Final report

Safety implications of the manufacture of minced meat from aged meat

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By

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1. Summary

The aim of this review was to critically look at the available scientific evidence that would support the newly imposed hygiene legislation regarding the regulatory limit on the age restriction of meat at time of mincing.

The following new requirements apply to the production of minced meat and meat preparations.

(a) Unless the competent authority authorises boning immediately before mincing, frozen or deep-frozen meat used for the preparation of minced meat or meat preparations must be boned before freezing. It may be stored only for a limited period.

(b) When prepared from chilled meat, minced meat must be prepared:

(i) in the case of poultry, within no more than three days of their slaughter;

(ii) in the case of animal other than poultry, within no more than six days of their slaughter; or within no more than 15 days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.

These requirements apply to establishments approved under Regulation (EC) No. 853/2004 that produce minced meat that is not sold directly to the final consumer. It does not apply to minced meat intended for heat treatment before sale, such as cooked pies.

These time limits have been carried over from the former Minced Meat and Meat Preparation (Hygiene) Regulations 1995 (S.I. 1995 No. 3205).

However, that legislation had an exemption for establishments that produced minced meat for the national market only, but it is not known how many food businesses operated under these conditions. The new legislation applies to the production of all minced meat, including that for the national market.

Food business operators that sell to the final consumer only (retailers) are exempt from the requirements of Regulation (EC) No. 853/2004. This means that the time limits do not apply to meat that is minced by a retail butcher, in a supermarket, in a restaurant for mince or steak tartare, or to minced meat intended for use in foods that are heat treated before sale, such as cooked pies.

Overall, this review has found little scientific data to support the current restrictions on the age of the meat used to make minced meat. The main points to emerge from this brief review are that:

1. No clear scientific literature has been located to support the restrictions on the time between slaughter and production of minced meat.
2. No specific scientific publications have been located that look at the safety and quality of mince produced from cuts and carcasses that have been stored for different periods of time post-slaughter.
3. There are a small number of publications on the quality of steaks and chops produced from meat that has been stored for up to 35 days and 80 days for beef, 40 days for lamb and 63 days for pork. As would be expected they all show that bacterial numbers are higher on meat produced from older meat. However, an acceptable display-life is often achieved with cuts produced from the older meat. Few publications have been found on poultry.

4. No publications have been located that show that the safety (i.e. pathogen levels) of mince produced from older meat is compromised, or visa versa.
5. There is a surprising lack of data on the storage-life of chilled meat carcasses and bone-in-cuts. The classic studies indicate much shorter storage lives than current industrial practice as indicated in IIR tables etc. There is little data on the growth of pathogens on meat carcasses during ageing.
6. More data is available on the storage-life of some vacuum-packaged primal meat, however this covers a limited range of storage conditions. There is little data on the growth of pathogens on meat carcasses during ageing.
7. As would be expected, the data that does exist shows that initial bacterial numbers, and storage atmosphere and temperature are the main factors governing storage life. pH and RH also influence storage life, and in one publication the rate of initial chilling is claimed to make changes of up to 50% in storage life.
8. Predicting microbial growth from surface temperature data has potential, however current models tend to predict growth during the chilling process while measurements show either no growth or death. The discrepancy is most likely due to a combination of poor surface temperature measurement and other factors, such as a_w or inhibitory interaction of competing microflora, not being taken into account in the models. Most models predict no growth of bacteria (particularly pathogens) at low storage temperatures and are unable to predict survival at low temperatures, so are unable to predict the role of ageing times on pathogenic growth. They can currently be used to predict the risk of bacterial growth during cutting/mincing operations at elevated temperatures, and the effect of break-downs in the chill-chain.
9. Overall, data on the growth of psychrotrophic pathogens would indicate that there is theoretically a greater risk of psychrotrophic pathogens proliferating in meat held for a longer time at a temperature above the minimum for pathogenic growth than in meat stored for a short time. Since mincing is known to distribute bacteria throughout the meat, and mince is acknowledged to present more of a risk to public health than cuts and steaks, it stands to reason that theoretically mince from aged meat has a higher risk than that from non-aged meat. However, it can also be said that any aged meat must on this basis present more of a risk than unaged meat. It is not clear from the literature how much of an additional risk ageing presents. Some authors (Dykes *et al*., 2001) imply that that the long period of storage, of particularly *E. coli* O157, in a non-growing state would result in "an excessive recovery period in these cells before growth would occur".

As a result of this brief review a range of critical controls have been suggested based on available data. However, we would recommend that research is funded to specifically look at the influence of post-slaughter storage times and conditions on the safety and quality of mince produced. Initial studies should concentrate on the production of high quality mince from meat aged on the bone and vacuum-packaged for extended periods. Work is also required on poultry to fill the total gap in published scientific literature in this area.

Contents

2. Introduction

New European Food Hygiene Regulations, which are directly applicable across all EU Member States, have applied since 1 January 2006. They harmonise food hygiene legislation throughout the Community and apply to all food businesses, whether they supply the national market or export to other EU countries. Exemptions are available in certain circumstances.

These Food Hygiene Regulations were subject to extensive consultation with industry and other stakeholders.

Regulation (EC) No. 853/2004 imposes time limits on the production of minced meat.

Regulation 853/2004: laying down specific hygiene rules for food of animal origin.

Annex III, Section V, Chapter III: Minced meat, meat preparations and mechanically separated meat (MSM) - Hygiene during and after production

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The aim of this review is to critically look at the available scientific evidence that would support the hygiene legislation regarding the regulatory limit on the age restriction of meat at mincing.

3. Literature

A significant amount of meat is converted into mince. In the UK this is used in catering and the home for a variety of cooked dishes and in manufacture of products ranging from burgers and sausages to-canned products and chilled or frozen ready meals. While some minced meat is prepared from trimmings from the preparation of joints and cuts, much minced meat is prepared from parts of the carcass for which there is insufficient consumer demand as joints or cuts, e.g. forequarter beef. It is widely recognised that minced meats pose more of a health risk than intact muscle because they can be contaminated throughout during the mincing operation (ICMSF, 1998; Barlow *et al*., 2006).

After slaughter and evisceration, meat carcasses are primary chilled, either in the form of sides in the case of beef and pork, or whole carcasses in the case of ovines and poultry. Legislation generally requires fresh red meat and poultry to be chilled to <7°C and <4°C, respectively. Once the meat has reached the required temperature it can legally be cut. To improve tenderness and prevent muscle shortening there will usually be a delay between the meat reaching the desired temperature and cutting. This may be as short as 10 hours in the case of chicken or typically 48 hours for beef before cutting, packing and retail distribution. To improve eating quality of the meat this delay may be substantially longer, a process known as ageing (or maturation). Alternatively once chilled the carcass or side may be split down to primal or sub-primal cuts and the more valuable primals (usually the hind quarters) aged while the other primals are cut into consumer units and distributed for retail. Ageing may be carried out in aerobic conditions ("dry-ageing") or the primals packaged in anaerobic conditions ("wet-ageing"). Following ageing the carcasses/sides/primals may be distributed direct to retail outlets for butchery, or more commonly cut into consumer portions, packaged and then distributed to the retailer.

3.1. Growth of pathogens and spoilage organisms on meat

3.1.1 Pathogens

A number of bacterial pathogens capable of causing food poisoning in humans are known to contaminate red meat. Those of most importance (in alphabetical order) are *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum*, *Clostridium perfringens*, pathogenic serotypes of *Escherichia coli* (principally *E. coli* O157:H7), *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica* (Nottingham, 1982; Mead & Hinton, 1996, ICMSF, 1998). *Listeria monocytogenes* is commonly associated with meat, but its public health significance in relation to raw meat is unclear (Mead & Hinton, 1996). The ICMSF (1998) quote that "there is no evidence that multiplication of *L. monocytogenes* on raw poultry during storage is a factor in human listeriosis", however they do cite a control study where undercooked chicken was a factor in human listeriosis.

The essential characteristics of pathogenic microorganisms can be found in numerous texts. There is a certain degree of conflicting data concerning the importance of various pathogens with regard to meat safety and the effect of specific temperatures or packaging atmospheres on their growth or inhibition. Inhibition temperatures for various species quoted by Rosset (1982) are shown in Table 1. Other minimum and optimum growth temperatures for pathogens commonly associated with meat are show in Table 2, and generation times shown in Table 3. Reviews of data on minimum growth temperatures for pathogens under a range of different atmospheric packaging conditions have been published by García de Fernando *et al*. (1995) and Nychas & Skandamis (2005).

Table 1. Effect of temperature on the inhibition of pathogens (source: Rosset, 1982)

10°C	Inhibition of *Staphylococcus aureus* toxin production Inhibition of *Clostridium botulinum* (type A and B) toxin production
6.7°C	Inhibition of *Staphylococcus aureus* growth
6.5°C	Inhibition of *Clostridium perfringens* growth
5.2°C	Inhibition of *Salmonella* growth
3.3°C	Inhibition of *Clostridium botulinum* (type E) toxin production

Table 2. Minimum and optimum growth temperatures for pathogens associated with red meats (sources: García de Fernando *et al*., 1995; Mead & Hinton, 1996; Doyle, 2002; Tamplin *et al*., 2005)

	Minimum temperature (°C)	Optimum temperature (°C)
Campylobacter spp.	30	42-43
Clostridium perfringens	12	43-47
Clostridium botulinum proteolytic	10	
Staphylococcus aureus	7	
Pathogenic *Escherichia coli* strains	7	35-40
Escherichia coli O157:H7	6 to 7	42
Salmonella spp.	5	35-43
Bacillus cereus	5	
Clostridium botulinum non-proteolytic	3	
Aeromonas hydrophila	-0.1 to 1.2	
Listeria monocytogenes	-1 to 0	30-37
Yersinia enterocolitica	-2	28-29

Table 3. Generation times for foodborne bacteria in raw meat

	Temperature	Time (h)		
Bacteria	(°C)	Lag	Generation	Ref.
Salmonella spp.	12.5		6.79	Mackey *et al*. (1980)
Clostridium perfringens	12		11.5	Lund *et al*. (2000)
Escherichia coli O157:H7, pH 5.7	12	16.2	6.0	Walls & Scott (1996)
Escherichia coli O157:H7, pH 6.3	12	2.78	3.9	Walls & Scott (1996)
Salmonella spp.	10		13.87	Mackey *et al*. (1980)
Salmonella typhimurium	10	45	9.65	Smith (1985)
Yersinia enterocolitica	10		12.73	Logue *et al*. (1998)
Escherichia coli SF	8.2	40	6.9	Smith (1985)
Bacillus cereus	5		8.3	Lund *et al*. (2000)
Yersinia enterocolitica	5		16.53	Logue *et al*. (1998); Lund *et al*. (2000)
Listeria monocytogenes	4		22.8	Lund *et al*. (2000)
Listeria monocytogenes	4		9.3	Pawar *et al*. (2000)
Escherichia coli O157:H7	2		no growth	Ansay *et al*. (1999)

Some pathogens, such as *L. monocytogenes*, are capable of growth at chill temperatures below 5°C. These are often cited as being of particular concern in relation to refrigerated meats since refrigeration can not be relied on to prevent growth (Doyle, 1987). On the other hand, psychrotrophic pathogens are not particularly heat resistant and adequate cooking should be sufficient to destroy any such pathogens. Illnesses caused by *L. monocytogenes* and *E. coli* are often due to inadequate cooking before ingestion.

Investigations into the effect of different storage atmospheres on pathogenic growth at low temperatures appear to show that Carbon dioxide (CO_2) enriched atmosphere produce the greatest inhibitory effect on psychrotrophic pathogens (*Y. enterocolitica*, *Aeromonas hydrophila* and *L. monocytogenes*). García de Fernando *et al*. (1995) concluded that "*at a normal meat pH (i.e. 5.5) and at a low temperature (e.g. 1°C) the growth of psychrotrophic pathogens is stopped when the CO_2concentration is 40%*". However, high pH meat (≥6) and/or higher storage temperatures will support growth of such pathogens.

3.1.2 Spoilage organisms

General data are available on the attainable chilled storage lives for many meats (Table 4). However, much is based on 'learned' opinion rather than peer reviewed scientific studies.

Table 4. Chilled storage times (source: IIR, 2000)

	Storage time (days (sd)) in temperature range (°C):			
	-4.1 to -1.1	-1 to 2	2.1 to 5.1	5.2 to 8.2
Beef	40 (26)	34 (32)	10 (8)	9 (9)
Lamb	55 (20)	41 (46)	28 (34)	
Pork	50 (58)	22 (30)	16 (16)	15 (18)
Poultry	32 (18)	17 (10)	12 (11)	7 (3)

In most cases the limiting factors that control the chilled storage life of meat are based on bacterial growth. 'Off' odours and slime caused by microorganisms are detected when populations reach *ca* 7 to 8 $\log_{10}$ cfu cm^{-2} (Gill, 1996). Temperature is the principal factor affecting the rate of microbial growth and hence the shelf-life of chilled meat. The lower the temperature, the longer the shelf-life. Shelf-life may also be extended by packaging under aerobic atmospheres rich in carbon dioxide, or by packaging under anaerobic conditions (Gill, 1996). The initial bacterial loading of the meat will always limit the maximum shelf-life (Blixt & Borch, 2002; Kennedy *et al*., 2004).

Reduction in temperature below the optimum causes an increase in generation time, i.e. the time required for a doubling in number. It is an accepted crude approximation that bacterial growth rates can be expected to double with every 10°C rise in temperature (Gill, 1986). Below 10°C, however, this effect is more pronounced and chilled storage life is halved for each 2 to 3°C rise in temperature. Thus the generation time for a pseudomonad might be 1 hour at 20˚C, 2.5 hours at 10˚C, 5 hours at 5˚C, 8 hours at 2˚C or 11 hours at 0˚C (Harrigan & Park, 1991). In the usual temperature range for chilled meat, -1.5 to 5°C, there can be as much as an eight-fold increase in growth rate between the lower and upper temperature. Storage of chilled meat at -1.5±0.5°C would attain the maximum storage life without any surface freezing.

3.2. Bacterial quality of carcasses

The initial level of bacterial contamination will of course affect the storage life. Over forty years ago Ayres (1955), in his comprehensive review of microbiological contamination in slaughtering, concluded that an aerobic population of 4.0 to 5.0 $\log_{10}$ cfu cm^{-2} and an anaerobic population of between 3.7 and 4.7 $\log_{10}$ cfu g^{-1} would be reasonable for wholesale cuts of meat. Surveys over the past 20 years have shown that in general levels of between 1 and 4 $\log_{10}$ can be expected on meat carcasses prior to chilling. There are very little data on the effect of current commercial chilling rates and conditions on changes in spoilage and pathogenic bacterial numbers during the chilling process. In most cases no change or a small reduction (0.5 to 1 $\log_{10}$ cfu cm^2) in number of organisms on the surface has been measured. There is a great debate regarding the role of surface drying during primary chilling. It is also

not clear whether the new emphasis in the Meat Industry on more traditional methods in meat manufacture which is seeing a move away from rapid chilling systems to delayed chilling systems is having any effect on bacterial numbers during chilling.

3.2.1 Effect of rigor changes on microbial growth

The way in which animals are handled before slaughter will affect the bio-chemical processes that occur before and during rigor mortis. The resulting metabolites influence the growth of microorganisms on meat.

During the onset of rigor mortis, which may take up to 24 hours, oxygen stored in the muscle is depleted and the redox potential falls from above +250 mV to -150 mV. Such a low redox value combined with the initial muscle temperature of 38°C provides ideal growth conditions for mesophilic microorganisms. Stress and excitement caused to the animal before slaughter will cause the redox potential to fall rapidly, possibly allowing proliferation of such microorganisms before cooling (Dainty, 1971).

Concurrent with the fall in redox potential is a fall in pH from an initial value in life of around 7 to a stable value around 5.5 (in beef), the 'ultimate pH' (Table 5). This is due to the breakdown of glycogen, a polysaccharide, in the muscle tissue to lactic acid. Lactic acid cannot be removed by the circulation system nor oxidised, so it accumulates and pH falls until the glycogen is all used or the breakdown stops. The pH has an important role in the growth of microorganisms; the nearer the pH is to the ultimate value the more growth is inhibited (Dainty, 1971).

Table 5. pH of meats

	Time to ultimate pH (h)	Ultimate pH	pH	Reference
Beef		5.5		Dainty, 1971
Beef		5.4-5.7		
Lamb		5.4-5.7		
Pork		5.4-5.7		
Poultry breast		5.8		ICMSF, 1998
Poultry leg		6.4-6.7		ICMSF, 1998
Poultry skin		≥6.6		ICMSF, 1998
Chicken	5	5.7-6.0		Thielke *et al*., 2005
Chicken mince			6.18	Saucier *et al*., 2000
Turkey		5.9		El Rammouz *et al*., 2004
Turkey mince			5.95	Saucier *et al*., 2000

Stress or exercise before slaughter can deplete an animal's glycogen reserves, consequently producing meat with less lactic acid and a relatively high ultimate pH, this gives the meat a dark, firm, dry (DFD) appearance. Alternative terms are 'dark cutting' and 'high-pH meat'. The condition occurs in pork, beef and mutton, but is of little economic importance in mutton (Newton & Gill, 1981). DFD meat provides conditions that are more favourable for microbial growth than in normal meat (Dainty, 1971; Newton & Gill, 1981). The preferred substrate for growth of pseudomonads, the dominant spoilage bacteria in meat stored in air at refrigerated temperatures, is glucose. Only when glucose is exhausted do they break down amino acids, producing the ammonia and sulphur compounds that are detectable as spoilage odours and flavours. In meat containing no glucose, as is the case with some DFD meat, amino acids are broken down immediately and spoilage becomes evident at cell densities of 6 $\log_{10}$ cfu cm^{-2} (Gill, 1982). This is lower than in normal meat, where spoilage becomes apparent when numbers reach *ca*. 8 $\log_{10}$ cfu cm^{-2}. Thus given the same storage conditions

DFD meat spoils more rapidly than normal-pH meat. The microbiology of DFD meat has been comprehensively reviewed by Newton & Gill (1981).

There is little significant difference in pH or chemical composition between PSE and normal meat. There is no evidence that the spoilage of PSE meat is any different to that of normal meat (Gill, 1982).

3.2.2 Ageing

The terms 'conditioning', 'ageing', 'ripening', 'maturing' and 'the resolution of *rigor*' have all been applied to the practice of storing meat for periods beyond the normal time taken for cooling and setting, to improve its tenderness after cooking. Consumer assessments of unaged beef are variable, ranging from 'moderately tough' to 'moderately tender' whilst beef conditioned for 9 days at 1°C receives largely favourable reactions, being scored 'moderately' to 'very' tender (Dransfield, 1985). Ageing imposes a severe limitation on processing conditions because it is a slow process.

The deficiencies in the commercial conditioning of meat were clearly illustrated by replies to a questionnaire to sections of the trade in the UK in 1977/8 (Dransfield, 1986). At the time a period of storage for wholesale meat was often not specified by retailers. When specified the duration of storage had much to do with distribution and turnover of meat and could often be shortened by commercial pressures. At retail, beef was kept for 1 to 4 days and most beef was sold 3 to 6 days after slaughter (Palmer, 1978). The majority of beef therefore had been only partially aged and tenderness would have been improved if the beef had been stored for a further week. Many retailers nowadays age beef for longer periods, but economic factors still often dictate the time of conditioning.

The major change that takes place in meat during ageing occurs in the muscle fibre. Ageing is caused by the presence of proteolytic enzymes in the muscle that slowly catalyse the breakdown of some of the muscle proteins. This causes weakening of the muscle so that the meat is more readily pulled apart in the mouth and is therefore tenderer. Two groups of enzymes are thought mainly responsible; calpains, which are active at neutral pH shortly after slaughter; and cathepsins, which are active at acid pH after *rigor* (Offer *et al*., 1988).

Rates of ageing differ widely between species. The tenderness of meat improves approximately as the logarithm of the storage time. Most of the improvement in tenderness therefore takes place in the initial storage period and tenderness eventually reaches a maximum. Table 6 shows the 1st order rate constants derived from the exponential decay of toughness of cooked muscles with time (Dransfield, 1986). Beef, veal and rabbit have a rate constant of 0.17 per day, which means that 80% of the theoretically-possible tenderising occurred in 10 days at 1°C. Although beef and veal condition at the same rate, veal is tenderer and therefore can reach an acceptable tenderness in 5 days at 1°C. Lamb conditions slightly faster than beef, and pig meat about twice as fast as beef. Chicken has a much higher rate and 80% of the tenderising will occur in about 10 hours.

Table 6. Variation in rate of ageing among species (source: Dransfield, 1986)

	Rate (day^{-1})	Time for 50% tenderising (days)	Time for 80% tenderising (days)
Beef	0.16 (0.04)	4.3	10.0
Veal	0.17 (0.03)	4.1	9.5
Rabbit	0.17 (0.06)	4.1	9.5
Lamb	0.21 (0.05)	3.3	7.7
Pork	0.38 (0.11)	1.8	4.2
Chicken	5.23 (1.68)	0.1	0.3

Longissimus muscles from four species were stored at 1-4°C (cf Dransfield *et al.*, 1980b) and rates calculated (cf Dransfield *et al.*, 1980a). Values are the rate of tenderising with standard errors and the time taken after stunning for 80% of the complete tenderising to occur.

