Shelf life of Australian red meat

Second edition

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Shelf life of red meat

What is shelf life? The Oxford English Dictionary defines shelf life as: *The length of time that a commodity may be stored without becoming unfit for use or consumption.*

Australian meat has a reputation around the world for excellent shelf life. The Australian meat industry produces meat products with shelf lives ranging from a few days (entire cuts, roasts and ground meats) to several months (vacuum-packed primals) to more than one year (frozen manufacturing meat). The industry services domestic and export markets in the retail and further processing sectors, all of which impose specifications or standards which relate to shelf life.

The purpose of this book is to explain the important elements that contribute to shelf life so that everyone in the supply chain can do their part to maintain a superior standard. It is also intended for Australian meat customers so that they can understand the technical aspects of the product, what to expect of Australian meat and how to set appropriate criteria for product acceptance.

As will be seen from the Contents page, the scope of this book is wide. It provides up-to-date information on the shelf life of Australian meat for a range of users who operate in the technical, regulatory and marketing spheres. To satisfy this broad spectrum of readers, each section is 'paced', progressing from a basic level and ending with the latest research work; a reference list is provided at the end of each section for those who wish to read further.

This second edition contains numerous research updates from CSIRO and University of Tasmania on shelf life of primals and sub primals, and on interventions. The shelf life predictor has also been developed to a stage where it has great potential value, especially for emerging markets where the cold chain infrastructure is not well developed.

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1 Introduction

1.1 History

1.1.1 Australia's meat industry – where did we begin and where are we now?

A great deal is known about the early Australian meat industry thanks to two definitive texts: *A Settlement Amply Supplied* by Dr Keith Farrer and *A Review of Research since 1900* by Dr Jim Vickery. This introduction owes much to their work, which shows how shelf life was optimised, initially by techniques that stemmed from historical times (salting, boiling) and then with 'new' technologies such as thermal processing and refrigeration.

1.1.2 The first fleet

In early 1788, Australia's meat industry came into being when seven Zebu cattle landed with wobbly legs after months at sea below decks. The eleven vessels of the First Fleet also carried 44 sheep, 19 goats, 32 pigs and various poultry. Initially stabled at what is now Sydney Botanical Gardens, then driven to Parramatta, the beef herd wandered off into the bush. Seven years later they were found near the Nepean River numbering 61, and in much better condition. From these small beginnings, the Australian livestock herd was steadily built as ships landed at Port Phillip Bay and Tasmania (Van Diemen's Land).

1.1.3 Immediately after colonisation

Australia's meat industry was based on freshly-killed game and salted pork imported from Norfolk Island where there was a large wild pig population. Tahiti, then regarded as a dependency of New South Wales (NSW), also supplied large quantities of salted pork. It was almost a quarter of a century before cattle numbers supported the needs of the colonists and, in 1813, the Windsor community in western Sydney suggested exporting salted beef in barrels to Britain for use by the navy.

1.1.4   The first exports

By 1830 the industry was experiencing its first over-supply crisis. The price of livestock collapsed and as a result a group of pastoralists decided to develop an export trade in salted meat. The first shipment was made that year and a steady trade developed with Britain and Mauritius. The Sydney Salting Company was set up in 1843, patenting a method of rapid impregnation of the meat tissues with brine. The salted meat trade thrived for the next half century with annual exports as high as 1400 tonnes.

Australia's next venture in meat processing was 'sheep boiling' to produce tallow. At the time, sheep sold for as little as sixpence whereas rendering increased their value 10-fold. Process development was explosive and by the mid-1840s, sheep were being processed into tallow, mutton hams, pig feed, meat meal and bone meal for fertiliser, glue, bone oil and portable soup (a dried bouillon cake). Within a year there were 56 boiling-down works in NSW and a further export industry was established.

1.1.5   Meat canning

Meanwhile, in Europe, heat processing was being developed. In 1810, Nicolas Appert developed the first shelf stable food products, hermetically sealed in glass containers. Across the English Channel, tinplate containers were developed and Appert's methods were used to put food into 'metal boxes'. By 1812, trial shipments were being evaluated by the armed services and, in 1822, British emigrants to Van Diemen's Land were advised to take with them preserved meats, implying that canned foods were commercially available.

The meat canning industry in Australia became truly commercial in 1848 when Moses Joseph opened the Patent Preserved Provision Manufactory in Camperdown and Henry Dangar and Sons opened the Newcastle Meat Preserving Works. Canneries, or meat preserving works as they were known, sprang up all over Australia and achieved market domination for the next two decades.

1.1.6   Refrigeration – new export opportunities

Mechanical refrigeration ('cold on demand') revolutionised the food industry, replacing an existing global trade in natural ice. For the Australian meat industry, refrigeration offered an alternative to canned meat. Refrigeration in Australia was commercialised by James Harrison in 1859 with the opening of ice plants in Geelong and Melbourne, and the Sydney Ice Company in 1860. The ability to freeze meat followed and in 1873 the ship *Norfolk* was loaded with a trial shipment to England. The trial was unsuccessful when the circulating brine system failed.

In 1879, the *SS Strathleven* was fitted with mechanical refrigeration and loaded with meat in Sydney and Melbourne, which was then frozen on board. After a 60-day voyage the *Strathleven* arrived in London with a 34-tonne cargo in excellent condition. More importantly, the venture was a commercial success stimulating a frozen meat trade that was to dominate beyond the middle of the 20th century. Australia continues to ship large quantities of frozen meat to many destinations – over 1.4 million tonnes in 2015.

1.1.7   The chilled meat trade

While the good news was that an export market had been opened, the cliché 'tyranny of distance' had its impact when South American countries such as Argentina and Paraguay successfully landed chilled meat in London in 1907 after a 14-day voyage. The product was markedly superior to Australian frozen meat because there was no 'drip', and it attracted a price premium.

For the next 60 years, Australian research concentrated on trying to deliver chilled meat to distant markets. Various processes were investigated and scientists from the forerunner of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) found that carbon dioxide atmospheres ultimately gave sufficient shelf life even after voyages of up to 60 days. The first trial shipment of chilled meat under carbon dioxide took place in 1933, with forequarters from FJ Walker's Aberdeen works being held in gas-tight cargo spaces. While the trial was successful, validating the effectiveness of modified atmospheres on meat spoilage organisms, commercialisation did not take place until the advent of flexible packaging.

During the late 1960s, because of advances in packaging films and technology, it became possible to reach distant markets, particularly Japan, with chilled primals and subprimals. Vacuum packaging technology has now progressed to the point where shelf lives well over 100 days at -1°C are regularly achieved. Over 400,000 tonnes of chilled bovine, ovine, and caprine meats were exported in 2015.

The packaging system was not without problems. Early on growth of spoilage organisms on meat with a pH >6 resulted in product undergoing greening and developing a strong odour. Scientists at the CSIRO Meat Research Laboratory in Brisbane determined the cause – sulphmyoglobin produced by *Pseudomonas* (now known as *Shewanella) putrefaciens* – and the use of high pH (dark-cutting) meat for vacuum packing (VP) was discontinued.

1.2 Shelf life: what is it and when does it end?

The Oxford English Dictionary defines shelf life as: *The length of time that a commodity may be stored without becoming unfit for use or consumption.*

So, how can 'unfit' be judged? The Codex Alimentarius Commission (a body that sets international food standards, set up jointly by the World Health Organisation and the Food and Agriculture Organisation of the United Nations) has developed a *Code of Hygienic Practice for Meat.* Codex has, as its first principle, that: *Meat must be safe and suitable for human consumption and all interested parties, including government, industry and consumers, have a role in achieving this outcome*. The ideas about shelf life are summarised in Figure 1.1, and explained in the following paragraphs.

Figure 1.1 End of Shelf life: Aspects of product that may result in the end of shelf life.

Processing SECTION 2 → Packing SECTION 4 →
PATHOGENS - Section 1.3
SPOILAGE - Section 5.3
MICROBIAL GROWTH - Section 5.2
MEAT QUALITY - Section 3
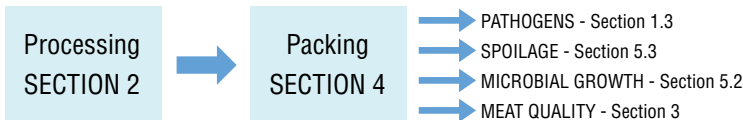
Meat is safe for human consumption if it has been processed according to all regulatory requirements and does not contain hazards that are harmful to human health. Sometimes the term 'safe for its intended use' is used which, in the case of meat, means it is intended for consumption after cooking. If processed according to regulatory requirements, frozen, vacuum packed or over-wrapped product always remains safe for consumption after cooking, provided that it has been stored with good temperature control.

Shelf life refers to the deterioration in characteristics such as colour, odour and taste that occur once a product is processed and is being stored. These are called organoleptic or sensory characteristics i.e. characteristics that can be sensed by eye, nose and mouthfeel. At all points along the marketing chain, shelf life is assessed using sensory criteria: do the product and the package containing it both look as they should and, at the time of cooking and consumption, is all well?

Meat and Livestock Australia (MLA) has conducted surveys of consumers in major markets, both nationally and internationally. These surveys tell us that consumers value the freshness of a product, which they judge by colour and odour. They also value taste and tenderness (depending on market) highly. Features such as colour and tenderness can be affected by many conditions, including those which can be controlled during processing. Thus, product can be dark in colour at the time of packing rather than having become darker during storage. Tenderness is affected by the breed and condition of the animal as well as processing conditions, but can also improve as product is stored (ageing under refrigeration).

All meat and meat products have a shelf life that is determined by the length of time that the characteristics of the product are expected to remain acceptable (i.e. suitable for human consumption) under specified conditions of packing and storage. It is possible that bacteria may grow in the product and cause a premature end to product acceptability, which is called spoilage. This situation should be unusual and can be controlled through the use of good processing standards. Table 1.1 lists criteria that define the usual end of shelf life for a range of red meats packed in aerobic (overwrap), vacuum and modified atmosphere (MA) packs.

Table 1.1 Sensory criteria at the end of shelf life

Retail meat package	Positive quality attributes	Attributes at end of shelf life
Over-wrapped tray (non-aged meat)	Pink-red bloom. Odour of fresh meat.	Loss of bloom, brown discolouration. Off-odours, off-flavours, slime.
MAP – high oxygen (non-aged)	Pink-red bloom. Odour of fresh meat.	Loss of bloom, brown discolouration. Off-odours, off-flavours, slime. Excessive purge (drip).
Vacuum-pack	Purple meat colour, tight pack.Short-lived confinement odour. Minimal drip.	Unacceptable, persistent confinement odour. Meat discoloured (brown, grey or green) in intact pack. Excessive purge (drip).
Over-wrapped (from VP-aged meat)	Good pink-red bloom. Odour of fresh meat. Minimal drip.	Loss of bloom, brown. Sour odour, flavour. Excessive purge (drip).
MAP – high oxygen (from VP-aged)	Good pink-red bloom. Odour of fresh meat. Minimal drip.	Loss of bloom, brown Sour odour, flavour. Excessive purge (drip).
Frozen ground beef	Pink-red.	Rancid odour, flavour when cooked. Surface desiccation, sponginess (freezer burn).
Frozen lamb chops	Pink-red.	Rancid odour, flavour when cooked. Freezer burn.

1.2.1   Expectations along the marketing chain

For a meat processor there is a chain of customers who have expectations for the product:

- Brokers or wholesalers, who store the product before moving it to the next purchaser
- Further processors, who portion meat for retail or food service use
- Retailers, who display product for sale
- Food service operators, who prepare meat for consumption
- Shoppers, who purchase meat

1.2.2   Customer perceptions

For the shopper, there are three occasions when quality is assessed:

- At purchase, when the meat appearance and package are evaluated
- On opening, when odour is the main sensory criterion
- On consumption, when odour, flavour and texture are assessed

If all expectations of the consumer are met, all links in the processing and marketing chain will have performed satisfactorily. If there are problems and the consumer complains to the retailer, repercussions will ramify along the chain, possibly even back to the processor.

As indicated in Table 1.1, sometimes shelf life ends because of production of off-flavours, odours, slime or colour change. Microbiological growth causes many of these changes.

1.3 Food safety and shelf life

One condition, taken as a given by customers, is that the product will be sound microbiologically. Food safety cannot be assessed by the consumer who relies completely on all participants in the processing and marketing chain to guarantee this factor. Meat will be safe for human consumption if it has been processed according to all regulatory requirements and does not contain hazards that are harmful to human health and is properly handled and cooked prior to consumption. Sometimes we talk about 'safe for its intended use' which, in the case of meat, is intended for consumption after cooking.

If processed according to regulatory requirements, frozen, vacuum packed or over-wrapped product remains safe for consumption after cooking provided that it has been stored with good temperature control and is consumed prior to reaching its stated shelf-life.

Sometimes shelf life is ended because of the presence of pathogenic bacteria, making the product unsafe for its intended use. Thus, finding *Listeria monocytogenes* in a 25 gram sample of ready-to-eat meat means that the product no longer has any remaining shelf life and must be destroyed because it is unsafe for consumption, even though the use-by date may be six weeks. Similarly, finding *Escherichia coli* O157:H7 in a 375 gram sample of frozen manufacturing meat destined for grinding into hamburger patties in the USA also signals the end of a product's shelf life, which otherwise may exceed 12 months, because it has failed to meet regulatory standards in the market.

A case of food poisoning that can be attributed to a particular product will also mean that the shelf life of other units from that production lot, and possibly other lots produced at a similar time or under similar conditions, will be over – the affected lot will be recalled and destroyed.

1.4 Product suitability and shelf life

As indicated in Table 1.1, most factors associated with end of shelf life can be perceived by using the senses: meat has lost its typical colour; unsightly drip and/or gas are present in the pack; or an objectionable odour persists when the pack is opened. The first two defects will prevent the product being purchased while the final one will prevent it from being consumed.

Identifying the time when any one defect renders the product suspect is an important step for a meat processor intending to set the shelf life for a particular product. A range of objective and subjective tools are available.

- Chemical methods can be used to measure the build-up of deleterious chemicals e.g. chemicals associated with rancidity in frozen meats
- Physical methods can be used to measure colour or texture (particularly toughness) of meat
- Sensory methods involving trained and untrained panels are routinely used in meat processing, focusing on criteria identified in Table 1.1.

1.5 References

Farrer, K. (1980). A Settlement Amply Supplied: Food Technology in Nineteenth Century Australia. Melbourne University Press, Melbourne.

Vickery, J. (1990). Food Science and Technology in Australia: A Review of Research since 1900. CSIRO Publishing, Sydney.

2   Processing and Microbiological Profiles

As will be illustrated throughout this book, meat quality, both in microbiological and sensory terms, is affected by a large number of factors both pre- and post-slaughter.

In this section we follow how livestock are transported, slaughtered, dressed and boned and see how microbiological profiles of the meat can be affected.

This section also reports on typical microbiological profiles of product at the beginning of their shelf life (i.e. at about the time of processing).

2.1   On the farm

Australia cattle and sheep are grazed extensively year round. Herds are mustered for routine production tasks such as castration, identification and animal health treatments. Other than shaded areas in feedlots, cattle are not housed for production purposes.

Lot-feeding of cattle is widely practiced, with around 800,000 head 'on feed' (Meat and Livestock Australia, 2014). For the domestic market, heifers and steers are held on feed for 70–80 days and for export, from 100–300 days.

2.2 Transport to the abattoir

Pointon *et al*. (2012) have reviewed the influence of transport and lairage on the microbiology of the animal presented for slaughter (see Figure 2.1). They found that the manner in which livestock are handled has a great effect on the pathogen status, potential shelf life and the chemistry/texture of the meat derived from the animal after slaughter. The major influences are:

- Distance and time over which stock are transported to the abattoir
- Withholding of feed and/or water prior to slaughter
- Holding times in the lairage.

Livestock are sometimes sourced far from the abattoir and it is not uncommon for cattle to be transported more than 1,500 km on journeys that exceed 24 hours. Transport can have major effects on the microbiological condition of livestock and the hygienic quality of the carcases derived from them. These factors include:

- stress levels in living animals
- degree of hide contamination.

Stress results in the depletion of glycogen in the living animal which, when the carcase undergoes *rigor mortis*, results in meat with a high pH (>5.7). High pH meat is termed DFD (Dry, Firm, Dry) or dark-cutting meat. More detail on meat chemistry and how it affects end-use and eating quality is presented in Section 3.

Figure 2.1 Pre-slaughter process steps showing internal and external contributing factors to animal and carcase contamination (after Pointon *et al.* 2012).

Internal Microbial Contributing Factors		External Microbial Contributing Factors
■ High shedding ■ Animal age ■ Seasonal shedding	**FARM**	■ Pasture type: grass/grain ■ Runny faeces ■ Season: wet/dry ■ Long/short hair/wool ■ Agitated at loading

■ Transport stress shedding	**TRANSPORT +/- SALEYARDS**	■ Cross contamination
		  – transport
		  – lairage/saleyard

■ High-shedding
■ Total time off feed
■ Long haul – rest period: short/long
	ABATTOIR	■ Cross contamination
		  – lairage
		  – stun box/shackling

■ Saliva and spillage from gut and respiratory tract ■ Increased external contamination	**CARCASE DRESSING CONTAMINATION**	■ Knives/hands ■ Fleece roll back ■ Hides and hooves ■ Aerosol

2.3 Hide contamination

Under its section on ante-mortem inspection and disposition, the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (AS 4696:2007) states that *"animals that are not clean are not passed for slaughter or slaughtered subject to conditions that ensure they do not contaminate animals, carcases"*.

The ability of the Australian transport industry to present cattle with a low level of 'tag' (mixture of soil, mud and faeces, frequently referred to as dags in Australia) was monitored using a technique developed for evaluating tag levels on North American cattle (Jordan *et al*. 1999). Cattle at three high-throughput abattoirs (>800 head/day) in Eastern Australia were scored at three locations (belly, leg and flank) immediately after cattle slaughter. Both grass-fed and feedlot cattle were assessed and the three site scores for each animal summed to give a total score on a scale of 0 to 9.

The distribution of tag scores from cattle slaughtered at the three abattoirs is shown in Figure 2.2. The data indicate a high standard of cleanliness of animals presented for slaughter with 94.5% of animals having tag score of 0 and the maximum from 400 animals in the survey being score 2. Data for Australian cattle are combined and overlaid with equivalent data obtained in the study of US and Canadian cattle (Jordan *et al*. 1999; van Donkersgoed *et al*. 1997). The data show a marked disparity in the cleanliness of the two study groups with Australian cattle having much lower and less variable tag scores.

The survey, while preliminary and undertaken over a short timeframe, indicates that abattoirs in Australia have greater flexibility in controlling the primary source of microbial contamination and the resources allocated for achieving hygienic dressing are far less likely to be overwhelmed by excessively contaminated animals.

Figure 2.2 Comparison between tag scores for cattle slaughtered in Eastern Australia (2003) and in North America (1998).
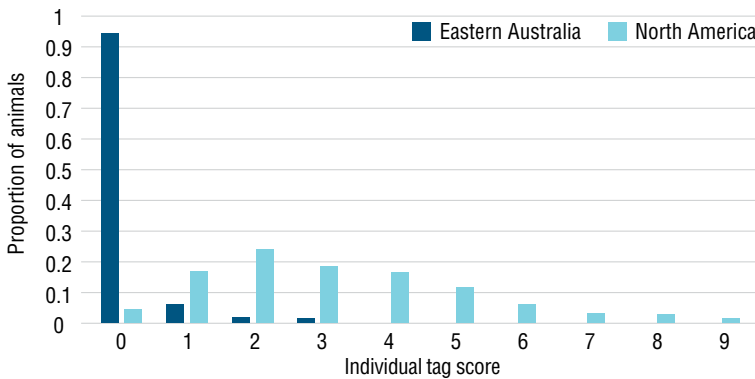
2.4   Withholding feed and/or water prior to slaughter (feed curfew)

2.4.1   Effect on *Salmonella* prevalence/concentration

Work done by CSIRO in the 1960s established that when cattle were fed regularly, the rumen produced volatile fatty acids that eliminated *Salmonella*. In contrast, when feed was withheld, both *Salmonella* and *E. coli* grew rapidly to counts of 1–2 log per mL of rumen fluid. Resumption of feeding after fasting for more than 48 hours was found to further stimulate growth of these organisms and to increase counts in the rumen and faeces (Brownlie & Grau 1967; Grau *et al.* 1968).

Fegan and co-workers surveyed *Salmonella* in the faeces of cattle presented for slaughter in Queensland, finding 67% (47/70) of grass-fed animals and 50% (35/70) of grain-fed animals positive for *Salmonella* with counts ranging up to almost 10,000 MPN/gram of faeces (Fegan *et al*. 2005a). An almost identical picture exists for sheep, with Grau *et al.* (1969) finding a 3-log increase of *Salmonella* in sheep starved for 72 hours.

Dewell *et al*. (2008a, b) estimated that the likelihood of having cattle hides positive for *Salmonella* at slaughter was increased two-fold for each of the following variables:

- Cattle from positive farms
- Transported in positive trailers
- Long haul to the abattoir (>160 km)
- Contaminated lairage pens
- More than 18 hours off-feed and agitated at loading

It is believed that many of these factors are common in transport regimes in Australia.

2.4.2 Effect on Shiga-toxic *E. coli* (STEC) prevalence/ concentration

While *Salmonella* colonises the rumen, Shiga-toxic *E. coli* (STEC) colonise the rectal wall (Chase-Topping *et al*. 2008). There doesn't appear to be a relationship between STEC shedding and feed curfew.

The occurrence of cattle super-shedders of *E. coli* O157:H7 (defined as having $>10^4$cfu/g of the pathogen in faeces) is of particular significance for hygienic dressing to prevent cross-contamination between subsequent carcases. In an Australian study of four lots each of 25 cattle, Fegan *et al*. (2005b) found that one animal shedding 7.5 x 10^5 MPN/g of *E. coli* O157:H7 in its faeces was one of six closely-grouped carcases from which O157 was isolated.

When pathogens are detected as part of a regulatory or customer required testing program by which lots of meat are passed for further processing, the shelf life of that lot is effectively terminated as far as its original purpose is concerned. The product is not spoiled but its shelf life is terminated prematurely for potential food safety reasons.

2.5   Holding times in lairage

The finding that pathogens survived on hides, in faeces and on lairage materials for more than one week indicated that pathogens could be carried over from one batch to another and/or from one day to the next (Small *et al*. 2002). In support of this pathway of contamination are the findings of Midgley & Desmarchelier (2001) who report contamination at the abattoir with STEC strains different from those at the farm and propose these strains are most likely from contamination within the abattoir environment. The same authors also observed that rain may influence the rate of STEC being excreted within a feedlot.

It is clear that pre-slaughter regimes on farm, in stock yards, during transport and during lairage at the abattoir can all have marked effects on the microbial loading of the finished carcase. Because of global market requirements the focus has been, and remains, on controlling pathogens, particularly pathogenic *E. coli*. However, cleanliness of stock presented for slaughter has impacts on the shelf life of meat cuts by influencing the initial microbial loading.

2.6   Processing at the abattoir

Since the early 1970s, when the international trade in chilled, vacuum-packed (VP) primals began, there has been anecdotal evidence that Australian product has a longer shelf life than that of its competitors. In recent years the benchmark has been 100 days at −1°C. Recently, shelf life trials undertaken by researchers from CSIRO have shown that VP striploins and cube rolls can be acceptable for up to 200 days after packing (see Section 6). One important factor in limiting shelf life is the initial microbial loading transferred to the meat during slaughter, dressing and boning. In this section, the ways in which beef and sheep are processed in Australian abattoirs to minimise contamination are summarised.

2.7   Beef slaughter and dressing

Very small plants may dress animals on a cradle or a frame with one operator carrying out all operations, this is referred to as solo butchering. The vast majority of calves and cattle, however, are slaughtered and dressed on a moving chain with each operator carrying out one or a small number of operations (figure 2.3).

Figure 2.3 Operator working on beef carcases

The major slaughter and dressing stages for beef are shown in Figure 2.4. Calves (mostly 1–2 weeks of age) are processed similarly to sheep (Figure 2.5) by either inverted or conventional dressing, usually on the sheep floor. The sole difference is that blood is drained via a thoracic stick directly into the heart of the calf.

Unit operations for slaughter and dressing are substantially the same throughout the global beef industry. However, the rate at which these operations are performed in Australia is much slower than in the USA. To determine chain speeds, Meat and Livestock Australia surveyed 40 Australian export abattoirs. The mean chain speed was 75 head/hour with a maximum of 330 head/hour, compared with large North American establishments, which exceed 300 head/hour.

In general, Australia is drier than North America or Europe and stock tend to have cleaner hides as a result. This along with slower chain speeds may assist in minimising the transfer of bacteria onto the surface of the carcase during dressing. The Controlling Authority in Australia requires that plants have documented procedures for carcase dressing and it approves and audits compliance against them. This has also helped focus the processing sector on the correct application of good hygienic practice.

Figure 2.4 Process flow chart for beef lairages, slaughter floors and boning rooms in Australian abattoirs.
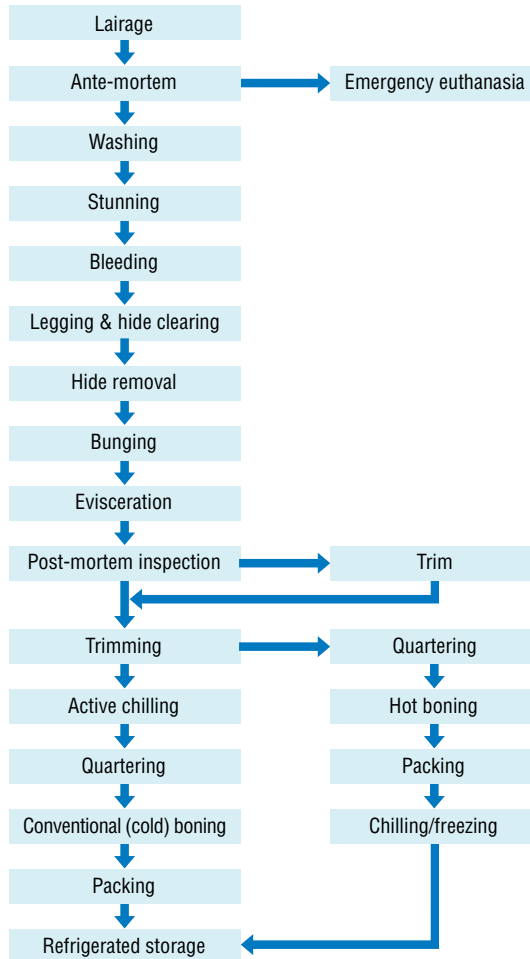
2.7.1 Contamination during pre-slaughter

The declaration by the US of seven serotypes of *E. coli* as adulterants (O26, O45, O103, O111, O121, O145 and O157) has increased the importance of presenting animals for slaughter with as little faecal contamination (sometimes termed 'tag') as possible. In Australia, this generally involves spraying cattle with chlorinated water, since more aggressive chemicals may have animal welfare impacts.

2.7.2 Contamination during slaughter

Before they are killed, cattle are usually stunned by shooting them in the head with a captive bolt. This method can drive extraneous matter into the brain and muscles in the head that may be used for human consumption. Other available stunning methods are less invasive i.e. mushroom head stunners and electrical stunning.

2.7.3 Contamination during hide removal

The industry generally raises the carcase to a rail where bleeding is completed and skinning and evisceration carried out. Contamination from hide to carcase occurs each time the hide is incised through its surface. Contamination is tracked from the hide along the cutting line and is unavoidable at the few points where such cuts need to be made, usually early in the skinning process. Once the hide is opened, subsequent cuts can be made from inside-out (so-called 'spear cuts'). Contamination can also occur from the operator's hands during opening cuts and later clearing of the hide.

Removal of the hide from cattle is done mechanically, the hide dragged from the carcase by a chain which winds around a drum. For cattle, the hide may be removed using either an upward or a downward hide puller. In both operations, the hide 'flaps' as it leaves the body; during this operation it is believed bacteria are liberated in dust and aerosol over the largely sterile carcase. Burfoot *et al*. (2006) suggest that this source of contamination is likely to be much less than that from direct hide contact. However, Schmidt *et al*. (2012) found that the airspace around hide pullers was a source of *E. coli* O157:H7 and *Salmonella*.

2.7.4 Contamination during evisceration

Once skinned, the carcase is susceptible to contamination from bacteria in the intestine, from ingesta in the paunch and from saliva and mucus in the head. Accidental incision of the gall bladder or bile duct has the potential to spread bacteria, as does spillage of milk or urine.

