

Meat technology update

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Lactic acid bacteria

- Lactic acid bacteria are used in starter cultures for fermented products such as yoghurt, soft cheeses and some fermented sausages. They contribute to the flavour of these products and lower the food pH, thus restricting the development of other spoilage organisms or pathogens.
- In a vacuum pack, the meat surface develops a high carbon dioxide (CO₂), low oxygen (O₂) environment. This suppresses aerobic bacteria and allows anaerobic lactic acid bacteria to grow.
- Lactic acid bacteria reach a stable level of around 7 log cfu/g within a few weeks.
- Lactic acid bacteria utilise glucose at the meat surface. When the glucose is exhausted, they begin to metabolise amino acids in the meat, and start to produce the offensive odours associated with spoilage. This occurs some weeks after the maximum numbers of lactic acid bacteria have been reached.
- Packaging in 100% CO₂ slows the growth of lactic acid bacteria, so can delay the onset of spoilage.
- High pH (dark cutting) meat encourages the growth of other spoilage organisms that out-compete lactic acid bacteria, which prefer a lower pH. These other spoilage organisms include those that cause putrid odours.

It is generally accepted that muscle tissue is sterile until the surface is exposed during the skinning, dressing and boning process. The microbes that then colonise the meat surface may originate from the skin/coat, gut or faeces of the animal; from the workers' hands and tools; and from the processing environment—for example from the air or from water used during processing. This means that freshly prepared meat carries a wide range of microbes.

Unless the meat is stored at temperatures below minus 10°C some growth of microbes can be expected. Different microbes prefer different environments, so chill storage will be unsuitable for microbes that like hotter or warm temperatures (thermophiles or mesophiles), but will be suitable for growth of cold-tolerant microbes (psychrotrophs). Similarly, the amount of available oxygen (O₂) will affect the types of microbe that can grow on the product. Aerobic organisms require O₂, while anaerobic organisms prefer a lack of O₂. Between these two extremes there is a range of O₂ tolerances, so some microbes like a small amount of O₂ (microaerophilic), and others still prefer to have O₂, but can cope with anaerobic environments (facultative anaerobes). The exact storage conditions of meat will determine which types of bacteria can grow, and how



quickly they can multiply. As a result, the microbial flora of the meat present at the end of storage can be dramatically different from the flora present at the point of production.

Chill storage reduces the rate of microbial growth, and vacuum packaging has long been used in conjunction with chilling to extend the storage life of fresh meat. In a vacuum pack, residual O₂ is used up by the natural respiration of the meat, and carbon dioxide (CO₂) is produced. The reduction in O₂ inhibits the aerobic microorganisms, and selects for anaerobic

organisms. In normal circumstances, the population that develops is dominated by microbes belonging to the lactic acid bacteria group (LAB).

LAB are a related group of bacteria. They are Gram-positive organisms that do not form spores, they are negative to the catalase and oxidase tests, and they are anaerobic organisms—although some strains can tolerate low levels of O₂. LAB utilise carbohydrates during multiplication, and produce lactic acid as a by-product. Homofermentative LAB produce mainly lactic acid, while the heterofermentative strains also produce acetic acid, and some minor acids e.g. formic acid, citric acid and malic acid. Lactic acid bacteria are used in starter cultures for fermented products such as yoghurt, soft cheeses and some fermented sausages. They contribute to the flavour of these products and lower the food pH, thus restricting the development of other spoilage organisms or pathogens. In vacuum packed meat, the predominant LAB include *Carnobacterium divergens*, *Carnobacterium piscicola*, *Lactobacillus sakei*, *Lactobacillus curvatus*, *Leuconostoc gelidum*, and *Leuconostoc carnosum*.

When meat is produced under good hygienic conditions, the number of LAB on the surface is very low (<100/cm²) at the time of packaging. The LAB, however, multiply very quickly, and can reach levels of between 6 and 8 log₁₀ cfu/cm² within the first two weeks of storage at 5°C. The counts stabilise at this level, but the population of the LAB changes as time goes on. Usually, in the first 2–3 weeks, *Carnobacterium* spp predominates. As this organism continues to produce lactic acid, the surface and drip pH decreases. Slowly, the *Carnobacterium* spp are replaced by *Leuconostoc* spp, which are more acid tolerant, and these in turn are replaced by *Lactobacillus* spp as the predominant organisms. There have been very few studies published on the different LAB in vacuum packed meat, and it seems that the specific LAB present can be quite variable, even between primals produced at the same processing plant or from the same class of animal (e.g. bull or steer). This indicates that the predominant microflora of meat during storage is influenced, not only by the composition of the meat and its storage conditions, but also by the strains of bacteria present initially.