3.3. Bacterial growth on carcasses during storage

General data are available on the attainable chilled storage lives for many meat carcasses (Table 7). However, as previously mentioned, much is based on 'learned' opinion rather than peer reviewed scientific studies.

Table 7. Practical Storage Life of aerobically chilled meats, PSL is the time that the product is still of acceptable quality, assuming good initial bacteriological quality and normal pH (IIR, 2000)

Product	Temperature (°C)	Packaging	PSL d = day w = week m = month
Beef carcasses	4	Unwrapped	10-14 d
Beef carcasses	–1.5 to 0	Unwrapped	3-5 w
Veal	–1.5 to 0	Unwrapped	3 w
Pork carcasses	4	Unwrapped	8 d
Pork carcasses	–1.5 to 0	Unwrapped	3 w
Lamb carcasses	–1.5 to 0	Unwrapped	3-4 w
Chicken, eviscerated	4	Perm. Plastic	1 w
Chicken, eviscerated	0	Perm. Plastic	2 w
Chicken, eviscerated	–2	Perm. plastic	3-4 w

There appears to be surprisingly little data on the growth of bacteria on meat carcasses during ageing.

Temperature is the prime factor controlling the storage-life of, and bacterial growth on, unwrapped carcass meat. Classical literature (Ingram & Roberts, 1976) says that odour and slime will be apparent after approximately 14.5 and 20 days respectively with beef sides stored at 0°C (Figure 1). At 5°C, the respective times are significantly reduced to 8 and 13 days. These data are contradicted by the 21 days or more that beef now is kept for traditional "dry-ageing".

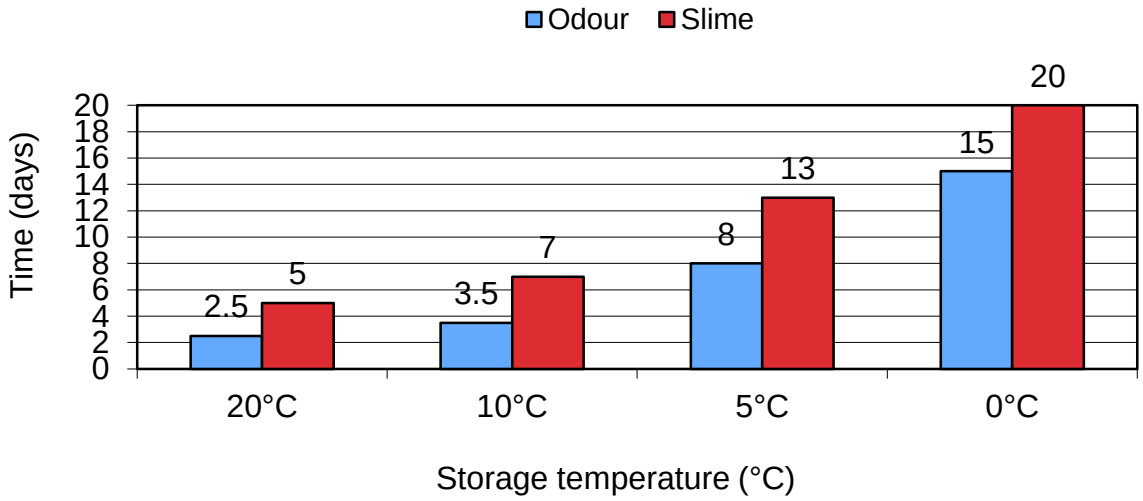


Figure 1. Time (days) for odour or slime to be detected on beef sides with average initial contamination stored at different temperatures (source: Ingram & Roberts, 1976)

Similar literature on chicken carcasses (Regez *et al*., 1988) show that odour will be apparent after approximately 12 days with chicken carcasses stored at 0°C (Figure 2). Longer storage times are reported by Mielnik *et al*. (1999) of 17 to 19 days at -1°C, and 8 to 10 days at 4°C.

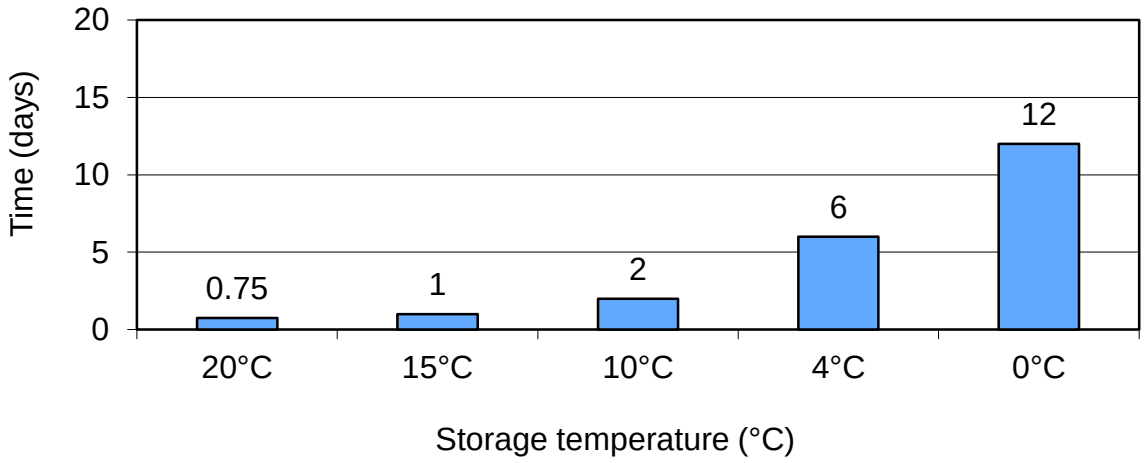


Figure 2. Time (days) for odour to be detected on chicken carcasses with average initial contamination stored at different temperatures (source: Regez *et al*., 1988)

High pH will limit the storage-life of beef carcasses. UK Meat Research Institute (Hudson & Roberts, 1972) work showed that bacteria grow faster on the surface of high pH sides than low pH (Figure 3).

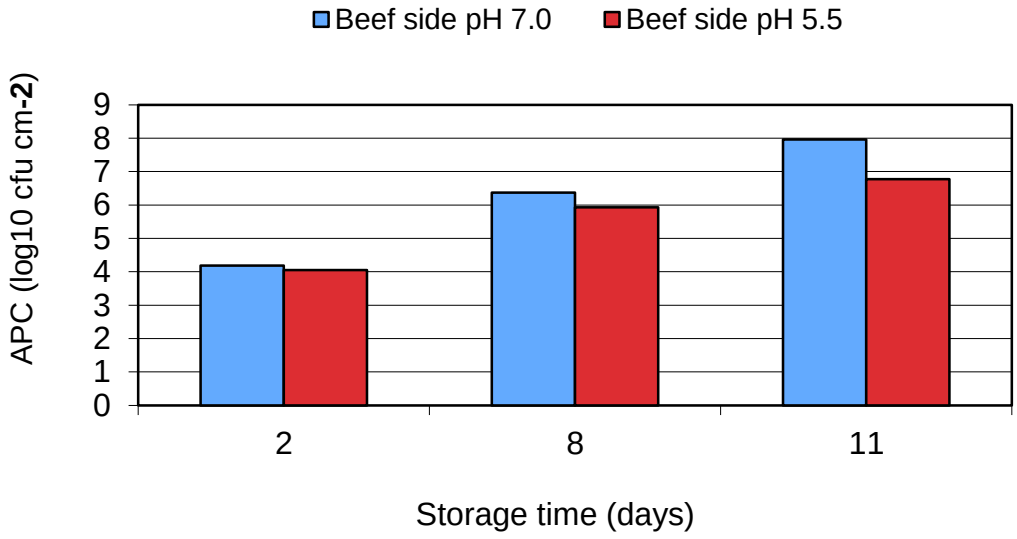


Figure 3. Growth of bacteria on beef sides with different pHs stored at 1°C (source: Hudson & Roberts, 1972)

Greer *et al.* (1990) found bacterial numbers to hardly change on conventionally and spray chilled beef carcasses aged for 7 days at 1°C. Initial APCs on conventionally chilled carcasses reduced from 2.92 to 2.87 $\log_{10}$ cfu cm^{-2}, while counts on spray chilled carcasses slightly rose from 2.52 to 2.54 $\log_{10}$ cfu cm^{-2}.

Specific surfaces of a carcass can have very high levels of initial contamination. Beef subcutaneous fat has been shown to have a high initial microbial load and a capacity to support extensive bacterial growth (Lasta *et al*., 1995). Initial values of total viable counts increasing from an initial value of 5.4 $\log_{10}$ cfu cm^{-2} to 10.0 after 11 days in a moist environment at 5°C (Figure 4). No noticeable deterioration in appearance of the sample was found after 14 days which was worrying. This type of material is often incorporated in manufactured products or could provide a cross contamination source.

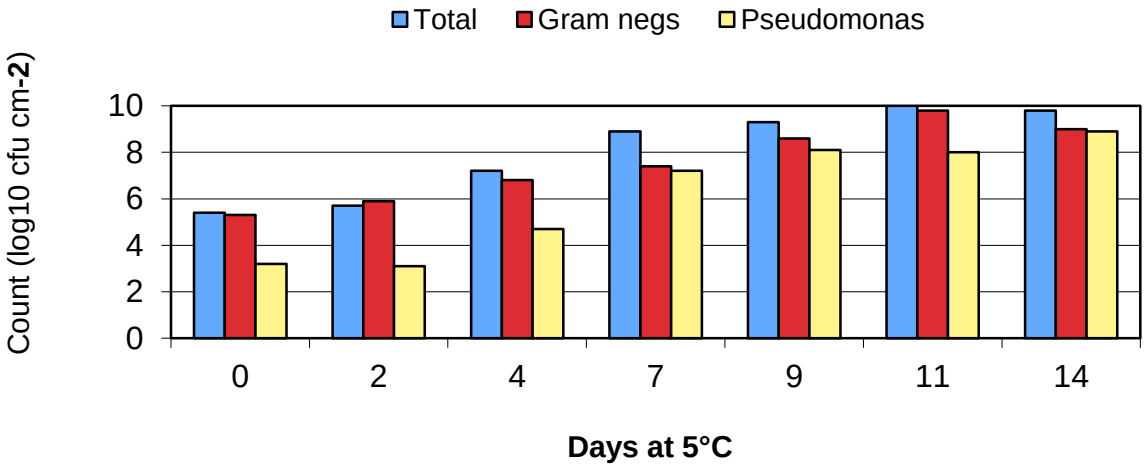


Figure 4. Growth of bacteria on naturally contaminated beef brisket fat stored at 5°C (source: Lasta *et al*., 1995)

The above results were obtained on the surface of samples stored in a near saturated air. There is much industrial belief that the surface of meat carcasses must be allowed to dry or

storage-life will be compromised. There appears to be no clear scientific studies that stored carcasses under a range of industrial conditions to prove or disprove this belief.

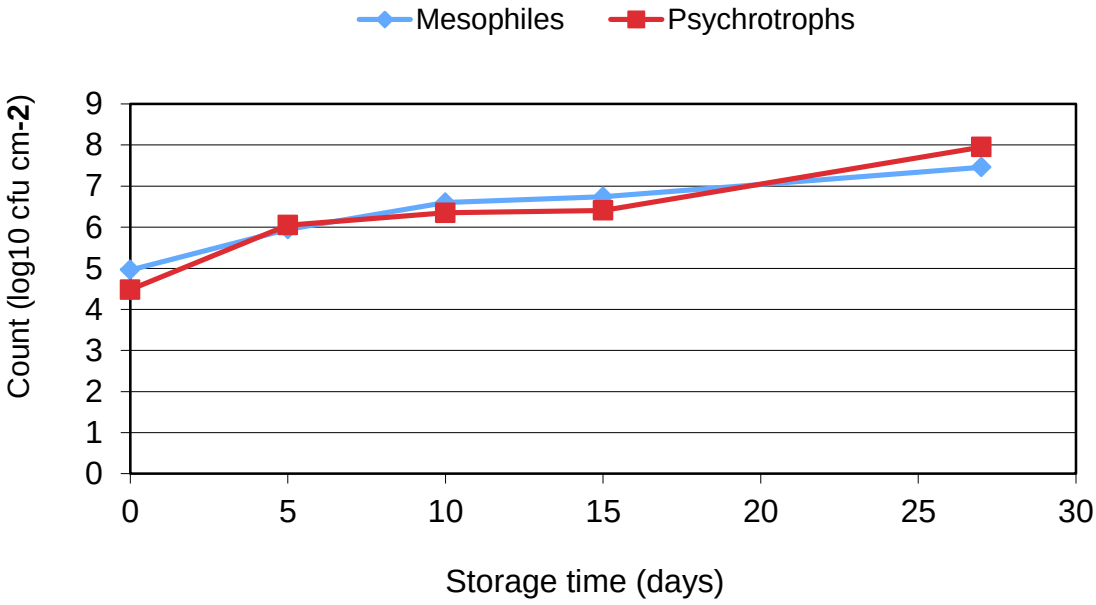


Figure 5. Growth of bacteria on lamb carcasses stored at 3±1°C (source: Prieto *et al.*, 1991)

Lamb carcasses typically are believed to have a shorter storage-life than beef carcasses. One reason for this is the belief that initial levels of contamination are higher on sheep carcasses than those of beef. However, Prieto *et al*. (1991) recorded storage-lives of between 23-29 days for lamb carcasses (pH >5.8,) stored at 3±1°C and 95±5% RH despite relatively high initial mesophilic counts of 5 $\log_{10}$ cfu cm^{-2} (Figure 5). Both mesophiles and psychrotrophs increased throughout the storage-life.

3.4. Bacterial quality of cuts

After the animal has been slaughtered, dressed and chilled, the resulting carcass or part-carcass (e.g. beef quarter) is subjected to further treatment before the meat is used. Butchery (cutting) subdivides the carcass or part-carcass into smaller portions, joints, cuts, etc. Simple processing including packaging may follow. Butchery can take place in premises adjacent to the abattoir. Alternatively, carcasses can be transported to a large centralised butchery operation or to catering or retailing premises, either directly from the abattoir or via a meat market. Numerous studies show that the breaking up of the carcass or side is a Critical Control Point (CCP) and substantial cross contamination occurs during these processes. This is relatively unsurprising since the initial breaking up of the carcass or side involves a large number of stages and the side is extensively manually handled. Gill & Jones (1999) quote 16 separate operations in the breaking of a beef carcass at one processing plant. Cutting and boning should be carried out at ambient temperatures below 12°C to comply with EU regulations. At this temperature salmonella requires at least 8 to 15 hours to double in number assuming there is no lag phase, and *L. monocytogenes* would double in 6 to 9 hours (Mackey & Roberts, 1991). At 10°C the generation times of *E. coli*, *Staphylococcus aureus*, or *L. monocytogenes* are all greater than 5 hours (Sumner & Krist, 2002). After an analysis of salmonella growth on pork cuts and in pork mince Mann *et al*. (2004) recommended that processors should ensure that the time product spends in the processing area should be no more than 12 hours when operating at 10°C and no more than 6 hours when operating at

room temperatures. Mackey & Roberts (1991) were of the opinion that boning operations should normally be completed within about 2 hours so there is insufficient time for extension proliferation even of the more psychrotrophic listeria. Similarly Sumner & Krist (2002) were of the opinion that the practice of re-warming beef to 10°C for "a few hours" prior to boning (to soften the fat) did not present a significant safety risk, particularly if a lag phase occurred.

According to Greer *et al*. (1983) the case-life of retail beef steaks are related to the psychrotrophic bacterial content on the wholesale ribs and steaks and to the degree of sanitation at the retail level. While, Chandran *et al.* (1986) showed that producing beef steaks under strict sanitary cutting procedures could improve their microbiological and sensory characteristics. There was no statistical difference in APCs on steaks from carcasses processed under strict sanitary slaughter and dressing conditions compared with those produced conventionally. However, at all storage intervals, steaks cut under strict sanitary procedures had lower bacterial counts than those obtained from the conventional cutting procedure (Table 8). The differences in APCs were approximately 2 log at the first four storage intervals (0, 7, 14, 21 days) and about 1 log at the next three storage intervals (28, 35, 42 days).

Table 8. Mean APCs (25°C/48 hrs; log_{10} cfu cm^{-2}) of vacuum-packaged steaks as influenced by hygiene of the slaughter-dressing and cutting procedures (source: Chandran *et al.*, 1986)

	Slaughter-dressing		Cutting	
Days of storage	Conventional	Strict sanitary	Conventional	Strict sanitary
0	2.34	2.04	3.25	1.13
7	3.48	3.66	4.79	2.01
14	4.31	4.71	5.83	3.19
21	5.27	5.67	6.45	4.50
28	6.18	6.07	6.77	5.53
35	6.75	6.46	7.11	6.03
42	6.67	6.75	7.06	6.36

The distribution of microflora was also affected. At day 0, the microflora of steaks produced under conventional conditions (Table 9) had a higher percentage of typical gram-negative spoilage bacteria (*Pseudomonas, Moraxella, and Acinetobacter*) than those produced under strict sanitary procedures. The microflora of steaks produced under conventional conditions was dominated by *Pseudomonas* spp., whereas both *Micrococcus* and *Pseudomonas* spp. were major parts (=25%) of the microflora of steaks produced under strict sanitary conditions. The microflora of steaks stored for 42 days was dominated by lactic acid bacteria, particularly *Lactobacillus cellobiosus*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. In parallel, a sensory evaluation revealed that steaks produced under strict sanitary practices had generally less off-odour. It was concluded that this difference in off-odour was most likely related to the fact that bacterial counts on steaks produced under strict sanitary conditions were lower than those on conventionally produced steaks.

Table 9. Distribution (%) of microbiological types on day 0 of storage of vacuum-packaged steaks obtained by conventional and strict sanitary slaughter-dressing and cutting procedures (source: Chandran *et al.*, 1986)

Microbiological type	Conventional slaughter-dressing		Strict sanitary slaughter-dressing	
	Conventional cutting	Strict sanitary cutting	conventional cutting	Strict sanitary cutting
Pseudomonas spp.	66.8	25.0	56.0	35.7
Acinetobacter-Moraxella spp.	12.7	3.5	1.2	
Staphylococcus spp.	0.2	17.7	1.2	
Micrococcus spp.	2.3	30.2	13.4	41.4
Yeasts	<0.1		11.1	
Brochothrix thermosphacta	4.7	1.7	6.2	6.2
Coryneform bacteria	0.7	16.7	1.7	16.7
Lactobacillus cellobiosus	3.2	3.1	8.7	
Lactobacillus coryneformis		2.1		
Lactobacillus plantarum	2.5		0.5	
Leuconostoc paramesenteroides	6.9			

A study by Jericho *et al.* (1996) on beef again showed the cutting operation to be a significant source of microbial contamination. During cutting there was a significant increase in APCs, by as much as 2 log, only 20 min after carcasses had left the chill room. Slightly lower counts were measured on "cut" surfaces than surfaces "not cut" in the cutting room. This implied that it was the general handling during cutting that caused much of the contamination. Similar finding were made by Gill & McGinnis (2000), they found that APCs, coliforms and *E. coli* were about 1, 3 and 3 log units more, respectively, after breaking than on carcasses entering the process. The large number of coliforms recovered was of particular concern but unfortunately the researchers were unable to establish a source of this contamination.

A number of studies have shown that bacterial counts increase on pork during cutting operations (Homann *et al.*, 1992; Bouvet *et al.*, 2002). Most authors conclude that some factor other than contamination during slaughtering-dressing is influencing the level of contamination on cut meat, such as cross-contamination from the hands of personnel, or inadequate cleaning and sanitising of equipment and contact surfaces. The storage-life of vacuum packaged pork has been shown to be directly related to the initial numbers of bacteria present and the degree of sanitation at the processing plant. To achieve a storage life of ≥7 weeks, initial APCs of ≤2 $\log_{10}$ cfu cm^{-2} and a storage temperature of -1.5°C are quoted by Holley *et al*. (2004). In their trials vacuum-packaged boneless pork loin pieces with initial counts around 3 log could be stored at -1.7±1°C for up to 8 weeks. They recommended using the fraction of Enterobacteriaceae in the bacterial population in vacuum-packaged pork stored at -1.5°C as a useful indicator of plant sanitation and product storage-life. Less than 5% incidence of Enterobacteriaceae would indicate acceptable sanitation, and would allow a "prediction of product quality ≥30 days in advance of the end of the desired product storage life".

3.5. Bacterial growth on primals and cuts during storage

The shelf-life of meat can be greatly extended by packaging under various atmospheres (vacuum, 100% carbon dioxide (CO_2), MAP (CO_2 rich atmosphere (20-30%) etc) and storage at low temperatures. Vacuum-packaging is the most widely used method of extending the storage-life of fresh meat, and is often used for the purpose of ageing primals (so called "wet ageing"). However, vacuum-packaging has an effect on the colour of the meat, hence meat is usually displayed for retail in various Modified Atmospheres (MA) containing oxygen to

give a "fresh" appearance to the meat. Carbon monoxide (CO) and nitrogen (N_2) rich atmospheres are also receiving some attention. For practical reasons such a practice is restricted for red meat to primals, sub-primals and cuts.