Early operations for cattle include closing off the weasand, preventing contamination from ingesta. Meat and the tongue can be contaminated by saliva and mucus during operations on the head chain.

Removal of viscera begins by freeing the anus ('ringing the bung') and then preventing it from leaking faeces by covering it with a plastic bag sealed with an elastic band around the rectum, an operation which minimises faecal contamination (Nesbakken *et al*. 1994). The abdominal cavity is then opened by making an incision large enough for the knife to be inserted and an inside-out cut made to open the abdomen. Intestines are removed for further processing usually onto a viscera table or, more rarely, into a barrow.

The cut margins of the abdomen are usually contacted by the forearms of the operator, particularly when the paunch is removed. The rib cage is incised by using a brisket saw that allows the heart, lungs and liver to be removed for inspection and further processing. Kidneys are also removed for inspection and further processing.

The body is split by sawing into two halves ('sides') using a large saw that is flushed with a constant supply of water during operation. The operation has become important because of the inclusion of spinal cord as Bovine Spongiform Encephalopathy (BSE) Specified Risk Material (SRM).

The carcase is inspected, then visible contamination and excess fat removed during trimming operations. Cattle sides may undergo a final wash before passing for active chilling.

There have been many studies on hygiene during slaughter and dressing, mainly from North America (reviewed by Gill, 2005). A number of studies from New Zealand have relevance because processes are similar to those in Australia. Bell (1997) studied contamination on beef carcases during the process and showed that the areas most heavily contaminated during skinning were the crotch, brisket and hind hocks. Bell also monitored the hygiene of operators' hands and contamination levels on knives, showing that 90% of contamination on knives was removed by pre-rinsing followed by a momentary dip in 82°C water.

2.8 Sheep slaughter and dressing

A comprehensive description of the stages and steps associated with sheep production in Australia is provided by Horchner *et al*. (2006). Primary production is based on an extensive grazing system. The major slaughter and dressing stages for sheep are shown in Figure 2.5.

Very small plants may solo butcher on a cradle or a frame. However, the vast majority of sheep are processed in medium and large abattoirs, some of which slaughter up to 10,000 animals/day. Animals are processed suspended from a moving chain, with operators undertaking only one or two operations. One of two methods is used – conventional dressing where the bodies are suspended by their hind legs, or inverted dressing where the animal is suspended by its forelegs. Chain speeds vary according to the stock being processed; they typically range from 350–750 bodies/hour.

Given their positions as major global exporters of sheep meat, much of the published work on the microbiology of sheep slaughter and dressing emanates from Australia and New Zealand. This includes Grau & Smith (1974), Grau (1979) and Nottingham (1982), who described sources of carcase contamination. More recently, Biss & Hathaway (1995) and Bell & Hathaway (1996) provided microbiological assessments of the two current dressing systems (conventional and inverted) used by Australian and New Zealand industries.

In conventional dressing, initial hide incisions and removal are from the rear end, prompting the suggestion that conventional dressing results in higher levels of contamination of bacteria of faecal origin. However, Bell & Hathaway (1996) found this premise to be confounded by other operational influences. Irrespective of whether bodies are dressed by conventional or inverted methods, there are a number of key influences on ultimate contamination levels:

- Opening Y-cut on forequarters
- Clearing shoulders and foreleg
- Removing, clearing, rodding and clipping the weasand
- Freeing the bung (anus)
- Opening abdomen and removal of the gastrointestinal tract
- Removing pluck (thoracic viscera)

In some establishments individual unit operations are undertaken by two operators, which effectively halves the line speed because each operator has twice as much time to perform the task.

A number of important management and operational variables have been evaluated by Kiermeier *et al*. (2007).

Figure 2.5 Process flow chart for sheep lairages, slaughter floors and boning rooms in Australian abattoirs.

```
                    ┌─────────────────────┐
                    │       Lairage       │
                    └─────────────────────┘
                               ↓
          ┌─────────────────────────────┐      ┌─────────────────────┐
          │   Ante-mortem inspection    │ ───→ │ Emergency euthanasia │
          └─────────────────────────────┘      └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │     Restraining     │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │ Electrical stunning │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │      Bleeding       │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │    Head removal     │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │ Pelt incision & cleaning │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │    Hide removal     │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │      Bunging        │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │    Evisceration     │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │ Post-mortem inspection │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │      Trimming       │
                    └─────────────────────┘
                               ↓
          ┌─────────────────────────────┐      ┌───────────────────────────┐
          │       Active chilling       │ ───→ │ Load out in carcase form  │
          └─────────────────────────────┘      └───────────────────────────┘
                               ↓
                    ┌─────────────────────────────┐
                    │ Carcase splitting at bandsaw │
                    └─────────────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │       Boning        │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │       Packing       │
                    └─────────────────────┘
                               ↓
                    ┌─────────────────────┐
                    │ Refrigerated storage │
                    └─────────────────────┘
```
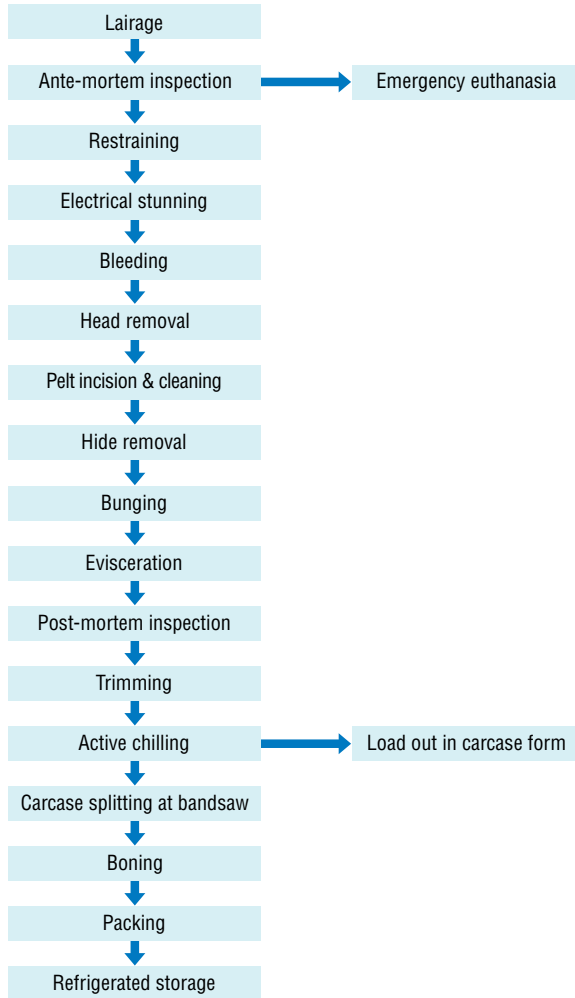
2.9 Antimicrobial Interventions

Australian meat processors have generally relied on good hygienic practices during processing to ensure that fresh meat is safe and wholesome. However, international markets for red meat are increasing their expectations and levels of evidence required to demonstrate that pathogenic *Escherichia coli* and other pathogens are not present on products. It is becoming increasingly difficult for processors to design systems that can show consistent results in meeting market expectations, particularly requirements with zero tolerance. To this end, Australian processors have begun to implement decontamination processes (antimicrobial interventions) to reduce carcase contamination during processing. Meat & Livestock Australia commissioned CSIRO and the University of Tasmania to review a number of interventions that may be applied along the red meat production chain, including those that are currently available and those that are being developed. This review is available on the MLA website (http://www.mla.com.au/off-farm/Food-safety/Food-safety-interventions)

Interventions are generally categorised as physical interventions (e.g. knife-trimming, washing), thermal interventions (hot water, steam pasteurisation) or chemical interventions (e.g. organic acid washes). They can be applied alone or sequentially at various stages during the production chain. However, implementing an intervention depends on a number of factors, including the required outcome, processes used, space availability and infrastructure in the existing premises, plus environmental factors such as waste and effluent disposal. It is also important to consider the constraints of the market (i.e. export and domestic regulatory status of the intervention) because regulatory agencies in some countries do not accept the use of intervention technologies as part of the fresh meat processing (Meat and Livestock Australia, 2015). For example, products destined for the EU market must not be treated with a non-approved intervention. By contrast, the US Food Safety and Inspection Service (FSIS) published '*E. coli* O157:H7 contamination of beef products' and accompanying guidance documents (USDA-FSIS, 2002 and 2003) which state that beef slaughter establishments should consider interventions that can be validated and verified as 'Critical Control Points for reducing or eliminating *E. coli* O157:H7'.

Interventions have their effect in:

Removing hazards by:

- Washing in water
- Trimming

Inactivating hazards by:

- Heat
- Cold
- Organic acids
- Others (e.g. radiation, high pressure)

Comprehensive reviews of decontamination strategies are available for physical (Bacon, 2005), chemical (Acuff, 2005) and emerging technologies (Guan & Hoover, 2005). A critical review of the effectiveness of such interventions has been made by Gill (2005). Interventions sometimes "fail" either because the population of the hazard exceeded its capacity to inactivate, or the active agent did not come into contact with the hazard. For example, Gill & Baker (1998) found no appreciable difference in the bacterial loading before or after steam vacuum treatment and pointed out that, to have any pasteurising effect, the meat surfaces must be raised to at least 85°C for at least 5 seconds, an impossibility at modern meat chain speeds.

2.9.1 Current Antimicrobial Interventions

Ideally an intervention will be a CCP and either:

- Prevent the hazard, or
- Eliminate the hazard, or
- Reduce it to an acceptable level (note that a zero tolerance specification doesn't allow an acceptable level)

In microbiological terms, an intervention should reduce the target hazard by at least 1 log unit to be considered a "real" improvement, such is the imprecision of microbiological testing.

Interventions can be made at various locations along the processing chain. It is common for North American operations to have multiple interventions though, in Australia, only interventions 1 and 3 are undertaken.

1. Hide-on carcase – Twin Oxide on cutting lines
2. Pre-evisceration – Organic acid +/- heat
3. Carcase sides – Trimming, Steam Vacuum, Organic acid +/- heat
4. Quarters at boning – Organic acid
5. Meat pieces – Organic acid
6. Boning room surfaces – Organic acid, UV light

No intervention can be expected to correct a highly contaminated product though steam vacuum is used to remove visible contamination. It is still necessary to follow strict hygiene practices and proper temperature control throughout the meat supply chain to produce safe and wholesome meat.

Table 2.1 Summary of the efficacy of the interventions currently available for Australian meat processors (from Meat and Livestock Australia, 2015).

Antimicrobial interventions	Claimed microbial efficacy (log reduction)
Hide wash and sanitise	1.5 – 2.0
Steam vacuum	1.0 – 2.0
Pre-evisceration acid rinse	1.0 – 1.5
Hot water or steam pasteurisation	1.5 – 2.5
Chilled carcase acid rinse	1.0 – 1.5

2.9.2 Proposed Antimicrobial Interventions

Current research by the University of Tasmania under projects A.MFS.0127 and G.MFS.0289. extends existing research findings on the effects of chilling on pathogenic *E. coli* physiology and behaviour on beef carcases. It emerges that *E. coli* is susceptible to oxidative damage during certain stages of chilling, a finding which has become a novel intervention during spray chilling (Kocharunchitt, 2012).

Trials in the laboratory and on-plant have shown chlorine dioxide, (ClO_2) to be very effective against *E. coli* and *Salmonella* when included in the spray chilling regime. Using chlorine dioxide in the final 20 cycles of the spray chilling process results in an approximately 3 log reduction in *E. coli* at the end of chilling (i.e. 14 h after commencing the chilling process). *E. coli* concentrations also appear to reduce further by at least 1 log within 9 days of storage at –1°C (Figure 2.6). The research highlights the potential for the proposed intervention to be implemented as a decontamination step for *E. coli* and related pathogens during carcase processing.

Figure 2.6 Population changes of a five-strain cocktail of non-pathogenic *E. coli* on untreated forequarter (blue squares) and forequarter treated with 200 mg/kg ClO_2 (red open squares) during commercial spray-chilling (left of dotted line) and subsequent storage (right of dotted line).
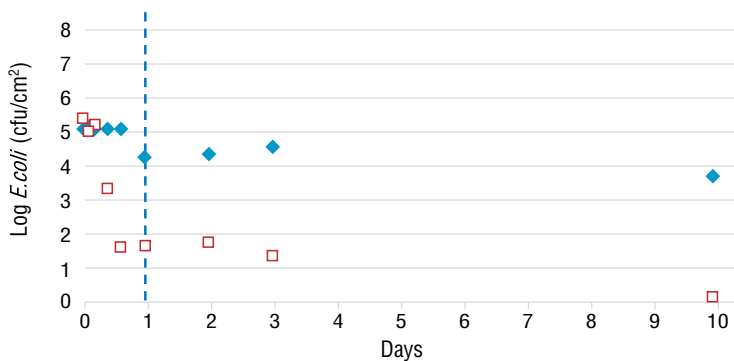
As well as inactivating target pathogens it was evident that ClO_2 application during spray chilling suppressed the growth of the total plate count (TPC), which prompted the idea that shelf life might be extended by the intervention.

The researchers followed the growth of lactic acid bacteria (LAB) on vacuum packed samples during storage at –1°C and found reductions of at least 1.5 log cfu/cm² TPC and 2 log cfu/cm² of LAB on samples treated with ClO_2 over the 30-day storage trial, a reduction which may prove important in complying with domestic supermarket specifications (see Section 8).

Figure 2.7 Increase in total bacteria count (TPC; A) and lactic acid bacteria (LAB; B) on vacuum packed meat during spray chilling (left of dotted line) and subsequent storage at –1°C (right of dotted line) for three independent trials. Data for untreated meat are blue, whereas data for meat treated with ClO_2-based intervention are red. ClO_2 treatment was applied at 200 ppm during the last 20 cycles of the spray chilling process.
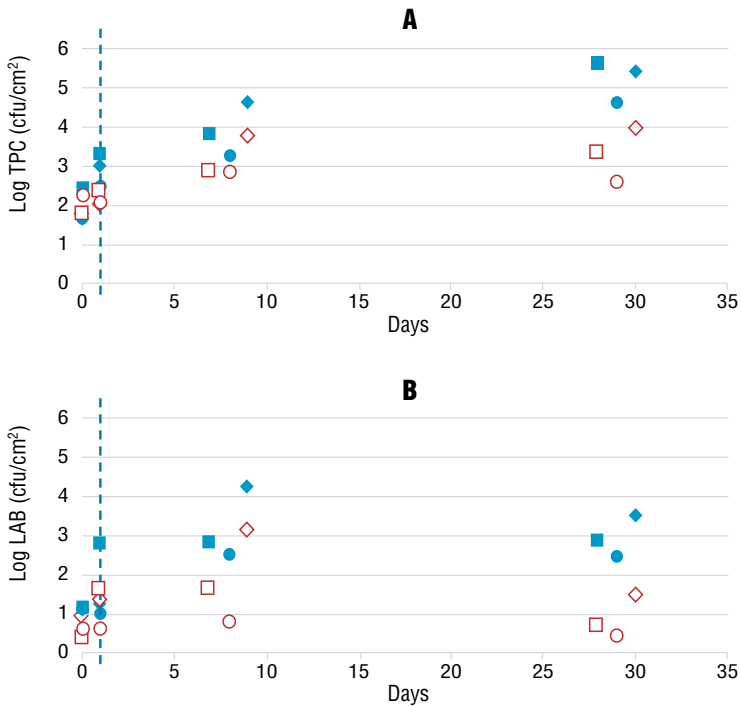
Taken together, the results of the trials to date indicate that spray chilling water containing chlorine dioxide can also be an effective intervention against *E. coli* and *Salmonella* on beef carcasses, and the intervention is being developed in the commercial setting.

2.10 Boning (Fabrication) of beef and sheep

In beef boning, one of two processes is followed. In quarter boning, each side is divided into two pieces – a forequarter and a hindquarter, each of which is further processed into primals, specific cuts and trim. In many rooms, though, side boning is undertaken where whole sides are processed along a single chain into specific cuts and trim. All boning operations have staff who bone, slice, trim and pack products. However, depending on the volume of product boned and the level of product quality control required, carcase breaking varies in complexity. At its simplest, portions from the body pass to a table where they are sliced, trimmed and packed into cartons. At its most complex, pieces of meat are boned onto conveyors and transported to stations where they are processed and then conveyed for packing.

In sheep boning each carcase is frequently divided by sawing into six pieces ('6-way cut'), a process which may be done manually or by robotic equipment. Legs, shoulders and loins are then transferred to boning stations for further processing.

In boning of beef and sheep carcases sources of contamination include:

- Carcases entering the boning room
- Knives and equipment of boners, slicers and trimmers
- Hands of packers
- Surfaces over which product passes

Boning rooms usually operate at 10°C or colder, a temperature chosen because *E. coli* will only increase two fold (one generation) at this temperature during an eight-hour shift.

Beef carcases are processed and packaged as:

- Carcases sent for further processing
- Primals or sub primals packed under vacuum or modified-atmosphere
- Pieces of trim packed in cartons and frozen for grinding
- Pieces of trim packed in tubs and despatched chilled for grinding

From the abattoir there are trading routes to:

- Retail butcher shops, which receive carcases and cartonned meats.
- Processing plants, which bone and package meat in cuts for retail sale and then transport them to supermarket distribution centres from whence they are withdrawn as needed.
- Smallgoods plants, which use trim and primals as components of sausages, cured, cooked and fermented meats.
- The export trade, which includes frozen cartons of primal cuts, trim and fancy meats, and chilled vacuum-packed and MAP meats.
- Pet food manufacturers for incorporation into canned or vacuum-packed products.

2.11 Chilling and freezing

2.11.1 Chilling and chilled storage

The objective of chilling carcase meat is to cool the meat quickly enough to prevent bacterial growth but not so quickly to cause cold shortening (toughening) of the meat. Most HACCP plans for production of chilled carcases and carcase parts, including cartoned meat, nominate chilling as a critical control point (CCP). Critical limits have to provide assurance that the control at the CCP is occurring reliably. Some critical limits are specified in the Australian Standard AS4696:2007 and in the Export Control (Meat and Meat Products) Orders. These include refrigeration index (RI) criteria.

Assessments against RI limits are determined by a predictive procedure that is described in some detail in Section 10.3.1 of this book and on the Food Safety Centre website *(http://www.foodsafetycentre.com.au/refrigerationindex.php).* Details of scientific information for establishing and reviewing critical limits and validation of chilling are also provided.

Factors that influence the adequacy of chilling include:

- Capacity of refrigeration system
- Pattern of loading of carcase and carton chillers
- Promptness of commencement of active chilling
- Size of carcases and cartons of product being chilled

Chilling of heavy beef sides that are too closely loaded into a poorly performing chiller may be too slow to prevent some bacterial growth. Hard fat on heavy beef and mutton carcases and the consequent physical difficulty with boning them can require the careful design and monitoring of chilling programs. More information can be found in Appendix 2: List of Meat Technology Updates.

The time for which meat can be stored at chill temperatures is influenced mainly by the species of animal, pH, initial level of bacterial contamination, storage temperature and the type of packaging.

High pH meat will spoil faster than meat with a pH of 5.3 to 5.7. The storage life can also be reduced by high initial levels of bacterial contamination on the surface of the meat because spoilage numbers of bacteria are reached sooner. The initial load on carcases is made up of a wide range of bacteria many of which do not grow on meat and others that will not grow under refrigerated conditions. Further, the types of bacteria initially present can change with geographical location and climate. Generally however, beef will keep longer than lamb, because lamb has a higher pH and lamb carcases tend to have higher initial numbers of bacteria due to differences in slaughter and dressing processes.

Chilled meat should be stored as cold as possible to maximise the storage period. A temperature of -1°C to 0°C is desirable and practical.

Vacuum packaging and packaging in a modified atmosphere of 100% CO_2, will greatly extend storage life. The practical storage life of different chilled meat products are listed in Table 2.2.

Table 2.2 Practical storage life of chilled meat.

Product	Storage Life
Carcases/quarters etc. in air (0°C to 2°C)	
Beef (stockinette)	3 – 4 weeks
Beef (overwrapped)	12 days
Lamb & mutton	10 – 13 days
Offals	7 days
Primal cuts – vacuum packed (0°C)	
Beef	20 weeks
Lamb & mutton	12 weeks
Beef & lamb offal	3 – 4 weeks
CO_2 (100%) gas flushed (0°C)	
Lamb & mutton carcases and cuts	Up to 16 weeks
*Primal – vacuum packed (–1°C)	
Beef	24 – 26 weeks
Lamb Shoulder	12 – 14 weeks
*See data presented in Section 6.	

2.11.2   Freezing and frozen storage

Although their growth is very slow, some psychrotrophic species of Gram-negative bacteria can still grow at -5°C or lower if the growth medium is not frozen (Partmann, 1975). However, in practice the crystallisation of ice in fresh meat tissue begins at -1.1°C (Calvelo, 1981) and very rarely would meat be considered not frozen at -5°C. Deterioration in palatability and colour of frozen meat can still occur due to fat oxidation and denaturation of proteins.

The current Australian regulations do not specify a temperature for frozen product – only that it must be hard frozen without delay and that the controls that are specified in the approved arrangement are achieved.

Various studies on the effect of different storage temperatures on fat oxidation and palatability of frozen meats indicate that a temperature of -20°C or lower is desirable for long-term storage.

The International Institute of Refrigeration recommends a temperature of -18°C for 'deep frozen food'. The Codex Alimentarius Commission's 'Recommended international code of practice for the processing and handling of quick frozen foods' states that cold stores should be operated so as to maintain a product temperature of -18°C or lower with a minimum of fluctuation.

Many of Australia's international customers follow these guidelines and require frozen meat to be delivered at a temperature of -18°C or colder. The benefit of storage under these conditions is illustrated by storage times in Table 2.3. The Australian industry's experience is that these shelf lives are very conservative. For example, frozen manufacturing beef usually has 24 months shelf life at -18°C.

Table 2.3 Practical storage life (months) at three storage temperatures.
Source: International Institute of Refrigeration (2006).

Product	−12°C	−18°C	−24°C
Beef carcase (not packaged)*	8	15	24
Veal carcase (not packaged)*	6	12	15
Lamb carcase (not packaged)*	18	24	>24
Pork carcase (not packaged)*	6	10	15
Beef cuts, steaks	8	18	24
Beef minced (ground)	6	10	15
Veal cuts, steaks	6	12	15
Lamb steaks	12	18	24
* Carcase may be wrapped in stockinette			

While some smallstock carcases and beef quarters are frozen for storage
and transport, most meat and meat products are frozen in cartons in either
air blast freezers or plate freezers. Contributing factors to quality problems
with frozen meat and meat products are usually delayed/slow rates of
freezing, interruptions to refrigeration or excessive fluctuations in frozen
storage temperature.

In a 1990s study of offal freezing for the Meat Research Corporation, the key
effects on cooling and freezing rate were the depth and weight of the filled
carton, the temperature of the product at the time of packing and the type of
freezer used. The best results were achieved when:

- There were minimal delays between evisceration and the
  commencement of active carton freezing
- There was some cooling of the offal before it was packed into the
  carton
- Plate cartons were not greater than 155 mm in depth and
- Plate freezing was used

Most of the beef for manufacturing is exported from Australia in shipping containers as cartons of frozen product. Some departures from best practice that can potentially affect frozen storage life include:

- Cartons being loaded into containers before the meat temperature has been reduced to the carriage temperature (–18°C or colder). Refrigeration units on containers are designed primarily to maintain shipping temperature. Reduction of temperature of meat in cartons that are closely packed in containers is slow. It is particularly important that hot-boned meat and meat products (beef livers for example) that are packed warm into cartons are fully frozen before the cartons are transferred to containers.
- Container doors not being closed immediately after completion of loading, leading to higher temperatures at the beginning of the voyage. Cartons have been known to take around two weeks to reach a carriage temperature of –20°C.
- Cartons being loaded too close to the doors thereby restricting air flow. This results in higher meat surface temperatures at the door end of the container.
- Off-power periods during road or rail transport to shipping ports or on board the ship during the voyage. Periods of 12 hours or so have been shown to have an insignificant effect on meat temperature, but longer periods off-power can lead to significant rises in meat temperature, particularly in cartons in corners of containers.

The results of an investigation to assess the effect of breaks to the frozen cold chain were summarised in Meat Technology Update 07-1 (Appendix 2), where severe cases of temperature abuse were simulated. While some cartons were held at a constant –20°C, others were allowed to warm in a manner similar to that in a container off-power for two days, and a third group was exposed to ambient temperature of 25°C for five hours. At the end of 12 weeks storage, samples were analysed for fat content, anti-oxidants and products of lipid oxidation. Neither the chemical analyses nor taste panel assessment detected any differences between the temperature-abused groups and the product stored at a constant –20°C.

Another study in the container test facility at the North Ryde, NSW site of Food Science Australia, found that when cartoned meat was at –18°C or colder, a shipping container may be off power for 19 hours on a typical sunny summer day before the meat temperature at the corners rises to –10°C and for 56 hours before it reaches –5°C.

Desiccation can be a problem with frozen meat. It is exacerbated by inadequate packaging and by fluctuating temperatures during storage. Freezer burn is one consequence of this water loss. The ice evaporates directly from the frozen state, leaving voids where the ice crystals were and a honeycomb structure. Extensive freezer burn is irreversible because the meat proteins have been denatured and will not rehydrate. This denaturation may be accompanied by the development of off-flavours due to oxidation of the fat. Products with a large surface area, e.g. patties, are particularly susceptible. More information on water loss, frosting and freezer burn in packs of frozen product can be found in Meat Technology Update 00-6 (Appendix 2).

2.12 Microbiological profiles of product

2.12.1 Microbiological profile of beef primals

The microbiological profile of indicator organisms and *S. aureus* on Australian chilled beef primal cuts was undertaken at 29 establishments (Table 2.4) as part of the 4th national baseline survey (Phillips *et al*. 2012a).

E. coli O157:H7, *Campylobacter* and *Salmonella* were not recovered from any of 1144 primal cut samples, while *Listeria spp*. were recovered from one of the 1144 (0.1%) samples at a concentration of 1 cfu/cm^2.

Table 2.4 Microbiological profile of Australian beef primal cuts (n=572 striploins, n=572 outsides).

| | Prevalence (%) | Concentration (log cfu/cm^2)[a] | | | |
		Mean	Median	SD	Maximum
TPC					
striploin	99.1	1.3	1.1	1.0	5.3
outside	99.1	1.5	1.3	1.0	4.2
Coliforms					
striploin	33.0	−0.5	−0.6	0.7	2.3
outside	43.5	−0.3	−0.5	0.8	2.4
E. coli					
striploin	10.7	−0.5	−0.6	0.7	2.3
outside	25.2	−0.3	−0.5	0.9	2.4
Coagulase-positive *staphylococci*					
striploin	7.7	0.2	0.2	0.7	1.4
outside	8.4	0.2	0.3	0.7	1.6

[a] Limit of detection 0.08 cfu/cm^2 and counts are for positive samples only

Comparisons may be made with international studies of primal cuts. In New Zealand during the late-1990s shelf life studies of hot-boned primals found striploins sampled just prior to vacuum-packaging had a mean TPC of 2.5 log cfu/cm² (Bell *et al*. 1996; Penney *et al*. 1998); with the latter study also finding that conventionally (cold) boned striploins had a mean TPC of 3.0 log cfu/cm². In Switzerland, beef primals (n=200) sampled at an EU-approved establishment had a mean TPC of 2.5 log cfu/cm² (Zweifel *et al*. 2005).

In the USA, clod (shoulder) and top butt (top sirloin) were sponged on the fat and lean surfaces to give mean TPCs of 2.25 log and 2.27 log cfu/cm², respectively (Ware *et al*. 2001). Also in the USA, Stopforth *et al*. (2006) carried out a six-month study on beef primals from two establishments in which 1022 samples were taken from 10 primal cuts. TPCs ranged from 4.0 log cfu/g for butts and rib eye rolls to 6.2 log cfu/g on club ends; on striploins the mean TPC was 5.9 log cfu/g. *E. coli* O157:H7 was isolated from 3/1022 (0.3%) and *Salmonella* from 22/1022 (2.2%) of samples; *E. coli* O157:H7 was not isolated from striploins but 5/52 (9.6%) yielded *Salmonella*.

