LONG-TERM STORAGE OF CHILLED FRESH MEATS

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INTRODUCTION

Fresh meats stored in air spoil rapidly due to the growth of Gram-negative bacteria which cause putrefaction. Even with a low initial population of contaminating organisms, the storage life of fresh meats at 0°C is only about two weeks. This is inadequate to allow the export of chilled fresh meats from Australia and New Zealand, which have large quantities of beef and lamb available. As a result, significant components of meat research in these countries have been programs aimed at developing methods to maximise the storage life of these products.

The storage life of fresh meats may be extended by several methods including vacuum-packaging, storage in modified gas atmospheres and irradiation. These procedures may be combined with decontamination of the meat to reduce the initial bacterial contamination. However a number of procedures of potential value (eg. irradiation) cannot at present be used in commercial practice, because their use is either not legal in a number of countries, or results in the meat being considered as processed. This paper summarises the current situation with respect to vacuum- packaging and modified atmosphere storage of primal cuts of meats. These are the two methods currently available to processors to maximise the storage life of beef, pork and lamb. It is not possible to discuss these techniques in detail here. Background information may be found in the articles of Ingram and Simonsen (1980), Dainty et al. (1979, 1983), Egan (1984) and Gill (1986a).

Spoilage of fresh meats is usually caused by bacterial growth, but under conditions of prolonged storage chemical changes may be a factor. An atypical flavour develops in beef stored vacuum-packaged at 0°C even in the absence of a significant population of bacteria (so called "sterile meat"). The rate of spoilage depends upon the permeability of the packaging film but even in film of very low permeability (i.e. in the absence of rancidity), the changes are significant after 14-16 weeks. The atypical flavours which develop are described by panellists as bitter and liver-like.

Vacuum-packaged meat is known to become more tender during storage and the chemical and biochemical changes occurring during the ageing process possibly contribute to the development of the atypical flavour. Further studies to clarify this are needed. Experiments of this type demonstrate that there is a limit to the storage life of chilled meat even in the absence of bacteria.

Assessment of spoilage

There are a number of factors to be considered in deciding whether meat which has been stored vacuum-packaged or in a modified gas atmosphere is spoiled. The first impact on the customer is when cartons are opened. In our experience, downgrading or rejection is usually based upon a visual assessment of the meat and any unusual colour or change in appearance may result in rejection.

In the absence of visual spoilage, packs are opened and checked for the presence of unusual, atypical or "off" odours. In the case of vacuum-packaged meat, any odour needs to be distinguished from the "confinement" odour or "vacuum-pack" odour which is characteristic of this product but which is not an indication of bacterial spoilage. In laboratory studies, storage life is usually determined by cooking the meat under controlled conditions and using a taste panel to evaluate its acceptability.

It is important to note that, especially in the case of product being exported, the definition of spoilage may depend upon the market. In commercial situations, appearance is still the major criterion used.

Effects of environmental conditions on bacterial spoilage

Under conditions of high relative humidity *Pseudomonas* outgrow other types of bacteria on fresh meats stored in air and cause putrefactive spoilage. Their growth is inhibited by 20% carbon dioxide and this is probably the main reason for their failure to grow on vacuum-packaged meats or meats stored in carbon dioxide. Low concentrations of oxygen (%) also contribute to inhibition (Shaw and Roncaroli 1982).

Brochothrix thermosphacta is resistant to inhibition by carbon dioxide and growth can still occur in high concentrations. Oxygen availability affects growth and this is probably the most important factor controlling growth in situations where the concentration of carbon dioxide is higher than about 40%. This organism does not grow on meat under anaerobic conditions if the meat pH is 5.8 or lower. This inhibition is caused by the lactate present in the muscle. Not only does the concentration of lactate in the meat increase as the pH falls but, a greater proportion of it is in the undissociated form, which is the active inhibitor. Further this bacterium is more sensitive to inhibition by lactate under anaerobic conditions.