General data are available on the attainable chilled storage lives for many meats are shown in Table 10. However, again much is based on 'learned' opinion rather than peer reviewed scientific studies. There is a large variation in published shelf-lives of meats due to the nature of the investigations. Some investigations are of long-term storage-life at low storage temperatures (-1.5 to 1°C) whilst others are of short-term display-life of retail packs under retail conditions (3-4°C). As may be expected, bulk storage-lives are many weeks, while display-lives are a matter of days.

Table 10. Practical Storage Life of chilled cuts of meat, PSL is the time that the product is still of acceptable quality, assuming good initial bacteriological quality and normal pH (IIR, 2000)

Product	Temperature (°C)	Packaging	PSL d = day w = week m = month	Notes
Beef, boneless joints	-1.5 to 0	Vacuum packed	12 w	
Beef, retail cuts	4	Oxygen permeable pack.	2-5 d	
Beef, retail cuts	4	Vacuum packed	2 w	
Beef, retail cuts	2	MAP	9-12 d	80%O_2+20%CO_2
Pork, joints	-1.5 to 0	Vacuum packed	3-5 w	
Pork, retail cuts	4	Oxygen permeable pack.	3 d	
Lamb	-1.5	CAP (100% CO_2)	16 w	
Lamb and mutton	-1.5 to 0	Vacuum packed	10 w	

Differences in the meat pH, tissue composition (adipose or muscle), environmental composition (oxygen concentration) and initial microbial population and numbers probably account for the differences in storage-life between beef, lamb and pork. The microflora of vacuum-packaged meats radically change during storage and essentially vacuum-packed beef undergoes a natural fermentation process due to the rapid growth of lactobacilli that prevents the growth of other spoilage bacteria. In lamb the growth of lactobacilli does not appear to be sufficient thus possibly limiting its storage-life (Gill, 1984). In general vacuum-packaged beef has the longest storage-life, of 11 to 12 weeks (Gill & Penney, 1985), followed by lamb, 6 to 8 weeks (Gill & Penney, 1985), followed by pork, 4 to 6 weeks (Egan *et al*., 1986). However, more recent studies have shown much longer storage-lives, up to 8 weeks now for vacuum-packaged pork (Holley *et al*., 2004), are possible for all meats. The degree of vacuum used can have a great effect on shelf-life with levels above 600 mm Hg being recommended (Newton, 1977).

As mentioned earlier, pH can have a significant effect on storage-life. In New Zealand studies, microbial numbers on high pH (>6.0) beef cuts, vacuum-packaged in polyvinylidene chloride (PVDC) reached maximum levels in 6 weeks at +1°C compared with 12 weeks for normal pH beef (Gill & Penney, 1986). In metalized polyester or aluminium foil laminate vacuum packs times were respectively 9 and 15 weeks.

Greer *et al*. (1990) found little difference between the storage-life of vacuum-packaged beef primals from spray or conventionally chilled carcasses stored for 10 weeks at 1°C. Bacterial generation times were higher on primals from spray-chilled carcasses (4.16 days compared to 3.87 days) but lag times were longer (15.04 days compared to 12.86 days).

Bell *et al*. (1996) detected no major off odours after 14 weeks at -0.1°C from hot boned bull beef that had been cooled and stored in vacuum or CO_2 packs. At opening the appearance of the striploins was also acceptable. However, over ageing was believed to have reduced the retail display life of the meat. The authors thought that the process could produce high quality beef for catering use with a storage life of 10 weeks.

Generally recognised storage-lives of chilled pork stored in different atmospheres at 0°C are shown in Figure 6. The average storage-life of vacuum-packaged, North American, chilled pork imported into Japan in the mid-90s was 6 weeks. This gave the meat a limited residual storage life in Japan of 2 to 5 days, making it difficult to distribute and merchandise the product. A test shipment produced under high hygienic standards was received in Japan 8 weeks after slaughter and found to have a residual storage life of 4 to 6 weeks in Japan (Jeremiah, 1997).

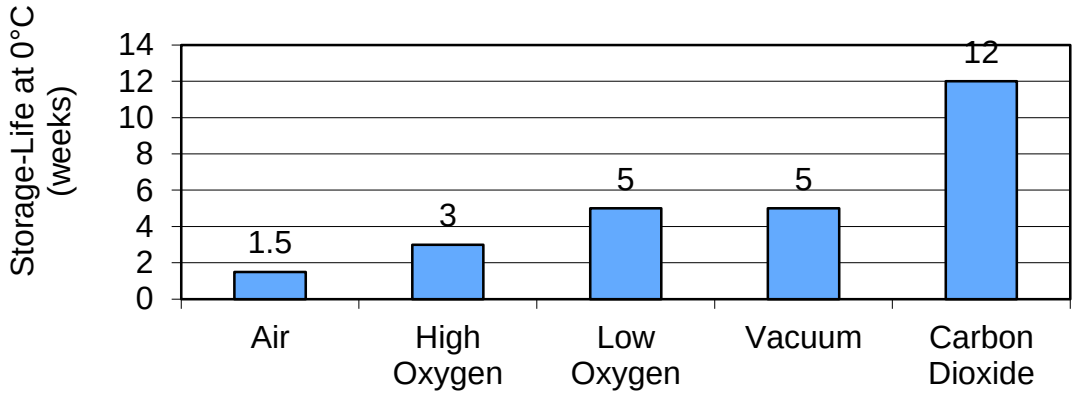


Figure 6. Storage-life of chilled pork stored in different atmospheres at 0°C (source: Jeremiah, 1997)

In vacuum-packaged pork primals, Egan *et al*. (1986) have also shown that the temperature of storage and pH determines both the storage-life and the nature of the changes during storage (Table 11).

Table 11. Storage-life and nature of spoilage of vacuum-packaged pork (source: Egan *et al.*, 1986)

	0°C		5°C	
Meat pH	Storage life (weeks)	Spoilage characteristics	Storage life (weeks)	Spoilage characteristics
5.4 to 5.8	6	Flavour changes souring	3 to 4	Flavour changes souring
6.2 to 6.5	4 to 5	variable	2 to 3	Greening, odour of H_2S, putrefaction

At a lower temperature Jeremiah *et al*. (1995a, b) have shown that off-flavour development, coinciding with lactic acid bacteria reaching maximum numbers, currently restricts the storage-life of CO_2 or vacuum packaged pork to 9 weeks at -1.5°C. Based on appearance, CO_2 packaged pork loin had a storage life of over 15 weeks and vacuum-packaged slightly over 12 weeks. Only small differences were found between pork loins from DFD, PSE and normal quality groups. They believed that reducing the current levels of microbial contamination would allow storage life to be extended to meet all domestic and export requirements.

The effect of temperature and packaging on the storage-life of pork was clearly demonstrated by Lee *et al*. (1985) and Gill & Harrison (1989). Only small changes in microbial numbers (Figure 7), pH, drip and off-odour were vacuum or vacuum plus gas flushed packs of pork after 49 days storage at -4°C (Lee *et al*., 1985). Whilst green discolouration was significant after 2 weeks at 3°C and 7°C, and 4 weeks at 0°C. The amount of drip loss increased substantially with both length and temperature of storage (Figure 8).

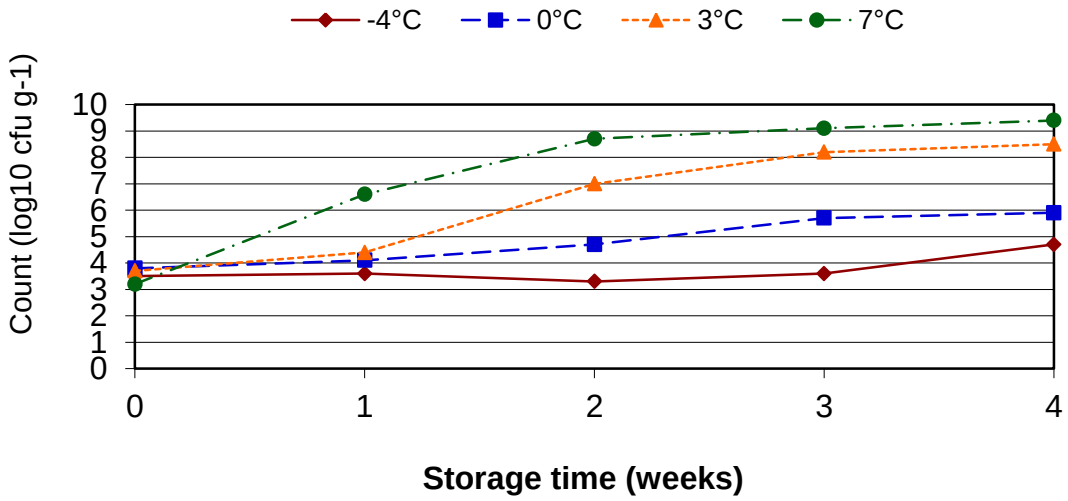


Figure 7. Growth of psychrotrophic bacteria on vacuum-packaged cubed pork at -4, 0, 3 and 7°C (source: Lee *et al*., 1985)

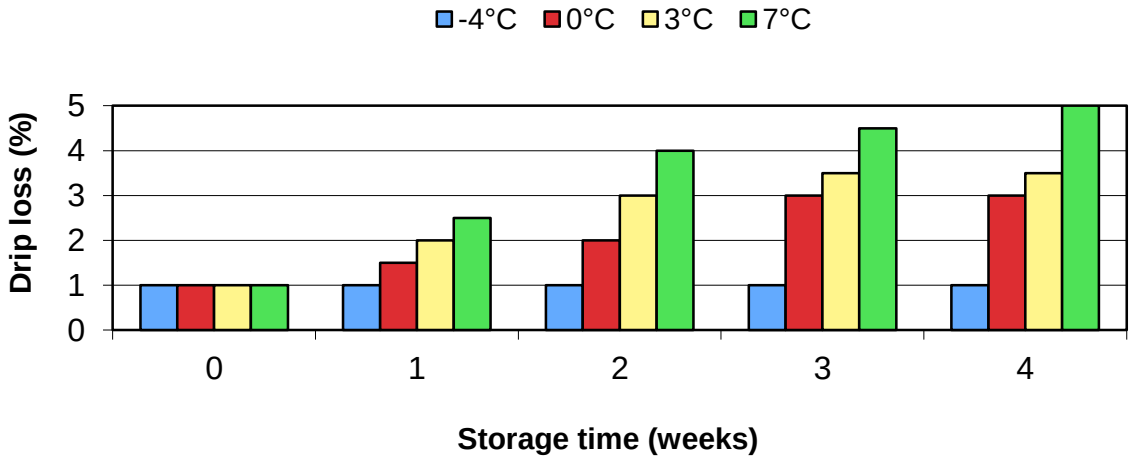


Figure 8. Drip loss from vacuum-packaged cubes of pork stored at -4. 0. 3 and 7°C (source: Lee *et al*., 1985)

Gill & Harrison (1989) found that vacuum-packaged cuts of pork *longissimus dorsi* muscle (skin on) were grossly spoiled by *Brochothrix thermosphacta* after 2 weeks storage at 3°C compared with 5 weeks at -1.5°C. Cuts packaged under CO_2 spoiled after 5.5 weeks storage at 3°C. Growth of *B. thermosphacta* was suppressed when the pork was stored under CO_2 at -1.5°C. Growth of Enterobacteriaceae caused gross spoilage of an increasing proportion of cuts between 18 and 26 weeks. Until spoilage occurred the eating quality of the pork was little affected by the length of storage.

An evaluation of different packaging systems for extending the storage-life of pork loin cuts by Scholtz *et al*. (1992) showed that a storage-life of 3, 2 or 1 week at 0°C could be achieved

using 100% CO_2, MAP (25% CO_2 75% O_2), or vacuum packaging, respectively. Enterobacteriaceae counts remained low for all the packaging treatments during storage, particularly in the case of the CO_2 treatment. The colour of the cuts were affected somewhat by the high CO_2 atmosphere but were considered acceptable.

Table 12. Growth of bacteria (log_{10} cfu cm^{-2}) on pork loin cuts stored at 0°C (source: Scholtz *et al.*, 1992)

Packaging	Storage (weeks)	Total count	Lactic acid bacteria	Pseudomonads	Enterobacteriaceae
100% CO_2	0	3.6	3.4	1.6	ND
	1	4.1	3.4	3.5	1.0
	2	4.2	3.4	3.8	1.2
	3	5.8	4.6	4.2	1.5
MAP	0	3.4	3.0	2.9	1.1
(25% CO_2 75% O_2)	1	4.7	3.5	4.6	0.9
	2	6.7	4.5	5.3	2.5
	3	8.0	6.0	7.2	4.4
Vacuum	0	3.4	2.8	2.6	ND
	1	6.6	4.5	5.5	2.9
	2	7.1	5.8	6.8	4.4
	3	7.8	6.8	7.1	4.9

Storage-life of as long as 8 weeks for vacuum-packaged boneless pork loins stored at -1.7±1°C (Figure 9) have been reported by Holley *et al*. (2004).

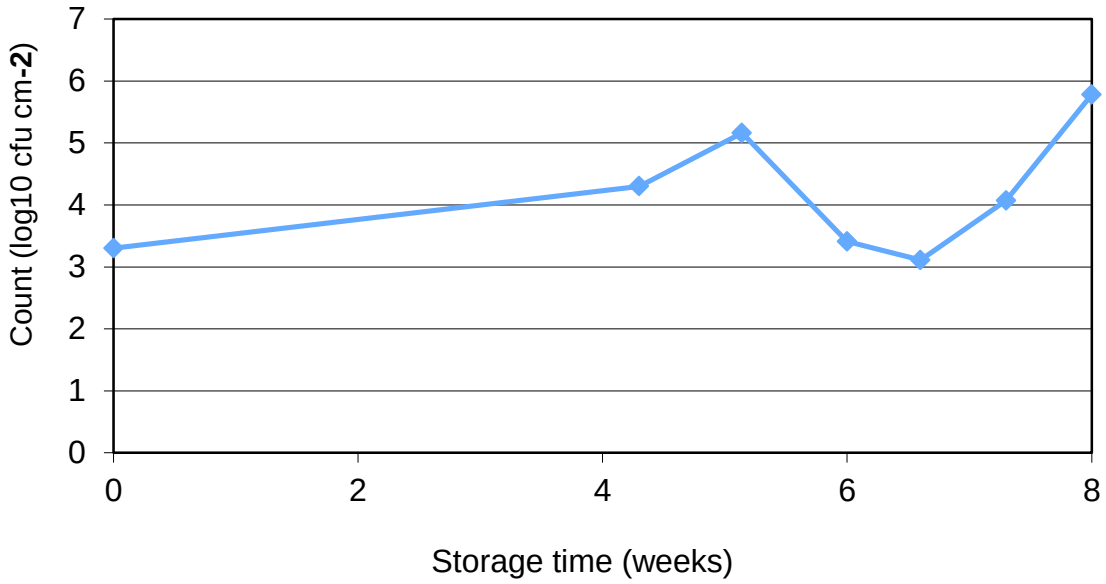


Figure 9. Growth of aerobic bacteria on vacuum-packaged fresh boneless pork loins during storage at -1.7±1°C (source: Holley *et al.*, 2004)

While the use of Carbon Monoxide (CO) has been shown to benefit the maintenance of a bright, pink-red fresh pork colour, it does not aid the storage-life of pork (Wilkinson *et al*., 2006). A comparison of the storage-life of pork chops packaged in either a 100% CO_2 atmosphere or a mixture of 80% CO_2, 19.6% N_2, and 0.4% CO stored at 3°C for up to 8 weeks showed a greater growth of aerobes and anaerobes on meat stored in the CO atmosphere (Figure 10).

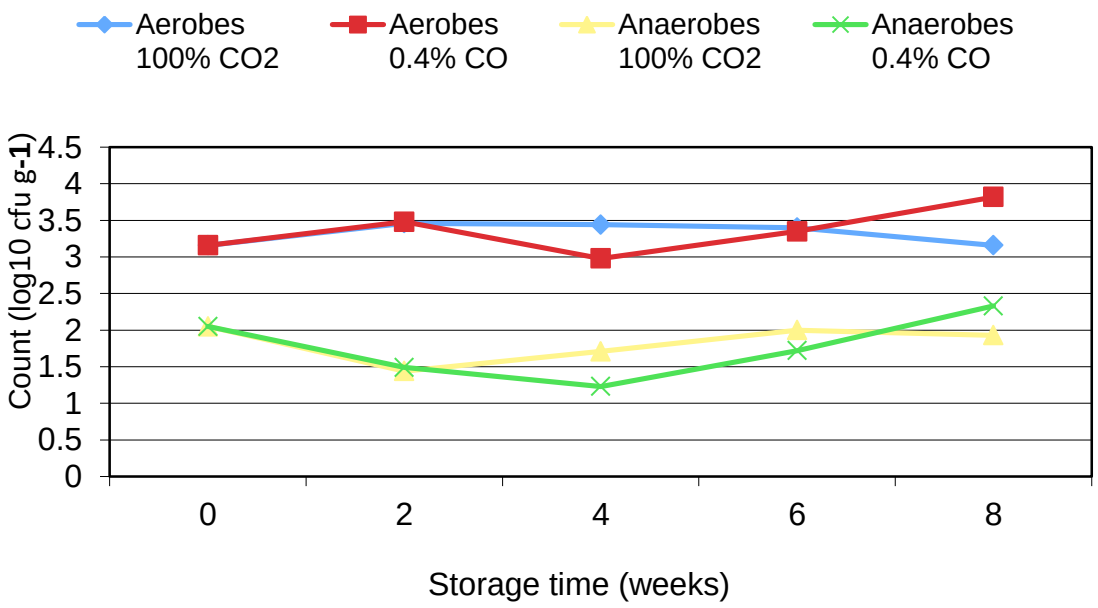


Figure 10. Effect of gas atmosphere on the growth of aerobic and anaerobic bacteria on pork chops stored at 3°C (source: Wilkinson *et al*., 2006)

In audits carried out in New Zealand to improve the storage-life of vacuum-packaged chilled lamb, changing the chilling practice was found to have the largest effect (Gill, 1987). It was found that the significance of the relatively small numbers of organisms added to carcasses during dressing was greatly magnified by their growth during carcass cooling. Small changes to the chilling practices alone extended the storage life by up to 50%. Studies on lamb have shown that it is possible to ensure a storage-life of at least 12 weeks for vacuum-packaged lamb cuts (Gill & Penney, 1985). Some MIRINZ studies (Newton *et al*., 1976) have reported even longer storage-lives with up to 15 weeks (at a vacuum level of 300 mm Hg) and 19 weeks (at a vacuum level of 580 mm Hg) for vacuum-packaged lamb legs, loins and shoulders stored at -1°C. A delay of 24 hours between cutting and packaging appeared to favour the growth of *Microbacterium thermosphactum* rather than *Lactobacillus*.

Sheridan *et al*. (1997) investigated the effect of vacuum and modified atmosphere packaging (80% O_2, 20% CO_2; 50% CO_2, 50% N_2; and 100% CO_2) on the storage-life of lamb primals stored at 5 and 0°C. They showed that in general there was little difference in total bacterial counts, irrespective of atmosphere, in primals held at 5°C after 4 weeks. There were significant differences in counts on primals packaged in different atmospheres at 1°C after 4 weeks, with the lowest counts on primals held in a 100% CO_2 atmosphere. In the case of *B. thermosphacta*, pseudomonad and Enterobacteriaceae counts there were significant differences in counts between the different atmospheres at either storage temperature. Again the lowest counts were generally on primals held in a 100% CO_2 atmosphere.

3.6. Bacterial quality of cuts from aged meat

A number of studies have shown that there is an interaction between storage time of primals/sub-primals and display-life in retail display. The type of packaging, atmosphere and temperature will also have a large effect on shelf-life and display-life.

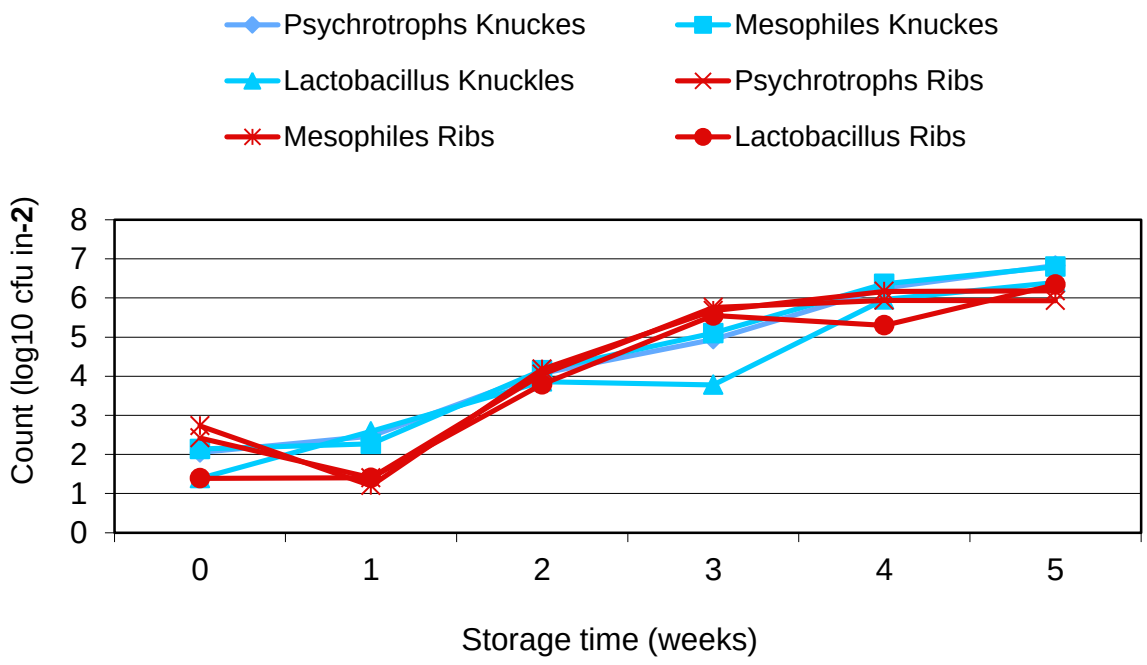


Figure 11. Growth of bacteria on vacuum-packaged beef knuckles and ribs stored at 1 to3°C (source: Seideman *et al*., 1976)

Figure 11 show the relationship between bacterial counts on vacuum-packaged beef knuckles and ribs kept at 1 to 3°C for up to 5 weeks and Figure 12 counts on steaks cut from these primals after different storage periods and then displayed for 5 days.