2.12.2   Microbiological profile of sheep primals

The microbiological profile of Australian sheep primal cuts at 12 meat processing establishments was studied (Table 2.4) as part of the 4th national baseline study (Phillips *et al*. 2012b). The mean TPC was 2.02 log cfu/cm² for legs and 2.29 log cfu/cm² for shoulders; maxima were 4.64 and 6.21 log cfu/cm², respectively. *E. coli* were detected on 42.9% and 34.6% of leg and shoulder samples, respectively. Coagulase positive staphylococci were detected on 4.2% of leg samples and 5.2% of shoulder samples.

E. coli O157:H7 was detected on 2/613 (0.3%) leg and 1/613 (0.2%) shoulder samples. *Campylobacter* was detected on 1/613 (0.2%) shoulder samples. *Salmonella* was detected on 17/613 (2.8%) leg and 5/613 (0.8%) shoulders samples. *Listeria sp*. were isolated on 1/613 (0.2%) leg samples.

Table 2.5 Microbiological profile of Australian sheep primal cuts (legs n=613; shoulders n=613).

	Prevalence (%)	Concentration (log cfu/cm^2)[a]			
		Mean	Median	SD	Maximum
TPC					
leg	100	2.02	2.00	0.81	4.64
shoulder	100	2.29	2.15	0.96	6.21
Coliforms					
leg	52.0	−0.36	−0.48	0.72	2.36
shoulder	46.2	−0.54	−0.77	0.63	3.10
E. coli					
leg	42.9	−0.44	−0.60	0.69	2.36
shoulder	34.6	−0.63	−0.77	0.56	1.82
Coagulase-positive staphylococci					
leg	4.2	−0.21	−0.09	0.60	1.15
shoulder	5.2	0.34	0.21	0.93	2.96

[a] Limit of detection 0.08 cfu/cm^2 and counts are for positive samples only

2.12.3   Microbiological profile of frozen boneless beef

The microbiological profile of indicator organisms and *S. aureus* on Australian frozen boneless beef trim was undertaken at 29 establishments (Table 2.6) as part of the 4th national baseline survey (Phillips *et al*. 2012a). The mean TPC (n=1165) was 2.22 log cfu/g. The maximum was 5.53 log cfu/g. *E. coli* were detected in 2.1% of the samples. Coagulase positive staphylococci were detected in 3.4% of the samples.

Table 2.6 Microbiological profile of frozen boneless beef trim (n=1165).

	Prevalence (%)	Concentration (log cfu/g)[a]	
		Mean	Maximum
TPC	94.9	2.22	5.53
E. coli	2.1	1.32	2.51
Coagulase-positive staphylococci	3.4	1.93	4.76

[a] Limit of detection 10 cfu/g and counts are for positive samples only

2.12.4 Microbiological profile of frozen boneless sheep meat

The 4th national baseline study (Phillips *et al*. 2012b) also included samples of Australian frozen boneless sheep meat taken from 12 establishments (Table 2.7). The mean TPC (n=551) was 2.80 log cfu/g. The maximum was 5.51. *E. coli* were detected in 12.5% of the samples. Coagulase positive staphylococci were detected in 1.8% of the samples.

Table 2.7 Microbiological profile of frozen boneless sheep meat (n=551).

	Prevalence (%)	Concentration (log cfu/g)[a]	
		Mean	Maximum
TPC	99.1	2.80	5.51
E. coli	12.5	1.51	3.30
Coagulase-positive staphylococci	1.8	1.66	2.32

[a] Limit of detection 0.08 cfu/g and counts are for positive samples only

2.12.5 Microbiological profile of ground meat

Ground or diced beef and lamb are manufactured by three distinct groups of establishments:

- Central processing and packing plants
- Supermarkets back-of-house
- Butcher shops

Raw material used for grinding in Australia is based on frozen or chilled manufacturing trim (as distinct from carcase trimmings) plus trimmings from carcases and primal cuts and vacuum-packed primals. Ground meat is sold in bulk or formed into hamburger patties or rissoles, packed either in aerobic film or in modified atmosphere packs for retail, or frozen for fast food restaurants.

The microbiological quality of ground meat is influenced primarily by the source material used for grinding (Table 2.8):

- Trim from boning operations – generally called meat for manufacturing.
- Meat trimmed from primal cuts, sometimes called bench trim. Comprises largely surface meat with a higher bacterial load compared to primals, in which the internal muscle is sterile (Sumner *et al*. 2011).
- Meat trimmed from vacuum packed primal cuts, where the count will vary according to the age and storage temperature of the cut. To obtain sufficient shelf life at retail, large grinding operations usually limit the age of primals to no more than 28 days.

Table 2.8 TPC and *E. coli* prevalence of raw materials used for ground beef

Beef mince	Mean total count/g	*E. coli* prevalence (%)
Made from primals	25,000	46
Made from bench trim	316,000	52

As part of a national baseline survey of ground beef and diced lamb Phillips *et al*. (2008) purchased 360 samples of each product type from retail outlets in Brisbane, Sydney and Melbourne. As shown in Table 2.9 mean total bacteria approached 1 million/g in both products with maxima around 100 million/g. It should be emphasised that all products were within their use-by date when purchased.

Table 2.9 TPC and *E. coli* prevalence of Australian chilled ground beef and diced lamb at retail outlets (n=360)

	Ground beef		Diced lamb	
	TPC [a]	*E. coli*	TPC [a]	*E. coli*
Prevalence (%)	100	17.8	100	16.7
Mean log cfu/cm^2	5.79	1.49	5.71	1.67
Maximum	8.00	2.98	8.49	4.18

[a] Limit of detection 10 cfu/cm^2 and counts are log cfu/cm^2 of positive samples only

2.13 Conclusions

Australian carcases, chilled or frozen primal cuts and bulk-packed trim have low bacterial loadings and are only occasionally contaminated with pathogens. This compares favourably with products manufactured overseas and accounts for the anecdotal evidence that Australian vacuum packed primals have a longer shelf life than those of their competitors. However, storage temperature and conditions are still significant factors in maximising storage life.

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3   Meat Quality

In the living animal, the energy for normal body functions is supplied by Adenosine Tri Phosphate (ATP) molecules, produced by the breakdown of glycogen and glucose (a process known as glycolysis). After slaughter, blood circulation to the muscles ceases and, with it, the supply of oxygen. As residual oxygen is used up, lactic acid accumulates in the muscles and rigor mortis begins.

Key influences of meat quality are colour, tenderness and flavour. A number of factors affect meat quality; these relate both to the living animal as well as how it is slaughtered and the carcase chilled. In this section we cover those factors.

3.1   Meat colour

Meat colour has the primary influence on customer choice, mainly because a bright red colour is linked with freshness and quality. On exposure to air, meat pigment, myoglobin, absorbs oxygen, forming the bright red, oxymyoglobin and producing the 'bloom' expected by the consumer. Prolonged exposure to air results in oxidation of myoglobin to metmyoglobin and a brown meat colour. In the absence of oxygen, for example in a vacuum-pack, meat is purplish-red, being in the form deoxymyoglobin (Figure 3.1).

Figure 3.1 Interconversion of different forms of muscle myoglobin.
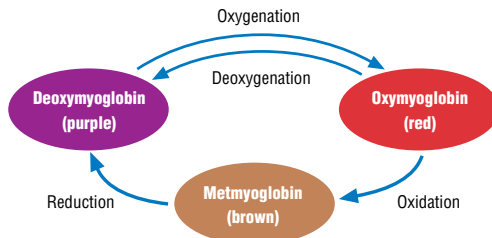
There are two important aspects of meat colour: blooming and colour stability. Blooming is the oxygenation of myoglobin to oxymyoglobin. When meat is removed from a vacuum-pack, deoxymyoglobin is converted (oxidized) to oxymyoglobin. Oxygenation occurs progressively and blooming takes up to 30 minutes. The red, oxygenated layer is only 3 – 4 mm at 0°C, and narrower at higher temperatures. Because the bright red colour is enhanced by elevated concentrations of oxygen, most meat on retail display is either wrapped in oxygen-permeable film, or sealed in a modified atmosphere package high in oxygen (around 80%).

3.1.1   Browning

When meat is exposed to air, it gradually loses its red colour, as oxymyoglobin is converted to metmyoglobin, and becomes brown (Figure 3.2). Unfortunately, consumers equate such browning with end of shelf life. Equally unfortunately, metmyoglobin is a stable pigment and is only slowly reconverted to deoxymyoglobin.

Figure 3.2 Left photo shows when meat is exposed to air (oxymyoglobin) and right figure shows the development of brown discolouration (metmyoglobin).

Muscles vary in their ability to reconvert metmyoglobin to deoxymyoglobin, with muscles such as the striploin *(longissimus dorsi)* being relatively colour-stable compared with other muscles, such as the cube roll *(longissimus thoracis)* muscle.

Tenderloin (*psoas major*) is one of the least stable muscles; it loses red colour rapidly in retail display. The ability to reconvert metmyoglobin is gradually lost as meat ages, such as in storage of vacuum-packed meats. This means that, when the pack is opened and meat is cut for retail display, the older the meat, the faster it will lose its red colour. As shown in Table 3.1, when primals have been stored for more than four weeks, the retail display time of portions cut from them is reduced to two days in overwrap and four days in MAP.

Table 3.1 Retail storage time (days) of portions cut from vacuum-packed primals.

	Storage time in vacuum-pack (weeks at 0°C)				
	0	2	4	6	8
Overwrapped trays (days)	3	3	2	2	1
MAP (high oxygen) (days)	7-10	5-6	4	3	2

3.1.2   Two-toned (pale and dark) meat

Two-toning usually refers to meat in which there are undesirable gradations in meat colour within a cut, usually with the deep meat tissue being paler than the normal red meat closer to the surface. Colour loss (paleness) is caused by denaturation of meat proteins at relatively high temperatures (30°C) when the pH is low, due to acidity resulting from accumulation of lactic acid in the early stages of *rigor mortis*.

It normally occurs in the deep muscle (e.g. of topsides) of heavy carcases where chilling is slow.

Two-toning can also occur at the cut surface of a piece of meat. Different muscles have different biochemical activities, so the rate of discolouration varies between muscles and this can lead to different colours in a single cut (Figure 3.3).

Figure 3.3 Two-toning of lamb leg steaks.

3.1.3   Dark-cutting meat

In the living animal, muscle pH is around 7 and, as lactic acid accumulates, pH falls to around 5.4 – 5.6 within 24 hours *post-mortem*. Stress prior to slaughtering affects glycogen levels in the muscle and ultimate meat pH, which in turn affects colour. Generally meat colour gradually darkens with increase in pH and if beef has a pH of 5.8 or more it is usually classified as 'dark cutting', 'high-pH', or DFD (dark, firm, dry) beef.

High pH meat has lower acid level, greater water holding capacity and less oxymyoglobin formation at the surface. Reduced myoglobin under the surface gives a dark appearance to the meat and dark cutting meat does not bloom when exposed to air.

If vacuum-packed, high pH meat spoils more rapidly than normal pH meat. This is partially because of spoilage bacteria such as *Serratia liquefaciens* and *Alteromonas putrefaciens* (now known as *Shewanella putrefaciens*). These bacteria utilise amino acids as a carbon source resulting in production of sulphmyoglobin (greening) on the surface of the meat (Gill & Newton, 1981).

3.1.4   Colour of vacuum-packaged and MAP meat

The bright red oxymyoglobin colour of fresh meat disappears in vacuum-packs and in CO_2-flushed packs as the pigment reverts to its purplish-red form. This is the normal and desirable colour of vacuum-packed meat. Within a short time of the pack being opened, the purple myoglobin at the meat surface changes to oxymyoglobin and the meat blooms again to a bright red colour, in response to the oxygen present in air.

Not all the air will be evacuated from a vacuum-pack, but residual oxygen should be used up by respiration in the meat tissue. How well this residual oxygen is used up is dependent on the age of the meat when packed, because its respiration ability declines with time. If the meat is older than around 48 hours post-slaughter at the time of packing, there may be insufficient meat respiration to consume the residual oxygen. If there is residual oxygen in the pack, or if there are poor seals, pinhole punctures, or poor air evacuation at the time of packing and sealing, the meat will turn brown during storage.

Colour problems will also occur with beef and lamb stored in high concentrations of carbon dioxide unless oxygen is excluded from the pack. As with vacuum-packs, if 0.5 – 1% oxygen is present, the rate of browning will be higher than in air. In addition to discolouration of the lean surface, problems with the appearance of fat surfaces may occur (grey-brown discolouration). With lamb, a brownish discolouration of the fascia (connective tissue) surfaces may develop after several weeks' storage at 0°C if there is too much oxygen present.

3.1.5   Greening

Green colour (sulphmyoglobin) develops in meat when myoglobin reacts with hydrogen sulphide (H_2S) generated by the growth of *Shewanella putrefaciens*. This organism requires meat pH >5.9, plus low levels of oxygen (Figure 3.4). The good oxygen barrier provided by most modern films and temperature control below 0°C, means that sulphmyoglobin greening is uncommon. The industry practice of not using DFD primals for vacuum packing has also minimised the likelihood of greening. When sulphmyoglobin green packs are opened, the green colour often disappears, because the pigment is reconverted to oxymyoglobin (red).

Figure 3.4 Greening in vacuum-packed lamb cuts (below) compared with 'normal' pack (above).

3.1.6 Brown and black spots

Brown discolouration in the form of spots on fat surfaces has been attributed to the yeasts *Yarrowia lipolytica* (formerly *Saccharomycopsis lipolytica)* and *Candida zeylanoides*. Yeasts can survive and grow on chilled meat stored in air and in vacuum-packed meat if the oxygen transmission rate of the packaging film is too high. Brown spot was a problem on vacuum-packaged beef in the 1970s, but is rare today.

Black spots may develop on frozen meat stored at –5°C for 40 days or more, and this has been associated with a number of yeasts and moulds mainly *Cladosporium* spp. These organisms penetrate into the meat surface, and must be trimmed.

3.2 Factors affecting meat colour

Beside animal age, species and muscle type there are a number of other *ante-mortem* and *post-mortem* factors that affect meat colour. These can occur through different mechanisms, such as changes in pH and reduction-oxidation chemistry of the muscles. Diet is known to affect antioxidant levels, which affect colour stability, together with glycogen reserves, thereby influencing lactic acid production and ultimate pH.

3.2.1 Finishing diet

Vitamin E is a powerful antioxidant that affects meat colour by influencing oxidation-reduction conversion of three forms of muscle myoglobin. Its supplementation in animal diet is known to improve both bloom and colour stability of meat. Grass is a good source of vitamin E, but animals can become deficient in vitamin E in the absence of an ample supply of green grass, especially during the dry season. This seasonal effect can be avoided by supplementing the finishing diet (two to four weeks before slaughter) with vitamin E.

In addition to vitamin E, the energy content of the finishing diet is also important for meat quality. Energy content affects the amount of glycogen reserves in muscle, thus influencing the amount of lactic acid and ultimate pH of meat post-slaughter.

3.2.2   Carcase chilling

It is important to reduce the carcase temperature as quickly as possible during processing to reduce microbial growth. However, fast chilling can sometimes result in cold shortening, which occurs when carcases are chilled below 15 – 16°C or frozen before the completion of *rigor* as demonstrated in the Figure 3.5 below. Electrical stimulation is used to prevent cold shortening and accelerate the rate of tenderisation. Another unfavourable condition can be hard fat, particularly in larger beef sides and mutton carcases, which makes boning operations difficult.

Figure 3.5 Acceptable pH decline in loin muscle during chilling.
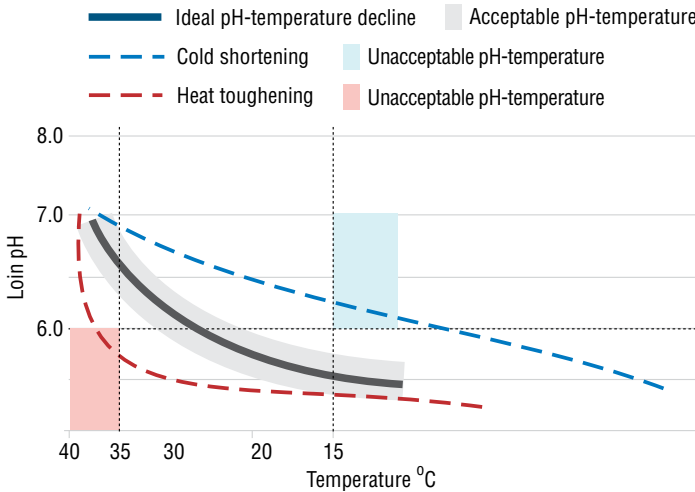
3.2.3   Electrical stimulation

Electrical stimulation is used to accelerate the rate of tenderisation during ageing and to prevent 'cold shortening'. These effects are mediated by an increase in the rate of pH decline *post-mortem*. A fast rate of pH decline will generally make the bloom colour lighter and more attractive to consumers.

Over stimulating can reduce colour stability by causing a very low pH when the carcase temperature is still high. However, this condition, known as 'heat toughening' (see Figure 3.5), occurs mainly in beef carcases that cool very slowly (e.g. heavy, grain-fed cattle) compared with lamb carcases that cool more quickly.

3.2.4   Ageing

Holding meat above its freezing point for a period of time is known as conditioning or ageing and has long been associated with improved tenderness and flavour. The ageing period refers to the time from slaughter to retail sale and will vary from a few days for meat sold domestically, to more than 100 days for some export markets. While, tenderness improves over a period as long as 30 days, ageing can reduce colour stability. The greatest improvement in tenderness occurs during the first two weeks of ageing, primarily from the structural weakening of key myofibrillar proteins.

3.2.5   Packaging to extend shelf life

If meat is to be stored/aged for lengthy periods, methods such as vacuum or modified atmosphere packaging are recommended to extend shelf life. Meat stored in vacuum or in 100% CO_2 MAP is purplish-red, a normal, desirable colour for these sorts of packaging. During vacuum packaging, air is removed from the package resulting in an atmosphere that usually contains less than 0.5% O_2, around 20–40% CO_2 with the remainder being N_2. Within minutes of opening the pack, purple myoglobin at the surface changes to bright red oxymyoglobin i.e. blooming takes place; however, aged meat does not hold its colour in air as well as non-aged meat. Beef and lamb stored under higher CO_2 levels appear to have greater colour stability than vacuum-packed beef and lamb. Retail display cuts prepared from lamb primals/carcases that have been stored at 0°C in a CO_2 atmosphere for 12 weeks have a display life in over-wrapped packs of around two to three days.

3.3   Meat tenderness

Meat tenderness is a complex quality parameter; it is usually measured though sensory evaluation by consumers, or in the laboratory as the amount of force necessary to bite through a piece of meat and fragment it (Kerth, 2013). Components of tenderness include:

- Protein tenderness (sarcomere (the basic unit of muscle) state and amount of myofibrillar protein degradation)
- Amount of connective tissue
- Background effect (flavour and juiciness)

3.3.1   Protein tenderness

During *rigor*, muscles shorten – the shorter the muscle, the less tender it will be. The extent of this shortening/contraction depends upon the stretching or restraint applied to individual muscles as the carcase goes through *rigor*, and the rate of chilling. Besides physical factors, muscle biochemistry also influences muscle contraction. Muscle is highly sensitive to its energy reserves (ATP) and calcium, as both are involved in the contraction-relaxation process.

Lack of ATP in muscles results in their shortening even before *rigor* completion. On the other hand, if excess calcium is present when ATP is also present, excessive shortening known as cold shortening takes place. This occurs when the carcase is chilled too quickly, before significant reduction in pH. Electrical stimulation applied to the carcase after slaughtering, prior to carcase chilling can help to prevent cold shortening.

3.3.2   Protein degradation

During post-mortem ageing, myofibrillar proteins (the structural proteins of an animal) start to degrade through the action of enzymes known as proteolytic enzymes. There are a number of proteolytic enzymes, such as cathepsins and calpains, naturally present in muscles. The major sites for structurally important proteolysis are the intermediate filaments (primarily titin, which anchor myosin to the Z-disc), M-filaments (primarily skelimin, which link adjacent M-lines), and the costamere structures (link Z-discs to the sarcolemma and to each other) (Figure 3.6). However proteins actin and myosin are not degraded during ageing. The activity of these enzymes especially calpains is dependent upon the amount of calcium and other enzymes present in post-mortem muscles. Nutrition, farm practices and genetics also affects levels of these enzymes and thus meat tenderness.

Figure 3.6 Muscle structure including actin (thin filament) and myosin (thick filament)
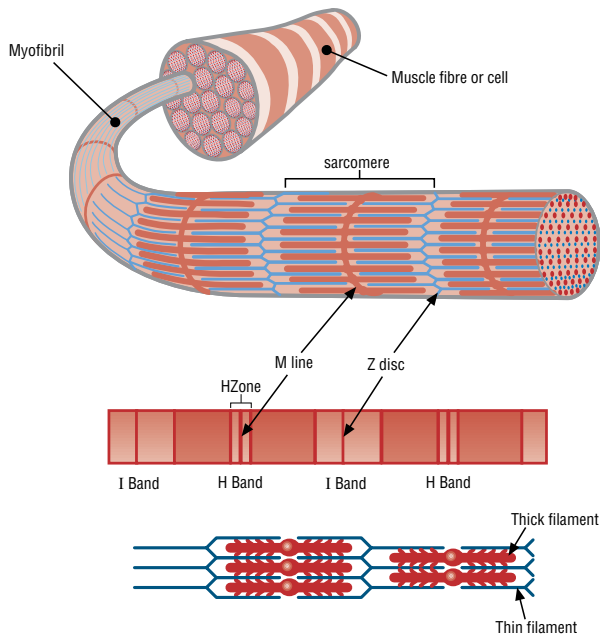
3.3.3   Connective tissue (collagen)

Different muscles have different types and amounts of connective tissues, thus differ in tenderness. Collagen is the major constituent of connective tissue. In collagen fibres chemical bands form between collagen molecules and link them together. Such crosslinks stabilise the collagen molecule and impart tensile strength.

In a calf, the amount of collagen is the same as an adult animal, but meat from the calf is more tender as there are fewer crosslinks. As the animal matures, the changes in connective tissue are not fully understood. However, there is a decrease in solubility of the connective tissue as the divalent crosslinks form.

Muscles used for movement tend to have higher amounts of collagen (thus less tender) than postural muscles or muscles used for fine movements (thus more tender). Muscles found in loin and ribs are examples of postural muscles.

3.3.4   Background effect

Background factors such as intramuscular fat (IMF)/marbling affect meat tenderness indirectly. IMF affects meat tenderness by influencing the juiciness and flavour characteristics of meat. This is consistent with earlier results, which suggested that increased marbling stimulates the salivary glands in the mouth and so the human perception of juiciness increases.

3.4 Fat hardness and fat oxidation

3.4.1 Hardness

The fatty acid composition of fat and its triacylglycerol structure plays the predominant role in determining lustre, texture and the tactile properties of fat in meat. Some cattle breeds (e.g. Wagyu) have higher levels of oleic acid and lower levels of stearic acid than other breeds resulting in considerably softer fatty tissue, desired by some markets such as the Japanese. Diet is known to affect fat hardness. For instance the inclusion of cotton-seed meal in cattle diets some years ago resulted in fatty tissue that was unacceptably hard for the Japanese market.

3.4.2 Oxidation

Although oxidation of lipids during storage is usually considered to produce off-flavours and rancidity, there are notable exceptions. For example in dry-cured hams and some fermented sausages, the desirable flavour does not occur until hydrolysis of some of the fat and a certain degree of oxidation has taken place during ripening. Further, lipid oxidation during cooking may be a source of intermediates which react with other components to give important constituents of the desirable flavour of normal cooked meat (Ladikos & Lougovois, 1990).

Cooked meats held in a refrigerator develop rancid odours and flavours which can become apparent within 48h at 4°C. These flavours are particularly noticeable after reheating the meat and referred to as warmed-over flavour.

A major cause of meat quality deterioration is lipid oxidation and changes associated with it. Lipid oxidation is a complex process. Unsaturated fatty acids react with oxygen and form peroxides as primary products of the oxidation. Primary oxidation is followed by a series of secondary reactions which lead to the degradation of the lipid and the development of oxidative rancidity. It is generally accepted that any process causing disruption of the muscle membrane system, such as grinding, cooking and deboning, results in exposure of the labile lipid components to oxygen, and thus accelerates development of oxidative rancidity.

Lipid oxidation is enhanced by metals such as iron, cobalt and copper, which facilitate the transfer of electrons leading to increased rates of free radical formation. The most common way that metal ions enter food is via the water used and in some instances via salt and spices. Lipid oxidation may also be accelerated by a variety of haem compounds (Ladikos & Lougovois, 1990). Sodium chloride accelerates oxidation of the triglycerides, although the mechanism of salt catalysis is still uncertain. Salt induces rancidity in freezer-stored, cooked, cured meat, and in raw and cooked beef, both during cooking and subsequent storage.

The most obvious precaution against oxidative deterioration is to remove the air. Packaging raw meat in oxygen-impermeable film prevents metmyoglobin formation and lipid oxidation during storage, if sufficient enzymic reducing activity is present in the meat.

Vacuum packaging or modified atmosphere packaging (carbon dioxide and nitrogen, no oxygen) of meat and meat products are very satisfactory measures taken to prevent colour and rancidity problems. Lipid oxidation can also be reduced and shelf life can be extended through dietary vitamin E supplementation above requirement levels (Morissey *et al*. 1994).

3.5 References

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4 Meat Packaging

In the early 1970s two developments: flexible packaging and vacuum machines changed the way meat was marketed. Previously, distant markets could be serviced only with frozen carcases – now it became possible to land chilled, vacuum-packed primals in cartons with ample shelf life for retail processing and distribution.

Soon after, the rise of supermarkets prompted development of centralised processing and packaging facilities and the 'retail ready' packaging which occupies much of the shelf space in the modern market.

In this section we describe how modern packaging and storage systems have extended the shelf life of vacuum-packed and modified atmosphere packed meats. The bacterial communities which influence shelf life and the sensory quality of meat are also covered.

4.1 The early days

Until the advent of refrigeration it was necessary for meat to be slaughtered and consumed with little or no delay, especially in the summer months. Until the middle of the 20th century most homes in Australia had rudimentary refrigerators called Coolgardie Safes in which perishable products such as meat, fish and milk were stored. The safes, basically boxes with fly screen walls to prevent flies contacting the food, were stored on the verandah to catch any breeze. A wet hessian bag was draped over the walls of the safe and, as water evaporated, heat was withdrawn from within the safe and therefore from the food.

Butchers typically slaughtered their own animals or received them from the local slaughterhouse and usually sold the meat the same day. Likewise, the consumer purchased meat and consumed it the same day or the next day, depending on the ambient temperature; packaging was usually in greaseproof paper. In some ways retail butcher shops maintain this basic model of purchasing carcase meats to suit peak retailing periods, breaking them into specific cuts and packing customers' purchases in a plastic bag.

Meat retailing changed radically in the middle of the 20th century when domestic refrigerators became affordable and when supermarkets began to replace high street shops. As recently as the end of the 20th century it was commonplace for supermarkets to load-in carcases each morning, for boning and packaging by butchers in a back-of-house facility. This is reflected in AS 4696 (the "Meat Standard") where load-out regulations specify surface temperature no warmer than 7°C for carcases. In the 21st century meat is transported increasingly in cartons, with a temperature no warmer than 5°C specified.

4.2   Vacuum packing primals

The early 1970s saw the commercial acceptance in Australia of 'flexible packaging' based on placing meat in bags and using machines to withdraw the air prior to sealing. Important for maintaining a vacuum was that the bag should have a low transmission rate for oxygen. Also important was the continued respiration of the vacuum-packed meat with the conversion of oxygen in the air remaining after vacuum packing to carbon dioxide. The concentration of carbon dioxide (about 20%) prevents growth of Gram-negative spoilage bacteria. However, growth of facultatively anaerobic, CO_2-tolerant bacteria – mainly lactic acid bacteria – can still occur.

The technology enabled the development of new, distant markets although early problems for vacuum-packed meats had to be solved.

These included:

- Improving temperature control during shipping, dry ice is sometimes used to maintain temperatures within the container – a process known as 'snowing'
- Maintaining the integrity of the seal
- Avoiding trapping pockets of air within the bag
- Vacuum packing only low pH meats (to avoid greening)
- Avoiding piercing the bags by bone-in cuts
- Preventing partial loss of vacuum with attendant increase in drip (weep)

The oxygen transmission rates of bags have been improved, as has their puncture resistance to allow bone-in cuts to be marketed. Vacuum machines have also been improved to allow rapid throughput with minimal failure rate due to seal failure and loss of vacuum.