Thus the effect of oxygen limitation on the growth of *B.thermosphacta* is largely indirect, confirmed by the observation that it can grow to a high population (10^7/cm) on vacuum-packaged meat of high pH, even when the meat is in packs made of film of extremely low gas permeability. *B.thermosphacta* causes chemical and dairy odours when oxygen is present but souring in its absence.

Aeromonas spp. and *Alteromonas hydrophila* are unable to compete with the faster growing *Pseudomonas* if oxygen is readily available. When only traces of oxygen are present they may grow and become significant in spoilage provided the pH is high. They cause the putrefactive spoilage and greening of vacuum-packaged meat of high pH, but fail to grow when meat pH is normal.

Enterobacteriaceae are inhibited by carbon dioxide but probably only in quite high concentrations. Oxygen limitation almost certainly contributes to the inhibition of their growth on vacuum-packaged meats. Again, whilst carbon dioxide may have some influence on their growth,

TABLE 1: The effect of oxygen availability and pH on the growth on lean fresh meat of the major types of meat spoilage bacteria at 0-5°C.

	pH 5.5-5.7		pH 6.0 or higher	
	Oxygen	No oxygen	Oxygen	No oxygen
Pseudomonas	+	-	+	-
Enterobacteriaceae	+	-[1]	+	+
Brochothrix thermosphacta	+	-[1]	+	+
Lactic acid bacteria	+	+	+	+
Aeromonas	-	-	+	+
Alteromonas putrefaciens	-	-	+	+

1 Some growth may occur, especially at intermediate pH values (5.8-5.9) and at 5°C, but this is usually not sufficient to contribute significantly to spoilage.

it may not be present in high enough concentrations to cause significant inhibition. The extent of growth of fermentative Gram-negative bacteria on vacuum-packaged meat is also affected by muscle pH, greater populations being reached in meat of high pH; lactate may once again be involved. Storage at 4-5°C rather than 0°C, particularly favours the growth of this group of organisms. They cause putrefactive spoilage and may contribute to greening.

Lactic acid bacteria grow readily in the absence of oxygen, are very resistant to inhibition by carbon dioxide and grow over the range of pH values which occur in meat. Their presence does cause spoilage, usually by souring, but this occurs much later in storage than spoilage caused by equivalent populations of the other types of bacteria discussed above.

The effect of oxygen availability and pH on the growth of the major types of meat spoilage bacteria on lean fresh meat is summarised in Table 1.

Strategies used in packaging fresh meats

The properties of the various groups of spoilage organisms suggest strategies to be used in attempting to preserve meats by the use of packaging systems. The practical end result to be aimed at in all cases is the creation of conditions under which lactic acid bacteria become the only group which grow readily. The presence of a flora dominated by lactic acid bacteria can normally be expected to signify a maximum storage life for packaged fresh meats.

Vacuum-packaging

Vacuum-packaging is a form of modified atmosphere storage, but one in which the composition of the gas atmosphere is neither known nor controlled. When fresh meat is vacuum-packaged in bags made of film of low gas permeability, the oxygen is consumed and carbon dioxide is produced (Enfors and Molin 1984). Providing the packaging has been correctly done, there is very little headspace within packs which contain boneless primal cuts. This makes an accurate analysis of the headspace gas difficult, but it will typically contain 20-40% of carbon dioxide, less than 1% oxygen with the remainder being nitrogen. To achieve a maximum storage life the following criteria must be met:

- the meat must have been produced using good manufacturing practice and packaged immediately after boning out from the carcass;
- the packaging film used must have a low permeability to gases (should not exceed about 30 ml/m/24 h/atm measured at 25°C, and be even lower if possible);
- there must be good control of temperature throughout the storage period.

When these criteria are met the storage life obtained will then depend upon:

- whether the meat is beef, pork or lamb;
- the surface to volume ratio;
- the temperature of storage; and
- the pH of the lean muscle, or if it is not possible to measure this, the average pH in the environment in which bacterial growth occurs.