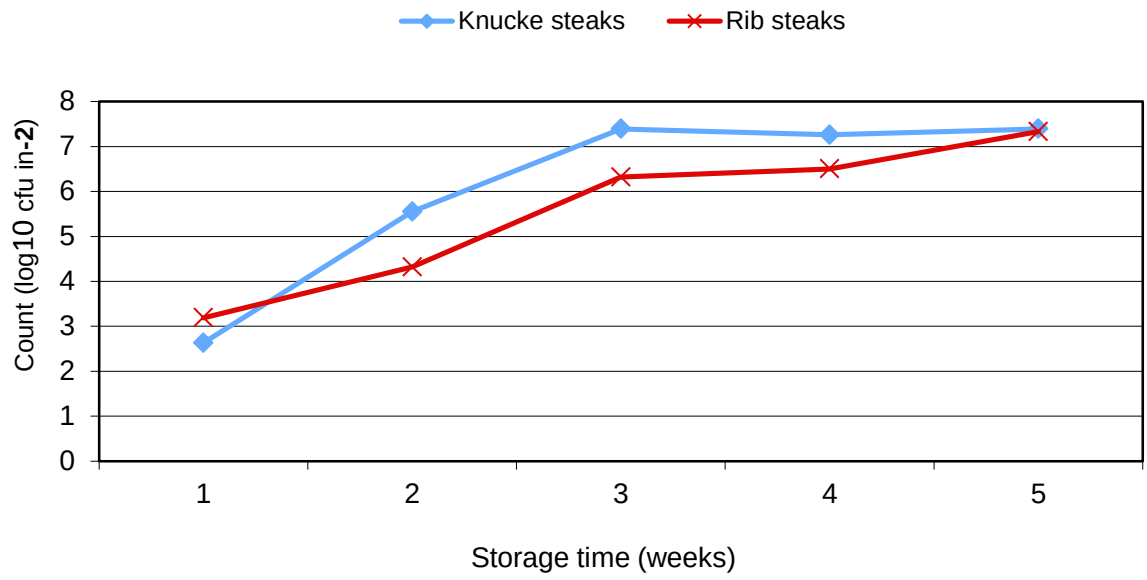


Figure 12. Psychrotrophic bacterial counts on beef knuckle and rib steaks after 5 days of retail display (1 to 3°C) according to the storage of the vacuum-packaged primal (source: Seideman *et al*., 1976)

Dixon *et al*. (1991) showed that vacuum-packaged beef sub-primals from carcasses processed under strict sanitary procedures plus the use of a hot lactic acid intervention could be stored for 80 days at 1°C and produce acceptable steaks, however they only had a 1 day acceptable

display shelf-life (Figure 13). Cuts from sub-primals produced under standard procedures were only acceptable from sub-primals stored for 20 days.

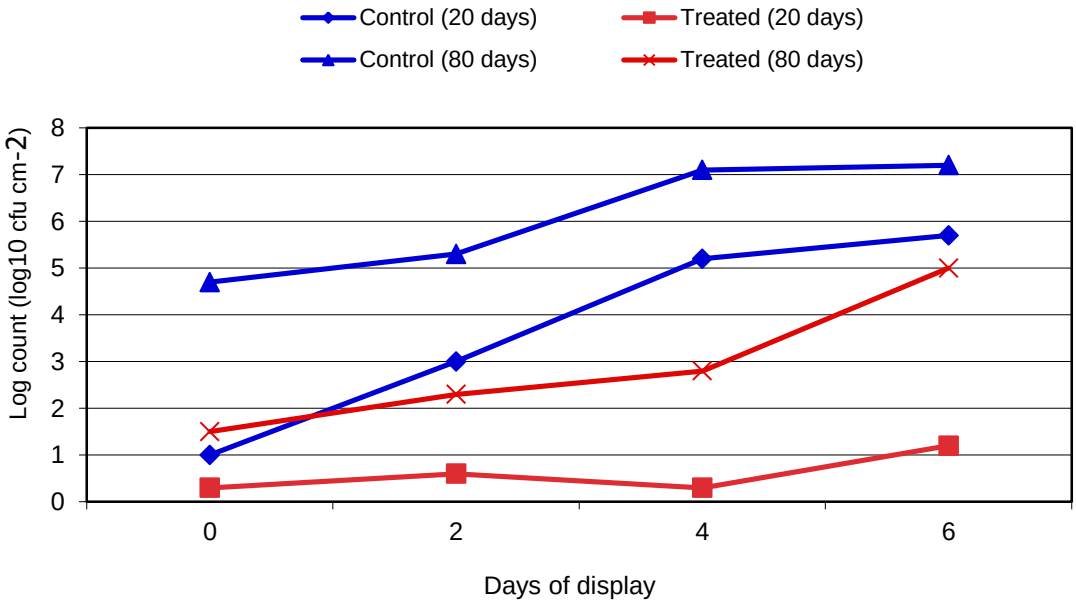


Figure 13. Mean aerobic plate counts on beef rib eye steaks, fabricated from control and treated sub-primals stored for 20 to 80 days at 1°C, displayed in PVC film for 0 to 6 days at 4±1°C (source: Dixon *et al.*, 1991)

Nortjé & Shaw (1989) reported that beef loin steaks from primals that had been aged for 3 weeks in vacuum packs discoloured more rapidly and off-odours developed sooner than those from meat that had been hung in air for one week or vacuum packed for one week. The poorer storage stability was explained by higher initial levels of bacteria due to growth during aging. Rancidity development was only detected in the 3 week aged steaks that were stored at 6°C.

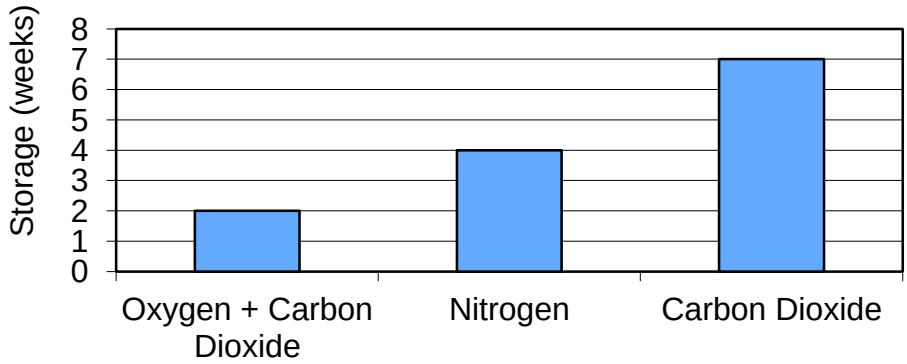


Figure 14. Storage periods of beef loins stored in different atmospheres at -1.5°C capable of producing steaks with a display-life of 2 days or longer (source: Gill & Jones, 1994a)

A study by Gill & Jones (1994a) showed that master packs with a CO_2 atmosphere could be stored for up to 7 weeks at -1.5°C and provide steaks with a display-life of 2 or more days (Figure 14).

A study by Reagan *et al*. (1971) implied that the display-life of lamb chops from vacuum-packaged primals is reduced in comparison to "freshly" prepared chops. Fresh lamb chops fabricated 8 days post-mortem and displayed immediately showed a 1.25 day advantage of increased display-life in comparison to chops from loins vacuum-packaged 8 days post-mortem, stored under vacuum for 8 days, fabricated and subsequently displayed. However, there was little overall difference in the average display-life (3.5 days) of either fresh chops or those from vacuum-packaged loins stored for up to 40 days at 0°C.

Greer *et al*. (1993) published a relationship between the retail display-life of pork from CO_2 packaged primals and the length of time the primals had been stored:

Essentially, there was a 1 day reduction in display-life for every 6 weeks in CO_2 storage.

- On appearance criteria: display-life (days) = 4.60 - 0.15 × (number of weeks in storage in CO_2 at -1.5°C)
- On odour criteria: display-life (days) = 5.03 - 0.17 × (number of weeks in storage in CO_2 at -1.5°C)

Pork loins stored for 24 days at had only 1 days display-life. For practical purposes loin primals in stored in CO_2 at -1.5°C had a storage-life of around 9 to 15 weeks.

While there have been a number of studies that have looked at the impact of ageing times on the display-life of meats few appear to have looked at the effect of ageing time and display on pathogenic growth.

In one of the few studies Dykes *et al*. (2001) investigated the growth of inoculated (at two different levels, 10^3 and 10^5 cfu g^{-1}) *E. coli* O157 and salmonella (*S. typhimurium* and *S. brandenberg*) on beef steaks stored under ageing and retail conditions. Vacuum or 100% CO_2 packaged beef steaks were stored at -1.5°C for 6 weeks (to simulate ageing in pack) followed by 2 weeks at 4°C (to simulate retail display). They reported no significant changes in numbers of any of the inoculated pathogens during storage at -1.5 or 4°C in either of the packaging atmospheres. The authors noted that similar studies have shown slight reductions in numbers of these pathogens during storage. The authors also concluded that the long period of storage, of particularly *E. coli* O157, in a non-growing state would result in "*an excessive recovery period in these cells before growth would occur*".

3.7. Bacterial quality of mince

The microbiological quality of minced meat is largely determined by the microbiological quality of the meat used in its production. The ICMSF (1998) note that minced meat prepared at retail often have greater microbial loads than those produced centrally, since they are often prepared from scrap meats and trimmings that have been stored for several days, rather than produced from fresh or frozen meat with lower counts. During mincing microorganisms present on the surface of the meat are distributed throughout the minced meat. Mincing itself may also increase the temperature of the meat. The extent of this increase depends on the process. The mincer itself may constitute a significant source of cross-contamination if not effectively cleaned before use and between batches. The effects of temperature and time on pathogen growth discussed earlier relating to cutting are equally relevant here. As with boning if mincing is completed within a few hours, and carried out under semi-refrigerated conditions, there is insufficient time for extension proliferation of pathogens, even of the more psychrotrophic listeria (Mackey & Roberts, 1991; ICMSF, 1998; Mann *et al*., 2004).

Eisel *et al.* (1997) carried out a microbiological survey of the relationship between microbial levels for incoming meat on levels in finished minced beef in a US red meat processing plant. It showed that while environmental sources of contamination existed in the processing plant most of the microorganisms came from the incoming raw meat. The survey highlighted the need to reduce microbiological populations on highly contaminated areas of the carcass, such as the brisket and skirt areas. Average APCs ranged from 3 $\log_{10}$ cfu g^{-1} for the retail cuts to 6.9 $\log_{10}$ cfu g^{-1} for the brisket area of beef carcasses. For carcass beef, the brisket and skirt areas were more contaminated compared with the round and flank. The authors postulated that the brisket and skirt areas were probably more susceptible to microbiological contamination during slaughtering because cattle are hung by the hindlegs. This may promote contamination on anterior parts of the carcass due to closer proximity to floor (splash) and rinsing liquid travelling from the posterior down to the anterior. Boxed beef, the other ingredient of ground beef, also had a comparatively high APC, generally near 4.7 $\log_{10}$ cfu g^{-1}. Mean *E. coli* counts were generally low, ranging from 1 to 2 $\log_{10}$ cfu g^{-1}. Microbiological concentrations for frozen samples of carcass beef and boxed beef from different suppliers, were similar. There was no correlation between a high APC and a high coliform count. Overall APCs on the finished minced beef were very similar to counts on the incoming meat with an average of 4.6 $\log_{10}$ cfu g^{-1}. There was no indication of an increase due to the mincing process itself.

A survey of the microbiological quality of beef trimmings on the quality of retail mince by Gill & McGinnis (1993) indicated that display temperatures had a significant effect on the overall quality of the mince. It also showed that there was often a significant time between the trimmings being vacuum-packaged and the meat being minced. This could be greater than 14 days, and was mainly due to the need of wholesalers and retailers to build up stocks of raw materials to cope with fluctuations in supply and demand. During storage of up to 18 days before mincing most trimmings developed a flora of lactobacilli, of up to 7 $\log_{10}$ cfu g^{-1}. Though numbers of coliforms and *E. coli* increased little or not at all, respectively. The survey showed a wide range of storage conditions and temperature fluctuations during chilling, transport and storage of the trimmings prior to mincing and display. Increased total counts and numbers of coliforms and *E. coli* increased in displayed mince indicating poor temperature control.

A survey of hamburger manufacturers and suppliers of manufacturing beef suppliers by Gill *et al*. (1996; 1997) led to a recommendation that manufacturing beef for such products should have no more than 1 $\log_{10}$ cfu g^{-1} of *E. coli.* Gill *et al*. (1997) showed that, as might be expected, there is a clear relationship between the microbial quality of the incoming raw material used for the manufacture of hamburger patties and the microbial quality of the finished hamburger patties.

A survey of the microbiological quality of beef trimmings and final minced beef by Scanga *et al*. (2000) showed that final minced beef samples had a 13.6 and 1.5% incidence of *L. monocytogenes* and *Salmonella* spp., respectively. Trimmings with higher fat content, had higher APCs, those that had, nominally, 30% fat the highest APCs. The authors believed that this was due to greater amount of exposed surface on such trimmings rather than a characteristic of tissue type. However other authors have noted higher rates of growth on adipose surfaces than muscle surfaces (Lasta *et al*., 1995). The authors recommended overall that processors "*focus their efforts on reducing the microbial counts on incoming raw materials, especially those containing large proportions of subcutaneous fat*".

Following an analysis of salmonella growth in pork mince Mann *et al*. (2004) recommended that raw materials and finished product should spend should be no more than 12 hours in the

processing area when operating at 10°C, or no more than 6 hours when operating at room temperatures. Mann & Brashears (2006) suggested the same limit when operating at room temperatures with regard to potential for growth of *E. coli* O157:H7.

While studies have looked at the microbiological quality of the in-coming meat used for mince production, and the effect of delays during mincing, no publications have located that have looked at the effect of length of meat storage before mincing on the subsequent storage-life or safety.

3.8. Bacterial growth on mince during storage

3.8.1 Spoilage organisms

It is generally considered that beef mince has a longer storage-life to lamb and pork, and that poultry mince has a shorter storage-life than red meat mince. This has been attributed to either a lower hygienic status during processing and/or a higher incidence of high pH meat in such meats (Blixt & Borch, 2002). A study of the shelf-life (at 4°C) of vacuum-packed minced pork and beef by Blixt & Borch (2002) showed that samples with the same initial bacterial loads did show differences in the rates of spoilage and bacterial growth, but they were more related to other intrinsic factors of the meat than species. These factors were the pH and concentrations of L-lactate and glucose-6-phosphate. Stern *et al*. (1992) also found no significant difference between the spoilage rates of beef and turkey mince, regardless of treatment or origin of species. Saucier *et al*. (2000) noted a slight difference in the numbers and growth of total aerobic mesophilic counts between chicken mince (higher) than in turkey mince throughout storage at 1°C.

There is a large variation in published storage/shelf-lives of minced meats (Table 13). Some of these variations are due to the nature of the investigations. Some investigations have been of long-term storage-life of "mother" packs of mince held at low storage temperatures (-1.5 to 1°C) whilst others are of short-term display-life of retail packs under retail conditions (3-4°C). As may be expected, bulk storage-lives can be up to 4 weeks, while display-lives are a matter of days.

Table 13. Storage-life of packs of minced meat

Meat	Temperature (°C)	Atmosphere	Shelf-life (days)	Reference
Beef	4	Oxygen permeable pack	1-2	IIR, 2000
Beef	4	Vacuum	7-14	IIR, 2000
Beef	2	$80\%O_2+20\%CO_2$	3-5	IIR, 2000
Beef	-1.5	Vacuum	32	Gill & Jones. 1994b
Beef	1	$24\%O_2+50\%CO_2+25\%N_2+1\%CO$	29	Lüno *et al*., 1998
Goat	4	Vacuum	28	Babji *et al*., 2000
Goat	4	Aerobic	3	Babji *et al*., 2000
Chicken	3	Vacuum	8	Linton *et al*., 2004
Chicken	1	$60\%CO_2+8\%O_2+30\%N_2$	>15	Saucier *et al*., 2000
Chicken	1	$20\%CO_2+80\%N_2$	>15	Saucier *et al*., 2000
Turkey	1	$60\%CO_2+8\%O_2+30\%N_2$	>15	Saucier *et al*., 2000
Turkey	1	$20\%CO_2+80\%N_2$	>15	Saucier *et al*., 2000
Ostrich	4	Vacuum	6	Seydim *et al*., 2006
Ostrich	4	High Nitrogen	6	Seydim *et al*., 2006
Ostrich	4	Aerobic	6	Seydim *et al*., 2006
Ostrich	4	High Oxygen	3	Seydim *et al*., 2006

Gill & Jones (1994b) compared the storage-life and display-life of vacuum-packaged minced beef stored at -1.5°C, with retail packs master packaged under atmospheres of N_2, CO_2 or O_2 + CO_2 (2:1) stored at 2°C. The appearance of the product displayed after storage in a vacuum-pack, for times up to 32 days, became unacceptable within 48 hours in a retail cabinet at 4±2°C. A product stored in any of the master packs for 1 day appeared unacceptable after 6 h of display. The display life of products stored under N_2 or CO_2 was similar to that of the vacuum-packaged products when storage times were between 2 and 24 days but the display life was shorter when the storage times were 28 or 32 days. The spoilage flora on products stored in vacuum pack or under O_2 + CO_2 did not attain the maximum numbers of 7 $\log_{10}$ cfu g^{-1} during either storage or display. Those maximum numbers were attained on products stored under N_2 and CO_2 after 16 and 28 days storage respectively. Some products stored under N_2 for 16 days or longer developed moderate or strong off-odours during display that were ascribable to microbial action. Other products developed only slight, non-microbial off-odours during display. The authors concluded that retail-ready packs or ground beef master-packaged under an oxygen-depleted atmosphere could then have a useful storage life of about 30 days in commercial circumstances.

A combination of MAP (70% O_2 + 20% CO_2 + 10% N_2) high oxygen / carbon monoxide (70% O_2 + 20% CO_2 + 9% N_2 + 1% CO) and low oxygen / carbon monoxide (24% O_2 + 50% CO_2 + 25% N_2 + 1% CO) were investigated for packaging fresh minced beef by Luño *et al*. (1998). The atmosphere containing low oxygen / carbon monoxide was found to give the best all round effects on storage-life. Psychrotrophic counts were greatly reduced, so that $\log_{10}$ cfu cm^{-2} was under 7.5 at 29 days of storage at 1°C.

The display-life of goat mince may be as short as 3 days for aerobic packages, whereas vacuum packed goat mince will last 28 days, at 4±1°C (Babji *et al*., 2000). High pH and an initial heavy carcass contamination, promotes the rapid multiplication of facultative anaerobes leading to spoilage of the mince. During storage putrid odours in aerobically packed mince and sulphide odours in vacuum packs were observed.

Work by Saucier *et al*. (2000) shows how gas mixture that can maintain a desirable colour in mince poultry meat may be less effective than others with respect to the microbial profile of meat. The storage-life of ground chicken and turkey meat at 1°C packaged under a modified atmosphere containing O_2 and a high level of CO_2 (62% CO_2, 8% O_2, and 30% N_2) was compared with a gas mixture without O_2 (20% CO_2 and 80% N_2). Meat packaged under no O_2 had a more appealing colour than the meat packaged under O_2 + high CO_2. While meat packaged under either of the gas mixtures tested had similar counts for presumptive pseudomonads, *Staphylococcus aureus*, and lactic acid bacteria after 15 days at 1°C, coliforms and *E. coli* counts were lower in meat packaged under O_2 + high CO_2.

Oxidation has been found to be the main limiting factor for the display-life of minced ostrich meat (Seydim *et al*., 2006). Ostrich mince was "below saleable quality" in less than 6 days displayed at 4±1°C under either high N_2, vacuum or aerobic atmospheres, under a high O_2 atmosphere the display-life was less than 3 days.

3.8.2 Pathogens

Mackey *et al*. (1980) quote published minimum growth temperatures for salmonella in pork and beef mince ranging from 4 to 7°C. Their studies did not show salmonellas to growth at 7 to 8°C. Ingham *et al*. (2004) found a sight increase (0.2 log) in the growth of inoculated salmonella in minced beef held at room temperature for 2 hours. There was no growth in minced beef held for up to 4 hours at 10°C.