4.2.1 Purge

Purge, 'weep' or 'drip' is the exudate which oozes from the cut surfaces of meat and while production of purge during chilled storage of vacuum packaged red meat product is generally accepted as normal, certain export markets have rejected Australian product suggesting that its presence means the meat is no longer 'wholesome'.

CSIRO considers that a 'normal' amount of drip in a vacuum pack of chilled primals is 1-2%, particularly for pieces with cut or trimmed surfaces. Smaller pieces of meat, with a higher surface area: volume ratio may also have a higher proportion of purge (CSIRO, 2002). Images of 'normal' and 'excessive' drip are presented in Figures 4.1 and 4.2.

Figure 4.1 Vacuum packed primal

Figure 4.2 Excessive purge in Vacuum packed primal

In addition, poor packing, where pieces of meat are crammed into a carton, creating pressure, is a major influence on drip formation.

In a storage trial of sub primals (brisket, eye round and topside) commissioned by MLA (Barlow *et al*. 2016) the increases in purge over a 32 week storage period are presented in Fig 4.3 and, in the case of eye round, ranged up to 6%.

Figure 4.3 Purge percentages (mL/g meat) in vacuum packaged brisket, eye round and topside stored for up to 32 weeks.
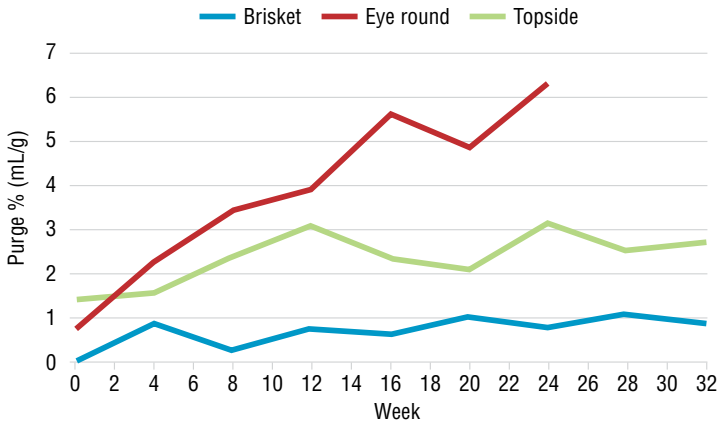
4.3 Packaging for retail

The modern retailing chain is based on centralised processing and packaging of meats so that, increasingly, the supermarket receives retail-ready products which are unpacked into refrigerated displays. A considerable amount of available shelf life is used up during centralised processing, packaging, transport to and storage in the supermarkets' distribution centres (DCs), and transport to the individual stores.

This business model has led to developments in packaging technology to further extend the available shelf life of retail raw meats. Form-fill packaging machines have been developed that are capable of:

1. Forming a tray from a bottom web into which product is placed
2. Adding a lid from a top web
3. Removing air from the pack
4. Injecting a specific gas mixture or evacuating all gases
5. Sealing the pack

This packaging technology and packaging films allow the atmosphere above the product to be modified and maintained (modified atmosphere packaging, MAP). Packaging with MA greatly extends the shelf life of retail meats by incorporating carbon dioxide to inhibit bacterial growth, together with oxygen to enhance the red colour of the meat.

Psychrotrophic clostridia can spoil vacuum-packed meats by producing cheesy odours and gas, which 'blows' the pack. Yang *et al*. (2014) have identified a range of clostridia, particularly *Clostridium estertheticum*, which "blow" anaerobic packs containing beef steaks when meat pH is >5.9; this occurrence of blown packs is uncommon in Australia.

Oxygen may be used at around 80% to give the red oxymyoglobin colour of meat. When CO_2 is used in high concentrations (20–30% or higher) it inhibits the growth of Gram-negative spoilers such as *Pseudomonas* spp. and *Acinetobacter* spp. But for extended shelf life a concentration of CO_2 near 100% with minimal oxygen (<0.2%) has been found to be the optimal achievable by MAP packing. The volume of CO_2 in the package compared to meat i.e. gas to meat ratio is an important consideration because CO_2 is highly soluble in meat. A headspace gas to meat volume ratio of 2–3 is generally observed to prevent package collapse (Gill & Gill, 2005).

Nitrogen is an inert gas and can be used as filler to prevent pack collapse. It has no antimicrobial effect and does not affect meat colour, but retains package structural integrity as CO_2 is absorbed into the meat.

4.4 References

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5   Meat Microbiology

Bacteria present on meat can play a major part in ending shelf life by producing off odours, forming slime and (rarely) by causing colour changes. To do this, spoilage bacteria must:

- *Grow to very high numbers*
- *Have the ability to break down chemicals in the meat (be biochemically active)*

In this section we cover the basics of meat microbiology so that we will be equipped to deal with more advanced work on meat shelf life.

5.1   Basic meat microbiology

Bacteria are often stained in order to better see them under a microscope. More than a century ago the Danish microbiologist, Christian Gram, devised a stain that divides bacteria into two groups: Gram-positive and Gram-negative, based on their colour under the microscope after staining. The distinction is useful in that Gram-positive bacteria tend to have more resilient cell walls than Gram-negative, making them more resistant to processing conditions including salt, sanitisers and inactivation and freezing temperatures.

Bacteria that grow on meat are influenced greatly by the gas atmosphere around them, the temperature and pH. Bacteria fall into four groups according to the environment required for their growth:

- Aerobes need oxygen to be present e.g. *Pseudomonas*
- Anaerobes need oxygen to be absent e.g. *Clostridium*
- Microaerophiles need a trace of oxygen in order to grow – *e.g. Campylobacter*
- Facultative anaerobes have no oxygen requirement although many grow better in the presence of oxygen e.g. *Salmonella* and *E. coli*

Carbon dioxide inhibits some bacteria, particularly *Brochothrix thermosphacta* and Gram-negative spoilage bacteria such as *Pseudomonas* and *Shewanella putrefaciens* – this is a primary reason why vacuum-packed meats have much longer shelf-lives than meat packed in overwrap.

Bacteria fall into three groups according to their temperature growth range.

- **Psychrotrophs** grow well at refrigeration temperatures (-5 to +5°C), have an optimum growth range of 25 – 30 °C and can grow at temperatures of up to 30 – 35°C. Important pathogens are *Yersinia* and *Listeria* while important spoilage bacteria include *Pseudomonas* (Gram-negative) and *Lactobacillus* (Gram-positive)
- **Mesophiles** grow best around 30 – 45°C, can grow at temperatures up to 35 – 47 °C and have a minimum growing range from 5 – 15°C. Most of the important human pathogens are mesophilic including *E. coli*, *Salmonella* and *Staphylococcus*
- **Thermophiles** grow well between 55 – 75°C and stop growing between 40 – 45 °C with a maximum temperature range of 60 – 90 °C. *Campylobacter* is an important thermophile although its temperature range is considerably narrower than those quoted above

For almost all its shelf life, meat is stored under refrigeration, either frozen (preferably around -18°C) or chilled between -1 and +5°C. Bacteria cannot grow on frozen meat, but chilled storage allows psychrotrophs to grow.

Bacteria increase their population by doubling: first they grow in size, then they divide by building a wall down their centre, and finally they split into two. This is an efficient way of increasing the population, and the rate of increase depends greatly on the temperature at which the meat is stored. Even a small increase in temperature in the refrigeration zone can cause a great increase in bacterial growth rate, which shortens product shelf life.

Figure 5.1 shows how bacteria double their population. Under optimal conditions one bacterium will multiply to 1,000,000 cfu/g or /cm^2 in about seven hours.

Figure 5.1 How bacteria double their population by simple cell division.
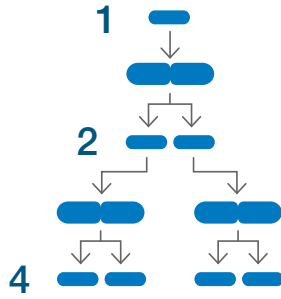
As the bacterial population increases, it eventually covers the entire surface of the food. A typical spoilage population is shown in Figure 5.2.

Figure 5.2 Electron micrograph of spoilage bacteria on a meat surface.
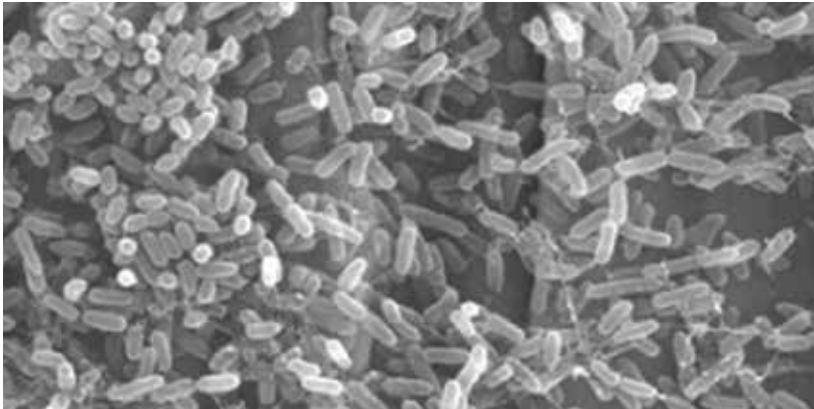
Microbiologists usually express bacterial counts on a logarithmic (log) scale. This is a convenient way of expressing the population, without using lots of zeroes.

Table 5.1 Logarithmic scale.

Arithmetic count (/g or cm²)	Log count (/g or cm²)
0.01	-2
0.1	-1
1	0
10	1
100	2
1,000	3
10,000	4
100,000	5

Log counts also take account of the fact that methods for counting are not very precise. For this reason, if microbiologists want to compare counts, for example counts on meat produced by Shift 1 compared to Shift 2, they always look for a 'real' difference of more than 0.5 log.

For example, suppose carcases produced by Shifts 1 and 2 have average counts of 250 cfu/cm^2 and 500 cfu/cm^2, respectively. It might be thought that Shift 2 is twice as bad as Shift 1. However, the log counts (2.4 cfu/cm^2 for Shift 1 and 2.7 cfu/cm^2 for Shift 2) would be considered very similar by microbiologists. This reflects the fact that a doubling in numbers represents only one generation of bacterial growth.

Figure 5.3 illustrates how the bacterial population in aerobically stored meat increases over time; the population is usually measured as the Total Plate Count (TPC) and the vertical axis is log count/cm^2.

Figure 5.3 How the bacterial population increases on meat stored aerobically at 5°C.
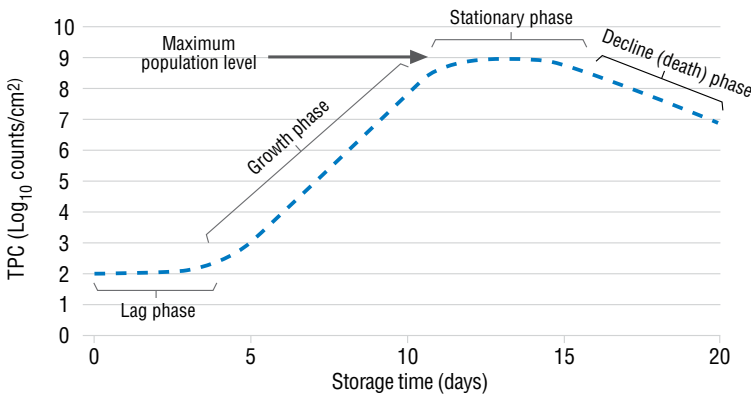
Figure 5.3 is a typical bacterial growth curve for meat being stored aerobically. Initially, the population doesn't increase. This is called the lag phase, and occurs as bacteria adjust to their new environment. When lag is completed, bacteria grow (double) at a regular rate and enter the growth phase. If counts are expressed on a log scale this phase is a straight line. At the end of the growth phase, when conditions begin to deteriorate due to overcrowding, food shortage or build-up of toxins, the population becomes stable and enters the stationary phase, where the number of bacteria dying is equal to the number being produced. Typically this occurs when the TPC reaches around 1,000,000,000 colony forming units (cfu/g or /cm^2) or, as microbiologists express it, 9 log cfu/g or cfu/cm^2. Finally, the population may enter a decline phase where the number dying exceeds those being produced, and the growth curve takes a downward path. In practice this doesn't occur in packages of meat – spoilage occurs before the population enters decline phase.

In Section 10 of this book, this basic information on growth curves is used to develop predictive models. One commonly used predictive model is the Refrigeration Index (RI). This predicts the growth of *E. coli* on carcase and meat during chilling and contains an allowance for lag phase.

5.2 Bacteria and shelf life

The development of new packaging technologies results in new microbial niches which are exploited by a range of 'new' spoilers. Figure 5.4 shows the range of breakdown products which affect the colour and odour of the meat, as well as the integrity of the pack, which is lost if gas is produced and the pack is distended.

Figure 5.4 Breakdown pathways for meat spoilage in aerobic and anaerobic packs.
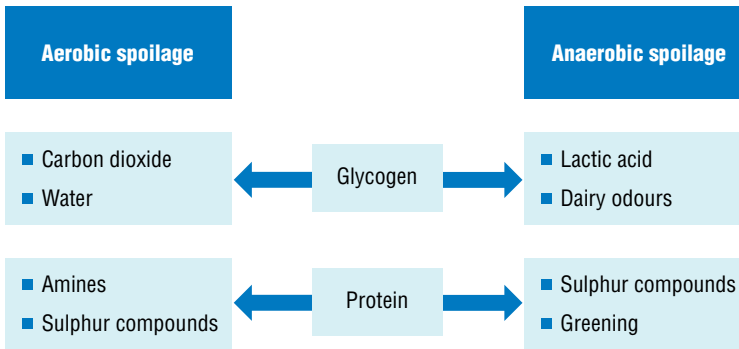
5.2.1 Bacterial growth of aerobically-packed meats

Traditionally, retail chilled meat has been packed in trays with an overwrap of low water permeability to retard moisture loss, and high oxygen permeability to enhance colour retention. Held under refrigeration, the product is susceptible to spoilage by psychrotrophic, aerobic bacteria such as *Pseudomonas* and *Shewanella* that can result in rapid spoilage of the product. Generally however colour is the determining factor in the shelf-life of aerobically stored meat.

In order to grow, bacteria need an energy source, which they obtain from glycogen in the meat. Glycogen is a glucose polymer and when the glucose units are broken down they yield water and carbon dioxide as by-products, which do not contribute to spoilage although gas can result in blown packs.

However, pseudomonads and other Gram-negative bacteria are active biochemically and also break down meat protein into two sets of compounds that have obnoxious odours: amines, which are associated with rotting flesh and sulphur compounds, which smell like rotten eggs.

When aerobic spoilage of meat occurs, odour becomes apparent when the Total Count of bacteria exceeds around 7 log cfu/cm^2 although spoilage has been noted at much lower levels (6 log cfu/cm^2). A second consequence of spoilage is slime formation (this is when bacterial colonies become so dense they join up to form a slime), which occurs around 8 log cfu/cm^2 or /g. Pseudomonads start from a low population, but multiply quickly at low temperatures to become the dominant microflora.

5.2.2   Bacterial growth of vacuum-packed meats

Around the mid-20th century, flexible packaging was developed with low oxygen permeability, enabling packing of meat primal cuts into bags. Vacuum machines and hot water or hot air shrink tanks or tunnels were developed so that bags could be sealed and the film shrunk tightly onto the meat surface. Within the bag, sufficient CO_2 was released from the meat to form an atmosphere of around 20% in the headspace leading to a shelf life sufficient to service the most distant markets.

In vacuum packs the oxygen level is too low for aerobic spoilage bacteria such as pseudomonads to grow and the carbon dioxide level is high enough to prevent growth of other Gram-negative spoilage bacteria, allowing Gram-positive bacteria, particularly lactic acid bacteria (LAB), to become the main microflora. In vacuum-packed meats, LAB grow slowly and tend not to break down protein, focusing instead on glycogen, which they convert to lactic acid; souring is the product of LAB growth in vacuum packs.

Because the breakdown products of LAB growth are less obvious, the total count of bacteria on vacuum-packed meat can reach 8 log cfu/cm^2 without the meat being obviously spoiled. Spoilage at 0°C does not become apparent until some 2-4 weeks after the maximum count has been reached.

Towards the end of shelf life, LAB produce odours which accumulate in the small headspace of a vacuum-pack building up into a confinement odour, which is obvious on opening the bag but dissipates quickly.

Figure 5.5 was developed from work done at SARDI (Kiermeier *et al*. 2013) where vacuum-packed lamb was stored at around -1°C. Initially, LAB (dotted line) are only a small proportion of the total bacteria (solid line). After about 40 days storage, LAB became dominant in the vacuum-pack and, at around 80 days the population reached 7–8 log cfu/cm^2 – almost all LAB. This pattern is repeated for other vacuum-packed products, though the bacterial counts at different times are likely to differ.

Figure 5.5 Growth of Total bacteria (TPC – solid line) and Lactic acid bacteria (LAB – dotted line) on vacuum-packed lamb stored at -1°C (after Kiermeier, *et al.* 2013).
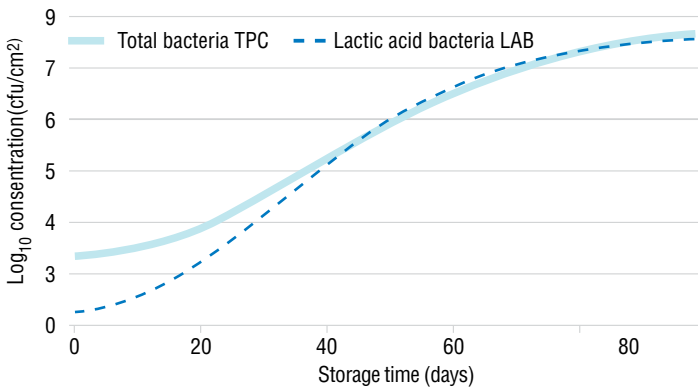
Sometimes if high pH meat (>5.9) is vacuum-packed, instead of LAB becoming dominant, *Brochothrix thermosphacta* grows in the vacuum-pack and causes objectionable 'dairy' odours or souring when levels reach around 10^7 cfu/cm^2.

Shewanella putrefaciens (a relative of *Pseudomonas*) can also grow if the pH is high and, because there is little or no glycogen in high pH (dark cutting) meat, the bacteria use protein and other carbohydrates as an energy source.

This results in formation of amines and sulphur compounds and early spoilage of the product, often at a TPC of around 6 log cfu/cm^2; sulphmyoglobin is produced that leads to greening of the meat. Some LAB are also capable of producing hydrogen sulphide resulting in greening of vacuum packaged meat.

5.2.3   Microbial populations of VP and MAP meats

Because aerobes such as *Pseudomonas* are inhibited by high concentration of CO_2, the shelf life of VP and MAP meat is extended. The group includes microbes that becomes dominant in VP and MAP meat is the lactic acid bacteria (species of *Carnobacterium, Lactobacillus* and *Leuconostoc*)and sometimes *Brochothrix thermosphacta*.

Pseudomonads typically do not form a significant proportion of MAP meat microbial populations. However, they can become commercially important because they can grow in poorly-sealed packs which allow oxygen to slowly enter the pack.

Lactic acid bacteria normally do not produce putrid substances like *Pseudomonas*. In MAP during storage a sour/acid/cheesy odour develops because of the production of organic acids from carbohydrates such as glucose by LAB after bacteria have attained maximum numbers.

The potential benefits of MAP in extending shelf life are apparent, but concerns had been expressed about the microbial safety of MAP meat, particularly the psychrotrophic pathogens capable of growing at high CO_2 and chilled storage: *Clostridium botulinum*, *Listeria monocytogenes* and *Yersinia enterocolitica*. However, the relative growth rate of LABs versus pathogens on meat in MAP favours the former, often leading to suppression of pathogens, a phenomenon called the Jameson Effect. More recently, concern about C. botulinum in MAP red meats as a potential cause of botulism has again been raised. Guidelines in some countries suggest that chilled product which is held above 3°C (such as on chilled display in a supermarket) should be held for no longer 10 days because the pH of the meat is too high to prevent the growth of C. botulinum and the product, prior to sale, has not been heat treated to destroy C. botulinum spores (Stinger et al. 2011).

5.3 Bacterial spoilage and shelf life

Since meat is typically stored at refrigeration temperatures, microbiological aspects of its shelf life are influenced by a number of important psychrotrophs:

- Gram-negative spoilers, such as *Pseudomonas, Shewanella* and *Alteromonas*, are closely related and are biochemically active, so they can break down amino acids into objectionable odours and flavours. Their growth is inhibited by high carbon dioxide concentrations (20%), which combined with low pH, is the basis for their inhibition in vacuum-packed primals.
- Lactic acid bacteria (LAB), such as *Lactobacillus*, *Carnobacterium* and *Leuconostoc* are tolerant of high carbon dioxide concentrations.
- *Brochothrix thermosphacta* produces odours redolent of sweaty socks, though only on meat above pH 5.9. Its growth is inhibited by high carbon dioxide concentrations.
- Clostridia produce a range of objectionable odours as well as gas, which distends the vacuum-pack.

All of these organisms are capable of growing at 0°C and many can grow at sub-zero temperatures. At –1.5°C, water in meat begins to freeze, making it impossible for bacteria to grow.

In the 1980s, CSIRO researchers identified the pre-requisites needed to optimise the shelf life of vacuum-packed meats (Egan *et al.* 1988):

- An initial count no more than $2-3$ log cfu/cm^2
- Packaging film with low oxygen permeability
- Good control of temperature throughout the storage period.

If these pre-requisites were met, Egan *et al*. (1988) predicted that shelf lives for VP lamb and beef stored at 0°C were $42-56$ days and $70-84$ days, respectively. However, shelf lives tended to the shorter end of the range because initial counts were higher than $2-3$ log cfu/cm^2 and when temperature control could not be guaranteed.

Over recent decades both slaughter/boning hygiene and temperature control have improved markedly leading to anecdotal evidence from within the trade that shelf lives have improved greatly compared with those of the 1980s. In the following sections we follow the developments in process hygiene, packaging technology and product storage temperatures which have led to greatly increased shelf lives compared with those established by Egan *et al*. (1988).

5.4   Spoilage bacteria

The following text is principally about lamb and is based very heavily on the work of Mills *et al.* (2014), which should be consulted for further information.

The initial objective of vacuum packaging was inhibition of the strictly aerobic bacteria such as *Pseudomonas*, which are the main cause of spoilage in meats packed in air. Consequently, the bacteria found in vacuum-packed chilled meat is determined by conditions in the vacuum pack including temperature, relative humidity, pH and the concentrations of O_2 and CO_2. It comprises anaerobic and facultatively anaerobic bacteria, usually dominated by psychrotrophic lactic acid bacteria (LAB). A summary of growth and spoilage characteristics of the six main groups of chilled meat spoilage bacteria is given in Table 5.2.

LAB are oxygen-tolerant anaerobes, which grow readily in the absence of O_2 and are not greatly inhibited by CO_2. The most common isolates from chilled meats are *Lactobacillus*, *Leuconostoc* and *Carnobacterium* spp. Based on studies with vacuum-packed beef, LAB can only ferment glucose and a limited number of the other carbohydrates that are present in small amounts on vacuum-packed meat muscle tissues. After packaging, the population of LAB is generally below the limit of detection (about 10 LAB/cm^2). Growth ceases when the bacterial numbers at the meat surface have consumed the available glucose – typically occurring when numbers reach about 10^8 cfu/cm^2. Some species produce only lactic acid (resulting in the slightly acidic taste of aged meat) but others can produce a range of other end products including ethanol, butyric acid and sulphides.

Table 5.2 Spoilage bacteria found in red meat (From Mills *et al.* 2014)

Microorganism	Oxygen requirement	pH requirement	CO$_2$ sensitivity	Spoilage potential	Common spoilage characteristics	Thresholds of spoilage	General remarks
Pseudomonas spp.	Strict aerobes	None	High	High	Sulphurous off odours	$10^7/cm^2$	
Shewanella putrefaciens	Facultative anaerobe	No growth below pH 6.0	Moderate	Very high	Sulphurous off odours and some discolouration of the meat		Major spoilage organisms of vacuum packed meat
Brochothrix thermosphacta	Facultative anaerobe	Growth reduced significantly below pH 5.8	Moderate	High	Some green drip, meat discolouration and pungent dairy odours, poor bloom, slight loss of vacuum, and bubbles	~$10^6/g$	Occasional major spoilage organism on vacuum-packaged meat
Psychrotolerant Enterobacter spp.	Facultative anaerobes	Some anaerobic growth below pH 5.8	Moderate	High	Some green drip, meat discolouration and strong sulphurous odours, poor bloom, slight loss of vacuum and bubbles	Varied depending on specific organism	Major spoilage organisms of vacuum-packaged high pH meat
Lactic acid bacteria	Aerotolerant anaerobes	None	Low	Low	Greening of meat, off-flavours include cheesy, malty, acidic, or liver-lie and production of slime	~$10^8/g$ depending on specific strain	Usually dominant organisms of vacuum-packaged meat
"blown pack" Clostridium spp.	Anaerobes but will survive in cool, aerobic environment	None	Low	High	Softening of meat, production of large amounts of exudates and offensive odours (dairy or sulphurous)	Initial loading of 1-10/g spoilage potential depends on temperature control	Occasional major spoilage organism in the absence of temperature abuse or packaging failure

Lamb has on average a higher pH than that of beef, and most vacuum-packed lamb products include both fat and muscle tissues. This means that the pH of many cuts will be ≥ 5.8, conditions that may enable bacteria with a higher spoilage potential to grow. However, LAB will still grow faster at chilled storage temperatures, and may continue to dominate the population if the initial numbers of other spoilage bacteria are low. The lower the storage temperature, the slower the growth rate of spoilage bacteria, with the optimum temperature for the storage of vacuum-packed meat for long periods being –1.5°C.

Some species of psychrotrophic *Enterobacteriaceae* cause deterioration of vacuum-packed meat, characterised by unpleasant odours and greening. These facultative anaerobes also use glucose preferentially, but when glucose becomes limiting they utilise amino acids, producing breakdown products resulting in putrid odours, and ammonia production, which raises the pH and can cause a pink-red colouration.

Greening is caused by the formation of hydrogen sulphide, with subsequent formation of green sulphmyoglobin anaerobically. Although these bacteria are more likely to cause spoilage under temperature abuse conditions (>5°C), there are a few species that can grow at chill temperatures in vacuum-packed meat.

Spoilage may be due to more than one organism. For example, spoilage of lamb primals stored in vacuum-packs at 0°C has been attributed to the growth of both *B. thermosphacta* and *Enterobacteriaceae*.

Growth of *Brochothrix thermosphacta* (producing cheesy or dairy odours), *Shewanella putrefaciens* (producing hydrogen sulphide and greening) and psychrotrophic *Enterobacter*ia (producing sulphurous odours) can occur under various conditions. For vacuum-packed meat these conditions include one or more of the following:

- Relative high pH (> 6.0)
- Storage temperature of 5°C to 10°C
- Packaging conditions resulting in the presence of residual oxygen (e.g. presence of large amounts of surface adipose tissue with only limited oxygen-scavenging potential)

In the case of *Brochothrix*, the amount of undissociated lactic acid is the effective inhibitor, rather than the pH itself, with around 50% and 90% reductions in growth rate reported in the presence of 0.5 mM and 2.0 mM undissociated acid, respectively, although how this might influence the different results seen on beef and lamb has yet to be determined.

Shewanella putrefaciens has been reported to cause extensive spoilage of chilled beef but has not been reported to spoil chilled lamb in scientific studies in recent years.

Blown pack spoilage of vacuum-packed chilled lamb can occur even if the temperature has been strictly maintained at the target temperature of –1.5°C (Mills *et al*. 2014). While *Enterobacteriaceae* may cause blown pack spoilage, the bacteria generally responsible are gas-producing cold-tolerant *Clostridium* spp., usually *C. estertheticum* or *C. gasigenes*. Other cold-tolerant clostridia have been associated with other (off-odours, but not blown) forms of lamb spoilage including *C. putrefaciens* and other species not previously associated with spoiled meat. *C. estertheticum* is the most common species found on lamb. Spores of cold-tolerant clostridia can be detected in animal fleeces and the slaughter room floor, and these are also detectable on chilled dressed carcases.