Common LAB associated with vacuum-packed meat

Microorganism	Microscopic appearance
<i>Lactococcus</i> species	Cocci (spheres) in chains
<i>Leuconostoc</i> species	Pleomorphic (cocci and short rods)
<i>Pediococcus</i> species	Cocci
<i>Lactobacillus</i> species	Rods
<i>Streptococcus</i> species	Cocci in chains
<i>Carnobacterium</i> species	Rods

In modified atmosphere packaging (MAP) for retail display, the gas mixture is usually 20–30% CO₂ and 70–80% O₂. The high concentration of O₂ is used to maintain an attractive red colour in the muscle myoglobin for as long as possible, while the CO₂ inhibits multiplication of *Pseudomonas* spp, the most important spoilage bacteria. As a result—particularly in packs prepared using vacuum-packaged meat—the group of microbes that becomes predominant is the lactic acid bacteria, with a small proportion of *Pseudomonas* spp, Enterobacteriaceae and sometimes *Brochothrix thermospecta*. Packs containing 100% CO₂ are sometimes used instead of vacuum packs, and these confer the longest shelf-life because they retard the growth of all the organisms present, including the LAB.

Enumerating LAB

The agar medium of choice is deMan, Rogosa and Sharpe (MRS). The plates are incubated anaerobically in CO₂ for 3 days at 25°C. LAB colonies are small (1–2 mm in diameter) and characteristically white or cream coloured.

LAB can also be enumerated using Petrifilm *aerobic count*[®] plates. The sample has to be diluted in MRS broth for plating, and the plates must be incubated under anaerobic conditions for 48 hours at 30–35°C. LAB colonies are reddish-brown in colour. Some strains (heterofermentative) produce gas bubbles.

LAB and Spoilage

In vacuum packed meat, the environment at the meat surface is usually microaerobic, rather than strictly anaerobic—this is because there is some small amount of O₂ penetration through the barrier bag, and some tissues (e.g. fat) do not utilise this small amount of O₂. Either way (microaerobic or anaerobic), the strictly aerobic organisms such as *Pseudomonas* are suppressed. The microflora on the meat becomes dominated by anaerobic or facultative anaerobic organisms. The type of organisms in this flora is determined by the pH of the meat and the storage temperature. Under normal conditions (pH 5.5, chill storage) the flora that develops is composed of Gram-positive LAB. They utilise glucose in the meat to grow, and when the glucose at the meat surface is used up, growth ceases. This is usually at a level of around 8 log₁₀ CFU/cm².

While they are fermenting glucose, the LAB do not produce offensive by-products. When the glucose runs out, the LAB begin to metabolise some amino acids, such as valine and leucine. This leads to the formation of volatile fatty acids which can impart acid/dairy flavours, and ultimately odours to the meat. These flavours and odours are unusual rather than grossly offensive, but ultimately render the meat unacceptable to consumers, four weeks or more after the LAB have achieved maximum numbers.

There is a very poor correlation between the level of LAB present in meat and the onset of development of the odours. Even the odours detected are poorly correlated with the microbial flora present. The odours are formed by a combination of LAB and their metabolites interacting—if you have a pure culture of one particular LAB on a sample for research purposes, the odour that develops is not the same as that which develops in normal meat, where there are a number of different organisms involved.

Some strains of LAB can metabolise sulphur-containing amino acids, slowly producing hydrogen sulphide (H₂S). This can react with

Spoilage characteristics associated with LAB

Microorganism	Spoilage Characteristics
<i>Lactobacillus</i>	Sulphide odours (rotton egg) Sour/dairy odours Gas production Discolouration
<i>Leuconostoc</i>	Sour/dairy odours and flavours
<i>Carnobacterium</i>	Sour/dairy odours and flavours Discolouration
<i>Pseudomonas</i> (not LAB)	Putrid odour

myoglobin to produce a green discolouration. In microaerobic conditions, some LAB may produce hydrogen peroxide (H_2O_2), which can also result in green discolouration.

Antimicrobial activity

LAB are often inhibitory to other microorganisms and this is the basis of their ability to improve the keeping quality and safety of many food products.

The principal factors which contribute to this inhibition are:

- low pH;
- organic acids;
- CO_2 production;
- H_2O_2 ;
- bacteriocins;
- nutrient depletion / competitive inhibition.