A comparison of salmonella growth in minced pork and boneless pork chops held at 4.4, 7.2 and 10°C by Mann *et al*. (2004) showed that salmonella grew at faster rates in minced pork. There was a lag in the growth of salmonella populations in minced pork for 24 and 32 hours at 10 and 7.2°C, respectively. Thus processing pork at 7.2 or 10°C would not lead to any significant growth of salmonella or increase in APCs provided the time spent in the processing area did not exceed 12 hours.

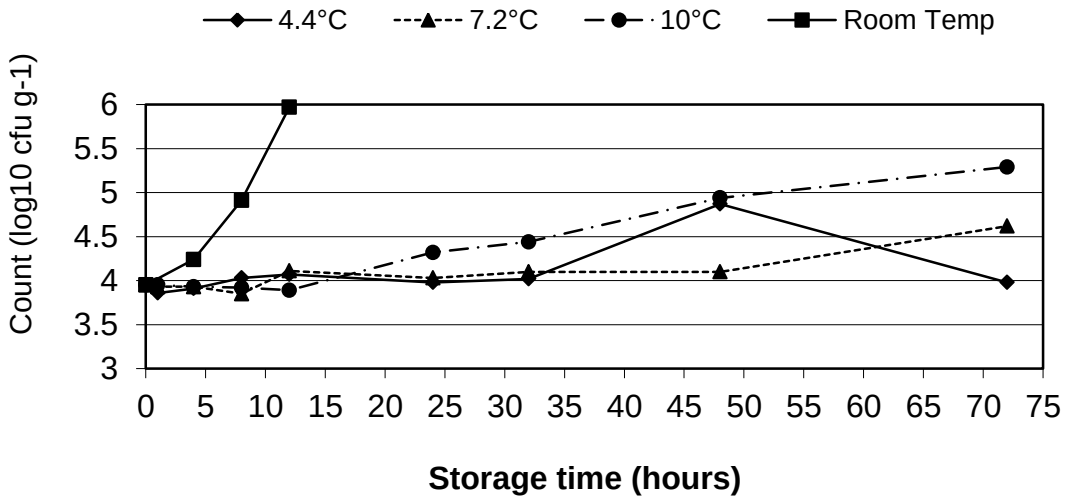


Figure 15. Growth of salmonella in minced pork at various temperatures (Mann *et al*., 2004)

Significant growth was observed at 6, 24, and 72 hours when samples were held at room temperature, 10 and 7.2°C, respectively. No significant growth was observed at 4.4°C. Background flora in ground pork samples increased significantly after 10 hours at room temperature and after 12 hours for samples held at 10 and 7.2°C. Background flora in samples held at refrigeration temperatures did not increase until 72 hours. Background flora in the boneless chops increased significantly after 6 hours at room temperature and after 24 hours when held at 10 and 4.4°C. These results illustrate that meat processors can utilize a variety of time and temperature combinations as critical limits to minimize Salmonella growth during production and storage of raw pork products.

A study of the growth of selected inoculated pathogens in wrapped minced beef by Goepfert & Kim (1975) showed no growth of *B. cereus* (5 strains), *Cl. perfringens* (5 enterotoxigenic strains), *Staph. aureus* (5 strains, including producers of A, B, C, D and E enterotoxins) stored at 1, 4.5, 7 or 12.5°C for up to 14 days. Only *E. coli* and *Salmonella* spp. (*S. typhimurium*, *S. Illinois*, *S. infantis*, *S. london* and *S. tennessee*) were able to grow, and then only at the highest temperature of 12.5°C.

The growth of *Y. enterocolitica*, *L. monocytogenes*, *E. coli* O157:H7 and strains of *Salmonella* were compared in minced beef packed in modified atmospheres of 60% CO_2/40% N_2/0.4% CO (high CO_2/low CO mixture), 70% O_2/30% CO_2 (high O_2 mixture) and in chub packs (stuffed in plastic casings) and stored at 4 and 10°C by Nissen *et al*. (2000). At 4°C the shelf-life, based on colour stability and background flora development, was prolonged (14 days) for the high CO_2/low CO mixture compared to the two other packaging methods, but at 10°C the shelf life was <8 days for all the packaging methods. Growth of *Y. enterocolitica* was nearly totally inhibited both at 4 and 10°C in the high CO_2/low CO mixture, while the bacterial numbers in the samples packed in the high O_2 mixture increased from about 10^2 bacteria/g at day 0 to about 10^4 at day 5 at 4°C and to 10^5 at 10°C. Growth in the chub packs

was even higher. *L. monocytogenes* showed very little growth at 4°C in all treatments. At 10°C there was slow growth from about 10^3 bacteria/g to about 10^4 at day 5 in the high CO_2/low CO mixture, while the numbers in the high O^2 mixture and the chub packs were about 10 times higher. Growth of *E. coli* O157:H7 at 10°C in the ground beef was nearly totally inhibited in both the high CO_2/low CO mixture and the high O_2 mixture. Growth in the chub packs was higher, as the number of bacteria increased 3 log in 5 days. The Salmonella strains (*S. typhimurium*, *S. dublin*, *S. enteritidis* and *S. enterica* 61:k:1,5,(7)) in the ground beef stored at 10°C for 5 and 7 days grew to a higher number in the high CO_2/low CO mixture than in the high O_2 mixture.

Background microflora was shown by Vold *et al*. (2000) to inhibit the growth of *E. coli* O157:H7 in ground beef stored either aerobically or anaerobically at 12°C. Under aerobic conditions and a background microflora *E. coli* O157:H7 grew to a maximum concentration of about 6 $\log_{10}$ cfu g^{-1} after 10 days, while with no background microflora growth reached this level after only 4 days. Tamplin (2002) compared real and predicted (using the Pathogen Modelling Program) growth of *E. coli* O157 in raw minced beef stored at 10°C. On retail minced beef the mean maximum population density (MPD) and exponential growth rate (EGR) for were 5.09 $\log_{10}$ cfu g^{-1} and 0.019 $\log_{10}$ cfu h^{-1}, respectively, and no lag phase was observed. Both the EGR and the MPD increased with decreasing fat levels, and the EGR and MPD decreased as the ratio of competitive flora to *E. coli* O157:H7 increased. Further studies (Tamplin *et al*., 2005) investigated the growth of 10 strains of *E. coli* O157:H7 on minced beef at storage temperatures from 5 to 46°C. Growth occurred from 6 to 45°C, with the absence of a lag period at 6, 8 and 10°C. At 6°C the mean MPD and specific growth rate (SGR) were 4.71 $\log_{10}$ cfu g^{-1} and 0.003 ln h^{-1}, respectively. Mann & Brashears (2006) recently published data showing a slight, though not statistically significant, rise in numbers of inoculated *E. coli* O157:H7 in minced beef at temperatures as low as 4.4°C after 72 hours. Less than 1 log of growth was observed after 48 hours at 10°C. Significant increases were observed in numbers after 6 to 8 hours at room temperature (22-23°C). Despite these studies that show growth of inoculated *E. coli* O157:H7 at low temperatures, Ingham *et al*. (2004) found no growth of inoculated *E. coli* O157:H7 in minced beef, and beef, pork and chicken pieces, held at room temperature for 2 hours or at 10°C for 4 hours.

Table 14. Mean inoculated *Y. enterocolitica* 0:3 and natural APC numbers ($\log_{10}$ cfu g^{-1}) at 28 days on lamb pieces and mince packaged in air and different gas atmosphere stored at 5 or 0°C (source: Doherty *et al*., 1995)

	Y. enterocolitica 0:3				APC			
Storage temperature (°C)	5	0	5	0	5	0	5	0
	Pieces		Mince		Pieces		Mince	
Air	9.54	5.82	9.40	4.75	12.27	9.41	11.98	8.20
Vacuum pack	8.11	5.88	6.50	2.68	8.55	6.14	9.07	5.25
80%O2/20%CO2	6.84	1.16	2.40	0.78	9.37	5.40	8.95	3.87
50%CO2/50% N2	8.52	3.86	5.25	1.29	8.93	5.11	7.75	4.59
100%CO2	5.56	1.56	1.05	0.00	7.70	4.03	6.44	2.68

Y. enterocolitica is known to grow at lower temperatures than other pathogens. *Y. enterocolitica* has been shown to increase in minced beef by 1 $\log_{10}$ cfu g^{-1} within 14 days at 1°C and 3.5 log within 14 days at 4°C (Kleinlein & Untermann, 1990). The presence of a heavy competitive flora inhibited the growth rate of yersinia, and CO_2 fully inhibited growth at 4°C. Work on lamb (Doherty *et al*., 1995) has shown that inoculated *Y. enterocolitica* serotype O:3 grows better on pieces than mince and that growth at low temperatures is

inhibited by atmospheres containing either a high concentration of O_2 or a high concentration of CO_2 (Table 14).

3.9. Microbial contamination of chill rooms

Psychrophilic spoilage microorganisms have been shown to persist on structural surfaces, including refrigeration coils, within chill rooms and have been shown to have a role in carcass contamination (Stringer *et al*., 1969; Ockerman *et al*., 1977; Newton *et al*., 1978; Gustavsson & Borch, 1989; Mafu *et al*., 1989; Nortjé *et al*., 1990; Evans *et al*., 2004).

The potential for the fans used in air chilling to disseminate moulds and bacteria has been identified in a number of reviews (Richmond, 1991; Houston, 1996) but very little work has been carried out to evaluate whether this is in fact the case. Stringer *et al*. (1969) noted higher airborne counts during chilling than after. Gustavsson & Borch (1989) found that a resident environmental microflora consisting mainly of *P. fluorescens* in a Swedish beef abattoir chiller contributed to carcass contamination through direct contact and by aerosols. An survey of a variety of chillers and chilled storage rooms across the food industry (including red meat and poultry processing plants) by Evans *et al*. (2004) found bacterial contamination on all evaporator cooling coils in all the 15 plants visited. In general counts were greatest in rooms with unwrapped product, and greatest in red meat and poultry plants than other food plants. While high counts of spoilage organisms were present in some cases (up to 5 $\log_{10}$ cfu cm^{-2}) very few pathogens were isolated.

Similarly, the chill room environment may be a potential reservoir for bacteria. Mafu *et al*. (1989) found a high prevalence of salmonella (12.5%) on chill room floor of a Canadian abattoir, they attributed this to the "*coming and going of workers*" between the slaughter area (25% prevalence) and this room. The floors were also found to be highly contaminated by an earlier study by Stringer *et al*. (1969). Counts were found to rise during an 18 hour chilling period.

Condensation in the chiller has also been identified as a possible source of cross-contamination. However, few studies have addressed this issue. A study by Ockerman *et al*. (1977) in the US suggested that condensate could potentially contribute significantly to the microbial load of a pork carcass but that "*condensation was not as big a problem as sanitation during the cutting operation*".

Since carcasses are exposed to the environment within chill rooms for such a long time, particularly in ageing (maturation) rooms, the sanitation of such rooms is particularly important. Some authors (Stopforth & Sofos, 2005) cite this as possibly more important than the cleaning of the slaughter and fabrication lines. Stopforth & Sofos (2005) recommend ideally using alternating chill rooms to allow enough time to thoroughly cleanse the room between unloading and reloading.

3.10. Predictive microbial growth modelling

Numerous mathematical models have been developed to predict the growth of bacteria on foods. These range from empirically-based curve fitting exercises at their simplest, to complex relationships describing the effect of environmental factors, e.g. temperature and pH.

For the growth process of bacteria at a given temperature, a simple model such as that shown below can be used (WHO, 2002):

$$N = N_0 \exp(\mu(t - \lambda))$$

Where N is the number of bacteria, N_0 the initial number of bacteria, μ the specific growth rate, t is time, and λ the lag time.

This type of model can be applied to published growth rate data such as that that can be found in the on-line ComBase database (http://www.combase.cc/) and can be used as an indication of growth of a specific bacteria at a static temperature.

A few specific models have been developed to predict the growth of bacteria on meats during chilling and chilled storage (a review of models relevant to the meat industry has been published by McDonald & Sun, 1999). A number of these use the Temperature Function Integration (TFI) technique to calculate the overall growth (Dickson *et al*., 1992; Gill *et al*., 1991a; Gill & Jones, 1992). This technique refers to the calculation of bacterial growth from product temperature histories and data relating bacterial growth rate to temperature. The numerical values are termed by some (Jones, 1993; Lovatt *et al*., 2006) as the Process Hygiene Index (PHI).

To use TFI, a time-temperature curve is used which represents that found in chilling. In general, this is measured experimentally. This curve is then integrated with a bacterial growth model. In general, the bacterial growth models have been derived by curve fitting growth data for specific bacteria under specific conditions under static temperatures. To date few of these models have been combined with dynamic heat and mass transfer models. Though recent versions of "*Food Product Modeller"* a commercial finite difference heat transfer based program developed by MIRINZ has began to incorporate these microbiological models.

A model for the growth of coliform organisms on lamb meat was derived by Smith (1985). Generation times at 40, 35, 30, 25, 20, 15 and 10°C were measured experimentally and equations derived relating generation time and lag to temperature using the method developed by Ratkowsky *et al*. (1982). The following models were derived:

Lag	Generation
$\sqrt{1/lag} = \frac{t - 3.0}{29.09}$	$\sqrt{rate} = \frac{t - 3.4}{18.58}$

Where $\sqrt{1/lag}$ expresses the lag rate (h), $\sqrt{rate}$ expresses the growth rate (as generations h^{-1}), and t the temperature (°C).

It was taken that the minimum temperature for growth was 8°C. Experimental results of generation and lag times for a strain of *S. typhimurium* treated in the same way gave longer generation and lag times at temperatures below 15°C. No reports of this model being used with the TFI technique have been located.

Dickson *et al*. (1992) calculated the following general model to describe lag and generation times as exponential-decay functions of temperature for *S. typhimurium* on beef surfaces:

$$y = D + E(e^{-Ft})$$

Where y expresses the lag rate or growth rate (as generations h^{-1}) and t the temperature (°C). D, E and F are derived parameters, thus:

Tissue	Lag	Generation
Lean	$y = 1.72 + 59.02(e^{-0.12t})$	$y = 0.188 + 7.65(e^{-0.09t})$
Fatty	$y = 1.68 + 338.27(e^{-0.167t})$	$y = 0.257 + 5.104(e^{-0.092t})$

Data was generated by incubating beef samples inoculated with *S. typhimurium* ATCC 14028 at 15, 20, 25, 30, 35 and 40°C and analysed at 2 h intervals. Data from each growth curve was fitted to the Gompertz equation. Validation studies of this model showed no significant difference between observed and predicted bacterial populations on isolated lean and fatty beef tissues cooled at either 6 or 9°Ch^{-1} (by a stepwise reduction in an incubator, 2 or 3°C every 20 min).

Three main models have been developed to describe the growth of *E. coli* and pseudomonads on the surface of meat carcasses during cooling and utilised in New Zealand for assessing carcasses cooling regimes.

Gill *et al*. (1991a) produced the following model to describe the relationship between temperature and the rate of aerobic growth of *E. coli*:

$y = 0$	when t is >47°C
$y = 2.66$	when t is between 40 and 47°C
$y = (0.027t + 0.55)^2$	when t is between 30 and 40°C
$y = (0.0513t - 0.17)^2$	when t is between 7 and 30°C
$y = 0$	when t is <7°C

Where y expresses the growth rate (as generations h^{-1}) and t the temperature (°C).

The model was developed from data for aerobic growth of a wild type strain in half-strength Brain Heart Infusion. It is an extension of that used by Lowry *et al*. (1988) in estimating *E. coli* proliferation during thawing of meat. Lowry *et al*. (1988) showed a good correlation between calculated and directly determined *E. coli* growth in bench scale studies. The average directly determined growth and the calculated growth generally differed by less than one generation. However, determined growth was significantly lower than the calculated growth when predicted growth exceeded 15 generations, since the actual flora was approaching maximum numbers by this point. Gill *et al*. (1991a) did not report any validation studies for the extended model on beef carcass surfaces, or how well the model matched measured microbial growth. This model has been used to assess the "hygienic adequacy" of air chilling (Gill *et al*., 1991a; Gill & Bryant, 1997) and spray chilling (Gill *et al*., 1991b; Jericho *et al*., 1998) of beef carcasses, air chilling of lamb carcasses (Gill & Jones, 1997) and spray chilling of pig carcasses (Gill & Jones, 1997). An investigation of two beef abattoirs by Gill & Bryant (1997) showed that *E. coli* generations calculated from temperature histories indicated that counts on carcasses would increase by about 1 log unit at abattoir A and 0.3 log units at abattoir B. However, enumeration of bacteria showed that cooling reduced mean numbers of APCs, coliforms and *E. coli* on carcasses at abattoir A by <0.5 log units. While at abattoir B APCs were reduced by about 0.5 log units and coliform and *E. coli* counts by 2 log units. The authors concluded that, while "temperature history data may be used to monitor the maintenance of standard operating procedures in such processes", microbiological data was required to properly access the hygienic effects of carcass cooling processes.

A model was also developed to describe the relationship between temperature and the rate of anaerobic growth of *E. coli* (Reichel *et al*., 1991):

$y = 0$	when t is >45°C
$y = 1.77$	when t is between 40 and 45°C
$y = (0.0163t + 0.676)^2$	when t is between 30 and 40°C

$y = (0.0433t - 0.15)^2$ when t is between 7 and 30°C

$y = 0$ when t is ≤7°C

Where y expresses the growth rate (as generations h^{-1}) and t the temperature (°C).

Gill & Jones (1992) calculated the following model to describe the relationship between temperature and the rate of growth of pseudomonads:

$y = 0$ when t is >35°C

$y = 1$ when t is between 25 and 35°C

$y = (0.033x + 0.27)^2$ when t is between -2 and 25°C

$y = 0$ when t is <-2°C

Where y expresses the growth rate (as generations h^{-1}) and t the temperature (°C).

Use of this model, as well as the aerobic *E. coli* model, on data collected during the air chilling of lamb carcasses and spray chilling of pork carcasses (Gill & Jones, 1997) predicted that *E. coli* growth would be undetectable on either types of carcass, but that APCs would increase by >1 and <1 log unit on the lamb and pork carcasses, respectively. However, counts on lamb carcasses showed that cooling reduced mean numbers of APCs, coliforms and *E. coli* on carcasses by 0.5, 1.5 and 2 log units, respectively. Though, counts on the pork carcasses behaved much as was expected from the predictions based on the temperature histories. This model was also used to assess the efficiency of storage during cross continental transport of beef sides and quarters (Gill & Phillips, 1993).

Table 15. PHI criteria for lamb and beef (number of generations)

	M	m	c	n	Reference
Beef	14	10	20%	≥20	Gill *et al*. (1991a)
Beef	14	9	20	≥20	Gill *et al*. (1991a)
Lamb	9	6	60%	≥5	Jones (1993)
Beef	19	14	60%	≥5	Jones (1996)
New Zealand regulations	14	10	80%		MAF (1997)

PHI criteria for sheep and beef (Table 15 and Table 16) have been published by a number of studies. In general, these criteria have been set by recording time-temperature curves in carcasses subjected to what have been considered to be carried out under Good Manufacturing Practice (GMP) and calculating the resulting TFI generations based on mainly the model proposed by Gill *et al*. (1991a). Lovatt *et al*. (2006) added an additional criterion based on the initial number of *E. coli* present:

Log_2(maximum acceptable number)=Log_2(initial number before cooling) + maximum allowable PHI

Table 16. PHI criteria and growth that may be allowed from the initial numbers of *E. coli*, while keeping predicted numbers below acceptable M and m values (source: Lovatt *et al*., 2006)

	M (log_2 cfu cm^{-2})	M (log_2 cfu cm^{-2})	I (log_2 cfu cm^{-2})	Maximum growth, M-I (generations)	60^{th} percentile growth, m-I (generations
Beef	13.3	6.6	1.1	12.2	5.5
Lamb	14.0	7.4	-3.7	17.7	11.1

The accuracy of the overall prediction is reliant on the accuracy of the temperature data and the accuracy of the model. Most models assume that time and temperature are the only factors limiting growth. However, there is concern that other factors are present that inhibit growth (such as surface drying; Jones, 1993) and that consequentially TFI methods over estimate microbial growth and "should not be seen as describing any "real" growth occurring at the monitored site (Jones, 1993). Jones (1993) argues that nevertheless the estimated growth will "assure the process because "actual" growth will not exceed the predicted number of generations". Similar observations have been made by Gill *et al*. (1991a, b), Armitage (1997) and Bell *et al*. (1998). Armitage (1997) reported a study comparing quantitative microbiological counts on lamb carcasses subjected to a range of ageing (conditioning) treatments with TFI predictions. TFI results showed a value of 10 generations (3 log_{10} potential growth), however the microbiological survey results showed only a slight rise (<0.5 log_{10}) in counts. Armitage (1997) put forward the following points as possible explanations for the discrepancy between the quantitative microbiological results and the TFI prediction:

1. Although the overall increase in mean APCs did not exceed one generation, at the 99.9th percentile (+3SD) the increase in APCs was larger and represented approximately 2.25 generations of growth.
2. Whilst it might be possible for *E. coli* to increase by a factor of 3 log_{10} (say -2 to +1 log_{10}) without a detectable change in the APC count, the process was not designed to select for mesophiles, i.e., the temperature parameters would be more likely to have potentially promoted growth of psychrotrophic bacteria. The APC results do not reflect any change in the composition of the flora that may have occurred during chilling.
3. The temperature history used to calculate the TFI was calculated for a PM grade of lamb. This type of lamb is moderately heavy with a heavy fat cover and represents approximately 20% of the total lambkill. Sixty percent of New Zealand lambs are lighter or have less fat cover and would, therefore, be expected to cool more rapidly than the PM grade, with a consequential reduction in the rate of bacterial proliferation.
4. TFI uses a model for *E. coli* growth that is only limited by temperature. The growth model makes no allowances for a reduction in available water that could be expected to occur as the surface of the carcass dries during cooling. Because the numerical increase in *E. coli* is dependent on moisture, the actual increase must be expected to be less than the predicted increase if any degree of surface drying takes place.
5. The temperature/time schedules that suggested a potential 3 log_{10} *E. coli* proliferation reflected physical conditions that might occur during the warmest months of the year. The microbiological survey results used represented two years production and included periods of the year where ambient temperatures were considerably less than the temperatures used in the TFI calculations.