C. estertheticum strains are capable of blowing packs at –1.5°C. In a New Zealand study, even low initial levels of *C. estertheticum* (≤10 cfu/cm^2) spores, on meat with 10^2–10^3 cfu/cm^2 total count and stored at –1.5°C blew within 40 days, whilst *C. gasigenes* caused slower onset of spoilage at 1 and 4°C. Mills *et al*. (2014) recommended that when storing lamb for long periods at –1.5°C, attention must be paid to minimising contamination, for example, by applying sporicidal agents to contact surfaces during cleaning of equipment. Some species of pyschrotolerant clostridia, for example *C. algidicarnis* are known to cause surface spoilage of chilled vacuum-packed lamb, as well as beef and venison. It was observed that whilst little or no gas accumulated on opening affected packs, sickly spoilage odours were detected and clostridia were isolated from both the drip and surface swabs.

Vacuum-packed meat can briefly be exposed to high temperatures if a heat-shrink process is applied during packaging. Heat shrink treatment may accelerate the onset of blown pack spoilage due to *C. estertheticum*, by stimulation of spore germination. Spore germination in *C. frigidicarnis* may be triggered by anaerobiosis (absence of air) in the presence of the amino acid L-valine and L -lactate, conditions that are present in vacuum-packed red meat. Hot and cold water washing of lamb contaminated with spores of *C. estertheticum* has been shown to extend the shelf life of vacuum packs by 12 to 13 days at −1.5°C.

5.5   References

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6 Shelf life of Australian Vacuum-packed Beef and Sheep Primals

Shelf lives of vacuum-packed beef and lamb primals, stored below 0°C are long enough to allow export from Australia to markets all over the world. When vacuum-packing was first introduced shelf life was accepted to be up to 56 days for lamb and 84 days for beef.

Improvements in hygiene, packing materials and maintenance of the cold chain have probably all contributed to longer shelf life being obtained by the industry. Over the past few years MLA has commissioned studies to demonstrate the shelf life of Australian chilled primals and found product acceptable for over 85 days for lamb and over 140 days for beef.

Since the early 1970s, Australia has developed markets for vacuum-packed beef and sheep primals, sending product in shipping containers at close to 0°C. As stated earlier, according to Egan *et al*. (1988), providing counts on meat cuts were low and product was packaged and stored well, shelf-lives for lamb and beef stored at 0°C were 42–56 days and 70–84 days, respectively. Gradual improvements in process hygiene led Grau (2001) to quote a shelf life for VP beef of around 90 days, similar to the anecdotal 'industry' life of 100 days at 0°C.

More recently, further anecdotal evidence from the market suggested that Australian VP beef and sheep primals had longer shelf lives than products from competing countries; possibly because the starting bacteria levels are lower (Phillips *et al.* 2012). To provide scientific backing for these anecdotal claims, MLA commissioned CSIRO and SARDI to undertake a series of storage trials on vacuum-packed beef and sheep primals. In addition, the University of Tasmania used modern microbiological techniques to identify the individual organisms which dominated the microbial populations at different stages of the shelf life.

In this section we document the work done by these three organisations in establishing the much extended shelf life for Australian VP primals.

6.1   Shelf life of lamb shoulders

6.1.1   Sensory quality of ageing of VP lamb shoulders

Several processors in Australia export vacuum packed lamb shoulders to overseas markets, including the Middle East, UK, USA and Japan. In 2009 concerns were expressed by a major Japanese importer that total bacterial loadings on VP lamb primals regularly exceeded a limit set by a large Japanese supermarket chain of 500,000 cfu/cm^2; the importer produced a large number of bacterial counts of product on arrival in-store to back up the claim.

As an immediate reaction it was thought that the high counts were due to temperature abuse during the export chain. To test this belief a trial was set up in which four Australian establishments stored VP lamb shoulders (n=25) in their own holding chiller. Bacterial counts were made on shoulders immediately before packing and after around 25 days storage, the approximate time of the transport chain from Australia to Japan; data loggers were inserted in the cartons to monitor temperature during storage.

At the end of storage close to 0°C at each establishment, none of the 100 packs from the four plants had obvious drip, bloom returned on pack opening and there was no deleterious odour. Mean Aerobic Plate Counts (APCs) at the four establishments varied from 1.8 to 2.3 log cfu/cm^2 at packing to 2.7–3.6 log cfu/cm^2 at the end of storage (Sumner & Jenson, 2011). However, 13/100 shoulders had counts which exceeded 500,000 cfu/cm^2, confirming the information supplied by the Japanese importer.

The sensory quality of the shoulders appeared excellent and MLA commissioned SARDI to investigate the microbial levels and sensory acceptability of vacuum packed boneless lamb shoulders. Two trials were conducted in which meat was aged to mirror the complete Japanese marketing, processing and consumer chain:

- VP lamb shoulders were stored at 0°C for 13, 31, 34, and 35 days
- Shoulders were sliced thinly (ca 5 mm) by a skilled operator then packed into retail trays overwrapped with cling film
- Trays were stored at 2°C for up to four days in a retail display
- Each day trays were withdrawn for microbiological testing (total plate count, TPC and lactic acid bacteria, LAB)
- A panel of 8–10 Japanese consumers assessed product for appearance, colour and smell (Figure 6.1)
- Lamb slices were then cooked by a chef experienced in Japanese cuisine and assessed by the Japanese panel for taste, texture and overall impression (Figure 6.1a and 6.1b)

Figure 6.1 Japanese taste panellists used in SARDI sensory evaluations.

Bacterial levels increased with the age of the vacuum packed product and ranged between 3 log and 6 log TPC/g with LAB forming the dominant population. Counts increased during retail storage by an additional 2–3 log cfu/g over the four-day storage period in overwrap trays.

Each day, the Japanese panel was assembled to assess both raw and cooked product quality. Although average sensory scores over the four days in retail display fell slightly, consumers considered the product acceptable irrespective of age or duration of storage after slicing.

The trial demonstrated that bacterial count was not linked with loss of sensory quality when meat was sliced, packed, retailed and consumed exactly as it is in Japan by Japanese consumers living in Australia.

Figure 6.1a Scatter plot of the mean sensory scores versus mean log APC (at 25°C). Trial 1 – different coloured points indicate different product ages.
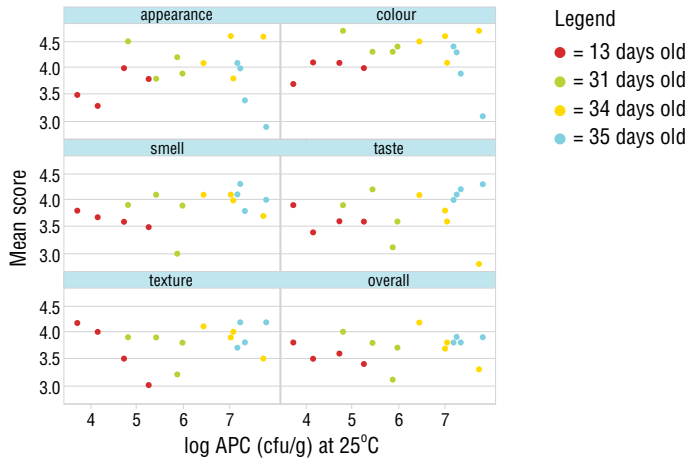
Figure 6.1b Scatter plot of the mean sensory scores versus mean log APC (at 25°C). Trial 2 – red crosses indicate product stored for two days and green dots indicate product stored for ½ day.
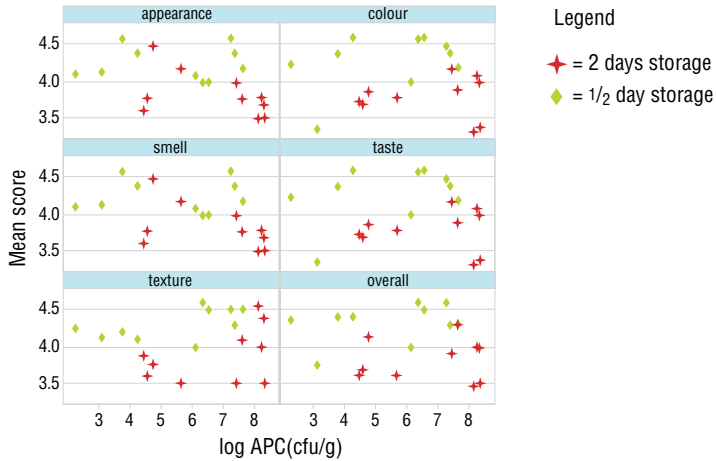
In a second trial, Long-term storage vacuum packed lamb shoulders were stored at -1 to 0°C for up to 78 days (Kiermeier *et al*. 2013). During this period, shoulders were removed from storage, sliced and packed for retail display before being presented to the sensory panel; sensory assessment was undertaken the same way as for Trial 1. Retail packs were either stored at 3.5 +/- 1°C for an additional two days or for half a day under refrigeration conditions, before sensory assessment which took place 22, 36, 50, 64, and 78 days after vacuum packing. Immediately prior to sensory assessments, sliced samples were removed for microbiological testing (TPC and LAB).

For freshly cut slices (½ day chilled storage) TPC increased from 3–7 log cfu/g over the 78-day storage period. These levels increased by approximately 1–1.5 log cfu/g on slices that had been cut two days earlier. Lactic acid bacteria on freshly sliced product were almost identical to TPC, increasing from around 3–7 log cfu/g over the same period. LAB counts increased by 1–1.5 log cfu/g on slices that had been refrigerated for two days.

Consumer acceptability did not decline over the 78-day storage period and this effect was consistent for all sensory characteristics assessed.

However, lamb shoulders that had been sliced two days prior to the sensory evaluation scored slightly lower than those that had been sliced that day.

The trial showed that aged vacuum packed lamb with TPCs and LAB at normal levels (7–8 log) was acceptable both as raw and cooked meat.

## 6.1.2   Shelf life of VP and MAP-packed lamb shoulders

While VP is a common practice in Australia, few processors pack product in MAP, which apparently is used more frequently in New Zealand. SARDI conducted a storage trial of bone-in and bone-out VP and MAP lamb shoulders to collect data on the shelf life of these products and demonstrate the potential value of this packaging system to the Australian meat industry (Kiermeier *et al*. 2013).

The shoulders were sourced from a single lot of lambs from an Australian processor. These were either vacuum-packed individually or in MA packs of four with a 100% CO_2 modified atmosphere, the latter using a ratio of 1:1.5, meat to CO_2. Product was stored for up to 12 weeks at an average temperature of -0.3°C. Shoulders were tested on a weekly basis for lactic acid bacteria (LAB) and total plate counts (TPC), and assessed for sensory properties (appearance, colour and odour) by a single trained panelist.

For VP product the log TPC increased from 3 to 8 log cfu/g over the 85 day storage period, for both bone-in and bone shoulder. Lag and stationary phases were not clearly apparent. Lactic acid bacteria grew in a similar manner from around 2 to 7.5 log cfu/g over the same period (Figure 6.2).

For MAP shoulders there was a slight increase in TPC over the storage period although TPC in some packs opened at the end of the storage had not changed from the initial level. Similarly, lactic acid bacteria did not grow well in MAP product, though some growth was observed toward the end of the storage period (Figure 6.2).

Figure 6.2 Growth of Total bacteria (TPC) and Lactic acid bacteria (LAB) on modified atmosphere and vacuum packaged bone-in (open circles) and bone-out (filled circles) lamb shoulders. The dashed lines indicate the general trend in bacterial counts data (after Kiermeier *et al.* 2013).
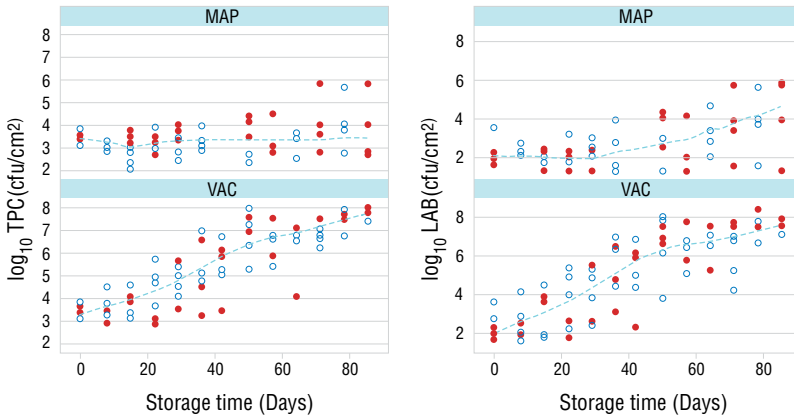
Throughout the storage period (85 days, when all product had been utilised for testing) all product types (VP, MAP and both bone-in and bone-out) scored high for meat colour and odour, indicating that both VP and MAP lamb shoulders had a shelf life that can exceed 85 days, provided temperature is well controlled at –0.3°C.

While VP product was consistently well packed, packaging problems were observed with MAP packs in this trial. Of the 19 packs received, four were not intact – one had not been properly sealed and three had holes in them caused by bone puncture due to excessive shrinkage. Shrinkage in MAP packs is as a result of CO_2 being absorbed into the meat. The excessive shrinkage in this study may have been due to the low CO_2 ratio of 1.5:1, used by the processor. This ratio is considerably less than the 2.5 – 3:1 used by CSIRO and Canadian researchers (Gill & Gill, 2005) in trials in the late 80s and early 90s. From the problems experienced with the MAP packs in this trial, a higher gas to meat ratio than 1.5:1 seems desirable. Alternatively, nitrogen, an inert gas used as a filler, will prevent pack collapse and thus prevent subsequent pack damage.

6.2 Shelf life of VP beef primals

MLA commissioned CSIRO to undertake a series of storage trials on vacuum-packed beef including primals (striploins and cube rolls) and sub primals (brisket, eye round and topside). Samples were withdrawn after intervals from CSIRO's chiller and microbiological and sensory testing carried out.

Trial 1 ran for 140 days, after which time the taste panelists established that both striploins and cube rolls were still of excellent sensory quality (Small *et al*. 2009). However, because all samples had been used after 140 days the actual shelf life of product could not be determined and therefore a second trial was undertaken.

Trial 2 used cube rolls and striploins from six abattoirs located from Tasmania to far-north Queensland and primals were stored at – 0.5°C for up to 30 weeks (Holdhus Small *et al*. 2012). Throughout the storage period, packs scored highly for vacuum integrity, panelists recording complete vacuum and tight package adhesion at 28 weeks, and moderate or good vacuum at 30 weeks.

Trial 3 studied the shelf life of sub primal cuts (brisket, topside, eye round) stored close to zero. Throughout the study chemical, microbiological and sensory testing on raw and cooked products was carried out.

Quality attributes of product during Trials 2 and 3 are discussed in 6.2.1 while microbiological changes are detailed in 6.2.2.

6.2.1   Sensory quality

In Trials 2 and 3 the researchers were able to test product to destruction i.e. to when shelf life was completed and product was no longer acceptable.

In both trials sensory panels scored visual appearance of packs as either very fresh with no discoloration, or as fresh with slight discoloration until 28 and 30 weeks, when there was a reduction in appearance score to marginally acceptable as the bloom score decreased and the quantity of drip increased.

Both primals and sub primals generally developed a confinement odour which became marked by 24 weeks, though dissipating during the 20-minute bloom period, Post 24 weeks, odour scores were summarised as typical or strong off odour, and characterised as 'sweet' or 'cheesy'. By 28 weeks and especially at 30 weeks, some persistence of odour was noted post-bloom. Post-bloom visual appearance scored highly, with a slight gradual decline, until week 28, after which the decline in score was more marked,

In Trial 2, panelists tasted steaks cooked from striploins and cube rolls stored for 24, 26, 28 and 30 weeks, when the meat aroma was rated as being slight to moderately strong for both striploins and cube rolls. Meat flavour was also assessed as being in the slight to moderately strong category for these sampling times with other flavours (sour, acidic) being detected at 28 weeks with an aftertaste at 30 weeks.

Despite these observations, over the storage period there was little change in the average acceptability score, which remained largely unchanged for cube rolls and striploins from all processors.

In Trial 3, grilled beef samples were prepared for each muscle type for the taste panel assessment. It was found that mean overall liking scores at week 20 were consistent with, and often better than, corresponding scores at zero, four and eight weeks of storage. Significant differences in mean overall liking scores were observed for brisket samples at weeks 24, 28 and 32 and topside samples from week 32 with these samples being adversely affected by elevated other flavour scores.

6.2.2   Microbiological changes

The storage life of striploins determined in 1982 and 2009 (the study discussed in the previous sections) are compared in Figure 6.4; both investigations were carried out by the CSIRO and product was stored in the same chiller facility. In 1982 there was a lag phase of around two weeks followed by an exponential phase progressing to stationary phase after around eight weeks. Odours and flavours indicating the onset of spoilage were detected about four weeks later. In those studies, stationary phase typically occurred at 7–8 log cfu/cm^2 at around eight weeks with the LAB counts being identical with the TPC. In the 2009 trial, the count never reached stationary phase, rising slowly to just over 6 log cfu/cm^2.

Figure 6.3 Microbial counts on vacuum-packed striploins stored in 1982 (at 0°C) and 2009 (at -1°C).
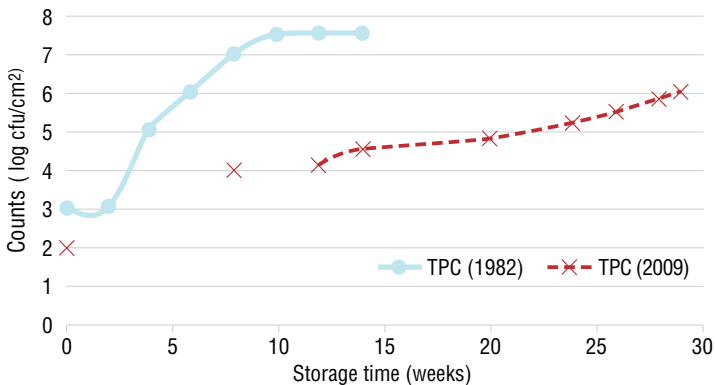
In the graph above (Figure 6.3), the data have been presented as the median (middle) count, which gives a smooth, gradual increase in populations. However, when the 2009 CSIRO shelf life trial data are presented to show their variability (box plot format) the picture is very different. In this graph (Figure 6.5) the median (middle) count is seen as a dark dot surrounded by a box into which counts from the 25th to the 75th percentile are included. Below each box are counts that dip to the minimum, while above it are counts which rise to the maximum. A feature of the data (not shown) is the large variation in counts within establishment and on each sampling day, often of the order of 4–5 log cfu/cm^2. This variability was similar for both Total and lactic acid bacteria (LAB) counts.

Figure 6.4 Variability in microbial counts of vacuum-packed striploins stored at –1°C (Trial 2 after Holdhus Small *et al.* 2012).
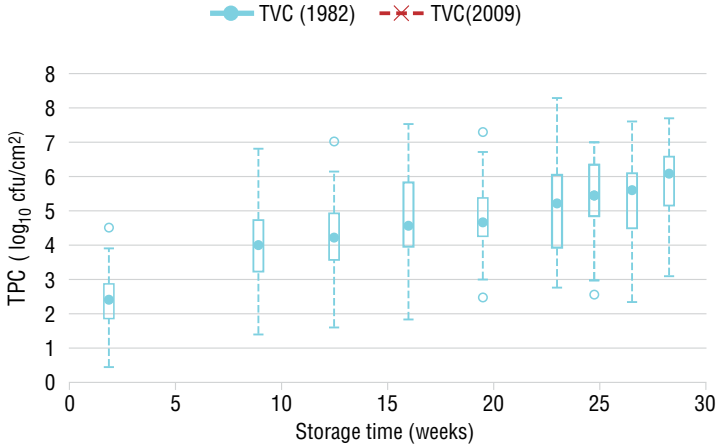
The researchers believe the low and variable counts may reflect the combined effect of a number of stressors that subject the bacteria to conditions close to their growth:no-growth interface. Such stressors include: meat pH, storage temperature and the low oxygen: high carbon dioxide atmosphere in the vacuum-pack. Thus, when temperatures are close to 0°C and the meat is close to pH 6.0, growth will be possible, as opposed to those times during storage below –1°C and where the pH is 5.4, when growth is restricted or may not occur. Since there are no definitive studies on the growth:no growth interface of chilled, vacuum-packed meat the researchers have no data against which to test their hypothesis.

In Trial 3, beef sub primals were stored at -0.5°C. Both TPC and LAB increased from an initial <2 log CFU/cm^2 to around 6 log CFU/cm^2 after 32 weeks for topside and brisket and to 7 log CFU/cm^2 for eye round; counts on fat and lean tissue were similar (Figure 6.10).

Figure 6.5 Average log TPC for briskets, topside and eye round lean and fat stored at –0.5°C to 32 weeks (Trial 3 after Barlow *et al*. 2016).
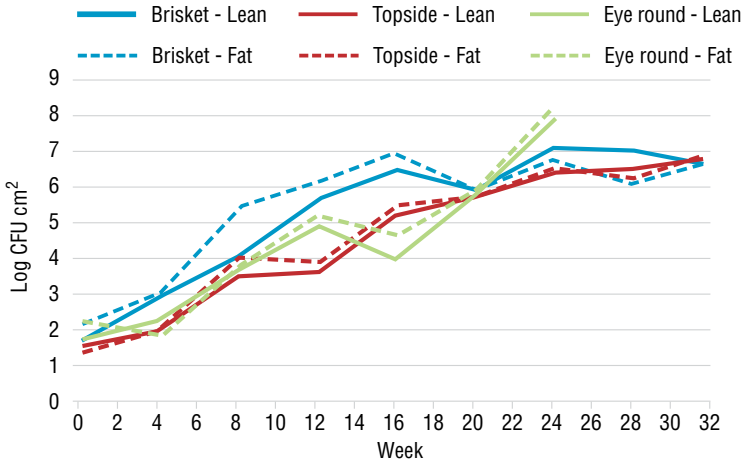
6.3 Effect of temperature on shelf life

The optimum temperature for storage of vacuum-packed primals was defined by Gill *et al*. (1988a) as -1.5±0.5°C. The same workers also established that small rises in temperature reduced shelf life significantly. At temperatures of 0, 2 or 5°C the storage life was reduced by about 30, 50 or 70%, respectively, compared with storage at -1.5°C (Gill *et al*. 1988b). One effect of a slightly warmer storage temperature (around 4–5°C) was that pseudomonads were favoured, particularly if the pH was 6.0 or above, a pH which also favours growth *B. thermosphacta.*

As stated previously, the shelf life of Australian vacuum-packed lamb primals was determined by Egan *et al*. (1988) as 42–56 days, providing meat had an initial bacterial level of 2–3 log cfu/cm^2, a level rarely attained in that era. However, a series of Australian baseline studies since 1993 indicates significant improvement in the hygienic status of lamb carcases, so that, in 2004, the mean TPC was 2.3 log cfu/cm^2 (Phillips *et al*. 2006).

Many exporters believe the shelf life for chilled, vacuum-packed lamb primals is now much longer than 60 days. To test the shelf life of vacuum-packed lamb primals, MLA assisted three companies with long-term trials in which boneless shoulders were held in the companies' chillers for up to 90 days (Sumner & Jenson 2011).

Sensory testing showed that, after 85 days storage, the appearance and odour of product from all three plants was still acceptable. At Plants A and B, all product was acceptable at 84/85 days, with the last samples being tested on this day. At Plant C, 85 days was the last acceptable time, with a persistent cheesy, sour odour characterising the 90-day sample.

Table 6.1 Total Plate Counts of vacuum-packed lamb boneless shoulders after storage at Plants A, B and C (after Sumner & Jenson, 2011).

| Plant | Storage | | Mean log TPC/cm^2 | | |
	Time (days)	Temperature (°C)	At packing	End of storage	Growth increase
A	84	−2.4	2.4	1.5	−0.9
B	85	−0.5	1.3	3.5	2.2
C	90	0	2.7	7.9	5.2

Microbiologically, there was a marked difference between bacterial counts in product from the three trials. There was also a significant difference in storage temperature among the three plants: at Plants B and C the temperature cycled close to 0°C, while at Plant A the room was at a mean −2.4°C. Such a difference in storage temperature was considered by Gill *et al*. (1988a, b) to have a large influence on microbial growth and, in this study, this is reflected in the growth increase at each plant, which varied from log 5.2 cfu/cm^2 at Plant C to log −0.9 cfu/cm^2 at Plant A. At the latter plant it must be emphasised that storage conditions were unusually cold and variable, and it is likely that the meat surface underwent freeze-thaw cycles, which may account for the bacterial population being similar, at least numerically, after 84 days to that at packing.

Effect of storage temperature on the shelf lives of vacuum packaged boneless lamb shoulders and beef striploins was further studied by University of Tasmania (Ross *et al*. 2016). Boneless lamb shoulders and beef striploins were sourced from three abattoirs over a wide geographic range from Tasmania to Queensland and were stored at -0.5, 2, 4 and 8°C. Samples were tested for TPC and LAB, and assessed for sensory properties (appearance, colour and odour).

The panelists considered product to be acceptable at each of the four temperatures for the times cited in Table 6.2. Sensory testing showed that, on average, lamb was acceptable for up to 100 days at −0.5°C, around 16 days at 8°C. In contrast, beef was acceptable for 28 weeks at 0°C and for 4 weeks at 8°C (Table 6.2).

Table 6.2 Length of time lamb and beef samples stored at different temperatures.

Boneless lamb shoulders		Beef striploins	
Storage temperature (°C)	Storage period (days)	Storage temperature (°C)	Storage period (days)
8	23	8	43
4	48	4	92
2	71	2	134
−0.5	107	0	213

The researchers calculated microbial growth rates for each storage temperature of the microflora of both meat cuts produced at each of the four abattoirs (Figures 6.6 and 6.7).

Figure 6.6 Square root of the growth rates of total viable bacteria on boneless lamb shoulder of three abattoirs stored at four different storage temperatures
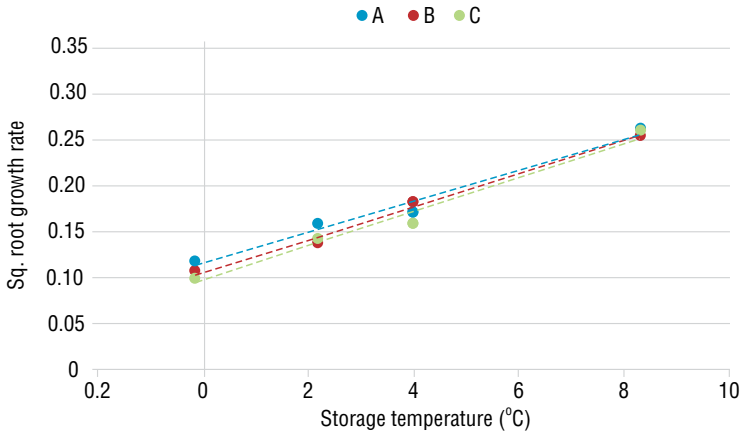
Figure 6.7 Square root of the growth rates of total viable bacteria on beef striploin of three abattoirs stored at four different storage temperatures.
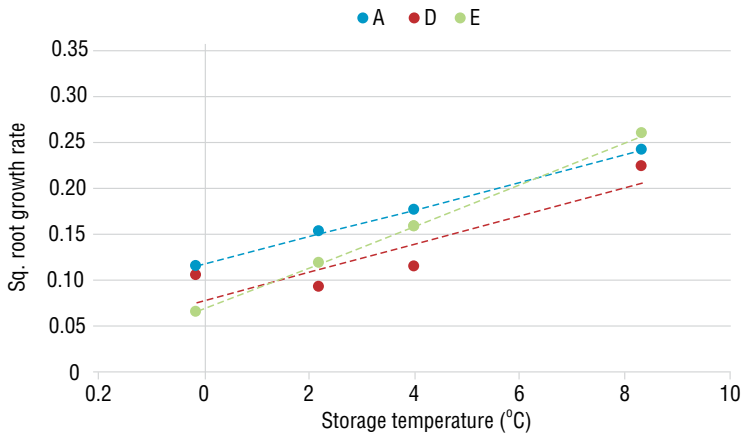
Growth rate increased with storage temperature, and between abattoirs, reflecting their wide geographical spread. Fastest growth rates were on product from establishment A, a Tasmanian abattoir possibly reflecting a higher psychrotrophic microflora. Bacterial growth rates were also faster on lamb primals, possibly due to a higher pH on ovine cuts.

6.4   Summary

Recent studies in Australia clarify that extension in shelf life for vacuum-packed beef and lamb primals has occurred over the past three decades probably because of:

- Lower bacterial populations when the meat is packed, reflecting improvements in process hygiene on the slaughter floor and in the boning room.
- Improved temperature control (0°C or colder) through the cold chain

In parallel with these trials, there have been studies on the microbial communities that develop in vacuum-packed meat and these will be investigated in the next section.