By far the most important are the production of lactic and acetic acids and the consequent decrease in pH.

pH and organic acids: LAB produce organic acids by fermenting metabolisable carbohydrates, e.g. glucose. The antimicrobial activity of organic acids is primarily associated with the chemical state of the acid, which is affected by pH. The antimicrobial activity of organic acids increases as pH decreases.

For this reason, the activity will be higher in fermented sausages with pH 4.5 than in cooked meat products at pH 6.3. On the other hand, it is possible to achieve an inhibitory effect by increasing the addition of organic acids to products with a high pH.

CO_2 production: During growth, LAB utilise the O_2 , reducing the O_2 tension and inhibiting the growth of aerobic bacteria. In addition, heterofermentative LAB produce CO_2 , which has specific antimicrobial activity. In meat preservation, a large amount of CO_2 production is undesirable, as it can cause blown packages, or make small holes in the product.

H_2O_2 : LAB produce H_2O_2 during aerobic fermentation of carbohydrates. They also have different enzymatic degradation systems that remove H_2O_2 . The efficiency of these enzyme systems varies among LAB, but often the production of H_2O_2 is greater than the degradation. H_2O_2 is inhibitory to bacterial cells because of its strong oxidative effect on cell membranes and proteins, but in meat it can result in lipid oxidation and green/brown discolouration.

Bacteriocins: are bactericidal peptides or proteins which are usually active against species closely related to the producing organism. They are produced by food-grade organisms and could therefore be regarded as natural, and hence are more acceptable to consumers as food preservatives.

Nisin is the only bacteriocin in commercial use at present. It was first discovered when a nisin-producing LAB caused problems in cheese-making by inhibiting the other starter organisms present. Nisin has been used as a food preservative in the UK since the 1950s, and was approved in the US in 1988, but is not approved in Australia and New Zealand. It differs from many other bacteriocins produced by LAB in having a relatively broad spectrum of activity against Gram-positive bacteria generally. Bacterial spores are

particularly sensitive, so nisin has been an important preservative for canned foods, and those that require milder heat-processing regimes. Nisin has also been used for decontamination of meat products. Although nisin has been reported to be unstable in raw meat, it has been shown to delay the growth of the indigenous LAB in vacuum-packed emulsion sausages, thus extending the shelf-life. A few papers report that bacteriocin-producing LAB can be used to prevent spoilage due to specific microorganisms. Some strains of *Lactobacillus sakei* are known to spoil vacuum-packed beef by producing sulphurous compounds within a few weeks. Researchers have found that the growth of these organisms can be avoided by adding *Leuconostoc gelidum* to the vacuum-packed beef at the beginning of storage. This technique has not yet been accepted in the commercial situation.

Competitive inhibition: The growth of bacteria within a food system depends on the amount of nutrients available and the number of hurdles added. If a product is inoculated with a high number of LAB capable of growth in the specific product, the growth of other bacteria using the same nutrients will be inhibited owing to competition, especially for the limiting nutrients.

Fermented meats

Traditionally, the use of starter cultures in the meat industry has been limited to fermented meats such as raw cured ham and sausages, where the cultures contribute to the texture, flavour and safety. However, research showing that bacteriocin-producing LAB can inhibit the growth of pathogens and spoilage organisms has increased the interest in using LAB in meat products such as cooked cured meat and sausages that are sliced and packaged under vacuum or MAP.

Fermented sausages are characterized by a rapid decrease in pH during fermentation accompanied by the development of colour and flavour. LAB such as pediococci and lactobacilli are responsible for the pH fall; micrococci, staphylococci and yeasts are responsible for the development of colour and flavour. A bacteriocin-producing strain of *Pediococcus* can reduce the number of *Listeria monocytogenes* by 3.4 log cycles, compared to a non-bacteriocin strain, which only reduces *L. monocytogenes* by 0.9 log cycles.

Three features complicate biopreservation of fresh meat. The first is the presence of proteolytic enzymes, which degrade the bacteriocins and reduce the antimicrobial effect of the bioprotective culture. The second is that most fresh meat is spoiled by Gram-negative bacteria, which are generally not sensitive to bacteriocins. Thirdly, fresh meat contains a large number of different bacteria, so the LAB starter culture needs to be able to compete with many different bacteria to become the dominant flora throughout the entire storage period.

LAB and health

LAB have occasionally been found in wounds and infections, but they have never been identified as causing infections; however, some LAB are known to produce biogenic amines by decarboxylation of the corresponding amino acid. Fermented foods such as cheese, sausages, fish and wine have been implicated in food poisoning due to biogenic amines. The production of biogenic amines depends of several parameters, such as: the specific strain, the composition of the food item and the temperature. If LAB are going to be added to a food product to extend shelf-life, its potential for producing biogenic amines should be investigated before its use.