6. The TFI calculations must be considered to be conservative in that whilst good agreement can be demonstrated between observed and predicted values in vitro, in practice the observed value is frequently less than the predicted value (Gill & Harrison, 1985). In carcass cooling studies where the surfaces of microbial concern were uncovered, the correlation between predicted and observed E. coli counts was poor and numerous counts extending through the 1 $\log_{10}$ range were observed for a given predicted value.

7. Except for temperature, all other characteristics that could be expected to favour the growth of *E. coli* are also assumed to be present, including *E. coli* having a selective advantage in the presence of competing organisms.

Despite the discrepancy between the quantitative microbiological results and the TFI results, Armitage was of the opinion that TFI was still a "rapid, cost effective method of quantifying a temperature dependent process in terms of the potential for microbial proliferation", though it "could not be relied on to validate a process outcome in the absence of quantitative microbiology".

McMeekin *et al*. (2002) criticise Gill's original model as based only on the temperature response of *E. coli* using a limited data set. They cite their own models for the growth of *E. coli* (Presser *et al*., 1997; 1998) as providing greater precision, however these more complex models require knowledge of the water activity, pH and lactate concentration:

$$\sqrt{rate} = 0.0247933 \times \sqrt{(a_w - 0.934)} \times (t - 4) \times \left(\sqrt{1 - \frac{10^{3.9}}{10^{pH}}} \times \sqrt{1 - \frac{[LAC]}{[10.7] \cdot \left(1 + 10^{pH - 3.86}\right)}} \times \sqrt{1 - \frac{[LAC]}{[823.4] \cdot \left(1 + 10^{3.86 - pH}\right)}}\right)$$

Where $\sqrt{rate}$ expresses the growth rate (as 1/generations min^{-1}), *t* the temperature (°C), LAC is the total concentration of lactic acid (mM) and a_w is the water activity.

Summer & Krist (2002) report that this model has been applied in Australia to the cooling process of hot (30-35°C) beef trim (using: lag 5 generations; pH 6.2; lactate 80 mM; a_w 0.992), as well as distribution and retail storage (using: no lag; pH 6.5; no lactic acid; a_w 0.992), and the assessment of the risk of warming of carcass meat to enable easy boneing.

This model has been further improved and refined (Ross *et al*., 2003) and expressed by Mellefont *et al*. (2003) as:

$$\sqrt{rate} = 0.2790 \times \left((t - 4.14) \times \left(1 - \exp(0.2636(t - 49.55))\right)\right) \times \sqrt{(a_w - 0.9508)} \times \sqrt{\left(1 - 10^{(3.909 - pH)}\right)} \times \sqrt{\left(1 - 10^{(pH - 8.860)}\right)} \times \sqrt{\left(1 - \left([LAC] / \left(10.433\left(1 + 10^{(pH - 3.86)}\right)\right)\right)\right)} \times \sqrt{\left(1 - \left([LAC] / \left(995.509\left(1 + 10^{(3.86 - pH)}\right)\right)\right)\right)} \pm 0.0054$$

Where $\sqrt{rate}$ expresses the growth rate (1/generation time (h)), *t* the temperature (°C), LAC is the total concentration of lactic acid (mM) and a_w is the water activity.

Cassin *et al*.'s risk assessment model on *E. coli* O157:H7 in hamburgers (Cassin *et al*., 1998) highlighted reducing bacterial growth during storage by reducing storage temperatures as the most effective hypothetical intervention for reducing food poisoning. A risk mitigation strategy based on storage temperature control was predicted to result in a 80% reduction in illness compared to 46% and 16% reductions achieved with strategies based on pre-slaughter screening and hamburger cooking, respectively. This approach could be used to assess the safety of mince produced from aged meat.

A number of models for predicting the growth of salmonella on chicken meat were critically assessed by the WHO when risk assessing salmonella in eggs and broiler chickens (WHO, 2002). They cited the model of Whiting (1993) for predicting salmonella survival at temperatures between 4 and 9°C, but overall concluded that there were no suitable models to estimate survival and die-off for salmonellas in or on chickens. For their risk assessment it was assumed that the salmonella population remains static below the growth rate. In their own risk assessment they used the growth model developed by Oscar (1999) for *S. typhimurium*:

$$LGR = \exp\left(-6.225 - \left[0.0114 \times NaCl\right] + \left[0.3234 \times Temp\right] + \left[0.002 \times \left\{NaCl \times Temp\right\}\right] - \left[0.0085 \times NaCl^2\right] - \left[0.0045 \times Temp^2\right]\right)$$

This model has a temperature range of 10 to 40°C so its usefulness for modelling growth during storage, distribution, retail and consumer handling is questionable, apart from perhaps modelling the effects of abuse of the cold-chain, particularly during transport from retail to the home. The WHO report cited the lack of suitable models for estimating bacterial growth during processing and chilling, and the lack of overall temperature data to base risk assessments on. The modelling approach used by WHO (2002) has also been used by Food Standards Australia New Zealand (2005) for the risk assessment of salmonella in chicken meat. A stochastic modelling approach was used for assessing the risk of campylobacteriosis from consumption of contaminated poultry meat. Growth of other pathogens were not modelled.

Venter *et al*. (2006) have generated a number of mathematical indices of the growth of specific bacteria on vacuum-packed beef stored at 5 and 18°C. This was carried out to investigate the proliferation of the various microorganisms at initial storage temperatures as well as to simulate conditions where a breach in the cold chain might occur. As may be expected, the results show that at these two temperatures, the various genera reacted totally differently with specific hazards originating from the predominance of certain groups. The initial microbial load played a pivotal role in the patterns of growth at both 5 and 18°C. The following relationships were generated for bacteria at 5°C:

Bacteria	Equation	Coefficient
APC	$Y = \frac{a}{\left[1 + e^{-(x - x_0)/b}\right]}$	$a = 2.5 \times 10^{8}$ $b = 1.23 \times 10^{-1}$ $x_0 = 4.98 \times 10^{0}$
E. coli	$Y = a(1 + x)^{b}$	$a = 4.7 \times 10^{2}$ $b = 3.3 \times 10^{0}$
Coliforms	$Y = y_0 + \frac{a}{\left[1 + e^{-(x - x_0)/b}\right]}$	$a = 4.87 \times 10^{7}$ $b = 7.25 \times 10^{-1}$ $x_0 = 9.89 \times 10^{0}$ $y_0 = 6.31 \times 10^{3}$

A number of studies have developed specific models for predicting the growth of pathogens (Tamplin, 2002; Tamplin *et al*., 2005; Oscar, 2006) and spoilage bacteria (Koutsoumanis *et al*., 2006) in minced meats during storage.

Tamplin and others (Tamplin, 2002; Tamplin *et al*., 2005) have compared real and predicted (using the Pathogen Modelling Program) growth of *E. coli* O157 in raw minced beef stored at different temperatures. Initial studies by Tamplin (2002) on the growth of *E. coli* O157:H7 in minced beef compared growth and predictions at 10°C. The version of PMP used (5.1) at pH 5.9 predicted a maximum population density (MPD) of 9.13 $\log_{10}$ cfu g^{-1}, an exponential growth rate (EGR) of 0.052 $\log_{10}$ cfu h^{-1}, and a lag time of 56.3 h. Similar parameter values were observed for the growth of *E. coli* O157:H7 sterilized minced beef; however, no lag phase was observed. However, on retail minced beef the mean MPD and EGR for were 5.09 and 0.019, respectively, and no lag phase was observed. Further studies (Tamplin *et al*., 2005) investigated the growth of 10 strains of *E. coli* O157:H7 on minced beef at storage temperatures. Growth occurred from 6 to 45°C, with the absence of a lag period at 6, 8 and 10°C. At 6°C the mean MPD and specific growth rate (SGR) were 4.71 $\log_{10}$ cfu g^{-1} and 0.003 ln h^{-1}, respectively. Discrepancies were found between observed growth and predictions using version 6.1 of PMP. Growth was observed at lower temperatures than those available in the PMP model. An extended Ratkowsky model (Ratkowsky *et al*., 1983) was suggested to model growth at temperatures below 10°C.

Oscar (2006) has developed a tertiary model for predicting the growth of *S. typhimurium* on minced chicken at temperatures from 10 to 40°C. This model allows for the effect of a low initial density of *S. typhimurium* and a competitive microflora.

Koutsoumanis *et al*. (2006) have developed a microbial model for the combined effect of temperature and pH on spoilage of minced beef and pork under dynamic temperature conditions. The changes in microbial flora and sensory characteristics of fresh ground meat (beef and pork) with pH values ranging from 5.34 to 6.13 were monitored at different isothermal storage temperatures (0 to 20°C) under aerobic conditions. At all conditions tested, pseudomonads were the predominant bacteria, followed by *Brochothrix thermosphacta,* while the other members of the microbial association (e.g., lactic acid bacteria and Enterobacteriaceae) remained at lower levels. The results from microbiological and sensory analysis showed that changes in pseudomonad populations followed closely sensory changes during storage and could be used as a good index for spoilage of aerobically

stored ground meat. The kinetic parameters of the spoilage bacteria were modelled by using a modified Arrhenius equation for the combined effect of temperature and pH:

$$\ln(\mu_{max}) = \ln(\mu_{ref}) - d_m \cdot (pH_{ref} - pH) - \frac{E_{Am}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)$$

$$\ln(\lambda) = \ln(\lambda_{ref}) - d_\lambda \cdot (pH_{ref} - pH) - \frac{E_{A\lambda}}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)$$

Where T is the absolute temperature (K), E_A the activation energy (kJmol^{-1}), R the universal gas constant, T_{ref} the reference temperature (T_{ref}=273K), pH_{ref} the reference pH condition (pH=5.7), μ_{ref} (h^{-1}) and λ_{ref} are the maximum specific growth rate and lag phase at reference storage conditions (T_{ref} and pH_{ref}), respectively, and d_μ and d_λ are parameters expressing the effect of pH on the maximum specific growth rate and lag phase, respectively. For the different spoilage bacteria in minced meat the parameters are the following:

Bacteria	Coefficient
Pseudomonads	$\lambda_{ref}(h) = 40.2$
	$E_{a\lambda}(kJ/mol) = 68.8$
	$d_\lambda = 1.22$
B. thermosphacta	$\lambda_{ref}(h) = 20.7$
	$E_{a\lambda}(kJ/mol) = 67.0$
	$d_\lambda = 1.73$
Lactic acid bacteria	$\lambda_{ref}(h) = 36.2$
	$E_{a\lambda}(kJ/mol) = 97.0$
	$d_\lambda =$ Not significant
Enterobacteriaceae	$\lambda_{ref}(h) = 63.5$
	$E_{a\lambda}(kJ/mol) = 93.5$
	$d_\lambda = 0.581$

The developed models were further validated under dynamic temperature conditions using different fluctuating temperatures. Graphical comparison between predicted and observed growth and the examination of the relative errors of predictions showed that the model predicted satisfactorily growth under dynamic conditions. Predicted shelf-life based on pseudomonads growth was slightly shorter than shelf life observed by sensory analysis with a mean difference of 13.1%.

3.10.1 ComBase Predictor and the Pathogen Modelling Program

ComBase Predictor and the Pathogen Modelling Program are readily available modelling programs that allow the estimation of the growth of a range of pathogenic, indicator and spoilage organisms under a range of intrinsic and extrinsic conditions. In order to assess the usefulness of the ComBase Predictor and the Pathogen Modelling Programs a series of predictions were carried out to estimate the growth of various pathogens on meats at different temperatures during the long storage times used commercially for ageing meats.

ComBase Predictor allows the estimation of the growth of a range of pathogenic, indicator and spoilage organisms at static temperatures under a range of intrinsic and extrinsic

conditions (Table 17). These models are based on the growth of these organisms in liquid media.

Table 17. Microbial growth models available on ComBase Predictor, in order of minimum growth temperature of microorganism

Microorganism	Temperature (°C)		pH	
	min.	max.	min.	max.
Clostridium perfringens	15	52	5	8
Clostridium botulinum (proteolytic)	14	40	4.7	7.2
Bacillus licheniformis	13	34	4	7.6
Bacillus subtilis	10	34	4.3	7.8
Escherichia coli	10	30	4.5	7
Staphylococcus aureus	7.5	30	4.3	7.1
Salmonella	7	30	3.9	7.4
Bacillus cereus	5	34	4.9	7.4
Clostridium botulinum (non-proteolytic)	4	30	5.1	7.5
Aeromonas hydrophila	2	25	4.6	7.5
Listeria monocytogenes/innocua	1	35	4.4	7.5
Yersinia enterocolitica	0	30	4.4	7.1
Brochothrix thermosphacta	0	30	5	5.7

Under conditions simulating meat (pH 5.8) ComBase Predictor estimates the following growths (log_{10} cfu g^{-1}) in salmonella, non-proteolytic *Cl. botulinum, L. monocytogenes* and *Y. enterocolitica* at a range of storage temperatures, aerobic conditions and times related to the recommended times for the storage of meat destined for mince, and those times used for ageing:

Salmonella	Temperature (°C)			Details
With CO_2 (0%)	7	10	12	
72 h (3d)	0.10 a	0.69	0.34	pH 5.8 / NaCl 0.5% / CO2 0%
144 h (6d)	0.49	2.54	1.37	pH 5.8 / NaCl 0.5% / CO2 0%
360 h (15d)	2.84	7.31	5.35	pH 5.8 / NaCl 0.5% / CO2 0%
504 h (21d)	4.50	7.52 c	7.23	pH 5.8 / NaCl 0.5% / CO2 0%
1008 h (42d)	7.50 b	7.52 d	7.52 e	pH 5.8 / NaCl 0.5% / CO2 0%

Saturation (h): a) 69.12, b)967.68, c)453.60, d) 463.68, e) 665.28

Salmonella	Temperature (°C)			Details
With CO_2 (30%)	7	10	12	
72 h (3d)	0.06	0.44	1.41	pH 5.8 / NaCl 0.5% / CO2 30%
144 h (6d)	0.23	1.65	3.78	pH 5.8 / NaCl 0.5% / CO2 30%
360 h (15d)	1.69	6	7.52 b	pH 5.8 / NaCl 0.5% / CO2 30%
504 h (21d)	2.9	7.43	7.52 c	pH 5.8 / NaCl 0.5% / CO2 30%
1008 h (42d)	6.79	7.52 a	7.52 d	pH 5.8 / NaCl 0.5% / CO2 30%

Saturation (h): a) 604.80, b) 345.60, c) 342.72, d) 342.52

Salmonella With CO_2 (40%)	Temperature (°C)			Details
	7	10	12	
72 h (3d)	0.05	0.35	1.21	pH 5.8 / NaCl 0.5% / CO2 40%
144 h (6d)	0.18	1.38	3.38	pH 5.8 / NaCl 0.5% / CO2 40%
360 h (15d)	1.38	5.39	7.51	pH 5.8 / NaCl 0.5% / CO2 40%
504 h (21d)	2.44	7.25	7.51	pH 5.8 / NaCl 0.5% / CO2 40%
1008 h (42d)	6.11	7.52 a	7.52 b	pH 5.8 / NaCl 0.5% / CO2 40%

Saturation (h): a) 665.28, b) 383.04

Salmonella With CO_2 (100%)	Temperature (°C)			Details
	7	10	12	
72 h (3d)	0.02 a	0.09	2.10	pH 5.8 / NaCl 0.5% / CO2 100%
144 h (6d)	0.04 b	0.34	5.07	pH 5.8 / NaCl 0.5% / CO2 100%
360 h (15d)	0.26	2.25	7.52 c	pH 5.8 / NaCl 0.5% / CO2 100%
504 h (21d)	0.57	3.68	7.52 d	pH 5.8 / NaCl 0.5% / CO2 100%
1008 h (42d)	2.24	7.38	7.52 e	pH 5.8 / NaCl 0.5% / CO2 100%

Saturation (h): a) 69.12, b) 126.72, c) 273.60, d) 272.16, e) 282.24

Clostridium botulinum (non-proteolytic)	Temperature (°C)						Details
	4	5	6	7	10	12	
72 h (3d)	0.00	0.00	0.00	0.00	0.10	1.97	pH 5.8 / NaCl 0.5% / CO2 0%
144 h (6d)	0.00	0.00	0.00	0.02	3.29	6.03	pH 5.8 / NaCl 0.5% / CO2 0%
360 h (15d)	0.00	0.14	1.28	3.49	6.04 c	6.04 f	pH 5.8 / NaCl 0.5% / CO2 0%
504 h (21d)	0.11	1.21	3.53	5.86	6.04 d	6.04 g	pH 5.8 / NaCl 0.5% / CO2 0%
1008 h (42d)	3.35	5.89	6.04 a	6.04 b	6.04 e	6.04 h	pH 5.8 / NaCl 0.5% / CO2 0%

Saturation (h): a) 826.56, b) 584.64, c) 244.80, d) 241.92, e) 241.92, f) 144, g) 151.20, h)161.28

Listeria monocytogenes/ innocua with CO2(%)	Temperature (°C)									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.01	0.02	0.03	0.05	0.08	0.14	0.26	1.27	2.53	pH 5.8 / NaCl 0.5% / CO2 0%
144 h (6d)	0.04	0.08	0.14	0.28	0.54	0.97	1.57	4.21	6.54	pH 5.8 / NaCl 0.5% / CO2 0%
360 h (15d)	0.47	0.93	1.60	2.48	3.56	4.86	6.30	7.52 d	7.52 g	pH 5.8 / NaCl 0.5% / CO2 0%
504 h (21d)	1.13	1.91	2.92	4.15	5.62	7.00	7.48	7.52 e	7.52 h	pH 5.8 / NaCl 0.5% / CO2 0%
1008 h (42d)	3.91	5.48	7.02	7.5	7.52 a	7.52 b	7.52 c	7.52 f	7.52 i	pH 5.8 / NaCl 0.5% / CO2 0%

Saturation (h): a) 866.88, b) 685.44, c) 604.80, d) 309.60, e) 312.48, f) 322.56, g) 216.00, h) 221.76, i) 221.76

Listeria monocytogenes/ innocua with CO2(30%)	Temperature (°C)									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.01	0.01	0.02	0.02	0.04	0.06	0.10	0.56	1.37	pH 5.8 / NaCl 0.5% / CO2 30%
144 h (6d)	0.02	0.04	0.06	0.11	0.21	0.40	0.73	2.57	4.41	pH 5.8 / NaCl 0.5% / CO2 30%
360 h (15d)	0.18	0.38	0.76	1.34	2.10	3.05	4.18	7.42	7.52 e	pH 5.8 / NaCl 0.5% / CO2 30%
504 h (21d)	0.48	0.95	1.64	2.53	3.62	4.93	6.37	7.52 c	7.52 f	pH 5.8 / NaCl 0.5% / CO2 30%
1008 h (42d)	2.35	3.52	4.95	6.54	7.42	7.52 a	7.52 b	7.52 d	7.52 g	pH 5.8 / NaCl 0.5% / CO2 30%

Saturation (h): a) 947.52, b) 766.08, c) 423.36, d) 423.36, e) 295.20, f) 302.40, g) 302.40

Listeria monocytogenes/ innocua with CO2(40%)	Temperature									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.01	0.01	0.01	0.02	0.03	0.05	0.08	0.41	1.07	pH 5.8 / NaCl 0.5% / CO2 40%
144 h (6d)	0.02	0.03	0.05	0.08	0.15	0.29	0.54	2.13	3.78	pH 5.8 / NaCl 0.5% / CO2 40%
360 h (15d)	0.13	0.28	0.56	1.04	1.71	2.55	3.57	7.16	7.52 d	pH 5.8 / NaCl 0.5% / CO2 40%
504 h (21d)	0.35	0.72	1.30	2.09	3.07	4.25	5.63	7.52 b	7.52 e	pH 5.8 / NaCl 0.5% / CO2 40%
1008 h (42d)	1.92	2.97	4.27	5.80	7.15	7.5	7.52 a	7.52 c	7.52 f	pH 5.8 / NaCl 0.5% / CO2 40%

