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# Bacterial spoilage of meat and cured meat products

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## Abstract

The influence of environmental factors (product composition and storage conditions) on the selection, growth rate and metabolic activity of the bacterial flora is presented for meat (pork and beef) and cooked, cured meat products.

The predominant bacteria associated with spoilage of refrigerated beef and pork, are *Brochothrix thermosphacta*, *Carnobacterium* spp., *Enterobacteriaceae*, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens*. The main defects in meat are off-odours and off-flavours, but discolouration and gas production also occur. Bacteria associated with the spoilage of refrigerated meat products, causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in pH, consist of *B. thermosphacta*, *Carnobacterium* spp, *Lactobacillus* spp, *Leuconostoc* spp. and *Weissella* spp.

Analysis of spoilage as measured by bacterial and chemical indicators is discussed. It is concluded that a multivariate approach based on spectra of chemical compounds, may be helpful in order to analyse spoilage, at least for spoilage caused by lactic acid bacteria.The consequences of bacteria–bacteria interactions should be evaluated more.

*Keywords:* Meat; Meat products; Bacterial spoilage; Spoilage indicator; Product composition; Storage condition

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## 1. Introduction

The shelf-life of meat and meat products is the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable bacterial level, or an unacceptable off-odour/off-flavour or appearance. The shelf-life depends on the numbers and types of microorganisms, mainly bacteria, initially present and their subsequent growth.

The initial mesophilic bacterial count on meat and cooked meat products is about $10^2$–$10^3$ cfu/cm$^2$ or gram, consisting of a large variety of species (Mol et al., 1971; Blickstad et al., 1981; Blickstad and Molin, 1983b; Jackson et al., 1992). Only 10% of the bacteria initially present are able to grow at refrigeration temperatures, and the fraction causing spoilage is even lower. Since meat products are heated to a temperature of 65–75°C, most vegetative cells are killed and post-heat treatment recontamination determines the shelf-life. The surface contamination of the cut meat and the meat products will determine the potential shelf-life.

During storage, environmental factors such as temperature, gaseous atmosphere, pH and NaCl will select for certain bacteria, and affect their growth rate and activity. The shelf-life of refrigerated meat and meat products may vary from days up to several months (Gill and Molin, 1991; Blickstad and Molin, 1983b).

The following presentation will focus upon bacteria able to grow and cause spoilage during the storage of meat (pork and beef) and cooked, cured meat products.

## 2. Meat

### *2.1. Environmental influences on bacterial growth and shelf-life*

Growth to high numbers is a prerequisite for spoilage. The expected shelf-life and growth ability of different bacteria under various environmental conditions, are presented in Table 1.

#### *2.1.1. Packaging*

Three different packaging types are in use: air, vacuum and modified atmospheres (MA). MA contain different levels of oxygen and carbon dioxide, balanced with inert nitrogen. Packages containing up to 80% oxygen and 20% carbon dioxide (high oxygen-MA) will reduce the colour deterioration of retail cuts of meat, but will only slightly increase the shelf-life, compared to aerobic storage. Pork is generally stored aerobically or in MA, and beef in a vacuum or MA due to the need for tenderization during an extended storage. Transitions between different packaging-types may be performed for retail cuts. The shelf-life of meat increases in the order: air, high oxygen-MA, vacuum, no oxygen-MA and 100% $CO_2$.

*Pseudomonas* spp. dominate on aerobically stored meat, and due to a high growth rate the shelf-life is a matter of days (Gill and Molin, 1991). The frequent domination of *Pseudomonas fragi* is suggested to be due to the ability of the

Table 1
Expected shelf-life under refrigerated storage, and growth ability of bacterial groups and specific bacteria on meat and meat products

| Product | Storage | Expected shelf-life | Growth[a] | | | |
|---|---|---|---|---|---|---|
| | | | *Pseudomonas* spp. | *Enterobacteriaceae* | Lactic acid bacteria | *B. thermosphacta* |
| Meat, normal pH | Air | Days | +++ | ++ | ++ | ++/+++ |
| | High $O_2$-MA | Days | +++ | ++/+++ | ++/+++ | +++ |
| | Vacuum | Weeks-months | + | +/++ | +++ | ++/+++ |
| | 100% $CO_2$ | Months | + | +/++ | +++ | + |
| Meat, high pH | Vacuum | Days | + | ++/+++ | +++ | ++/+++ |
| | 100% $CO_2$ | Weeks-months | + | +/++ | +++ | + |
| Meat products | Air | Days | +/++ | + | ++ | +++ |
| | Vacuum | Weeks | + | + | +++ | ++/+++ |
| | $CO_2+N_2$ | Weeks | + | + | +++ | + |