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7   Microbial Communities in Vacuum-packed Meat

When refrigerated meat is stored in aerobic films it spoils in about a week producing putrefying odours as the bacterial population breaks down proteins in the meat. Vacuum and MA packed meats, by contrast, have long refrigerated shelf lives and the bacterial population present causes much less objectionable odours even when it reaches very high levels.

Modern microbiology allows us more insight into the communities within the spoilage microflora and in this section we identify these communities.

7.1   Common culturing methods for spoilage bacteria

The spoilage of stored meat is mainly due to the growth and dominance of undesirable bacteria. The storage conditions (e.g. temperature and gaseous atmosphere) not only influence the type of microbes that grow, but also their growth rate. As mentioned in earlier sections, the pH of the meat post *rigor mortis* can also affect shelf life, particularly for meat stored in a vacuum-pack.

Under good processing and packaging conditions, the counts of LAB on the surfaces of primals at the time of packaging are low (<10 cfu/cm^2). However, their numbers increase during storage and can be expected to exceed 10^6 cfu/cm^2 after two to three weeks (Blixt & Borch 2002; Leisner *et al*. 1995).

The above information is gained using conventional microbiological methods – plating out dilutions taken from surface sponging or excisions on agars which support the growth of general bacteria – Total Plate Count (TPC), Aerobic Plate Count (APC) or Total Bacterial Count (TBC). More specific agars and gas atmospheres are needed to identify individual families or genera and some common selective media are included in Table 7.1.

Table 7.1 Commonly used culturing conditions to enumerate meat spoilage bacteria (extracted and extended from Corry, 2007).

Targeted group	Medium	Incubation (temperature/ time/ atmosphere)	Confirmatory test
TPC, APC	PCA (plate count agar), TSA (tryptone soya agar)	-	-
Pseudomonas spp.	CFC (cephaloridine-fucidin-cetrimide) agar	25°C, 48h, aerobic	Count oxidase-positive colonies
Lactic acid bacteria	MRS (de Man, Rogosa and Sharpe) agar	25°C, 5 days, anaerobic	Count catalase-negative colonies
Brochothrix thermosphacta	STAA (streptomycin-thallium acetate-actidione) agar	25°C, 48h, aerobic	Count catalase-positive, oxidase-negative colonies
Enterobacteriaceae	VRBG (violet-red-bile-glucose) agar	30°C, 48h, aerobic	Count all pink or red colonies

7.2 Monitoring shelf life by conventional microbiological methods

The microbiological profile of Australian vacuum packed beef was characterised by Grau (1979). He indicated that the stationary phase at 7–8 log cfu/cm^2 was reached after 5–8 weeks at 0°C with spoilage occurring at around 12 weeks, manifested by the appearance of sour/cheesy/acid odours due to the predominance of lactic acid bacteria, particularly species of *Lactobacillus*, some of which are now called *Carnobacterium*.

More recently the predominant organisms have been identified as *Carnobacterium divergens*, *C. piscicola*, *Lactobacillus sakei*, *L. curvatus*, *Leuconostoc gelidum, Leuc. carnosum* and *Brochothrix thermosphacta* (Ercolini *et al*. 2006; Fontana, Cocconcelli & Vignolo 2006; Jones 2004; Nissen & Sorheim 1996; Sakala *et al*. 2002).

While much can be gained by using conventional microbiological methods based on testing individual colonies growing on a culture plate, the 21st century has seen the development of new methods which enhance the information which can be gained by conventional methods.

Conventional culture methods often result in an incomplete picture of true microbial diversity, missing out information on other bacterial species that are difficult to culture or that make up less than 10% of the population. For example, the organism often associated with 'blowing' of vacuum and MA packs, *Clostridium estertheticum* is extremely difficult to grow. A rapid and reliable method of detecting this and other species is to use direct DNA extraction coupled with polymerase chain reaction (PCR) -based approach (Broda *et al.* 2000; Boerema *et al.* 2002).

7.3 Monitoring shelf life by new, culture-independent methods

It is becoming increasingly common to replace plate count methods with more rapid and selective detection and quantification tools, such as PCR-based, DNA fingerprinting. This provides a reliable identification and estimation of microbial diversity and dynamics during meat storage. However, each method has its own advantages and disadvantages, and often a combination of different culture-dependent and independent methods is needed to obtain a realistic picture of the microbial diversity on meat.

In this section we describe some of the new microbiological techniques that are being used by researchers.

7.3.1 Nucleic acid based methods

Polymerase chain reaction (PCR) is a widely used nucleic acid-based method used because of its speed, selectivity and sensitivity. The technique will be familiar to technicians who use Bax machines to identify *Salmonella* or *E. coli* O157:H7 in enrichments from meat. PCR can be used in shelf life studies, and Gribble & Brightwell (2013) used it to identify different species of *Brochothrix* in meat products.

7.3.2 Microbial community profile tools

A number of culture-independent molecular techniques such as Terminal Restriction Fragment Length Polymorphism (TRFLP), Denaturing Gradient Gel Electrophoresis (DGGE), cloning and next generation sequencing (Pyrosequencing) have been developed. These techniques have been used by University of Tasmania researchers on cultures gathered by CSIRO and SARDI during the shelf life studies of vacuum and modified atmosphere packaged beef and lamb presented in the previous sections.

7.4 Communities in vacuum-packed beef primals

In a CSIRO study on beef shelf life, vacuum-packed cube rolls and striploins from six abattoirs from Tasmania to far-north Queensland were stored at -0.5°C for up to 30 weeks (see Trial 2 in Section 6). A notable finding from this study was that TPC and LAB counts varied among the six abattoirs (Holdhus Small *et al*. 2012). Rinsates from vacuum-packed primals tested for TPC and LAB levels in the CSIRO study were further investigated by the University of Tasmania using DNA fingerprinting techniques such as TRFLP and DNA sequencing. This investigation showed that bacterial communities on the surface of primals differed at each abattoir in terms of their bacterial species and relative proportion.

Initially, both primals (cube rolls and striploins) were colonised by Gram-negative, aerobic bacteria (*Pseudomonas* species) particularly in meat from the Tasmanian abattoir. By week 16, various species of the lactic acid bacterium, *Carnobacterium*, was found in meat from each abattoir and, at 30 weeks, carnobacteria dominated.

In a more recent shelf life study conducted by University of Tasmania Ross *et al.* (2016). In this study beef striploins from three abattoirs from Tasmania, south east and far north Queensland were stored at four different temperatures (-0.5, 2, 4 and 8°C). There was great bacterial diversity at the start, which decreased as storage progressed, irrespective of temperatures and abattoir. At a storage temperature of -0.5°C, lactic acid bacteria belonging to *Carnobacterium*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* genera dominated the overall bacterial communities from week 6 to week 30 of storage.

The storage temperature also had a great effect on the bacterial communities. Lower temperatures significantly favored *Carnobacterium*, *Leuconostoc* and *Lactobacillus* abundance. Storage at higher temperature resulted in the greater prevalence of non-LAB; *Enterobacter*, *Serratia* and *Enterococcus*, bacterial species usually associated with meat spoilage Figure 7.1).

Figure 7.1 The effect of storage temperature at 0 and 8°C on the bacterial communities on vacuumed beef primals
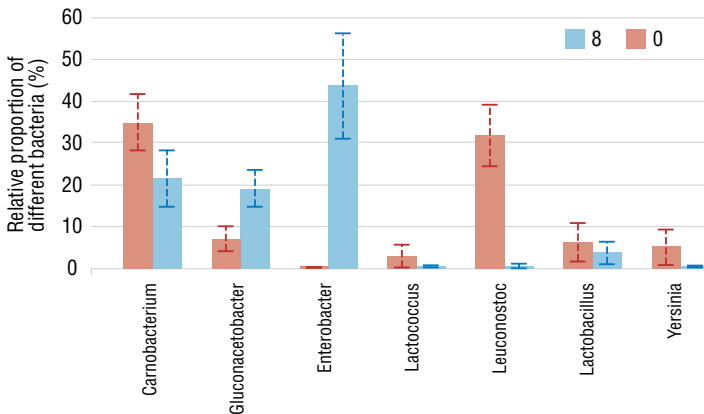
7.5 Communities in vacuum-packed lamb primals

Microbial growth, community and sensory characteristics of Australian vacuum-packaged lamb shoulders (both bone-in and bone-out) were evaluated by Kiermeier *et al*. (2013) using both traditional culture-dependent (TVC and LAB counts) and culture-independent DNA based microbial community profiling techniques (TRFLP and pyrosequencing).

DNA fingerprinting techniques showed an overall shift in bacterial community from an early mixed population of Gram-negative aerobic bacteria such as *Pseudomonas* to a predominantly Gram-positive facultative anaerobic LAB population such as *Carnobacterium* spp. in the later storage.

The study established that shelf life of vacuum-packaged lamb shoulders can exceed 85 days provided the storage temperature and packaging are well-controlled and beneficial microbes such as *Carnobacterium* spp. flourish.

A shelf-life study of boneless lamb shoulders from three different abattoirs (Tasmania, South Australia and New South Wales) was conducted at four different storage temperatures (–0.5, 2, 4 and 8°C) by University of Tasmania (Ross *et al*. 2016). As with beef primals, there was great bacterial diversity at the start, with lactic acid bacteria *Carnobacterium*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* dominating during storage.

Also, similar to beef, storage temperature also influenced bacterial communities. Lower temperatures favored *Carnobacterium*s. while higher temperature resulted in the greater prevalence of non-LAB; *Enterobacter*, and *Gluconobacter*, species usually associated with meat spoilage (Figure 7.2).

Figure 7.2 The effect of storage temperature at -0.5 and 8°C on the bacterial communities on vacuumed lamb primals
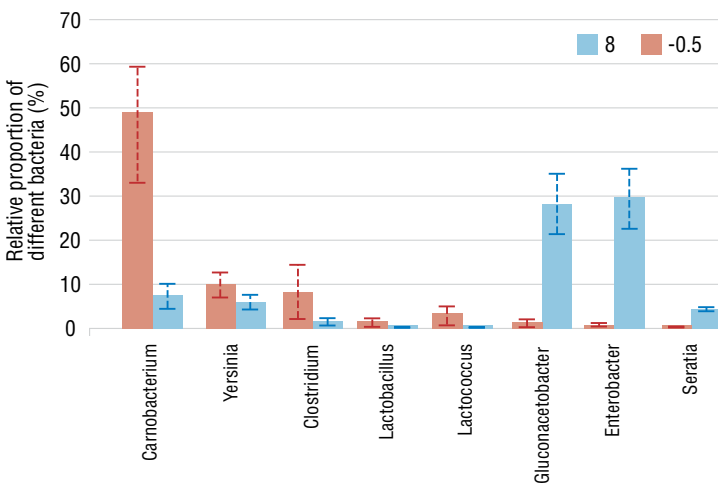
7.6 Summary

Carnobacteria are LABs and their growth in vacuum-packed meats has a number of positive effects (see review by Leisner *et al*. (2007)):

- They produce lactic acid, lowering the pH
- Some produce antibacterial compounds (bacteriocins) which may inhibit pathogens. *Carnobacterium divergens* and C. *maltaromaticum* have been studied extensively as protective cultures in order to inhibit growth of *Listeria monocytogenes* in fish and meat products
- Some produce probiotic (health promoting) compounds
- The finding of significant differences in the microflora at 'good' temperatures (–0.5, 2°C) and at 'bad' temperatures (4°, 8°) is particularly relevant for companies exporting to markets where the ambient temperature is very high (e.g. 40°C is not uncommon for many months of the Middle Eastern year) or where storage and distribution infrastructure is not able to maintain the cold chain close to zero.

7.7   References

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8 Export and Retail Meat Shelf life Expectations

Meat companies, whether suppliers to domestic customers, or to more than one hundred export markets, are required to meet a wide range of specifications and standards.

In this section we examine both microbiological and logistical standards e.g. getting meat landed so that there is sufficient time left for processing and retailing.

We also illustrate the importance of the cold chain in meeting microbiological criteria.

8.1 Shelf life regulations and customer requirements

Rules and requirements on shelf life may originate from government or commercial sources. Since suppliers need to meet both requirements, both are dealt with equally. Shelf life requirements may be expressed in arbitrary terms as a maximum number of days that a product may have before it has to be consumed or sold. Shelf life may also be expressed in microbiological terms, so microbiological specifications are discussed here also – not just for the microorganisms that are likely to limit shelf life by their growth, but all microorganisms for which standards and specifications may exist. So product has to meet arbitrary shelf life requirements as well as microbiological requirements.

Three terms are used in this section to describe the requirements for shelf life; they have slightly different meanings which are important for suppliers.

- **Standards**: a requirement that is in a law, or referenced by a law. A standard must be met.
- **Guideline**: a suggestion, by some government or non-government body, of the characteristics that should be achieved
- **Specification**: a commercial requirement that is agreed between the supplier and a customer

8.2   Arbitrary shelf life

Arbitrary shelf life is set in a number of countries, particularly in the Middle East, but also, informally, in Japan. Since many of these requirements are set out in national or regional standards that have the force of law in those countries, there is little option for the exporter but to comply with the requirement. These requirements are called 'technical trade barriers'. They are rarely justified under World Trade Organisation rules and it is not easy to have them modified. Industry organisations such as MLA and AMIC lobby and provide the Australian Department of Agriculture and Water Resources with information to help them address these barriers, and MLA Regional Offices often work with organisations and trade associations in the importing country in an attempt to have the requirements changed.

Gulf Cooperation Council criteria

The Gulf Cooperation Council develops a large number of standards covering the following countries:

- Bahrain – or Kingdom of Bahrain
- Kuwait
- Oman – or Sultanate of Oman
- Qatar
- Saudi Arabia – or Kingdom of Saudi Arabia (KSA)
- UAE – United Arab Emirates

The following expiry dates are mandated in the Gulf Standards Organisation Standard (GSO 150/2007 Expiration periods of Food Products): *Vacuum-packed meat stored at -0.5° to 0°C: no more than 70 days, with the exception of MAP lamb (CO$_2$ gas flushed), which is no more than 90 days from the date of slaughter.*

Unlike USA and Japanese standards, framed to accommodate products which are traditionally undercooked, Middle Eastern standards are concerned more with maintaining sensory attributes in a climate where very high temperatures exist for much of the year. As shown in Table 8.1, criteria have been set for the age of meat when it enters the country (e.g. Lebanon) and/or age when the shelf life is deemed to have expired. Some expiry dates appear extremely conservative, particularly for vacuum-packed beef primals which can approach 30 weeks when stored at -1°C (Holdhus Small *et al*. 2012). It may be that the conservative approach is considered necessary to accommodate possible shortcomings in the cold chain between unloading from the vessel and consumption in the home.

Egyptian criteria

The Egyptian Standard for chilled meat (3602/2008) considers that the usual temperature for storage is 1–4°C. The shelf life is defined by Egyptian Standardization Classification (ESC) no. 1546 'Packaged food products cards data'. The slaughter date and expiry date needs to be labelled in Arabic plus any other language. Bone-in cuts have a maximum shelf life of 28 days and boneless meat 49 days, which must enter Egypt no more than 14 and 24 days from the date of slaughter respectively. MAP packed (CO$_2$ flushed) is allowed a shelf life of 70 days from slaughter.

Lebanese criteria

Lebanese shelf life standards are shown in Table 8.1.

Table 8.1 Shelf life expiry dates for vacuum-packed meats in selected Middle Eastern countries.

Country	Product	Storage temperature (°C)	Expiry date (days)*	Entry date (days)*
Lebanon	Refrigerated meat (boneless vac pack)	–2 to +2	84	50
Lebanon	Refrigerated meat (boneless sheep meat vac pack)	–2 to +2	70	50
Gulf Cooperation Council	Chilled meat packed under carbon dioxide	-	90	-
Gulf Cooperation Council	Meat packed under vacuum	-	70	

* From date of slaughter

Jordanian criteria

Vacuum-packed meat (including MAP and gas-flushed) product has an expiry date from the date of slaughter of 90 days (Jordanian Standard and Measurement. Meat and meat products – fresh, chilled and frozen beef meat 2002/471). Storage temperature -1 to 7°C.

Japanese criteria

The Japanese Meat Traders Association (JMTA) shelf life guidelines are as follows:

- Australian beef – 77 days
- US beef – 62 days
- Domestic beef – 61 days

Some importers appear to ignore this guideline.

8.3 Microbiological criteria for raw meats

Since many microbiological criteria are set out in national or regional standards that have the force of law in those countries, there is little option for the exporter but to comply with the requirement. These requirements are called 'technical trade barriers'. Most requirements are rarely justified for the protection of public health and it is not easy to have them modified. Industry organisations such as MLA and AMIC lobby and provide the Australian Department of Agriculture with information to help them address these barriers, and MLA Regional Offices often work with organisations and trade associations in the importing country in an attempt to have the requirements changed.

8.3.1 Where have we come from?

When surveys in the early 1970s in USA and Canada established that much of the ground beef on sale had counts >10 million/g, microbiological standards were proposed for meat. One standard, a limit for *E. coli* of 50/g at retail in Oregon State, was particularly onerous as was demonstrated when an offending supermarket manager was sent to jail for failing to meet this standard (Carl, 1975).

When it was shown that it was often impossible to meet the standard and that the causes lay with stages upstream from the retailer (boning, trimming, grinding) the law was repealed and the standard replaced by a guideline (Winslow, 1975).

At about the same time in the UK, retailer Marks and Spencer Ltd (M&S) formulated a set of microbiological criteria for their suppliers (Goldenberg & Elliott, 1973). The criteria were revolutionary for several reasons because they were:

1. Standards, not specifications or guidelines – with all the rigour that comes with a standard
2. The first imposed by a retailer on its suppliers
3. Risk-based, with products sorted into categories: '*Satisfactory*', '*Acceptable but investigate*' and '*Stop production*' as descriptors for the three classes
4. Considered at the time to be unattainable by many suppliers

Goldenberg & Elliott (1973) were concerned that suppliers use microbiological testing to improve the safety and shelf life of their products. They quoted the famous microbiologist, Sir Graham Wilson: *"Bacteriologists are better employed in devising means to prevent or overcome contamination than in examining more and more samples. Their chief contribution to the supervision of our food supply should be to ensure that the preparation and processing are properly carried out. Control of processing is of far greater importance than examination of the finished article"* (Wilson, 1970).

Wilson's ideas were a forerunner to the HACCP concept, set out as 'Wilson's Triad' for milk processing:

1. Hygienic care of raw materials
2. Thorough pasteurisation
3. Prevention of post-process contamination and colonisation

The M&S standards came as supermarkets began to influence a meat trade that had changed little for almost a century. Frozen meat from Australasia and chilled meat from South America had supplied global markets, apparently without food safety problems until a Swedish salmonellosis outbreak in 1953 (Lundbeck *et al*. 1955).

Now, supermarkets were selling packaged meat cuts in refrigerated displays and needed to be sure that food would remain safe and of high eating quality over a specific shelf life, some of which had to be shared with the customer's refrigerated storage.

So microbiological criteria were born and the M&S standards became a forerunner for sampling schemes developed by the International Commission on Microbiological Specifications for Foods (ICMSF). Initial work by ICMSF focused on standardising methods and sampling for lot acceptance at a time when little (or nothing) was known about the microbiological quality of the product.

Microbiological criteria and sampling plans were developed for many foods in international trade (ICMSF, 1974), based on a broad concept of the degree of hazard, and comprising two components:

- Severity of adverse effects, ranging from no effect, to serious, life-threatening illness
- Whether subsequent processes would increase or decrease the level of hazard

Five levels of adverse effects and three levels of impact of subsequent processing were defined, creating a series of 15 'cases' with increasingly stringent sampling plans and criteria.

Each case was based on a sampling plan described by 'n' (number of samples required), 'm' (the acceptable microbiological limit), 'M' (the level that when exceeded leads to the product being rejected) and 'c' (the number of samples that may lie between 'm' and 'M"). For pathogens a two-class plan is advocated where m=0. For other organisms a three-class plan operates where 'c' defines how many samples may lie between 'm' and 'M'.

For raw meats, and possibly responding to the Swedish outbreaks of 1953, an early ICMSF sampling plan for *Salmonella* in chilled or frozen carcase meat was assessed as Case 10 where the hazard was adjudged to be serious, incapacitating but not usually life-threatening, and where the food is expected to be consumed cooked. In this case, 5 x 25 g samples (n=5) were required, with no more than one sample (c=1) allowed to contain *Salmonella* (m=0) but with the aspiration that industry improvement would lead to no sample (c=0) containing *Salmonella* (ICMSF, 1974).

Four decades hence, the latest edition of the ICMSF sampling plans (ICMSF, 2011) acknowledges that food safety control is no longer accomplished primarily through inspection and end product testing, but relies on the approach to risk management that has been developed through the work of the Codex Alimentarius Commission (CAC).

Emphasis is placed on testing of carcases during the process to assess hygienic process control and on testing the processing environment to ensure that cleaning has been adequate. End-product criteria are given for *E. coli* in raw meat (Case 4, n=5, c=3, m=10, M=100) and for *E. coli* O157:H7 in beef trimmings used for ground beef (Case 14, n=30, c=0, m=0), intended for use only by countries where beef is known to be a source of illness.

8.3.2   Microbiological criteria applied by regulators

Regulatory authorities use microbiological criteria as a mechanism for minimising the likelihood of their consumers either rejecting product, because it unacceptable from the sensory viewpoint, or of them becoming ill. In the latter case, criteria are often imposed following food poisoning outbreaks. Sweden set criteria for *Salmonella* in meats following their 1953 outbreak, as did the USA for *E. coli* O157 following a large outbreak involving hamburgers from Jack in the Box quick service restaurants.

USA criteria

The Jack in the Box outbreak became of prime importance to Australian processors when USA authorities imposed the Pathogen reduction; Hazard Analysis and Critical Control Point (HACCP) systems; final rule (Anon. 1996). The rule required that all establishments implement a HACCP plan supported by sanitation standard operating procedures (SSOPs) and good manufacturing practices (GMPs); a zero-tolerance was mandated for visible contamination with faeces and ingesta. Microbiological testing was introduced for both contact surfaces and products, in the latter case, both for indicator organisms and *Salmonella*. Later, the USA declared *E. coli* O157:H7 an adulterant and, more recently, added six additional serotypes of Shiga Toxin-producing *E. coli* (STECs). The declaration has resulted in testing of manufacturing beef for the presence of STECs becoming the significant measure of the control of this pathogen in the beef supply chain. This has led to the establishment's testing program becoming a 'disposition CCP' under which a unit (lot) of production cannot be released to the trade until there is confirmation that the pathogen has not been detected in the sampled units.

Early sampling plans involved a sample size of 25 gram (5 x 5 gram samples) per lot of production, later increased to 325 gram (5 x 65 gram samples), then to so-called N-60 or 'robust' testing involving the collection of 60 surface slices from the external carcase surface. Improvement in analytical techniques has also increased the sensitivity of testing for STECs. This end-product testing program has resulted in pressure being applied to processors to reduce the level of these contaminants to a prevalence at which it isn't routinely detected. Although end product testing is not considered ideal, this rather blunt regulatory instrument has had the effect of focusing the processor on reducing the risk and avoiding severe regulatory penalties.

A more pragmatic approach to setting microbiological criteria for food is to set limits that can be met by the industry operating to the best of its ability. Typically, limits are set to allow a high proportion of the industry to process without exceeding them, with the intention that the 'worst' establishments, say 10%, will be stimulated to improve their performance. A problem with setting the microbiological limit too low is that investigations into 'failures' become numerous, possibly without a significant statistical deviation from the mean, and the exercise becomes routine and possibly meaningless. An example of how criteria were set for the Australian meat industry is presented in Vanderlinde *et al*. (2005) in which criteria for *E. coli* and *Salmonella* on chilled beef carcases were set so that 95% of establishments could be expected to conform on a regular basis.

Thus, at any one time the stringency of microbiological criteria under which an industry is required to operate will reflect the performance of that sector. Many countries have introduced microbiological criteria into their regulations or have begun applying microbiological criteria to meat and meat products in international trade. Some of these criteria are focused on wholesomeness (i.e. indicator organisms) while others are focused on pathogens such as *Salmonella*, *E. coli* O157:H7 and specific STEC serotypes.

However, application of these criteria does not always follow the standard risk management framework. Sometimes product tested at the border is declared unsuitable if any pathogen is detected, even when that pathogen has not been declared an adulterant in the importing country.

Japanese criteria

The Ministry of Health Labour and Welfare conducts monitoring at the border, testing for *E. coli* O157, *E. coli* O104, *E. coli* O111 and *E. coli* O26. The protocol of detection for Vero toxigenic (analogous to Shiga toxigenic) *E. coli* O157, O104, O111 and O26 is described. Samples of meat (including offal meat), processed meat products and cheeses are examined by using only cultural methods, though it would be desirable if the existence of a verotoxin (VT) gene were established prior to cultural follow-up.

GCC criteria

The Gulf Standards Organisation Standard, GSO 997/1998 'Beef, buffalo, mutton, and goat meat, chilled and frozen' references a microbiological criteria standard GSO 1016/1998 'Microbiological criteria for foodstuffs - Part 1.'

In addition to arbitrary shelf life requirements, the United Arab Emirates also imposes a microbiological criterion for Aerobic Plate Count of n=5, c=3, m=10^6 and M=10^7, which applies to all chilled meat (AQIS Market Access Advice 1025, 2010). An APC in the UAE is performed by incubating the sample at 35°C for 48 hours. As shown in Section 6, this criterion should be regularly attainable for vacuum-packed beef, though less so for vacuum-packed sheep primals.

Egyptian criteria

According to the Egyptian Standard for chilled meat (3602/2008):

- *Salmonella* shall be absent in 25 gram of chilled meat
- *Shigella* shall be absent in 25 gram of chilled meat
- Total bacteria <1,000,000/cm^2 of surface
- Chilled meat shall be free of *Clostridium* and *Listeria monocytogenes*
- Chilled meat shall be free of *Staphylococcus aureus*

Jordanian criteria

In addition to the criteria listed in Table 8.2:

- Full carcases and boneless meat must be free of *E. coli*
- Full carcases and boneless meat must be free of *E. coli* O157:H7 (Jordanian Standard and Measurement. Meat and meat products-fresh, chilled and frozen beef meat 2002/471)
- Total Plate Count (ISO 4833:2003 method) maximum 10^6/gram
- Coliforms (ISO 4831:2006 method) maximum 10^2/gram
- *E. coli* (ISO 7259:2005 method) maximum 10^2/gram
- *Staphylococcus aureus* (ISO 6888-1:1999 method) maximum 10^2/gram
- *Clostridium perfringens* (ISO 7937:2004) maximum 10^2/gram
- *Salmonella* – not detected in 25 gram

Table 8.2 Microbiological criteria for meats imported to Jordan.

Acceptance	Bacteria	Meat products
The highest limit 6,000,000	Total bacterial count	Carcases chilled and frozen
The highest limit 6,000,000	Total bacterial count	Boneless chilled and frozen meat

Vietnamese criteria

Vietnamese criteria are stipulated in Circular 29/2010/TT-BNNPTNT – Food safety criteria and maximum levels thereof in certain foodstuffs of animal origin. They are shown in Table 8.3.

Table 8.3 Microbiological criteria for meats imported to Vietnam

Foodstuff	Testing point	Micro-organism	Sampling plan			
			n	C	m	M
Minced meat and mechanically separated meat	Border inspection posts	Total aerobic colony count	5	2	5×10^5/g	5×10^6/g
		E. coli	5	2	5×10/g	5×10^2/g
Meat preparations		*E. coli*	5	2	5×10^2/g or cm^2	5×10^3/g or cm^2
Minced meat or meat preparations made from other species than poultry intended to be eaten cooked	Throughout shelf life	*Salmonella*	5	0	Absence in 25g	

Russian criteria

Criteria in Table 8.4 have been stipulated by SanPiN 2.3.2.1078-01 and approved by the Chief Sanitary Officer of the Russian Federation on 14 November 2001.

Table 8.4 Microbiological criteria for meats imported to Russia.

Group of Products	Amount of mesophilic aerobic & facultative anaerobic microorganisms not more than KOE in 1 g[a]	Mass of product (g) to be free of bacteria[b]		Notes
		Coliforms	Pathogenic, including *Salmonella*	
Meat (all types of slaughter animals)	Test samples taken from deep layers			
Fresh meat in carcases, half-carcases, quarter-carcases, cuts	10	0.1	25	*L. monocytogenes* not detected in 25g
Chilled and semi-frozen meat in carcases, half-carcases, quarter-carcases, cuts	1,000	0.1	25	as above

a – believed to be equivalent to Total Plate Count
b – implies that an enrichment technique should be used

EU criteria

EU criteria relate to Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. This EU regulation has criteria for minced meat, mechanically separated meat and meat products intended to be eaten raw – but not for primals, beef trim and meat intended to be cooked.