Saturation (h): a) 866.88, b) 473.76, c) 483.84, d) 331.20, e) 332.64, f) 342.72

Listeria monocytogenes/ innocua with CO2(100%)	Temperature									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0	0	0.01	0.01	0.01	0.01	0.02	0.06	0.16	pH 5.8 / NaCl 0.5% / CO2 100%
144 h (6d)	0.01	0.01	0.01	0.02	0.03	0.05	0.08	0.42	1.09	pH 5.8 / NaCl 0.5% / CO2 100%
360 h (15d)	0.03	0.05	0.08	0.15	0.30	0.56	0.99	3.11	5.17	pH 5.8 / NaCl 0.5% / CO2 100%
504 h (21d)	0.06	0.10	0.20	0.40	0.77	1.31	2.01	5.02	7.19	pH 5.8 / NaCl 0.5% / CO2 100%
1008 h (42d)	0.35	0.72	1.31	2.10	3.09	4.28	5.66	7.52 a	7.52 b	pH 5.8 / NaCl 0.5% / CO2 100%

Saturation (h): a) 947.52, b) 665.28

Yersinia enterocolitica with CO2(0%)	Temperature									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.29	0.43	0.63	0.89	1.22	1.62	2.07	3.79	5.21	pH 5.8 / NaCl 0.5% / CO2 0%
144 h (6d)	1.29	1.76	2.30	2.93	3.65	4.46	5.35	7.22	7.30 s	pH 5.8 / NaCl 0.5% / CO2 0%
360 h (15d)	5.03	6.11	6.95	7.25	7.30 g	7.30 j	7.30 m	7.30 p	7.30 t	pH 5.8 / NaCl 0.5% / CO2 0%
504 h (21d)	6.93	7.26	7.30 c	7.30 e	7.30 h	7.30 k	7.30 n	7.30 q	7.30 u	pH 5.8 / NaCl 0.5% / CO2 0%
1008 h (42d)	7.30 a	7.30 b	7.30 d	7.30 f	7.30 i	7.30 l	7.30 o	7.30 r	7.30 v	pH 5.8 / NaCl 0.5% / CO2 0%

Saturation (h): a) 665.28, b) 564.48, c) 473.76, d) 483.84, e) 403.20, f) 403.20, g) 338.40, h) 342.72, i) 342.72, j) 295.20, k) 292.32, l) 302.40, m) 252.00, n) 252.00, o) 262.08, p) 165.60, q) 171.36, r) 181.44, s) 129.60, t) 129.60, u) 131.04, v) 141.12

Yersinia enterocolitica with CO2(30%)	Temperature									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.09	0.13	0.19	0.28	0.41	0.60	0.85	1.95	2.96	pH 5.8 / NaCl 0.5% / CO2 30%
144 h (6d)	0.40	0.61	0.89	1.26	1.70	2.23	2.83	5.11	6.74	pH 5.8 / NaCl 0.5% / CO2 30%
360 h (15d)	2.38	3.12	3.97	4.95	6.00	6.87	7.23	7.30 i	7.30 l	pH 5.8 / NaCl 0.5% / CO2 30%
504 h (21d)	3.83	4.85	5.95	6.88	7.24	7.30 e	7.30 g	7.30 j	7.30 m	pH 5.8 / NaCl 0.5% / CO2 30%
1008 h (42d)	7.23	7.30 a	7.30 b	7.30 c	7.30 d	7.30 f	7.30 h	7.30 k	7.30 n	pH 5.8 / NaCl 0.5% / CO2 30%

Saturation (h): a) 947.52, b) 806.40, c) 665.28, d) 564.48, e) 483.84, f) 483.84, g) 423.36, h) 423.36, i) 259.20, j) 262.08, k) 262.08, l) 201.60, m) 201.60, n) 201.60

Yersinia enterocolitica with CO2(40%)	Temperature									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.02	0.10	0.14	0.20	0.30	0.44	0.63	1.57	2.49	pH 5.8 / NaCl 0.5% / CO2 40%
144 h (6d)	0.28	0.43	0.64	0.94	1.32	1.77	2.30	4.37	6.08	pH 5.8 / NaCl 0.5% / CO2 40%
360 h (15d)	1.85	2.49	3.24	4.11	5.10	6.14	6.95	7.30 g	7.30 j	pH 5.8 / NaCl 0.5% / CO2 40%
504 h (21d)	3.09	3.98	5.01	6.11	6.97	7.26	7.30 e	7.30 h	7.30 k	pH 5.8 / NaCl 0.5% / CO2 40%
1008 h (42d)	6.86	7.25	7.30 a	7.30 b	7.30 c	7.30 d	7.30 f	7.30 i	7.30 l	pH 5.8 / NaCl 0.5% / CO2 40%

Saturation (h): a) 927.36, b) 786.24, c) 665.28, d) 564.48, e) 473.76, f) 483.84, g) 295.20, h) 302.40, i) 302.40, j) 223.20, k) 221.76, l) 221.76

Yersinia enterocolitica with CO2(80%)	Temperature									Details
	1	2	3	4	5	6	7	10	12	
72 h (3d)	0.03	0.04	0.06	0.08	0.11	0.17	0.24	0.75	1.38	pH 5.8 / NaCl 0.5% / CO2 80%
144 h (6d)	0.10	0.14	0.22	0.33	0.51	0.76	1.10	2.59	3.99	pH 5.8 / NaCl 0.5% / CO2 80%
360 h (15d)	0.69	1.06	1.53	2.11	2.80	3.60	4.54	7.15	7.30 e	pH 5.8 / NaCl 0.5% / CO2 80%
504 h (21d)	1.36	1.93	2.62	3.45	4.41	5.49	6.56	7.30 c	7.30 f	pH 5.8 / NaCl 0.5% / CO2 80%
1008 h (42d)	3.95	5.08	6.3	7.11	7.29	7.30 a	7.30 b	7.30 d	7.30 g	pH 5.8 / NaCl 0.5% / CO2 80%

Saturation (h): a) 866.88, b) 725.76, c) 433.44, d) 443.52, e) 316.80, f) 322.56, g) 322.56

The Pathogen Modelling Program (v. 7) also allows the estimation of the growth of a range of pathogenic, indicator and spoilage organisms at temperatures under a range of intrinsic and extrinsic conditions (Table 18 and Table 19). Again these models are primarily based on the growth of these organisms in liquid media.

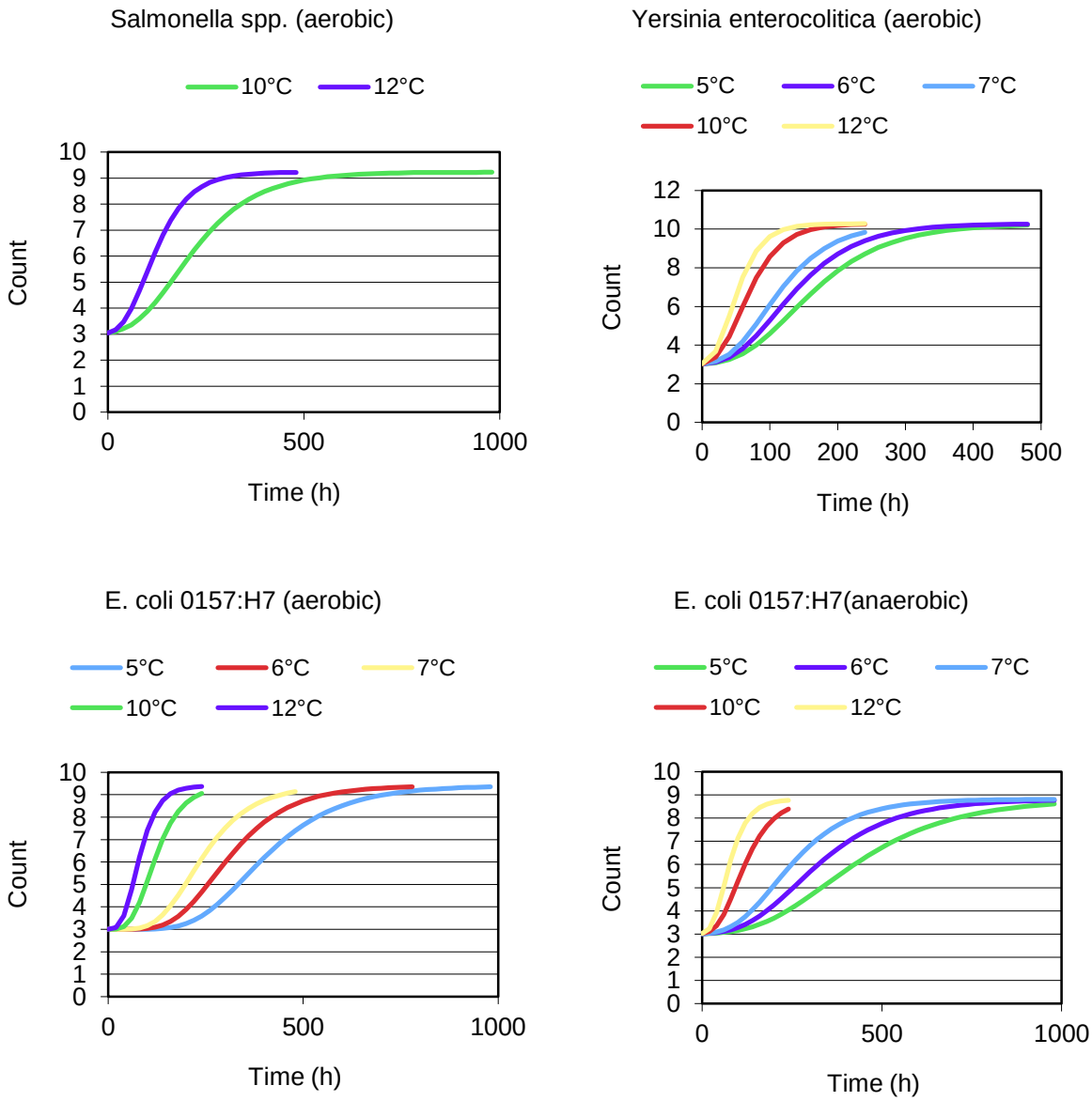
Table 18. Microbial aerobic growth models available on Pathogen Modelling Program (7), in order of minimum growth temperature of microorganism

Aerobic Growth	Temperature (°C)		pH	
Organism	min.	max.	min.	max.
Salmonella spp.	10	30	5.6	6.8
Staphylococcus aureus	10	42	4.5	9
Shigella flexneri	10	37	5	7.5
Aeromonas hydrophila	5	42	5.3	7.3
Bacillus cereus	5	42	4.7	7.5
Escherichia coli 0157:H7	5	42	4.5	8.5
Yersinia enterocolitica	5	42	4.5	8.5
Listeria monocytogenes	4	37	4.5	7.5

Table 19. Microbial anaerobic growth models available on Pathogen Modelling Program (7), in order of minimum growth temperature of microorganism

Anaerobic Growth	Temperature (°C)		pH	
Organism	min.	max.	min.	max.
Clostridium perfringens	19	37	6	6.5
Shigella flexneri	12	37	5.5	7.5
Staphylococcus aureus	12	42	5.3	9
Bacillus cereus	10	42	5	9
Aeromonas hydrophila	5	30	5.3	7.3
Escherichia coli 0157:H7	5	42	4.5	8.5
Listeria monocytogenes	4	37	4.5	8

Under conditions simulating meat (pH 5.8) the Pathogen Modelling Program estimates the following growths ($\log_{10}$ cfu g^{-1}) in *Salmonella* spp., *E. coli* O157:H7 and *Y. enterocolitica* at a range of storage temperatures and times:



Overall both ComBase Predictor and the Pathogen Modelling program (v. 7) are currently limited in their usefulness for assessing the growth/survival of pathogens in meat during ageing since they are unable to predict the survival of pathogens below their minimum growth temperature, and in many cases the minimum temperature that they are able to predict growth at is relatively high. That said, ComBase Predictor indicates that *L. monocytogenes* and *Y. enterocolitica* may potentially proliferate on meat during ageing even at low temperatures. However, few published data appear to support this hypothesis.

4. Risk analysis

In order to set critical limits for temperature-related Critical Control Points (CCPs) in the production of mince from aged meat in their HACCP plans processors and retailers need specific information on the growth of pathogens on their products under the conditions that the meat will be subjected to. As briefly noted previously there is a great amount of published data on the effect of temperatures on the growth rates of pathogenic and spoilage organisms under a range of processing conditions (Table 2 and Table 3). However, it can be a long process for a processor to extract the specific relevant data from such publications. The ComBase Database, available on-line, consists of thousands of microbial growth and survival curves of specific bacteria on meats. However currently there are gaps and conflicts in the data and it can be difficult to critically assess the data without referring back to the original source. Often it is difficult to identify whether the "meat" is in the form of a carcass, side, quarter, primal, or cut, and whether it was taken immediately from an abattoir or bought from a retailer and is referring to growth under actual/simulated commercial storage, retail display or domestic storage conditions.

Despite the wealth of data available on the survival and growth of bacteria on meat at present:

- There is little information on the growth/survival of specific pathogens on the surfaces of beef, pork, lamb and poultry carcasses under the refrigerated conditions used commercially during the ageing of meat on the bone.
- There is some information on the growth/survival of specific pathogens in packaged (vacuum, CO_2 etc.) beef, pork, lamb and poultry primals and sub-primals at the refrigerated conditions used commercially during the ageing of meat.
- There is little information on the effect of ageing time on the growth/survival of specific pathogens in beef, pork, lamb and poultry mince produced from aged meat.

Of what is known, it can be concluded with regard to the growth of specific pathogens that may be present on meats, that:

4.1. Campylobacter spp.

Campylobacter spp. will not grow at chill (<10°C) temperatures.

Even poor chilling regimes are unlikely to have much effect on the growth of *Campylobacter* spp. on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, will not increase the risk from growth of *Campylobacter* spp.

4.2. Clostridium perfringens

Cl. perfringens will not grow at chill (<10°C) temperatures.

Poor chilling regimes are unlikely to have much effect on the growth of *Cl. perfringens* on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, will not increase the risk from growth of *Cl. perfringens*.

4.3. Staphylococcus aureus

Staph. aureus will not grow in meats at temperatures below 7°C.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have a slight effect on the growth of *Staph. aureus* on the surfaces of meat carcasses during primary chilling. An

increase of storage time from slaughter to mincing, of properly chilled meat, will not increase the risk from growth of *Staph. aureus*.

4.4. *Salmonella* spp.

The likelihood of growth of salmonellae in meats at temperatures below 7°C is low, below 4°C there is little evidence of any growth occurring.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have a slight effect on the growth of *Salmonella* spp. on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, will not increase the risk from growth of salmonellas. Vacuum and MA packaging of meats present no increased risk of salmonellae growth, and indeed, may decrease the risk from inhibition by the lactic spoilage flora.

4.5. *Escherichia coli* O157:H7

E. coli O157:H7 will generally not grow in meats at temperatures below 6°C.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have a slight effect on the growth of *E. coli* O157:H7 on the surfaces of meat carcasses during primary chilling. On the basis of recent published data an increase of storage time from slaughter to mincing, of properly chilled meat, may theoretically increase the risk from growth of *E. coli* O157:H7, unless the meat is held below 6°C.

4.6. Bacillus cereus

The likelihood of growth of *B. cereus* in meats at temperatures below 5°C is low.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have a slight effect on the growth of *B. cereus* on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, may theoretically increase the risk from growth of *B. cereus*, unless the meat is held below 5°C.

4.7. *Clostridium botulinum* non-proteolytic

There is a risk of growth of *L. monocytogenes* at temperatures as low as 3°C. Below 3°C there is little evidence of any growth occurring.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have an effect on the growth of non-proteolytic *Cl. botulinum* on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, may theoretically increase the risk from growth of non-proteolytic *Cl. botulinum*, unless the meat is held below 3°C.

4.8. Listeria monocytogenes

There is a risk of growth of *L. monocytogenes* at temperatures as low as 0 to 1°C.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have an effect on the growth of *L. monocytogenes* on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, may theoretically increase the risk from growth of *L. monocytogenes*, unless the meat is held below 0°C.

4.9. Yersinia enterocolitica

There is a risk of growth of *Y. enterocolitica* at temperatures as low as 0 to 1°C.

Poor chilling regimes, particularly delayed chilled of large carcasses, may have an effect on the growth of *Y. enterocolitica* on the surfaces of meat carcasses during primary chilling. An increase of storage time from slaughter to mincing, of properly chilled meat, may theoretically increase the risk from growth of *Y. enterocolitica*, even if the meat is held below 0°C.

However, while *Y. enterocolitica* has been shown to grow at low temperatures, this growth is inhibited and large numbers (as much as 10^9) of yersinia are required to induce enteritis in healthy humans (Kleinlein & Untermann, 1990). Even if large numbers were to grow during storage these would be reduced by cooking so this pathogen is only likely to be of concern through the consumption of raw minced meat products.

Based on the known growth temperature limits of pathogenic bacteria associated with meats it is clear that there is a potential risk of the following psychrotrophic pathogens; *E. coli* H157:H7, *B. cereus*, non-proteolytic *Cl. botulinum*, *L. monocytogenes* and *Y. enterocolitica*, growing on meats during long term chilled storage and ageing under the temperatures used commercially at present.

5. Recommended Critical Controls

EU Regulations requires food business operators, including meat plant operators to implement and maintain hygiene procedures based on HACCP principles. There are 7 principles of HACCP each of which must be undertaken when a HACCP plan is being developed and implemented (Bolton *et al*., 2004):

1. Conduct a hazard analysis, i.e. prepare a list of steps in the process where significant hazards occur and describe the preventive measures.
2. Determine the critical control points (CCPs) or steps at which control can be applied and are essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.
3. Establish critical limit(s), which are the maximum or minimum value(s) to which a hazard must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of the identified food safety hazard.
4. Establish monitoring procedures to assess whether a CCP is under control and to produce an accurate record for future use in verification.
5. Establish corrective actions to be followed when a deviation occurs, which is a failure to meet a critical limit.
6. Establish verification procedures to determine if the HACCP plan is operating as intended.
7. Establish record-keeping and documentation procedures.

There are numerous publications available on the construction of HACCP plans for meat processing, many of which offer detailed models on which to base a plan. The purpose of this section is to highlight the issues and recommend specific controls that may be usefully incorporated into HACCP plans for the production of minced meat from aged meat.

On a general level it is clear from the literature that in order to ensure a safe and long storage-life it is important to:

- Produce carcasses with the lowest possible initial bacterial numbers.
- Chill carcasses as fast as possible.
- Keep an intact cold-chain throughout the entire production chain from the abattoir to the retail display cabinet.
- Maintain strict sanitary conditions throughout the whole production process, from slaughter through chilling, storage, and fabrication to packaging.
- Develop and implement a food safety management system based on the principles HACCP, with regular reviews.
- Introduce facilities, equipment and practices that should limit cross-contamination.
- Control (and record and regularly inspect) product and environmental temperatures.
- Determine and verify the storage-life and display-life of all products.

5.1. Slaughter

Few specific control measures are required for aged meats that differ from those of "unaged" meat. In both cases carcasses should be produced as hygienically with as low a microbial load as possible.

5.2. Primary chilling

The aim of the primary chiller is to reduce the temperature of the meat in a controlled manner.

At present, legislation has required that red meat carcasses be chilled to a maximum temperature of 7°C and poultry carcasses to a maximum temperature of 4°C. No time limits on achieving these times have been set. Since microbial contamination is primarily a surface phenomenon there is an argument to be made that surface temperatures are far more important than deep temperatures (Gill, 2005).

There is a body of data on the primary chilling of individual beef sides, which can be used to predict surface and deep temperatures under controlled conditions. There are less comprehensive data sets for pork and even less data on lamb chilling. There is very little published data on current commercial processes especially when it comes to accurate surface temperature determinations during primary carcass chilling.

There are very little data on the effect of current commercial chilling rates and conditions on changes in bacterial numbers during the process. In most cases no change or a small reduction (0.5 to 1 $\log_{10}$ cfu cm^2) in number of organisms on the surface has been measured. The classic work on the effect of surface drying during chilling on bacterial survival was carried out over a range of chilling rates that are far slower than current commercial practice. More recent work comparing high humidity with conventional chilling has failed to find any difference between the effect of dry and "wet" chilling regimes on bacterial numbers.

Generally:

- If the cooling rate is too slow then there will be problems with bone taint and high drip losses. At extremely slow rates toughening due to hot shortening and microbial problems due to growth of spoilage and potently pathogenic organisms can occur.
- If the initial cooling rate reduces the muscle temperature of beef or lamb to below 10°C within 10 hours of slaughter then irreversible changes will occur in the meat due to cold-shortening and the meat will remain tough even after extensive ageing and cooking. In pork cold-shortening occurs if temperatures between 3 and 5°C are reached before the onset of rigor (normally 3 to 8 hours). Chilling is seldom fast enough for cold-shortening to be a problem in chickens. There is little data on cold-shortening in turkeys.
- If the cooling rate is variable then an inconsistent product will be produced.
- The chilling system has to cater for a very wide range of carcasses types and weights.
- There should also be adequate air circulating around the carcasses. The classic reasons for poor air distribution are:
    - Incorrectly positioned fan coils.
    - Supporting structures deflecting airflow.
    - Roof structures stopping distribution.

- Rooms too long and/or to low in height for fans to be able to distribute air over load space.
- Large spaces between fan coils causing dead spots.
- Evaporator coils blocked with ice.
- Carcasses/sides hung in such a manner that they are in direct contact or produced small channels with still air in them.