[a] +++, dominant part of the microflora; ++, intermediate part of the microflora; +, minor part of the microflora.

bacterium to use creatine and creatinine (Drosinos and Board, 1994). In a mixed broth culture of *Ps. fragi*, *B. thermosphacta* and *Carnobacterium maltaromicus* [*piscicola*], all grew at the same exponential rates at 4°C and pH 6.1. *B. thermosphacta* dominated the flora at the early stationary growth phase, but was eventually outgrown by *Ps. fragi* (Drosinos and Board, 1995).

In high oxygen-MA, a variety of bacteria are able to grow to high final numbers, such as *B. thermosphacta*, *Pseudomonas* spp., *Leuconostoc* spp. and *Lactobacillus* spp. (Ordóñez et al., 1991; Jackson et al., 1992). Most bacteria are more or less inhibited by $CO_2$, and thus the growth rate is reduced, compared to air, and the shelf-life increased. When incubated as a mixed broth culture in 80% $O_2$ and 20% $CO_2$, the maximum counts decreased in the order: *B. thermosphacta* > *C. piscicola* > *Ps. fragi*. Two factors explain why *Ps. fragi* was outnumbered in the 20% $CO_2$-enriched atmosphere: the growth rate was reduced and the advantageous creatinine-metabolism was suppressed (Drosinos and Board, 1995).

In a vacuum-pack the composition of the gaseous phase changes during storage; the concentration of oxygen decreases while that of carbon dioxide increases (Gill and Molin, 1991). The bacterial flora is gradually selected towards a $CO_2$-tolerant but slowly growing one. Vacuum-packaged beef may have a storage life of 10–12 weeks at 0°C, until the off-flavour becomes unacceptable (Egan, 1983). The bacterial flora is dominated by lactic acid bacteria, mainly *Carnobacterium* spp., *Lactobacillus* spp. and *Leuconostoc* spp. (Shaw and Harding, 1984; Borch and Molin, 1988). The film permeability has been shown to affect shelf-life (Newton and Rigg, 1979). With increasing permeability, the growth rate and the maximum number of *Pseudomonas* spp. increases; the growth rate of *B. thermosphacta* is unaffected, but the maximum count increases; *Lactobacillus* ssp. growth is unaffected.

A long shelf-life may be attained in pure $CO_2$. The time needed to reach $10^7$ bacteria/cm$^2$ and off-odour, was 10 days in air, and 40 days in 100% $CO_2$ for pork stored at 4°C (Blickstad et al., 1981). The effect of $CO_2$ is enhanced by a low storage temperature, due to increased solubility of the gas. On pork loins stored under $CO_2$ at $-1.5$°C, a maximum bacterial number of $10^7$ cfu/cm$^2$ was reached after 63 days (Greer et al., 1993). Shelf-life extension by $CO_2$ results from an immediate selection, as opposed to a gradual one in a vacuum-pack, of lactic acid bacteria growing at a reduced rate (Blickstad et al., 1981; Greer et al., 1993). Depending on pH and storage temperature, other bacteria such as *Aeromonas* spp., *B. thermosphacta* and *Enterobacteriaceae* may grow (Blickstad and Molin, 1983a; McMullen and Stiles, 1993). However, the lower the temperature, the greater the inhibition of *Enterobacteriaceae* and *B. thermosphacta* (Blickstad et al., 1981; Greer et al., 1993).