8.3.3   Retail microbiological criteria

Major retailers set microbiological criteria for both food safety and shelf life, with the expectation that, on occasion, product will receive temperature abuse during the consumer phase. A conservative approach is used to 'build in' safety or, as was stated for M&S' original standards in the less politically correct times of the early 1970s, to incorporate an 'idiot factor'. The following pages contain summaries of the microbial requirements set by major retailers at time of writing (2016).

Retailer A

The shelf life requirements of beef, lamb, pork and game meat products were reviewed by Retailer A in early-2016 and included:

- Meat carcases
- Packaged primals and trim intended for further processing (e.g. grinding, slicing, packing)
- Packaged retail straight cuts and diced meats in overwrap
- Packaged retail straight cuts and diced meats in vacuum or MA packs
- Packaged retail comminuted and value added meats
- Packaged retail offals and bones

Microbiological limits are specified for each of the above categories for Start of Shelf Life (SOL) and End of Shelf Life (EOL) and, where possible, reference guideline levels specified in:

- Meat Standards Committee Guidelines
- IFST microbiological criteria
- MLA Shelf Life of Australian Red Meat.

Table 8.5 Microbiological criteria for raw retail meats: Retailer A.

	Start of Shelf Life (SOL)		End of Shelf Life (EOL)
	Target	Report	Reject
Packaged primals and trim for further processing			
Enterobacteriaceae	<100 cfu/cm²	-	≥100 cfu/cm²
E. coli	<10 cfu/cm²	-	≥100 cfu/cm²
SPC	<10,000 cfu/cm²	≥10,000 cfu/cm²	>100,000 cfu/cm²
Packaged retail straight cuts and diced meats (VP/MA)			
Enterobacteriaceae	<1,000 cfu/g	≥1,000 cfu/g	≥100,000 cfu/g + Sensory
E. coli	<100 cfu/g	≥100 cfu/g	≥100 cfu/g + Sensory
SPC	<1,000,000 cfu/g	≥10,000,000 cfu/g	≥100,000,000 cfu/g + Sensory
Packaged retail straight cuts and diced meats (OVERWRAPPED)			
Enterobacteriaceae	<1,000 cfu/g	≥1,000 cfu/g	≥100,000 cfu/g + Sensory
E. coli	<100 cfu/g	≥100 cfu/g	≥100 cfu/g + Sensory
SPC	<1,000,000 cfu/g	≥10,000,000 cfu/g	≥100,000,000 cfu/g + Sensory
Packaged retail Comminuted and Value added (fresh to be cooked) e.g. sausages, rissoles, burgers, meatballs, mince, pickled, marinated			
Enterobacteriaceae	<1,000 cfu/g	≥1,000 cfu/g	≥100,000 cfu/g + Sensory
E. coli	<100 cfu/g	≥100 cfu/g	≥100 cfu/g + Sensory
SPC	<1,000,000 cfu/g	≥1,000,000 cfu/g	≥10,000,000 cfu/g + Sensory
Mould	<10,000 cfu/g	≥100,000 cfu/g	≥1,000,000 cfu/g + Sensory
Yeast	<10,000 cfu/g	≥100,000 cfu/g	≥1,000,000 cfu/g + Sensory

Comment:

1. The specifications informally align with 3-class sampling (n, c, m and M) with the:
   - *'Target' level being equivalent to "m"*
   - *'Report' level allowing departure from "m", specified by "c"*
   - *'Reject' level being equivalent to "M" – applied at the End of shelf life*
   - *There is no minimum number of samples "n" specified, although typical industry practice would involve submitting a single sample of each product type*
2. The specifications recommend that, in the event of the Report level being exceeded at the Start of Life, the supplier contact the Product Technologist for Retailer A
3. Corrective action may include:
   - *additional testing to determine how many samples are above the Target level.*
   - *an investigation of the food production or handling practices to determine the source/cause of the results so that remedial actions can commence if necessary*

Retailer B

Criteria set by Australian Retailer B (Table 8.6) are for end of shelf life and appear impossible to achieve for primals, offals, diced and trim if they are vacuum-packed.

Table 8.6 Microbiological criteria for raw retail meats: Retailer B.

MEAT - COMMINUTED PRODUCTS (fresh to be cooked) e.g. sausages, rissoles, burgers, meatballs, mince	Criterion
Enterobacteriaceae	< 10,000 cfu/g
Escherichia coli	< 10 cfu/g
Standard Plate Count	< 1,000,000 cfu/g
MEAT – PRIMALS & PRODUCTS FOR FURTHER PROCESSING	
Enterobacteriaceae (Carcase/bones)	< 100 cfu/cm^2
Enterobacteriaceae (Primals/offals/diced/trims)	< 1,000 cfu/g
Escherichia coli	< 10 cfu/cm^2 or cfu/g
Standard Plate Count (Carcase/bones)	< 10,000 cfu/cm^2
Standard Plate Count (Primals/offals/diced/trims)	< 100,000 cfu/g

Retailer C

Australian Retailer C sets criteria for Total Plate Count (TPC) at packing and at the end of aerobic shelf life that are in a range that can be achieved by suppliers; the criterion for *E. coli* is also achievable (Table 8.7).

Table 8.7 Microbiological criteria of Retailer C for raw meats.

Product	Test	Criterion
Beef Scotch fillet	TPC at packing	<5,000 cfu/g
	TPC at end of aerobic shelf life	<10,000,000 cfu/g
	E. coli	<10 cfu/g

Retailer D

Australian Retailer D has a criterion for Standard Plate Count (SPC) at packing that is achievable for suppliers, though the end of shelf life SPC may be difficult; the criterion for *E. coli* is also achievable (Table 8.8).

Table 8.8 Microbiological criteria of Retailer D for raw meats.

Product	Test	Criterion
Marinated lamb leg (vacuum-packed)	SPC at packing	<500,000 cfu/g
	SPC at end of shelf life	<5,000,000 cfu/g
	E. coli	10 cfu/g

Retailer E

A major Japanese retailer, Retailer E, sets a criterion for SPC of not more than 500,000 cfu/g for vacuum-packed lamb primals at the point of entry to their further processing facility.

8.4 What does a realistic microbiological standard for shelf life look like?

The philosophy of setting a standard for shelf life differs from standards concerned only with food safety. In the latter, the standards setting body (usually a government agency) may need to react to a food safety incident and increase the stringency of the current criteria. Or the focus may be building to a medium-term goal of reducing the disease burden of a particular pathogen e.g. *Listeria monocytogenes* in ready-to-eat foods.

A standard for (non-pathogen aspects of) shelf life is governed primarily by the commercial realities of the last owner, the consumer, who will reject the product if:

- The appearance is poor because the colour is lost, there's excess purge or the pack is blown
- The odour is objectionable and persistent
- The cooked meat tastes off, or is tough.

Some of these negative attributes are a consequence of excessive microbial growth and retailers exert pressure on wholesalers or importers to provide product with a high likelihood of consumer acceptance.

From the wholesaler/importer viewpoint, quality criteria are a balance between what the supplier can achieve on a high proportion of occasions, and what the retailer needs to allow them to clear product from the displays to the satisfaction of every customer. Retailers incur losses when they have to discount product near the end of its shelf life and when they discard out of date product (termed 'shrinkage').

For a microbiological criterion for shelf life to be realistic, the product must incur no abusive temperatures (>5°C) during storage in the cold chain. Given good storage temperatures, the following criteria will fulfill the needs of all stakeholders (Table 8.9).

Table 8.9 Suggested criteria for end-of-shelf life testing of meat products.

Product	n	C	m	M
Ground beef, sausages in aerobic, VP or MA packs	5	2	1,000,000	10,000,000
Vacuum-packed subprimals and cuts	5	2	10,000,000	100,000,000

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9   Establishing and Managing Shelf life

All meat and meat products will have a shelf life that is determined by the length of time that the characteristics of the product are expected to remain acceptable organoleptically and safe for consumption. The lower the storage temperature, the slower the growth rate of spoilage bacteria, with the optimum temperature for the storage of vacuum-packed meat for long periods being −1.5°C (Gill et al. 1988a & b).

In this section, we describe the various testing methods and principles of how the end of shelf life may be determined.

9.1   Testing to establish end of shelf life

9.1.1   Raw meats – fresh

Establishments producing raw vacuum packed chilled bovine and ovine routinely evaluate the shelf lives of their products; this is required as a matter of course by domestic and international customers.

In 2016 an industry-based panel developed guidelines for estimating shelf life in order to standardize methodologies and to provide a scientific underpinning which may be used when liaising with customers.

9.2   Guidelines for developing a method for estimating shelf life of chilled raw vacuumed meat products

This section contains information designed to assist in the development of procedures for undertaking a shelf life estimation study. It is based on information from available publications and reports and intended primarily for use with chilled ovine and bovine vacuum packed meat cuts.

9.2.1   Defining end of shelf life

Shelf life ends when meat becomes unfit for use, human consumption, or sale; this may occur because of sensory reasons (appearance or smell) or microbiological reasons (a customer specification is exceeded).

Where unique customer requirements are specified, they will need to be incorporated into the shelf life design, which may also include chemical testing.

9.2.2   Design of a shelf life trial

The aim of a shelf life trial is to estimate, with reasonable accuracy, the number of days that sensory and microbiological criteria of the meat product remain acceptable.

To do that, meat samples need to be stored under defined conditions as close as possible to those to which they will be subjected in the marketplace. Samples need to be withdrawn at key times to estimate the time when the product will still meet customer requirements and expectations or when end of shelf life is reached.

The shelf life trial should challenge the product until spoilage occurs, which requires a sufficient number of samples to allow testing to proceed past the expected shelf life of the product.

a) Sampling days

If the last day on which the product is expected to be just acceptable is defined as 100% (i.e. the shelf-life), then sampling may be focused around this point:

Sampling time	1	2	3	4	5	6
Shelf life used	0%	90%	95%	100%	105%	110%

If a customer requests more frequent sampling intervals these will need to be added to the above table.

Note that samples at time zero i.e. immediately after packaging are included to establish the starting levels of the product. This is important to allow proper interpretation of the results obtained at later times.

b) Number of samples

Shelf life trials are a cost of doing business and can be expensive particularly when high-value cuts such as striploins, cube rolls or rumps are used. Laboratory and sensory testing also have their own associated costs.

Key considerations are that:

- There are sufficient samples to go to the end of shelf life and beyond.
- Replicate samples are taken at each sampling day – if only one sample is used and the pack turns out to be a leaker, no result will be possible for this sampling day.

Three replicate samples are considered a good number to ensure the integrity of the trial and this amount of replication used in trials on chilled meat in Australia (Holdhus Small *et al*. 2012; Kiermeier *et al.* 2013) and in Canada (Youssef *et al.* 2013).

As an example, if determining the shelf life for a vacuum packed (VP) beef primal, historical data may indicate a shelf life of around 160 days. Using this information, a total of 18 packs can be used and samples withdrawn at times around the expected shelf life (160 days) and beyond.

Sampling time	1	2	3	4	5	6
Shelf life used	0%	90%	95%	100%	105%	110%
Days after storage	0	144	152	160	168	176

If the trial was for the shelf life of VP lamb primals, a total of 18 packs could also be stored, with sampling focused around an expected shelf life of 90 days.

Sampling time	1	2	3	4	5	6
Shelf life used	0%	90%	95%	100%	105%	110%
Days after storage	0	80	85	90	95	100

Note that the above are suggested sampling times – actual schedules should be based on historical product knowledge and also on customer requirements.

It is also wise to include 2-3 'spare' samples in case leaker packs are found among the stored samples.

9.2.3   Type of cut and packaging

Ideally, cuts are taken from the boning room immediately before they are packed into cartons, as was done in the study by Holhus-Small *et al.* (2012) where strip loins and cube rolls from six abattoirs in Australia were tested.

To minimise expense, cuts are sometimes divided before packaging e.g. Canadian researchers divided striploins into two before packaging (Youssef *et al.* 2014). For this particular cut the procedure probably has no influence on the shelf life since the bag has the appropriate dimensions for the cut and sealing can be done without any impact on the heat seal and there should be no creases to trap air.

It is tempting to divide a primal cut into as many small pieces as possible e.g. cutting a striploin into 10-12 steaks. This should be avoided because the surface area to volume ratio of the steak is radically different from that of an entire striploin and this will adversely affect the shelf life. Consequently, the stored samples would be unrepresentative of the product.

9.2.4   Storage temperature

The choice of storage temperature depends on what the trial is designed to achieve; it may be the storage temperature recommended to the customer by the establishment prior to use, or the customer's expected usage requirements using their nominated parameters.

Without prior knowledge or customer specification, a good temperature is close to 0°C since this approximates what would normally be used at an establishment for storage and in a refrigerated container for overseas delivery.

For a domestic retailer a temperature of 5°C for a nominated number of days after removal from close to zero storage may also be required, since that will be their retail display temperature. Similarly, the retailer may also require shelf life to be established at an abusive temperature of 8°C.

Since temperature has such a large effect on shelf life, at least one data logger should be included in your trial, located securely between two individual cuts and maintained *in situ* for the entire trial. The data file from the logger should be kept with other documentation from the trial.

9.2.5   Sensory testing

a) **Training a sensory panel**

When meat is assessed senses are used: eyes, nose and mouth and, unlike machines or instruments, individuals don't all assess the same product in a uniform manner.

For this reason it is necessary to assemble a sensory panel/team. The number of panelists can vary, but three is the minimum number recommended.

Panels are more effective if each individual receives some training on how to interpret what they are seeing, smelling or tasting. This can be achieved by exposing panelists to products with a range of attributes so they become experienced in what each descriptor means, e.g. "Moderate odour".

The panel will also need training in how to fill in the assessment sheets and on doing the assessment without communicating their thoughts to the other panelists, at least in the first instance.

If sample packs are sent for evaluation to an off-site laboratory, then it is necessary to establish that the necessary skills are in place and that an acceptable procedure will be followed. It is also necessary to consider the time and temperatures of storage for samples sent to off-site laboratories.

b) **Creating a suitable area for assessment**

Product should be assessed in an area which is quiet, well-lit and spacious.

A laboratory is a good location as benches can be cleaned after the panel has finished, and any spilled liquids removed.

c) Assessing appearance of the pack

When a carton is opened to remove a sample the first criterion to assess is whether any packs have leaked, in which case these should be noted and removed from the assessment.

It is prudent to remove non-leaker packs from the carton and wipe them clean with a damp paper towel, and to remove the plastic liner and replace it before putting packs back in the carton.

The next criterion to assess is the amount of drip/purge/weep in the selected samples.

A 'normal' amount of drip is 1–6% of the weight of the cut, with seam-boned primals losing less than pieces subjected to trimming/cutting e.g. denuded knuckle,topside (Barlow *et al.*, 2016).

Drip may also increase if individual cuts have been packed into the carton so that those at the bottom are under pressure. Excessive drip is not equated with end of shelf life.

d) Bag integrity

Before opening the pack the seams should be examined to check whether there is any 'doubling-up' caused by the bag not being laid correctly on the heat seal bar.

Assess also whether there are folds in the bag. Air becomes trapped if the bag doesn't fit closely over the meat and this allows aerobic growth; folds also facilitate production of drip.

e) Opening the pack

The pack is opened by slitting just beneath and along the line of the seam.

There is almost always an odour detectable on opening the pack, usually slightly sour, which dissipates after a few minutes. This is called 'confinement odour', and is a normal occurrence as meat ages and should not be considered as part of the odour assessment.

The odour which must be assessed is that which persists around the meat when it has been removed from its packaging for a few minutes.

f) Assessing odour

Training will involve exposing the panel to various odours, which involves guiding them to assessing terms such as sour, acidic, cheesy, sweet, sickly, putrid and to other descriptors such as slight, moderate and extreme.

Experienced panel members with good industry and product experience are best suited to perform product sensory assessments; sales staff can be especially useful because they deal with customers and their perceptions.

The essential feature of odour assessment is that each panelist is 'grounded' in identifying unacceptable odours and this may take continuous coaching/guidance from the leader of the sensory team.

g) Meat colour and bloom

When samples are removed from the vacuum pack cut surfaces should quickly regain their bright, red colour (bloom).

h) Scoring the assessments

Panelists score their observations against a set of criteria for which they have received training on a score sheet.

All score sheets have a scale on which criteria gradually change from acceptable to unacceptable.

The number of points on the scale can vary from 4-point to 9-point.

Sensory score sheet with 9-point scale

Score	Vacuum	Appearance	Odour
8	Complete, tight package adhesion	Very fresh, no discolouration	Fresh, no off odour
6	Good vacuum	Fresh, slight discolouration	Slight off odour
4	Moderate vacuum	Good, acceptable	Medium odour
2	Poor vacuum	Poor	Strong off odour
0	No vacuum, probable leaker	Severe discolouration	Extreme off odour

The point when shelf life expires on the 9-point scale, above, is the time when either the appearance or odour reaches a score of 2.

Note that a score of 2 for vacuum leads to a consideration of whether the pack is a leaker before rating that the shelf life has expired; only intact packs should be used for shelf life assessments.

Sensory score sheet with 5-point scale

Score	Drip	Vacuum	Appearance	Odour
4	None	Complete adhesion	Deep red colour	Fresh
3	Slight	Good	Light red colour	Slight sour/dairy
2	Acceptable	Moderate	Slight discolouration	Sour/dairy
1	Heavy	Poor	Poor colour	Strong sour/dairy
0	Extreme	None/blown	Severe discolouration	Off odours

The point when shelf life expires on the 5-point scale, above, is the time when either the appearance or odour reaches a score of 1; note the previous remarks about leaking packs and excluding these from the assessment.

Similar criteria are covered in a 4-point sensory scale, where the cut-off point for acceptability is a score of 1 for either colour or odour.

Sensory score sheet with 4-point scale

Score	Vacuum	Colour	Odour
3	Seal intact, minimal drip	Purple/red	Fresh
2	Seal intact, normal drip	Purple/red	Slight stale
1	Broken seal, slack pack, excess drip	Two toning, browning	Strong stale/dairy
0	Broken seal, copious drip	Brown, grey colour	Putrid

i) Reaching a consensus

When the panel has completed its assessment the scores are compared and evaluated.

There will be occasions when discrepancies occur e.g. sometimes a panelist scores differently from other team members and the discrepancy will need to be investigated to determine if re-training of the panel is required.

On occasion, all panelists may agree that one of the three packs sampled is unacceptable and two are acceptable. In this case it is advisable to open three more packs to assist the decision on whether end of shelf life has been reached.

When the panel determines that all three packs are unacceptable, the end of shelf life has been reached. A safe shelf life is therefore the previous sampling day when all three packs were acceptable.

9.2.6   Microbiological testing

While some customers impose only sensory specifications or the proportion of shelf life remaining when the consignment is accepted, others set a microbiological criterion. Some criteria set by importing countries and Australian supermarkets are detailed in Section 8.

a) **Sampling meat for microbiological testing**

Methods for removing bacteria from meat surfaces fall into two categories:

Destructive or excision sampling, where tissue is removed

Non-destructive sampling, where the meat surface is swabbed, sponged or washed to remove bacteria into a surrounding medium (so-called 'meat-in-bag' technique).

Destructive or excision sampling

It is generally agreed that excising surface tissue, then blending or stomaching it, will result in greater recovery of bacteria than will non-destructive sampling methods (Capita *et al.* 2004).

For those establishments which have laboratory staff skilled in excising tissue and blending it using aseptic technique, excision sampling is considered the 'gold standard'.

Non-destructive sampling

The numbers removed by non-destructive sampling vary widely according to the vigour with which the tissue is rubbed and to the abrasiveness of the sponge or swab.

Gill & Jones (2000) found that recovery was lower when cotton wool swabs were used, compared with excision samples and with samples obtained using a sponge or abrasive gauze pads, with the difference in Aerobic Plate Count/cm^2 when chilled carcases were sampled, was around 0.5 log.

In Australia, Seager *et al.* (2010) monitored the recovery of bacteria from beef carcases using ten experienced samplers. On average, about 40% of the total bacteria on the meat surface was removed by using a Whirlpak sponge but the standard deviation at each site was high, reflecting the wide variation of recovery among operators (2.3 – 93.1%).

Using a Whirlpak sponge for shelf life testing may be a favoured method given that all establishments use this technique for ESAM testing.

An alternative non-destructive method was used by Holdhus Small *et al*. (2012) in which rinse samples were collected from the primal by placing it in a sterile bag with 500mL of sterile saline and massaging its surfaces for 2 minutes.

This technique is widely used in the poultry industry and has the advantage that bacteria are removed from all surfaces of the meat, and the disadvantage that converting the count on the plate to a count/cm^2 requires a mathematical formula – Holdhus Small *et al*. (2012) show how to do this for striploins and cube rolls.

b) **Media used in estimating microbial counts**

In research studies such as those quoted previously (Holdhus Small *et al*. 2012; Kiermeier *et al*. 2013; Youssef *et al*. 2013) a range of culture media are used to enumerate bacteria which dominate the population during the various stages of shelf life. Information on these bacteria is presented in Sections 5, 6 and 7.

In general, for the purpose of gathering microbiological information an Aerobic Plate Count (APC) is sufficient. If customers specify a suite of organisms to be tested for then their requirements must be met.

c) Incubation temperatures for shelf life estimation

Because shelf life is assessed by storing meat under refrigeration for many weeks the dominant microflora is composed of psychrotrophic bacteria.

Psychrotrophs generally have a temperature optimum of 15 – 25°C and a maximum growth temperature around 30 – 35°C (ICMSF, 1980). Therefore, it is logical to incubate cultures near their optimum growth temperature (25°C) for sufficient time (4 days) so that colonies are clearly visible and therefore countable on the culture plate.

This was captured by an Australian Standard (AS 1766.3.1-1991) Food microbiology Method 3.1: Examination of specific products – Meat and meat products other than poultry: *Standard plate count. Incubate at 25 ±1°C for 96 ±2 h. Examine the plates after 72 ± 2 h, and record the counts for those plates that are likely to be overgrown before the full incubation period has elapsed.*

While this Standard has been replaced by less prescriptive standards it is recommended that cultures are incubated for 4 days at 25°C.

The importance of using the correct incubation temperature in monitoring shelf life studies is captured by Pothakos *et al*. (2012) who incubated plate counts of stored food samples at either 22°C/5 days or 30°C/3 days and found that counts from the former temperature were 0.5 – 3 log cf u/g higher. The authors concluded: *"This study highlights the potential fallacy of the total aerobic mesophilic count as a reference shelf life parameter for chilled food products as it can often underestimate the contamination levels at the end of the shelf life."*

d) Interpreting micro counts for shelf life

Early work by CSIRO during the 1980s showed that APCs reach very high levels – between 7 and 8 log cfu/cm^2 (10,000,000 – 100,000,000 cfu/cm2) when VP beef was stored at around 0°C (Egan, 1983).

The meat was still acceptable to a taste panel even after bacterial numbers reach a maximum; only after several more weeks of storage did spoilage become apparent.

A very high count is 'normal' in vacuum packed meat storage as the product ages. The dominant bacteria are lactic acid bacteria (LAB). High counts towards the end of storage should not be of concern and customers should be reassured that such counts are not only normal but are the reason why vacuum packed primals have such long chilled storage lives.

9.2.7   Chemical testing

Chemical tests are not usually necessary as part of shelf life testing in the commercial setting, though knowing the initial pH may prove valuable in interpreting the final shelf life.

9.3 Optimising shelf life – cold chain management

For overseas markets, sea freight to Europe is around seven weeks with the need to maintain temperature close to –1°C. Many exporters include a data logger within a carton of meat to obtain a temperature:time record for the entire journey. One logger is usually included in a carton in the first row during container loading and another in the final row next to the door.

The importer recovers the logger and downloads the information, which provides key information e.g. average temperature during the trip. A temperature:time chart is also provided, which highlights any departures from the set temperature for the container.

The current generation of data loggers can trigger an alarm when product temperature departs from the prescribed range, the alarm being picked up when the logger is downloaded. The next generation of loggers is nearing release where temperatures are transmitted continuously and wirelessly, triggering alarms in real time, and potentially permitting corrective action before product is adversely affected.

Figure 9.1 is a temperature record of chilled, vacuum-packed lamb primals shipped from Australia to Europe. The product was maintained at – 0.7°C over the 41-day days from Australian establishment to European cold store, indicating optimal storage temperatures over the entire voyage.

Figure 9.1 Temperature record of chilled, vacuum-packed lamb primals shipped from Australia to Europe with optimal storage temperatures.
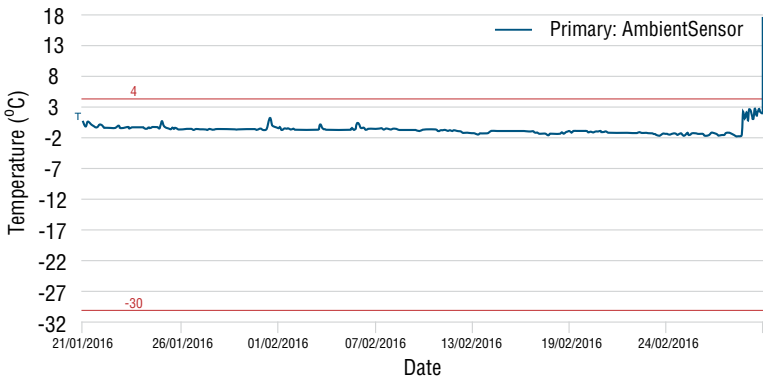
At intervals in Figure 9.1 there are temperature "blips" when the container is off power: at loading in Australia, during trans-shipping in Singapore and unloading in Europe. These short rises in temperature have little effect on shelf life.

Occasionally, however, problems occur and a container is either off power for an extended time or the set point results in a "high" temperature for the whole trip. The log for product in Figure 9.2 indicates that the temperature was always above zero, with an average temperature of +1.9°C over 31 days between establishment and customer.

Figure 9.2 Temperature record of chilled, vacuum-packed lamb primals shipped from Australia to Europe with poor storage temperatures.
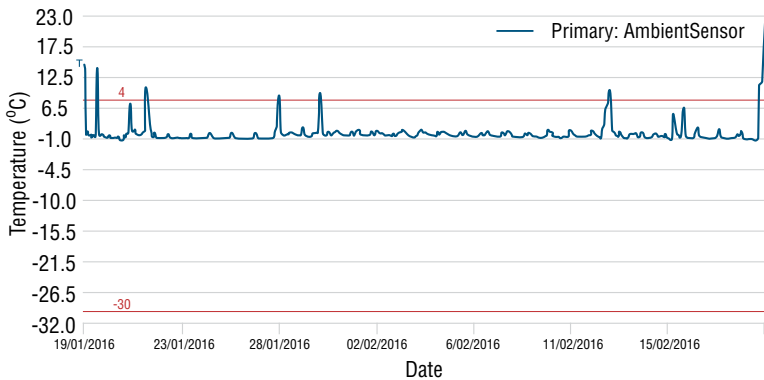
While there may seem little difference between temperatures on the voyages illustrated in Figures 9.1 and 9.2 there will be a significant loss of shelf life available to the importing customer.

How much shelf life is lost in product may be determined by inserting the temperature:time data into the UTas predictive tool for vacuum-packed lamb primals. In the next section predictive models and how they have evolved into powerful tools for assessing shelf life are examined

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10 Predictive Tools

Traditionally, if a company wanted to know how a bacterial population responded to different processing parameters,

they set up a challenge trial by inoculating a known number of bacteria onto meat before the process and measuring the changes in numbers during and after the process.

In the 1990s researchers discovered how to mimic thebehaviour of bacteria under certain processes in the laboratory– without the need to grow them on meat. So reliable are bacteria that it became possible to predict how they wouldgrow. This led to the development of predictive mathematical models and, from them predictive software tools, such as the Refrigeration Index.

In this section we describe how tools to predict shelf life are developed and how they can be used in the domestic and export trades.

10.1 What are predictive tools?

The behaviour of spoilage bacteria is predictable, which serves as the foundation of a field of food microbiology called Predictive Microbiology. In this research discipline, predictive tools (models) are produced by measuring and understanding how quickly bacteria grow (or die) in different food environments. Once understood, the data are converted into mathematical equations, which are then translated into software tools that help food companies manage the growth or death of bacteria in food processing systems and supply chains. The benefits of validated tools include reduced reliance on microbiological tests and greater flexibility in meeting performance standards.