Other factors affecting meat storage life

The storage-life of raw meat is affected by a few main factors.

1. The initial number of microbes present (particularly those with spoilage potential), and the particular microbes present.

The maximum numbers of potent spoilage organisms in vacuum packs are usually constrained by the LAB. The inhibition of other organisms by LAB only occurs when the LAB population reaches its peak. Before that, the various organisms grow at rates that are determined by the temperature and the environment provided by the meat, without any obvious interactions between types of bacteria. So, spoilage depends on the initial levels of these organisms. If the potent spoilers are present in numbers that are low relative to the LAB, their growth can be suppressed before they reach numbers sufficient to cause noticeable spoilage; but, if their numbers are relatively high, premature spoilage will occur.

2. The temperature of storage

LAB are psychrotrophic organisms, which means that they have a growth rate advantage at colder temperatures. That advantage reduces with increasing temperature. Where meat is held at higher temperatures—as in retail display—, *Brochothrix thermospacta* and *Shewanella putrefaciens* can compete effectively with LAB, and, at warm temperatures, the Enterobacteriaceae can dominate.

3. The gaseous atmosphere at the meat surface

LAB are anaerobic organisms, they are inhibited by O₂. Where the vacuum film has a poor barrier, *Pseudomonas* and *Brochothrix* are encouraged, leading to early onset of spoilage.

4. Meat characteristics

High pH meat is associated with preslaughter depletion of glycogen in the muscle. This means that there is very little carbohydrate available for the LAB to metabolise and they quickly begin to metabolise amino acids, leading to the development of off flavours and odours. A high pH also encourages growth of organisms such as *Brochothrix thermospacta*, *Shewanella putrefaciens* and/or Enterobacteriaceae. In high pH vacuum-packed beef, LAB levels are only around 60% of the total microbial population compared with 80--90% in beef of pH <5.8. *Shewanella*



and Enterobacteriaceae also metabolise amino acids when the glucose is exhausted, and produce offensive odours and flavours. H₂S is also produced which can lead to green discoloration. In MAP, the level of O₂ present allows *Pseudomonas* to grow if meat pH is above 5.8, leading to slime formation and a putrid smell. Researchers have shown that the shelf-life of dark cutting (DFD) meat in vacuum packs can be extended by adding glucose and acidifying with lactic and/or citric acid, but this process is not permitted in commercial meat production in Australia. Packaging in 100% CO₂ can also extend the storage life, by suppressing all microbial growth.

Fat has lower levels of carbohydrate and amino acids than muscle tissue, and a higher pH. This may lead to faster growth of spoilage organisms. Vacuum-packed lamb does not keep as long as beef and this may be related to the greater proportion of surface fat with raised pH. Vacuum-packed pork has a shorter shelf-life than beef because the glycogen and glucose decrease at a faster rate in pork than beef; so, the organisms start to metabolise amino acids and produce offensive byproducts at an earlier stage during storage.

Summary

The biggest potential for extending the storage life of fresh meat is in vacuum-packs or MAP without O₂. In vacuum packs, during storage, anaerobic or facultative anaerobic microorganisms multiply on the surface of the meat. In the majority of cases, a non-spoilage flora will develop; however, in some cases, the meat is spoiled due to the growth of psychrotrophic *Clostridium* species, LAB or Enterobacteriaceae. Non-spoilage LAB can out-compete other organisms, so it has been suggested that adding LAB to fresh meat before vacuum packaging could be used to extend shelf-life.

The information contained herein is an outline only and should not be relied upon in place of professional advice on any specific matter.

Contact us for additional information

Meat Industry Services is supported by the Australian Meat Processor Corporation (AMPC) and Meat & Livestock Australia (MLA).

Brisbane:

Food Science Australia
PO Box 3312
Tingalpa DC QLD 4173

Ian Eustace
T +61 7 3214 2117
F +61 7 3214 2103
M 0414 336 724

Neil McPhail
T +61 7 3214 2119
F +61 7 3214 2103
M 0414 336 907

Alison Small
T +61 7 3214 2109
F +61 7 3214 2103
M 0409 819 998

Sydney:

Bill Spooner
PO Box 181
KURMOND NSW 2757

T +61 2 4567 7952
F +61 2 4567 8952
M 0414 648 387
bill.s@bigpond.net.au

Adelaide:

Chris Sentance
PO Box 178
FLAGSTAFF HILL SA 5159

T +61 8 8370 7466
F +61 8 8370 7566
M 0419 944 022
Chrisfss@ozemail.com.au

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