Retail display often includes the opening of a package and exposure to air with subsequent storage at a higher temperature. On pork chops prepared from loins stored under $CO_2$ at $-1.5$°C, lactic acid bacteria dominated the flora during storage in air at 8°C, and in addition the rapid growth of *Pseudomonas* spp. to high numbers occurred (Greer et al., 1993). The retail case life (estimated as off-odour) decreased with increasing storage times in $CO_2$. In contrast, the storage time of vacuum-packaged beef at 4°C, did not directly affect the subsequent aerobic shelf-life. The retail shelf-life (estimated as time to $10^7$ cfu bacteria/cm$^2$), was 5–6 days when prepared from beef stored for two to six weeks in a vacuum (Borch, E., unpublished results). Prolonged storage in a vacuum-pack favoured the growth of slowly growing lactic acid bacteria and *Enterobacteriaceae* at the expense of rapidly growing *Pseudomonas* spp., during the subsequent aerobic storage (Table 2).

### 2.1.2. Temperature

The lowest cold-storage temperature for meat is $-1.5$°C, while the minimum growth temperature of psychrotrophic bacteria is $-3$°C (Gill and Molin, 1991). Decreasing refrigeration temperatures decrease bacterial growth, and affect the composition of the bacterial flora. For vacuum-packaged beef, a bacterial count of about $10^7$ cfu/cm$^2$ was reached after 14 weeks at $-1.5$°C, but as early as after three weeks at 4°C (Fig. 1a, Blixt and Borch, unpublished results). The growth of *Enterobacteriaceae* was drastically reduced at $-1.5$°C, but a transition to 4°C initiated the growth (Fig. 1b). This is in accordance with McMullen and Stiles (1994). On vacuum-packaged beef, *Hafnia alvei* dominated among the *Enterobacteriaceae* at 4°C, while *Serratia liquefaciens* dominated at $-1.5$°C (Blixt and Borch, unpublished results). Also among the lactic acid bacteria, the composition varies with the storage temperature. In the study by McMullen and Stiles (1994), *Carnobacterium* spp. dominated the microbial flora on pork in 100% $CO_2$ at $-1.5$°C, while homofermentative *Lactobacillus* spp. dominated at 4°C and 7°C.

### 2.1.3. Product composition

The meat pH and availability of nutrients affect the selection and growth of bacteria. Normally, the muscle pH decreases post mortem to values between 5.4 and 5.8. A high ultimate pH ($> 6.0$; DFD-meat, dark firm dry) may be the result of stress of the living animal. Adipose tissues also have a higher pH than normal meat. Meat contains about 0.2% glucose and 0.4% amino acids. In adipose tissue and high pH meat, the levels of bacterial nutrients are lower. High pH meat and adipose tissue spoil more rapidly than normal pH meat since amino acids are rapidly attacked.

Bacteria able to grow well on vacuum-packaged high pH meat are *B. thermosphacta*, *Enterobacteriaceae* such as *H. alvei*, *S. liquefaciens* and *Enterobacter* sp.,

Table 2
Microbial flora on beef steaks, prepared from beef loins stored for 1, 3 or 5 weeks in vacuum-packs at 4°C and stored in air at 4°C for seven days

| Storage in vacuum (weeks) | Composition of microflora in air (%) | | |
|---|---|---|---|
| | *Pseudomonas* spp. | Lactic acid bacteria | *Enterobacteriaceae* |
| 1 | 95 | 0 | 5 |
| 3 | 25 | 45 | 30 |
| 5 | 0 | 75 | 20 |