These factors are discussed in more detail below.

Microbiology of vacuum-packaged meat

The use of packaging films of low oxygen permeability, combined with the oxygen consuming capacity of the muscle tissue means that there is very little oxygen available for microbial growth. Yeasts and moulds are unable to grow and pseudomonads grow very little. Under these conditions the flora consists of bacteria which grow readily in the absence of oxygen; lactic acid bacteria, *Brochothrix thermosphacta*, *Alteromonas putrefaciens*, Enterobacteriaceae and *Aeromonas* spp. (Dainty et al. 1979; Gill and Newton 1978; Egan 1984).

The final composition of the flora of lean muscle surfaces and in exudate (weep) from them will depend upon pH. Fat and fell surfaces may be bathed in a thin film of weep which will be a factor in determining the types of organisms which grow there. Certainly the bacteria mentioned above have the capacity to grow on the fat surfaces of vacuum-packaged meats, however the significance in spoilage of bacterial growth on fat surfaces has been little studied.

If muscle pH is in the range considered as normal (5.4-5.8), the flora which develops is dominated by lactic acid bacteria. These organisms may grow to the virtual exclusion of all others and may reach populations of 2.5 x 10^7/cm with populations of other organisms limited to 10^4-10^5/cm. If muscle pH is higher than 5.8, there is increased growth of *Brochothrix thermosphacta* and the Gram-negative bacteria mentioned above. If lean muscle pH values are 6.2-6.5 these organisms may reach populations of 10^6-10^7/cm and contribute to spoilage. This is particularly the case when the storage temperature is 4-5°C rather than 0°C (Table 3).

Vacuum-packaged beef

In Australia the incidence of high pH (dark-cutting) beef is sufficiently low that it is readily excluded from export vacuum-packaging. It is routinely identified in export boning rooms on the basis of colour and a simple test of pH. When it is packaged it is not exported but is sold for local catering use, in which case it is used within two or three weeks.

TABLE 2: The storage life at 0°C of vacuum-packaged primal cuts of beef, lamb and pork

	Muscle pH	Storage life[1] (weeks)	Spoilage defect
Beef	5.5 - 5.8	10-12	Flavour (souring)
Pork	5.5 - 5.8	6	Flavour
	6.0 - 6.3	4- 6	Colour (greening)
Lamb	N/A[2]	6- 8	Colour, appearance of fat

1 Vacuum-packaged using films with oxygen permeabilities less than 50 ml/m²/24 h/atm (measured at 25°C and 98% RH)

2 Not applicable

TABLE 3: The effect of muscle pH and storage temperature on the microflora of vacuum-packaged* pork

pH	Temperature (0°C)	Total viable count∝	Lactic acid bacteria	B. thermosphacta	Gram-negative bacteria
5.4-5.8	0	7.9	7.9	4.2	4.8
	5	8.0	7.9	4.5	6.2
6.1-6.7	0	8.7	8.5	7.5	6.7
	5	8.6	7.9	7.2	8.0

* Packaging film oxygen permeability 20-30 ml/m²/24 h/atm measured at 25°C and 98% RH.

∝ Log_{10} number/cm of typical maximum populations present on the lean surface of meat stored for up to 6 weeks.

The storage life of vacuum-packaged primal cuts of beef of normal pH stored at 0°C is up to twelve weeks under commercial conditions (Newton and Rigg 1979; Egan 1983). Its acceptability is at a maximum after about four to eight weeks storage. After this time flavour changes may start to be detected. Spoilage is caused by the development of a flavour described by panellists as cheesy, sour and acid. These changes are attributed to the accumulation of end products resulting from the growth of lactic acid bacteria, together with endogenous chemical changes in the muscle.

The large size of primal cuts of beef (2-7 kg), and their relatively small surface to volume ratio compared to other packaged meats, is another factor contributing to the long storage life of this product.