Predictive microbiologists translate patterns of microbial behaviour (growth, survival, and inactivation) into mathematical models to be able to predict microbial behaviour under various environmental conditions. This is possible because parameters of microbial growth (lag phase, growth rate, maximum population density) and inactivation (death rate) change in a predictable way in response to environmental conditions (Figure 5.3, Section 5).

Developing a predictive tool requires several stages.

Stage1

Stage 1 involves understanding the rate of bacterial growth, or death, and requires a series of laboratory experiments conducted over a range of environmental conditions relevant to the food of interest. In this example, the growth rate of a spoilage bacterium, *B.thermosphacta* is measured over a range of storage temperatures in the laboratory in a broth similar to the chemical make-up of meat. At each temperature the number of *B. thermosphacta* is determined at several time intervals and the number of bacteria graphed against time. A line is fitted to the growth phase points (see Figure 5.3), and is called the Primary Model. The steepness of that line is used to calculate the rate of growth of *B. thermosphacta* at a specific temperature.

After conducting several experiments at different temperatures or other conditions (pH, aerobic or anaerobic etc.) the growth rates are plotted as shown in Figure 10.1, and described by a mathematical equation that is, in effect, the predictive model.

Figure 10.1 Change in the growth rate of *B. thermosphacta* in beef as a function of storage temperature.

$y = 0.016x + 0.0253$
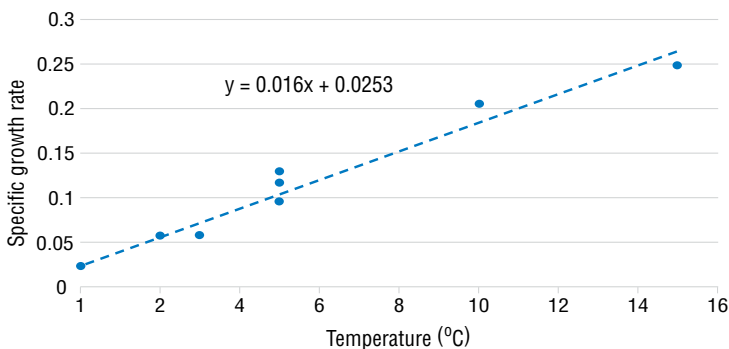
Stage 2

Stage 2 is to fit an equation through the data using a computer. The equation for that line forms the Secondary Model. As seen from Figure 10.1, the equation below allows us to predict the growth of *B. thermosphacta* at any temperature between 0 and 15°C.

Specific growth rate = (0.016 x temperature in °C) + 0.0253

Stage 3

For predictive models to become useful tools for industry, they must be tested (validated) under real food production conditions. Validation determines the accuracy of model predictions under real conditions and identifies whether the model should be adjusted to improve prediction accuracy; this comprises Stage 3. Validation is done by repeating the Stage 1 (laboratory) experiments on product in the meat plant. In this example, where a predictive tool for *B. thermosphacta* is being made, this involves inoculating it onto pieces of meat, vacuum packing them and storing them at temperatures which are relevant commercially e.g. –1°, 2°, 5° and 8°C. At intervals, samples are removed and the numbers of *B. thermosphacta* counted, as before, to measure the increase in population over time. Finally, the growth rate is calculated at each temperature and, if the rates from the model closely align with the rates obtained on meat, the model is considered "valid".

Stage 4

In Stage 4 computer software, or an 'app' is developed and the model becomes a tool. The software incorporates the validated secondary model and makes calculations of growth based on it, given information about time and temperature and other relevant conditions. The user interface for the Refrigeration Index (RI) is shown in Figure 10.2. The interface acts as a shop front where the result can be seen when data is inserted into the tool. Behind the interface, 'back of house', is where the software developer places the equations which translate the data the user enters into the tool into the final result. A well-designed interface requires a full understanding of how the model will be used by the industry.

10.2 How are predictive tools used?

Predictive models are used to estimate bacteria growth or death over time with respect to some environmental factor(s). For example, a company may be exporting vacuum-packaged lamb into an overseas market where an import criterion exists for maximum TPC levels. If the company knows the temperature of the product throughout the supply chain, and the level of the relevant population on the meat at the time of load-out, a predictive tool can be used to predict TPC levels when the product reaches the market, as well as indicating remaining shelf life.

The majority of models have been produced in response to meeting performance standards e.g. the US Department of Agriculture Pathogen Modeling Program and the UK Food MicroModel. These and other models can be used to predict bacterial growth/death for various conditions including temperature, pH, water activity, lactate, and packaging atmosphere and are useful in developing HAACP plans.

10.3 Examples of predictive tools

Currently, there are more models for bacterial pathogens than for spoilage bacteria and shelf life. As mentioned above, this is based on a historical response to industry needs related to HACCP programs. However, more models are now being produced to predict food spoilage.

10.3.1   Refrigeration Index (RI)

The Refrigeration Index (RI) is a predictive tool that is used every day by Australian export establishments to predict the potential growth of *Escherichia coli* during chilling of carcase and carton meat. The RI predicts the growth of 'generic' *E. coli* in raw meat products under dynamic cooling conditions (Figure 10.2). While the model overpredicts growth on carcases due to its failure to account for surface drying, it predicts reasonably accurately the growth of *E. coli* in carton meat. The user enters the product temperature profile from a data logger, selects the temperature measurement time interval, the form of meat product and several other parameters. The RI then predicts the potential level of *E. coli* growth which can be evaluated against the criteria set out in AS 4696:2007.

Figure 10.2 Example of output from the Refrigeration Index . Red line – predicted *E. coli* growth; blue line – product temperature profile.
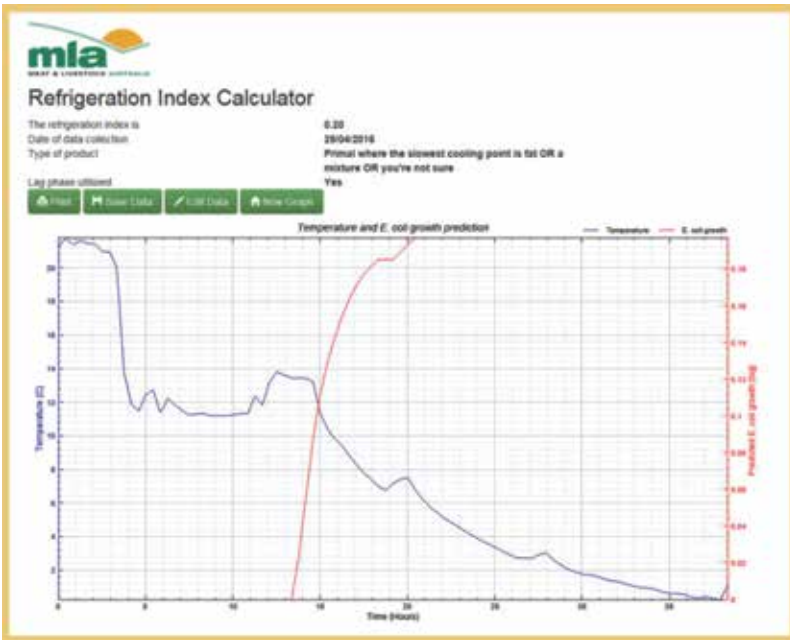
The RI has been of immense benefit to the industry because, without it, the effectiveness of the establishment's chilling regime would need to be verified daily by carrying out microbiological testing. This is not only expensive but would need meat to be held while lab tests are completed e.g. *E. coli* counts would require 24 – 48 hours. By contrast, once the establishment has validated its chilling regime the RI provides a real-time result at little cost.

The RI has also proved invaluable in resolving how product should be disposed of when refrigeration is interrupted. One extreme case involved Cyclone Yasi, which wreaked havoc over far north Queensland in February 2011. Knowing that the first casualty of the cyclone would be loss of refrigeration, technical staff at the establishment placed data loggers on carcases in each chiller. This allowed measurement of surface temperatures over the period while refrigeration was lost and calculation of the RI of meat in each chiller. The RI, supported by back-up sensory and micro testing, was pivotal in preventing the meat being destroyed and in saving the company around $500,000 (Sumner *et al*. 2012).

10.3.2   Danish shelf life tool

The Danish Meat Research Institute has recently released on-line software programs (Figure 10.3) to calculate the shelf life of vacuum-packaged beef cuts. It allows the user to enter the starting level of psychrotrophic bacteria, and then four separate time-temperature scenarios. The model then predicts growth of psychrotrophic bacteria and sensory score. Initial evaluation of the Danish tool indicates that the model underpinning the tool may overestimate the growth rate.

The model is based on storage trials performed in controlled conditions with meat from different commercial plants in, for example, Denmark, Sweden, Norway and Germany. Each individual storage trial includes as much natural variation as possible: different producers, different processes and different cuts. The shelf life models are highly robust due to the large range of selectable variables.

In principle, the shelf life model consists of two models:

- Growth curve for psychrotrophic bacteria (bacteria that grow at chilling temperatures); and
- Shelf life based on assessment of the odour of raw meat. The smell of raw meat is the sensory parameter that changes first during the storage period. For that reason, it is the most important factor when predicting shelf life.

The models can be accessed at: http://dmripredict.dk/

Figure 10.3 Danish Meat Research Institute tool for predicting shelf life of vacuum-packaged fresh beef cuts.
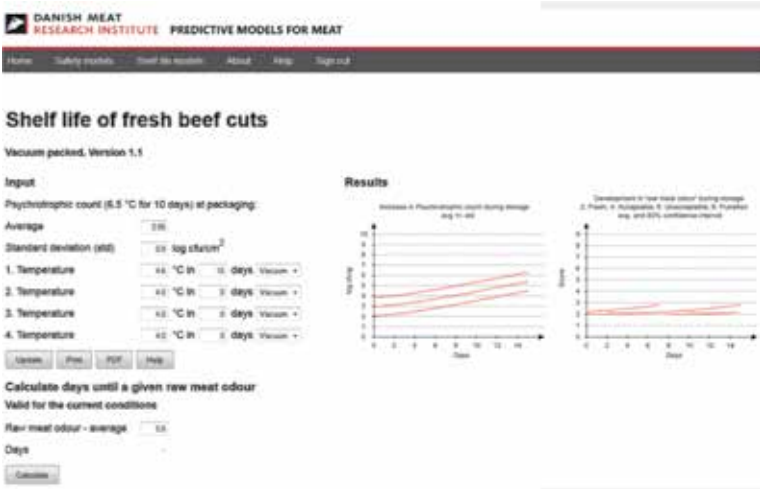
10.3.3 University of Tasmania shelf life tools

Recently, MLA commissioned University of Tasmania to develop spoilage predictor tools for vacuum packaged beef and lamb primals. The tools are based on the growth and development of TPC and spoilage odour as a function of storage temperature. An establishment will need to know the TPC at packing, plus the temperature:time record of meat in the container. When these parameters are entered into the tool, predictions of TPC at the end of the journey, together with days remaining until detection of off odours; this latter can be estimated for a range of temperatures

The meat spoilage predictor (Figure 10.4) consists of two models for each of VP beef and lamb:

- TPC growth curve
- Onset of off odour of raw meat.

Figure 10.4 MLA – University of Tasmania spoilage predictor for vacuum packaged beef and lamb primals.
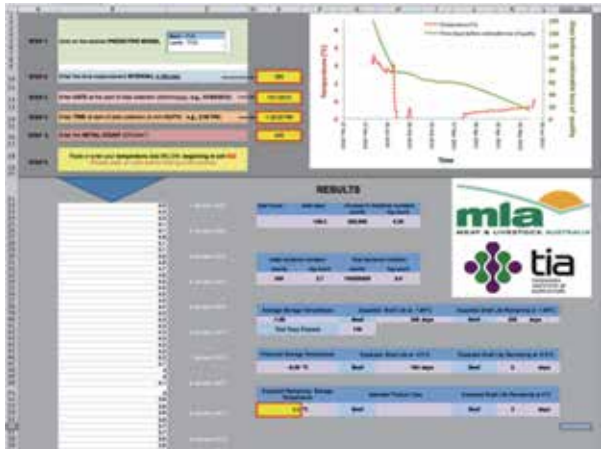
10.3.4 ComBase tools

Predictive models depend on large quantities of data and, as new data sets become available in the published literature, new models can be developed. The University of Tasmania is a partner in the ComBase initiative (www.combase.cc), the world's largest public database of microbial responses to food environments. ComBase contains tens of thousands of records, many describing the growth of spoilage bacteria in meat. ComBase also contains various models that can be used to predict the growth of spoilage bacteria in meat. In Figure 10.4 is shown the result of a ComBase search for growth rates for *B. thermosphacta.*

Figure 10.5 An individual data record in ComBase showing experimental detail of food and its composition and storage conditions, table of data and plot of time versus log cfu/g.
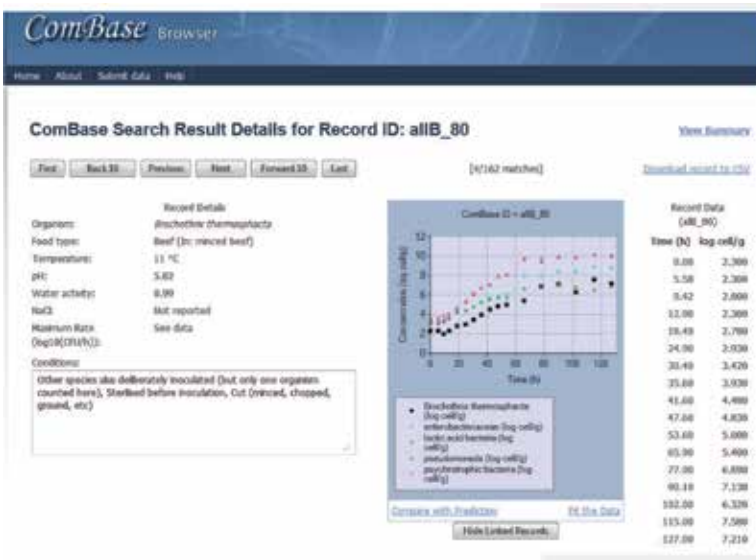
10.4 Limitations of models and tools

Models have limitations that are based on the range of variables tested and the food or laboratory broth used to generate the growth rate data. Some limitations are caused by:

Range of prediction

If the data for a model were produced in meat from –1 to +4°C, then the model can only make predictions for temperatures between, and inclusive of, –1 to +4°C (i.e. by interpolation). User interfaces will normally not let the user make predictions outside of the limits of the model (i.e. by extrapolation).

Type of food and packaging

The use of a model may be limited to the specific type(s) of food for which it was designed. To apply it to other types of food can result in over- or under-estimations of growth/death if the main factors affecting microbial growth in the relevant foods are not included in the secondary model. For example, bacteria grow very differently under aerobic versus anaerobic (vacuum-packaged) conditions. This limitation also extends to different pH values and environments: a model that doesn't include the effect of anaerobic conditions might produce poor predictions of microbial growth in vacuum-packed primals. Similarly, a model for *E. coli* that doesn't include the influence of pH, lactic acid and water activity/salt concentration would probably provide poor predictions of growth during fermentation of meats. In other words, the model must be 'fit for purpose'. Modelling the lag before growth occurs is also problematic as the lag can become highly variable (sensitive to small changes in experimental conditions) as the temperature decrease or the growth conditions approach the growth:no-growth interface become limiting.

10.5 References

Sumner, J., Jenson, I., Ross, T., 2012. Using predictive microbiology to benefit the Australian meat industry, in: Hoorfar, J. (Ed.), Case studies in food safety and authenticity: lesson from real-life situations. Woodhead.

Appendices

Appendix 1   Shelf life Troubleshooting Guide

Below is a guide on how to navigate the book to quickly troubleshoot common shelf life problems.

PROBLEM	→	CONTRIBUTING FACTORS	→	POSSIBLE CAUSES
Visual or Organoleptic Meat colour - **Section 3.1**	→	Temperature & Packaging - **Section 2.10**	→	Common Spoilage bacteria - **Section 5.4 & Table 5.2** Rancidity - **Section 3.4**
High microorganism count or temperature abuse - **Section 5**	→	Storage temperature and Predictive model - **Section 10**		
Food Safety and contamination	→	Hygienic processing & interventions - **Section 2**		
Organoleptic vs Microorganism count	→	Vacuum pack Lamb trial - **Section 6.1** Microbiological standards - **Section 8.3** Methods to assess and determine shelf life - **Section 9.2**		

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Appendix 2   List of Meat Technology Updates (MTUs)

</div>

Title	Year/Volume	Link
Microbiological Criteria for Raw Meat	1994/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_94-4.pdf
Measurement of Temperatures in Fresh and Processed Meats	1995/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_95-1.pdf
Chilled Cartoned Meat in Shipping Containers	1995/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_95-3.pdf
Facilities and Resources Necessary for Safe Microbiological Testing at Meatworks	1995/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_95-4.pdf
Measurement of Surface Temperatures of Chilled Carcases and Sides	1995/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_95-6.pdf
Factors Affecting Fat Hardness of Beef Carcases	1996/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_96-1.pdf
Carton Quality for Frozen Meat	1996/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_96-3.pdf
Tender Beef	1996/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_96-4.pdf
Tender Lamb	1996/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_96-5.pdf
Plate Freezers	1996/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_96-6.pdf
Principles of Chilling	1997/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_97-1.pdf
Safety in the Microbiological Testing Laboratory	1997/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_97-1.pdf
High-pH/Dark Beef	1998/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_98-1.pdf
Tenderstretch	1998/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_98-2.pdf
Refrigeration Principles	1998/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_98-4.pdf
A Critical Control Point Approach to Beef Eating Quality	1999/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_99-1.pdf
Taints in Meat	1999/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_99-3.pdf

Title	Year/Volume	Link
Post-slaughter Aspects of Beef Eating Quality	1999/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_99-4.pdf
Cattle Handling Design Principles	1999/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_99-5.pdf
Pre-slaughter Aspects of Beef Eating Quality	1999/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_99-6.pdf
Beef Fat Quality	1999/7	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_99-7.pdf
Prevention of Fresh Meat Colour Defects	2000/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_00-2.pdf
Sheep Meat Eating Quality	2000/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_00-3.pdf
The Chilled Vacuum-packed Meat Cold Chain	2000/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_00-4.pdf
Production Factors Affecting Beef Eating Quality	2000/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_00-5.pdf
Frozen Meat: Loss of Water and Freezer Burn	2000/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_00-6.pdf
Effect of Hot Boning on Meat Quantity	2001/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_01-1.pdf
Gas Bubbles in Vacuum Packaged Meat	2001/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_01-3.pdf
Modified Atmosphere Packaging of Meat	2001/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_01-4.pdf
Sheep and Lamb Dressing	2001/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_01-6.pdf
How Useful are Microbiological Criteria for Fresh Meat?	2002/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_02-1.pdf
Spray Chilling of Carcases and Sides	2002/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_02-3.pdf
The Causes of Drip in Meat	2002/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_02-6.pdf
Storage life of meat	2002/9	http://www.meatupdate.csiro.au/Storage-Life-of-Meat.pdf
Pathogen Reduction Interventions for Carcases	2003/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_03-2.pdf
Scientific information for validation of EMO chilling and freezing requirements	2003/8	http://www.meatupdate.csiro.au/Validation-of-EMOs.pdf
E. coli, *E. coli* O157 and *Salmonella*	2003/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_03-6.pdf

Title	Year/Volume	Link
Tenderstretch	2004/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_04-1.pdf
Visual Assessment of Marbling and Meat Colour	2004/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_04-2.pdf
Validation of Critical Limits During Chilling	2004/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_04-4.pdf
Predictive Microbiology for Comparing the Performance of Carcase Chilling Patterns	2004/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_04-5.pdf
Freezing of Hot Offals	2004/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_04-6.pdf
Literature for chiller validations	2004/9	http://www.meatupdate.csiro.au/Chiller-validation.pdf
The Cold Chain for Cartoned Meat Exports	2005/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_05-2.pdf
Marbling and Quality of Beef	2005/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_05-3.pdf
Risk Profiles and Risk Assessments	2005/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_05-4.pdf
Listeria in Fresh and Processed Meats	2005/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_05-5.pdf
Processing Factors Affecting Sheep Meat Quality	2005/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_05-6.pdf
Shelf life Testing: Methods for Determining the Claimable Life of Meat Products	2006/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_06-2.pdf
Spread of Foodborne Pathogens During Feeding, Transportation and Holding Prior to Slaughter	2006/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_06-3.pdf
Colour Defects in Meat - Part 1: Browning of Fresh Meat	2006/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_06-5.pdf
Colour Defects in Meat – Part 2: Greening, Pinking, Browning & Spots	2006/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_06-6.pdf
Do Breaks in the Cold Chain Affect Frozen Beef Quality?	2007/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_07-1.pdf
Beef Carcase Chilling – Opportunities for Customising Programs	2007/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_07-2.pdf

Title	Year/Volume	Link
Lactic Acid Bacteria	2007/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_07-5.pdf
Producing Quality Sheep Meat	2007/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_07-6.pdf
Hormonal Growth Promotants and Meat Quality	2009/4	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_09-4.pdf
Bulk-packed Frozen Meat for Further Processing: Alternatives to Current Practice	2009/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_09-5.pdf
Shelf life of Australian Chilled, Vacuum-packed, Boneless Beef	2009/6	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_09-6.pdf
Dry Ageing of Beef	2010/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_10-2.pdf
Shelf life of Australian Chilled, Vacuum-packed Lamb	2010/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_10-3.pdf
Sources of Contamination on Beef Carcases During Dressing	2010/5	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_10-5.pdf
Effect of Slaughter Method on Animal Welfare and Meat Quality	2011/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_11-1.pdf
Heat Toughening – Part 1: Effects of Heat Toughening on Quality of Beef, and the Incidence in Australia	2011/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_11-2.pdf
Heat Toughening – Part 2: Strategies for Reducing the Incidence of Heat Toughening in Beef Carcases	2011/3	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_11-3.pdf
The Effect of Diet on Sheepmeat Flavour	2012/1	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_12-1.pdf
Pathogenic Shiga Toxigenic *E. coli*—STEC	2012/2	http://www.meatupdate.csiro.au/data/MEAT_TECHNOLOGY_UPDATE_12-10.pdf

Appendix 3 List of Newsletters

Title	Year/Volume	Link
Microbiological Criteria for Meats	1969/6	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_69-6.pdf
Salmonellae	1969/8	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_69-8.pdf
Dark Cutting Beef	1969/10	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_69-10.pdf
Storage of Meat under Ultraviolet Light (U.V.)	1970/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_70-2.pdf
Surface Drying of Meat and Its Effect on Storage Life of Chilled Carcases	1970/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_70-5.pdf
The Use of Packaging Films for Chilled Fresh Meats	1970/6	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_70-6.pdf
The Use of Packaging Films for Chilled Processed Meats	1970/7	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_70-7.pdf
Shipment of Chilled Beef Cuts	1971/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_71-2.pdf
Bone Taint	1971/3	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_71-3.pdf
Greening of Vacuum-packed Fresh Meat - Causes and Prevention	1971/6	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_71-6.pdf
Greening of Cured Meat Products	1974/6	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_74-6.pdf
Guidelines for the Manufacture of Minced Meat of Good Microbiological Quality	1975/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_75-2.pdf
Storage Temperatures for Frozen Meats	1977/1	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_77-1.pdf
An Introduction to Microbiology	1977/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_77-2.pdf
Food Poisoning Bacteria	1977/3	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_77-3.pdf
Air Freezing and Thawing Lamb and Mutton Carcases	1980/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_80-2.pdf
Welding Torch and Internal Combustion Engine Gases Ruin Meat	1980/3	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_80-3.pdf

Title	Year/Volume	Link
Greening of Vacuum-packed Chilled Beef	1981/3	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_81-3.pdf
Packaging of Chilled Unprocessed Meat	1982/1	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_82-1.pdf
Taints in Meat and Other Foods - Chlorophenols and Related Compounds	1982/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_82-5.pdf
Dark Cutting Beef	1984/1	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_84-1.pdf
Recommendations for the Cooling of Hot-boned Meat	1984/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_84-5.pdf
Interpretation of Bacteriological Tests on Vacuum-packaged Meat	1985/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_85-2.pdf
Increasing the Storage Life of Chilled Vacuum-packed Meat by Acetic Acid Treatment	1986/3	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_86-3.pdf
Transport of Export Chilled Meat	1987/1	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_87-1.pdf
Cooked Meat Products Problems and Solutions	1987/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_87-2.pdf
Listeria monocytogenes	1987/4	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_87-4.pdf
Shelf-stable Edible Meat	1989/2	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_89-2.pdf
Bone Taint	1989/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_89-5.pdf
Preventing Contamination of Cooked Meats With *Listeria*	1990/1	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_90-1.pdf
The Microbiology and Packaging of Processed Meat	1990/4	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_90-4.pdf
Prevention of Colour Defects in Precooked and Cured Meat Products	1990/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_90-5.pdf
Hazard Analysis Critical Control Point (HACCP) Concept and the Production of Processed Meats	1990/6	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_90-6.pdf

Title	Year/Volume	Link
Prevention of Colour Defects in Uncooked Meat Products	1991/1	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_91-1.pdf
Listeria monocytogenes on Processed Meats	1991/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_91-5.pdf
Recommendations for the Production of Fermented Sausages	1992/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_92-5.pdf
Some Factors Affecting Fat Colour in Beef	1993/4	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_93-4.pdf
Displaying Fresh Meat for Maximum Return	1993/5	http://www.meatupdate.csiro.au/data/MEAT_RESEARCH_NEWS_LETTER_93-5.pdf

Appendix 4   Glossary of Terms

<	Less than, as in less than 10
>	More than, as in more than 10
Ambient temperature	Temperature of the air around you or the product
AMIC	Australian Meat Industry Council
Anaerobic	The absence of oxygen, a state which can exist in canned and vacuum-packed products
CFU	Colony Forming Unit, an estimate of viable number of bacteria
Cold chain	The process of maintaining foods under refrigeration, in either a chilled or frozen state, during storage, distribution and marketing
Comminuted	A meat product which is chopped or minced
Contaminant	Something which may make food unsafe or unwholesome. Examples of contaminants are micro-organisms, chemical residues or metal specks
Controlling authority	The Commonwealth, State or Territory authority which is responsible for the enforcement of standards
Critical Control Point	A point, procedure, operation or stage in a process at which a hazard is prevented, eliminat ed or reduced to an acceptable level
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point is the system which identifies and controls those hazards which pose a significant risk to food safety
Hazard	A biological, chemical or physical agent which may compromise or affect food safety
Log	Logarithm – used to express microbial counts e.g. log 2 is 100, log 3 is 1,000
Microbial count	The number of micro-organisms living in or on a food product
Microbiological limits	The maximum number of micro-organisms specified for a food product
Microorganisms	Viruses, yeasts, moulds and bacteria

Glossary of Terms continued...

MAP	Modified Atmosphere Packaging. Enclosure of meat in high gas barrier film, in which the gas environment around meat has been changed by removing all the air from pack and flushing it with a gas mixture of varying concentrations of O_2, CO_2 and N_2, different from air. Vacuum packaging (VP) where, most of the air is removed before sealing the pack, is sometimes included in MAP
MPN	Most Probable Number. Method used to determine bacterial numbers based on probability concept instead of counting colonies
Pathogen	A microorganism which causes illness
PCR	Polymerase Chain Reaction, molecular technique to amplify DNA
pH	A measure of acidity or alkalinity
RTE meats	Ready-to-eat meats are products that are intended to be consumed without further heating or cooking. They include cooked or uncooked fermented meats, pâté, dried meat, slow cured meat, luncheon meat, cooked cured or uncured muscle meat other ready-to-eat meat that is susceptible to the growth of pathogens or the production of toxins.
SARDI	South Australian Research and Development Institute
Shelf life	Length of time that a commodity may be stored without becoming unfit for use or consumption, due to loss of quality, the presence of undesirable chemicals, toxins, or growth of pathogens.
Spoilage bacteria	Bacteria which limit the shelf life of foods by producing objectionable odours and slime
Toxin	A chemical which can cause illness. Toxins may be produced in food by bacteria
TRFLP	Terminal Restriction Fragment Length Polymorphism. A DNA-based molecular microbial community analysis technique
Validate, validation	The process of obtaining evidence to demonstrate that hazards in a food process are controlled
Verify, verification	Monitoring used in food processing to demonstrate that critical limits and other important parameters have been complied with