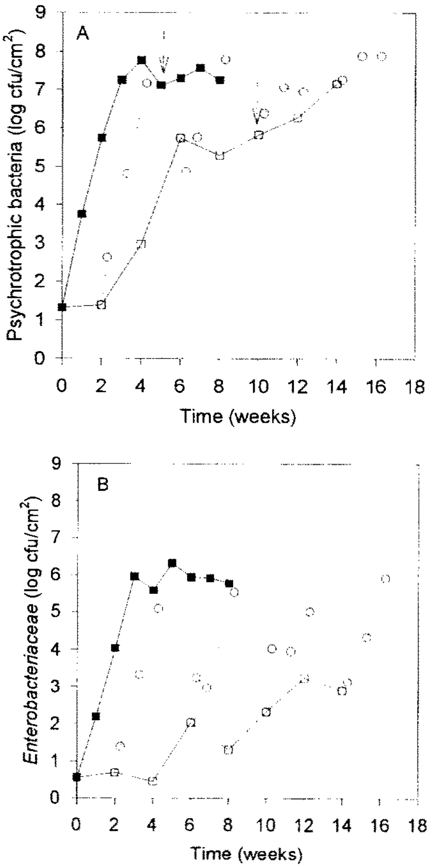

Fig. 1. Growth of (A) psychrotrophic bacteria, and (B) *Enterobacteriaceae* on vacuum-packaged beef stored at − 1.5°C (□), 4°C (■), and beef transferred from − 1.5°C to 4°C (○). The arrows indicate when off-odours were detected.

*Lactobacillus* spp., and *Shewanella putrefaciens* (Patterson and Gibbs, 1977; Gill and Newton, 1979). For high pH beef, the storage life may be extended by storage below 0°C (Shay et al., 1994) and storage in pure $CO_2$ (Erichsen and Molin, 1981). In 100% $CO_2$, homofermentative *Lactobacillus* spp. completely dominated the bacterial flora at 4°C (Erichsen and Molin, 1981). Neither *Enterobacteriaceae*, nor *B. thermosphacta* were able to grow on high pH meat stored in 100% $CO_2$ at − 1.5°C (Jeremiah et al., 1995).

Vacuum-packed pork is reported to have a shorter shelf-life than beef, even though lactic acid bacteria dominate on both types of meat. Glycogen and glucose decrease at a faster rate in pork than in beef, leading to an earlier initiation of amino acid degradation in pork. In addition, *Enterobacteriaceae* are reported to develop better on pork than on beef (Boers, 1992).

### 2.1.4. Other factors

Interactions between bacteria are important in a natural ecosystem such as meat. In addition to competition for nutrients, the production of antibacterial substances such as hydrogen peroxide, lactic acid and bacteriocins is likely to select the growing bacteria. Several lactic acid bacteria produce such metabolic products (Holzapfel et al., 1995). It has been proposed that specific bacteria may be used as a protective culture, delaying spoilage caused by other bacteria. Characteristics of such bacteria would be: growth and production of antagonistic compounds during prevailing storage conditions, and no off-odour production.

## 2.2. Bacteria associated with spoilage

The predominant bacteria associated with spoilage of refrigerated beef and pork, are *B. thermosphacta*, *Carnobacterium* spp., *Enterobacteriaceae*, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Sh. putrefaciens* (Dainty and Mackey, 1992). The main defects of meat are off-odour and off-flavour, but discolouration and gas production also occur.

### 2.2.1. Off-odours and off-flavours

Off-odours such as sweet and fruity, putrid, sulphury and cheesy, characterize aerobically stored meat (Dainty and Mackey, 1992). *Pseudomonas* spp., specifically *Ps. fragi* produce ethyl esters coinciding with the early stages of spoilage. Sulphur-containing compounds contribute to the putrid and sulphury odours. The responsible compounds are for example hydrogen sulphide formed by *Enterobacteriaceae*, and dimethyl sulphide formed by *Pseudomonas* spp. Cheesy odours are associated with acetoin/diacetyl and 3-methylbutanol formation, presumably by *Enterobacteriaceae*, *B. thermosphacta* and homofermentative *Lactobacillus* spp. (Borch and Molin, 1989; Dainty and Mackey, 1992).

The off-odour of meat packaged in high oxygen-MA is characterized as cheesy and rancid. In this atmosphere a variety of bacteria such as *Pseudomonas* spp., *B. thermosphacta* and lactic acid bacteria (Ordóñez et al., 1991; Jackson et al., 1992) are able to grow, and contribute to spoilage. The presence of oxygen will increase the spoilage potential of both *B. thermosphacta* and lactic acid bacteria, due to the formation of end-products such as acetoin and acetic acid (Dainty and Hibbard, 1980; Blickstad and Molin, 1984; Borch and Molin, 1988).