Table 2 lists the storage life at 0°C of vacuum-packaged primal cuts of beef, lamb and pork. Holding the meat at higher temperatures reduces storage life. There are few detailed studies of the storage of primal cuts at 5°C, but the available information suggests that it would be wise to assume a storage life of only about half that obtained at 0°C. Storage at about -1°C is recommended whenever possible. Again there have been few studies, but an increase in storage life of up to about 50% should be achievable.

Vacuum-packaged pork

In Australia, the incidence of high pH (DFD) pork is sufficiently high, to make the use of pH estimation as a basis for the selection of meat for vacuum-packaging, impractical under commercial conditions. The microbiology of vacuum-packaged pork has been studied in our Laboratory (Egan and Shay 1984). Pork of high pH usually spoils because of the growth of Gram-negative bacteria, particularly when stored at 5°C (Table 3). *Alteromonas putrefaciens* and *Aeromonas* sp. may grow to populations in excess of 10^6/cm and cause greening of the weep and fat surfaces. Putrefaction is also a cause of spoilage and the microbiology and spoilage of high pH pork thus parallels that of beef (Bem, Hechelmann and Leistner 1976; Taylor and Shaw 1977; Erichsen, Molin and Möller 1981).

Treatment of pork of high pH (6.2-6.5) with a dilute solution of acetic acid (immersion for 10 s in a 1.5% v/v solution of acetic acid at 55°C) prior to vacuum-packaging results in an extension of storage life to six weeks at 0°C. Preliminary studies have indicated that a similar treatment using lactic acid (2% v/v solution, 10 s at 55°C) does not appear to be quite as effective (Shay et al. 1988). Unfortunately neither acid is fully effective if the meat pH is above about 6.5 or if the meat is stored at 5°C. Under these circumstances, the inhibition of the growth of the Gram-negative bacteria is not sufficiently prolonged to result in a worthwhile extension to storage life.

The bacterial flora of vacuum-packaged pork of *normal* pH stored at 0°C is very similar to that of beef, and lactic acid bacteria are dominant. The reason for the shorter storage life is not clear, but it seems unlikely that the difference is due only to differences in surface to volume ratios. It is possible that the endogenous chemical and biochemical changes that occur during storage are more rapid in the case of pork.

Vacuum-packaged lamb

(i) **Primal cuts.** Because of the multiplicity of muscles in the cuts commonly packaged, it is not meaningful to talk about muscle pH for packaged lamb. The exudate from lamb commonly has a pH in the range 5.9-6.1. This, together with the more extensive area of fat surface that is usually present, results in a less restrictive environment for bacterial growth than is the case with beef. Vacuum-packaged cuts of lamb have a storage life of about six to eight weeks at 0°C and eight weeks may be guaranteed by storage at -1°C (Shaw et al. 1980; Gill 1986b).

(ii) **Telescoped carcasses.** Lamb carcasses may be reduced in size by a process known as telescoping. In this process the hind legs are folded up into the thoracic cavity and by this means there is a considerable volume reduction with significant savings in transport costs. Carcasses packaged in this way are exported from Australia. However the packs still contain a considerable central cavity and there are areas where the film does not make contact with the surface of the product, i.e. there is still a considerable void volume in the pack. Because the carcass has little cut lean surface exposed (the surface is predominantly fat and fell tissue) there is no significant respiratory activity and it is difficult to remove all the oxygen (1-3% is commonly detected). The presence of a considerable volume of exudate also contributes to microbiological problems.

Carcasses packaged in this manner have a storage life of about six weeks at 0°C. This may be extended to about

TABLE 4: Defects in the appearance of vacuum-packaged fresh meats[1]