- Consideration should be given to temperature rises that occur during loading, defrost cycles and in inactive chills.
- Red meat chilling is currently a batch operation. Consideration should be taken of the time it takes to fill the chiller, and that it takes longer to fill the chiller than to empty it (Gill, 2005). Thus carcasses/sides entering the empty chiller at the beginning of the day will in general receive a longer chilling time than those that enter at the end of the day (Figure 16). Often the chiller is filled up over a whole working day and then operated overnight after it is filled.

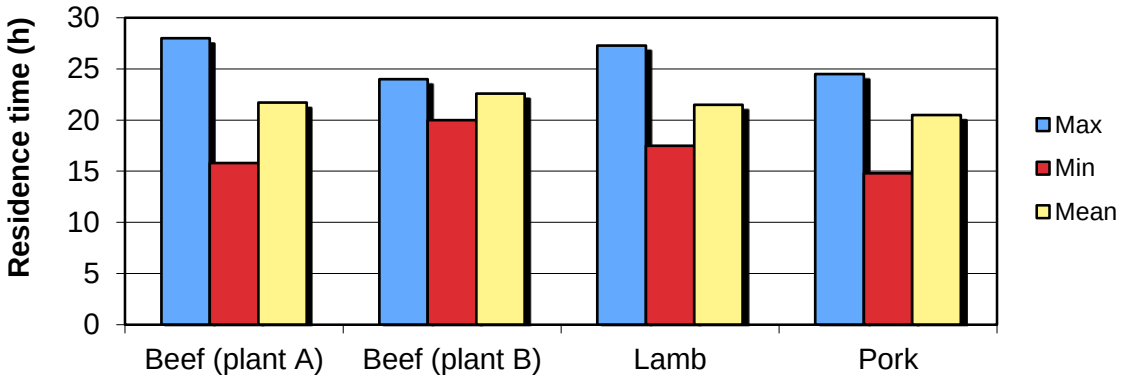


Figure 16. Residence times of carcasses in chillers during primary chilling (source: Gill, 2005)

5.2.1 Critical limits

The chilling parameters (air temperature, relative humidity, air flow, carcass grade and spacing) that achieve the greatest reduction/inhibition in microbial levels on the carcasses need to be determined so that these may be used as critical limits.

Chilling of all species should commence within 1 hour of slaughter.

Critical limits for chilling should be set to achieve:

1. A minimum rate of surface temperature decrease of 2.5°C per hour over the first 10 hours.
2. A minimum rate of surface temperature decrease of 1°C per hour over the next 10 hours.
3. An overall average temperature of 0±1°C in the meat (irrespective of species) within 48 hours of slaughter.

As a recommendation to meet all these requirements in an optimal but still cost effective manner the primary chillers should be designed and operated with the aim:

- To ensure that there is sufficient spacing around carcasses/sides to maintain an air velocity of $\geq 1.0 \pm 0.25$ ms^{-1} over all the exposed carcass/side surfaces.
- To reduce the air velocity to 0.25 ms^{-1} at the end of the chilling period or when operating in stand by mode.
- To have sufficient evaporator extraction capability to maintain the above in mid summer with the maximum load of meat envisaged.
- To have sufficient total refrigeration capacity to maintain all the required temperature performance in all the chillers when operating at maximum capacity
- To operate efficiently at minimum throughput.
- If at all possible, carcasses of similar proportions should be refrigerated together to achieve uniform results.

5.2.2 Monitoring

The temperature of the surface and the deep muscle should be checked regularly (or preferably, constantly monitored) in a set number of carcasses (e.g. 4) to give a set total (e.g. 40 carcasses) selected to represent the chilling performance in the entire chiller.

The abattoir could also establish the air chill pattern that consistently achieves the critical limits based on the temperature of the carcass surface and deep round muscle and monitor air temperature instead. Such an approach permits automation as the air temperature may be automatically monitored and controlled on a continuous basis using a System Control And Data Acquisition (SCADA) or similar system (Bolton *et al*., 2004) or using electronic or chemical Temperature Time Integrators/Indicators. This would also alert the production manager (or other designated personnel) when the critical limits are breached, automatically take immediate corrective action and produce an ongoing record of performance.

The accumulation of microbiological assessments over time may be used as a trend analysis to establish the hygiene and effect of chilling practice over time. Aerobic plate count (30°C incubation) or psychrotrophic counts may be a useful measure of the bacteriological status of carcasses after chilling or during storage (ICMSF, 1998).

5.2.3 Corrective action(s)

Carcasses that have not reached the target temperature should be chilled for an additional period until the target temperature is obtained. At present, this is the only corrective action available for chilling as a CCP.

5.3. Storage (Ageing)

At present, legislation has required that red meat carcasses be chilled to a maximum temperature of 7°C and poultry carcasses to a maximum temperature of 4°C. No time limits on achieving these times have been set. For ageing meat lower temperatures, ≤0°C, are to be recommended.

The aim of the ageing room(s) is to maintain the meat at a constant temperature, -1.5 to 0±0.1°C. It should therefore be designed on the understanding that the temperature of the meat on loading is at the required temperature.

Ageing rooms operate best if they can be rapidly loaded in one short operation, the room sealed and kept sealed until ageing is complete. The refrigeration system can then be

designed to rapidly extract the small amount of heat added during loading and then operate in a maintenance mode. During the maintenance mode it should be designed to isolate the meat from any heat ingress through the structure. A false ceiling or air sock air delivery system should be installed to produce a very constant temperature, low air movement throughout the load space. Consideration should be given to temperature rises that occur during loading and defrost cycles.

If weight loss and surface darkening is not thought to be a problem or is even desirable then the evaporator coils should be designed to maintain a relative humidity of approximately 75%. However, some of the best and most cost effective maturation rooms operate with filtered air at a relative humidity approaching 90%. In any case, it is very desirable that the evaporator coil and the structure is designed to be easily and completely cleaned. Studies have shown that dirty coils support bacteria and can act as a source of contamination to the ageing meat.

On-line monitoring of both ageing and bacterial growth would be a useful tool for monitoring and controlling the ageing process. A system such as that described by Yano *et al*. (1996) shows promise. They used a biosensor composed of a putrescine oxidase immobilized electrode (which measured putrescine and cadaverine which are produced by bacteria) and a xanthine oxidase immobilized electrode (which measures hypoxanthine and xanthine which accumulate in meat with aging) as detectors. This system was shown to be useful for quality control of beef ageing at 5 and 10°C, but not at 0°C.

5.3.1 Critical limits

Bone-in red meat and poultry (carcasses or parts of the carcass) should be the maintained at a temperature of between -1.5°C to 0°C ±0.5°C during ageing.

5.3.2 Monitoring

Temperature monitoring of the air temperature and / or product temperature should be continuous, or at least once every hour, as appropriate.

As for chilling the abattoir could also establish the air chill pattern that consistently achieves the critical limits based on the temperature of the carcass surface and deep round muscle and monitor air temperature instead. Such an approach permits automation as the air temperature may be automatically monitored and controlled on a continuous basis using a System Control And Data Acquisition (SCADA) or similar system (Bolton *et al*., 2004) or using electronic or chemical Temperature Time Integrators/Indicators. This would also alert the production manager (or other designated personnel) when the critical limits are breached, automatically take immediate corrective action and produce an ongoing record of performance.

The accumulation of microbiological assessments over time may be used as a trend analysis to establish the hygiene and effect of storage practice over time. Aerobic plate count (30°C incubation) or psychrotrophic counts may be a useful measure of the bacteriological status of carcasses after chilling or during storage (ICMSF, 1998).

5.3.3 Corrective action(s)

Carcasses/sides/quarters may be re-chilled if the core temperature has not risen above 7°C. If the former is obtained for an extended period of time (more than 6 hours), the carcass/side/quarter should be processed immediately and used for cooked product only, or discarded.

5.4. Cutting and mincing

Cutting/mincing facilities should be operated at ≤12°C (ideally 10°C). Temperatures lower than 10°C can make working conditions for staff uncomfortable. Localised environmental control should be considered to keep the meat isolated from a higher temperature environment. While these environmental temperatures still permit the growth of *L. monocytogenes*, growth is retarded. The growth of salmonella at 10°C will be inhibited providing product temperatures do not remain at this temperature for more than 12 hours.

Since bacteria are primarily present on meat surfaces manufactures should consider the ratio of trimmings containing surfaces that are minced. Adipose surfaces favour the growth of bacteria in comparison with muscle surfaces and thus the proportion of subcutaneous fat may effect overall bacterial levels.

The cleanliness of mincing and cutting equipment is particularly important to prevent cross-contamination.

5.4.1 Critical limits

The temperature history of the meat between cutting/mincing and cooling back to the storage temperature should be known and controlled.

During mincing the internal temperature of the meat should be kept at 7°C or less (if the mincing takes up to 1 hour) or at 4°C or less (if the mincing takes more than 1 hour).

The temperature of the processing area (boning hall, cutting area, minced meat preparation area, etc.) should be maintained at 12°C or less with an air velocity of $<0.5\ ms^{-1}$.

Chilled minced red or poultry meat and minced meat preparations should be stored at 0±0.5°C or lower.

5.4.2 Monitoring

Temperature monitoring of the air temperature and / or product temperature should be continuous or at least twice per day as appropriate.

The temperature history of the meat between cutting/mincing and cooling back to the storage temperature should be known.

The cleanliness of mincing and cutting equipment and the effectiveness of cleaning should be regularly assessed. This may be carried out either using traditional microbiological methods or using non-microbiological rapid methods.

The accumulation of microbiological assessments over time may be used as a trend analysis to establish the hygiene and effect of storage practice over time. Aerobic plate count (30°C incubation) may be a useful shelf-life test for minced meat (ICMSF, 1998). *E. coli* has been shown to be a useful indicator of plant hygiene (ICMSF, 1998). The ICMSF (1998) recommends that when it is known that the incidence of a pathogen is <1% in samples tested it is not advisable to carry out routine tests for such pathogens. For vacuum-packaged aged meats the fraction of Enterobacteriaceae in the bacterial population may be a useful indicator of plant sanitation and product storage-life (Holley *et al*., 2004).

5.4.3 Corrective action(s)

Meat, either raw material or finished material, may be re-chilled if their temperature has not risen above 10°C. If the former is obtained for an extended period of time (more than 6 hours), the meat should be used for cooked product only or discarded.

5.5. Storage and distribution

Environmental and storage temperatures must be maintained, controlled and monitored at all times. Storage areas should be designed to maintain the correct product temperature. Raw materials should be stored separately from finished products. Practices that involve the shutting down of power required for environmental and storage temperature maintenance should not be implemented under any circumstances. Contingency plans should be available to ensure the continued safety of products in the event of power failures.

It is particularly important that meat is at the correct temperature before loading since the refrigeration systems used in most transport containers are not designed to extract heat from the load but to maintain the temperature of the load. In the large containers used for long distance transportation meat temperature can be kept within ±0.5°C of the set point.

5.5.1 Critical limits

Critical limits for the storage and distribution of minced meat from aged meat should be the maintenance of a temperature of -1.5 to 0±0.1°C for both red meat and poultry mince.

5.5.2 Monitoring

Temperature monitoring of the air temperature and / or product temperature should be continuous, or at least once every hour, as appropriate.

Time-Temperature Integrators/Indicators on the packaging can be used to indicate adequate temperatures during storage and distribution, or abuse of the chill chain.

The accumulation of microbiological assessments over time may be used as a trend analysis to establish the hygiene and effect of storage practice over time. Aerobic plate count (30°C incubation) or psychrotrophic counts may be a useful measure of the bacteriological status of the meat during storage and distribution (ICMSF, 1998).

5.5.3 Corrective action(s)

Meat may be rechilled if its temperature has not risen above 7°C. If the former is obtained for an extended period of time (more than 6 hours), the meat should be used for cooked product only or discarded.

5.6. Retail

Products should not be stacked higher than the maximum level indicated in display cases, or in front of air ducts, or too close to heat generating lamps.

In case of breakdown of the refrigeration unit of the display case, the products should be moved to another case or to a cold room (Codex Alimentarius, 1999). If the breakdown of the refrigeration unit of the display case takes place when the establishment is closed, temperature of the products should be checked. If acceptable, the products should be moved to a suitable area; if not, they should be removed from the case, not offered for sale, and destroyed if necessary.

5.6.1 Critical limits

Critical limits for the retail display of minced meat from aged meat should be the maintenance of a temperature of -1.5 to 0±0.1°C for both red meat and poultry mince.

5.6.2 Monitoring

Temperature monitoring of the air temperature and / or product temperature should be continuous, or at least once every hour, as appropriate.

Time-Temperature Integrators/Indicators on the packaging can be used to indicate adequate temperatures during storage and distribution, or abuse of the chill chain.

The accumulation of microbiological assessments over time may be used as a trend analysis to establish the hygiene and effect of storage practice over time. Aerobic plate count (30°C incubation) or psychrotrophic counts may be a useful measure of the bacteriological status of the meat during retail display (ICMSF, 1998).

5.6.3 Corrective action(s)

Meat may be rechilled if their temperature has not risen above 4°C (in the case of poultry) or 7°C (in the case of red meat). If the former is obtained for an extended period of time (more than 6 hours), the meat should be discarded.

6. Conclusions

The aim of this review was to briefly critically look at the available scientific evidence that would support the newly imposed hygiene legislation regarding the regulatory limit on the age restriction of meat at time of mincing.

This new hygiene legislation requires that minced meat must be prepared: from poultry within no more than three days of their slaughter; in the case of animals other than poultry, within no more than six days of their slaughter; or within no more than 15 days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.

Overall, this review has found little scientific data to support the current restrictions on the age of the meat used to make minced meat. The main points to emerge from this brief review are that:

1. No clear scientific literature has been located to support the restrictions on the time between slaughter and production of minced meat.
2. No specific scientific publications have been located that look at the safety and quality of mince produced from cuts and carcasses that have been stored for different periods of time post-slaughter.
3. There are a small number of publications on the quality of steaks and chops produced from meat that has been stored for up to 35 days and 80 days for beef, 40 days for lamb and 63 days for pork. As would be expected they all show that bacterial numbers are higher on meat produced from older meat. However, an acceptable display-life is often achieved with cuts produced from the older meat. Few publications have been found on poultry.
4. No publications have been located that show that the safety (i.e. pathogen levels) of mince produced from older meat is compromised, or visa versa.
5. There is a surprising lack of data on the storage-life of chilled meat carcasses and bone-in-cuts. The classic studies indicate much shorter storage lives than current industrial practice as indicated in IIR tables etc. There is little data on the growth of pathogens on meat carcasses during ageing.
6. More data is available on the storage-life of some vacuum-packaged primal meat, however this covers a limited range of storage conditions. There is little data on the growth of pathogens on meat carcasses during ageing.
7. As would be expected, the data that does exist shows that initial bacterial numbers, and storage atmosphere and temperature are the main factors governing storage life. pH and RH also influence storage life, and in one publication the rate of initial chilling is claimed to make changes of up to 50% in storage life.
8. Predicting microbial growth from surface temperature data has potential, however current models tend to predict growth during the chilling process while measurements show either no growth or death. The discrepancy is most likely due to a combination of poor surface temperature measurement and other factors, such as a_w or inhibitory interaction of competing microflora, not being taken into account in the models. Most models predict no growth of bacteria (particularly pathogens) at low storage temperatures and are unable to predict survival at low temperatures, so are unable to predict the role of ageing times on pathogenic growth. They can currently be used to predict the risk of bacterial growth during cutting/mincing operations at elevated temperatures, and the effect of break-downs in the chill-chain.

9. Overall, data on the growth of psychrotrophic pathogens would indicate that there is theoretically a greater risk of psychrotrophic pathogens proliferating in meat held for a longer time at a temperature above the minimum for pathogenic growth than in meat stored for a short time. Since mincing is known to distribute bacteria throughout the meat, and mince is acknowledged to present more of a risk to public health than cuts and steaks, it stands to reason that theoretically mince from aged meat has a higher risk than that from non-aged meat. However, it can also be said that any aged meat must on this basis present more of a risk than unaged meat. It is not clear from the literature how much of an additional risk ageing presents. Some authors (Dykes *et al*., 2001) imply that that the long period of storage, of particularly *E. coli* O157, in a non-growing state would result in "an excessive recovery period in these cells before growth would occur".

It is interesting to note that the Richmond report in 1991 was concerned with draft EC legislation at the time laying down hygiene requirements for the production and placing on the market of minced meat. The purpose of the proposal was to harmonise rules for the sale of mince in the Single Market. However, since the proposed rules took account of the fact that minced meat is sometimes consumed raw in some member states, in their view it set requirements that were unnecessarily restrictive for minced meat that was to be cooked before consumption. It could be said that the same is true of the newly applied legislation.

As a result of this brief review a range of critical controls have been suggested based on available data. However, we would recommend that research is funded to specifically look at the influence of post-slaughter storage times and conditions on the safety and quality of mince produced. Initial studies should concentrate on the production of high quality mince from meat aged on the bone and vacuum-packaged for extended periods. Work is also required on poultry to fill the total gap in published scientific literature in this area.

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8. References

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9. Appendix: Aged mince

A quick trawl of the web shows that opinions are changing regarding the quality of the meat used to produce mince. A number of specialist suppliers of meat in the US make a point of stating that their beef mince is made from matured meat:

"*Even Konove's ground beef, aged along with the steaks and roasts, has full, rounded flavor. It's beef as we might remember from childhood*."

(http://www.boston.com/ae/food/articles/2005/07/06/local_beef_hold_the_anxiety/)

"*Aged Ground Beef: Our ground beef is uniquely lean and full of flavor, a result of our using the same top grade of aged meat for all cuts, including ground beef… Every cut of beef that we sell has been aged for 14-28 days*."

River Rock Farm, Brimfield, Massachusetts, USA

(http://www.riverrockfarm.com)

"*Ground dry-aged beef with unmatchable flavor and tenderness!!*"

Wilson Beef Farms, Upstate New York, USA

(http://www.wilsonbeeffarms.com/index.php?catID=Ground%20Beef)

"*Our great tasting ground beef is 88% lean and source verified - made from the trimmings of our dry-aged beef*."

McLean Beef, Benedict, Nebraska, US

(http://www.mcleanbeef.com/retail_beef.htm)

"*Certified Organic Ground Beef: Our delicious ground beef is made from dry aged steak and roast ends from our premium beef cattle--the same beeves you would buy a steak from. We do not use old or 'cull' cows or bulls for our ground; nor do we add any non-meat ingredients such as organs or water. 90-93% lean. We recently improved it even further by grinding twice with a special plate making it finer and more even grained*."

Alderspring Ranch, Idaho, USA

(http://www.alderspring.com/store/page3.html)

"*Ground Beef: This is the part of our business that really turns peoples heads. Our ground beef is twice hand ground from only the highest quality dry-aged trimmings. Customers remark daily about our smokey old fashioned ground beef which tastes like ground steak. Ground Beef is offered in several different lean-to-fat grind mixtures (88%, 85%, 80%)*."

Prather Ranch, California, USA

(http://www.pratherranch.com/glossary.php)

"*Ground Beef/ Ground Sirloin/ Ground Round: This is part of our business that really turns peoples' heads. Our Big Sky Ground Beef is twice hand ground from our Choice quality dry-aged trimmings and customers remark how it tastes like ground steak. Big Sky Ground Beef built our business due to the fact that it has minimal shrink and product loss during cooking. Our target is 90% lean and you will see the difference when compared to most consumer beef*."

Big Sky L Ranch, WI, USA

(http://www.naturalbeefatbigsky.com/glossary.shtml)

A number of companies in the UK also imply that their mince is made from matured meat:

"*Beef Mince (Frozen): All of our beef is supplied by the Cambrian Hills Company in Wales. Their traditionally reared Welsh beef has all the taste and texture of beef as it should be - especially their native Welsh Black. All beef is traditionally hung and matured for a full 3 weeks to guarantee it melts in your mouth. All our meat adheres to the strict requirements of the Soil Association, an endorsement of not only quality but of the highest standard of animal welfare and husbandry*."

Eatorganic, Rotherfield Peppard, UK
(http://www.eatorganic.co.uk/default.asp?id=338&ver=1)

"*Fresh beef mince: 2 weeks matured for a wonderful full flavour and tenderness*."

Woodhouse Farm, UK
(http://www.woodhousefarm.co.uk/product_details.asp?pid=38)

"*Beef Mince: Selected cuts of our matured, pasture reared beef ground to create a lean, versatile base for many delicious meals*."

The Real Meat Company, UK
(http://www.realmeat.co.uk/acatalog/Beef_Ingredients.html#a1310000)

"*Organic Lean Beef Mince: Product Code: DO37: Producer: Daylesford Organic: Daylesford's organic beef is from a closed, pedigree herd of Aberdeen Angus reared on the lush clover-rich pastures of the Staffordshire estate. The beef is hung for up to four weeks resulting in unbeatable quality and maximum flavour. All of our meat can be frozen*."

Provenance Fine Foods, UK
(http://www.provenancefinefoods.co.uk/shop.php?categories_id=20#DO37)

"*Sainsbury's Traditional Beef Mince, Taste the Difference: Matured beef delivering a fuller flavour*."

Sainsbury's, UK
(http://www.sainsburystoyou.com/webconnect/index.jsp?bmUID=1163497798491)

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