Defect	Symptom	Cause	Comments and/or prevention
Greening	Green discolouration of weep, fat surfaces	Production of H_2S by bacteria resulting in formation of sulphmyoglobin	
(a) high pH meat		Gram-negative bacteria <u>Alteromonas</u>, <u>Aeromonas</u> etc.	Do not vacuum pack meat of pH>5.9. Use films of low permeability[2].
(b) normal pH meat		Lactobacilli	Should only occur late in the storage period. Use films of low permeability[2].
Browning	Brown discolouration of lean surfaces and weep	Excess O_2 in pack resulting in metmyoglobin formation.	Use films of low permeability[2]. Check quality control procedures - seals, degree of evacuation, film quality.
Loose packs	-	Poor seals, punctures in films.	Check quality control procedures.
Blown packs	-	Excessive growth of bacteria which produce CO_2 (Enterobacteriaceae)	Indicative of temperature abuse and rare if temperature control is good.
Brown spots	Brown or blackish spots on fat surface (increasing in area during storage)	Growth and metabolism of yeasts such as <u>Saccharomycopsis lipolytica</u>	The brown colour is due to haem pigments, associated with yeasts growing in the surface layer of the fat. Usually no colonies visible on surface. Find source of contamination (eg. chiller condensate dripping onto carcasses). Improve cleaning procedures and use low permeability films[2].

1 All known to have caused rejections of commercial shipments of vacuum-packaged beef

2 Film permeability should not exceed 25-30 $ml/O_2/m^2/24$ h/atm measured at 25°C in 98% RH and should be even lower if possible.

eight weeks by gas flushing with 100% carbon dioxide (i.e. a cycle consisting of evacuation, filling with CO_2, evacuation, sealing).

Treatment of carcasses by immersion in a dilute solution of acetic acid results in an extension of three to four weeks in storage life. For maximum effectiveness this should be done "on line", i.e. before the carcasses are chilled. The characteristics of vacuum-packaged telescoped lamb carcasses have been discussed by Eustace (1984).

Table 4 lists defects which affect the appearance of vacuum packaged meats and which may result in rejection. The causes of these problems and ways of avoiding them are indicated.

Storage in 100% carbon dioxide

Meat contains about 75% water and when carbon dioxide is injected into packs of meat, some of it dissolves in the water and forms carbonic acid. Part of the anti-microbial action of carbon dioxide is thought to be due to this phenomenon and the reduction in pH that it causes. To be fully effective the gas must have access to all the meat surfaces during storage, i.e. there must be excess gas present.

The amount of carbon dioxide which dissolves in the product and hence the volume of the head space required depends upon factors such as storage temperature, the nature of the meat surface (lean or fat) and the surface to volume ratio. Lean meat surfaces have a higher water content than fat or skin and will absorb more gas. The effectiveness of carbon dioxide as an inhibitor increases as the temperature decreases. This is due in part to the fact that its solubility increases as the temperature decreases.

Generally speaking the storage life of fresh meat stored in an atmosphere of 90-100% carbon dioxide should be at least as long as that obtained by vacuum-packaging. Swedish workers found that pork stored in an atmosphere of 100% carbon dioxide does not spoil because of microbial growth during 3 months storage at 0°C. (Blickstad and Molin 1983). However taste panel studies are needed to determine the eating quality of pork after such a prolonged period of storage.

For primal cuts or whole lamb carcasses stored at 0°C, at least two months should be achievable. Again excess gas, above the volume which will dissolve in the meat, must be present. The volume of gas used should be 1-1 litres per kilogram of meat (at least 15 litres for a 12 kg lamb carcass) and the residual concentration of oxygen should be as low as possible. This can be achieved by including a flushing step in the process.

The use of atmospheres of carbon dioxide presents a number of technical problems. Holding meat in an excess of the gas can be achieved by using rigid sealed containers, but these present problems in commercial use. The meat may be stored sealed in plastic bags made of films of low gas permeability. After packaging, gas is absorbed by the meat, the amount depending upon the temperature, i.e. the volume of the pack depends upon storage temperature. An added complication is that it takes some hours for the contraction in volume to occur. Both these factors have significant implications for cartoning, final storage volume etc. It is possible to vary the gas to meat ratio such that just sufficient gas is added so that it is absorbed during the initial stages of storage, the pack tightens and has the visual appearance of a true vacuum pack. Unfortunately this amount of gas is not optimal microbiologically and further, if the temperature rises, some gas will be released from the meat and the packs will become slack, i.e. look like "leakers".

Two types of colour problems can occur with meat stored in high concentrations of carbon dioxide. One of these is browning which results from the presence of low concentrations of oxygen. If only 0.5-1% is present, the rate of formation of metmyoglobin is high and browning occurs. Under commercial conditions it is difficult to exclude all oxygen and colour changes remain a problem, especially with beef because of its high pigment content. Bleaching of the surface may also occur when meat is exposed to a high concentration of carbon dioxide. This is caused by denaturation of proteins in the surface layer of the meat resulting in a grey appearance.

In addition to discolouration of the lean surface, problems with the appearance of fat surfaces may occur (brown-grey discolouration). For example, with lamb a brownish discolouration of the fell surfaces may develop after several weeks storage at 0°C and if this occurs the appearance is inferior to that of fresh primals.

A system recently developed in New Zealand may overcome these problems. The lamb cuts are vacuum-packaged in permeable bags which are heat shrunk. These are then stored in a "master pack" consisting of a large bag made from very impermeable film that is filled with an excess of 100% carbon dioxide and sealed. (The packaging of the cuts in permeable film is to allow access of the carbon dioxide to the meat). Storage at -1°C is reported to yield a life of at least 15 weeks (Gill 1986b).

Retail display following vacuum-packaged storage of primals

Vacuum-packaged primal cuts are broken down to consumer cuts prior to sale. Whilst there have been many studies of the storage of vacuum-packaged meats, little attention has been paid to the quality of consumer cuts prepared from them.

Table 5 shows the results of preliminary experiments to determine the retail display life of consumer cuts of beef (stored at about 5°C) in conventionally overwrapped and modified atmosphere packs (20% CO_2/80% O_2) as a function of the time of storage of the meat in the vacuum pack at 0°C. The longer the period of prior storage in the vacuum pack, the shorter is the display life of the consumer portions. The advantage of longer display life achieved by the use of MAP, becomes less as the period of prior storage increases. Experiments of this type also suggest that there is little point in storing primal cuts of beef for longer than about eight weeks at 0°C, but further studies are needed to confirm this.

TABLE 5: The approximate retail display life of consumer cuts of beef as a function of the time the meat was stored vacuum-packaged

Storage time in the vacuum pack (weeks at 0°C)		0	2	4	6	8
Retail display life (days)	Overwrapped trays	3	3	2	2	1
	MAP	>7	5-6	4-5	3-4	2

The storage life required for export markets

Meat exported from Australia and New Zealand can reach the most distant markets using sea transport in about five weeks. Assembly of the product and distribution each require about one week and thus a storage life of about eight weeks is adequate.

Vacuum-packaged pork and lamb are at or near the end of their storage life after eight weeks storage at 0°C, and beef of this age already has a shorter retail display life than fresh product when broken down to consumer cuts. To overcome these problems storage at about -1°C is recommended. However at present most chilled meat exported from Australia goes to Asian or Middle-Eastern destinations. The transport time to these markets is only about three weeks and product needs to be in good condition for only about five weeks.

CONCLUSIONS

1. **Beef.** Vacuum-packaging in plastic films of low permeability and storage at 0°C gives a storage life (10-12 weeks) adequate for this product to reach Asian markets in good condition. High pH (5.9) meat should not be packaged for export.

2. **Pork and lamb.** A storage life of only about six weeks can be guaranteed for vacuum-packaged pork and lamb stored at 0°C. Storage at about -1°C and/or decontamination with acetic acid prior to packaging are recommended to extend this period.

3. Storage in 100% carbon dioxide is effective, especially for pork. Further studies to minimise colour problems with beef and lamb and to improve packaging are needed.

4. Further studies of the non-microbial factors causing organoleptic changes in red meats during prolonged storage at 0°C and -1°C are needed. Consumer acceptance studies of such meats in export markets would be useful.

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