The off-odours of vacuum and anaerobic MA-packaged meat are less offensive than of aerobically stored meat. The spoilage characteristics are sour and acid, and are typically associated with lactic acid bacteria and the production of lactic acid and acetic acid (Dainty and Mackey, 1992). Sulphur-compounds may also contribute to off-odour (Edwards and Dainty, 1987). During extended storage of meat a depletion of glucose is likely. A metabolic switch due to glucose depletion, leads to the formation of end-products such as acetic acid and hydrogen sulphide in homofermentative *Lactobacillus* spp. in anaerobic atmospheres (Egan et al., 1989; Borch et al., 1991).

### 2.2.2. Discolouration

The bacterial production of hydrogen sulphide converts the muscle pigment to green sulphmyoglobin. Hydrogen sulphide is produced from cysteine and is triggered by glucose limitation. *Lactobacillus sake* forms hydrogen sulphide, but only when the glucose and oxygen availability is limited (Egan et al., 1989). Sulphmyoglobin is, however, not formed in anaerobic atmospheres (Borch and Agerhem, 1992). Other bacteria able to produce hydrogen sulphide are *H. alvei* and *Sh. putrefaciens* (Dainty et al., 1989a). Greening is typically associated with high pH meat, but may also occur in normal pH meat.

### 2.2.3. Gas production

*Clostridium* spp. have been associated with the production of large amounts of gas ($H_2$ and $CO_2$) in vacuum-packaged beef, accompanied by foul off-odours (Dainty et al., 1989b). Gas production ($CO_2$) by lactic acid bacteria without extensive off-odours may be associated with vacuum-packaged beef and pork.

## 3. Heat processed meat products

### 3.1. Environmental influences on bacterial growth and shelf-life

The microbiological stability of cooked, cured meat products depends on extrinsic factors, mainly the packaging method and storage temperature, and on intrinsic factors, such as the product composition.

### 3.1.1. Packaging

Cooked meat products are chill-stored, usually in vaccum-pack or in MA-packs, but are also distributed unpacked, i.e. stored in an aerobic atmosphere. Furthermore, in retail shops slicing is performed after the opening of packages, with subsequent storage in an aerobic atmosphere.

During the aerobic storage of cooked, sliced meat products a mixed flora composed of *Bacillus* spp., *Micrococcus* spp. and *Lactobacillus* spp. is reported to dominate (Alm et al., 1961). In addition, *Pseudomonas* spp. may increase up to $10^5$ cfu/g (Krabisch et al., 1992). In cured, raw meat products, *B. thermosphacta*, *Moraxella* spp./*Psychrobacter* spp. and *Pseudomonas* spp. were retrieved. In addition good growth of yeast occurred (Dowdell and Board, 1968; Blickstad and Molin, 1983a).

Vacuum-packaging is frequently used for cooked meat products. The combination of the microaerophilic conditions, the presence of curing salt and nitrite favours the growth of psychrotrophic lactic acid bacteria (von Holy et al., 1991). *B. thermosphacta* may also be a dominant part of the bacterial flora; this will particularly be the case when the film permeability is high (Nielsen, 1983).

Storage under modified atmospheres ($CO_2$ plus $N_2$; often 20%:80%,) is also used for cooked, cured meat products. Comparisons of the shelf-life of cooked, cured meat products in vacuum-packs and in MA-packs have resulted in different findings. While some investigations indicated no extension of the shelf-life of MA-packaged meat products (Simard et al., 1983; Boerema et al., 1993), other studies reported an increase in shelf-life by MA (Blickstad and Molin, 1983b; Ahvenainen et al., 1989; Borch and Nerbrink, 1989). For meat products stored in MA, the shelf-life with respect to bacterial numbers was prolonged by 75% compared to vacuum-packaged, independent of the proportions of $CO_2$ and $N_2$ used (Borch and Nerbrink, 1989; Fig. 2). The effects on flavour and slimy spoilage varied, however, with the different gaseous atmospheres used, where 100% $N_2$ gave the best overall result followed by MA with an initial $CO_2$ concentration $< 50\%$. High concentrations of $CO_2$ may cause discolouration, off-odours and off-flavours, and a release of liquid from the meat products (Ahvenainen et al., 1989). The amount of drip from emulsion sausage stored in vacuum or modified atmospheres decreased in the order: 100% $CO_2 >$ vacuum $> 70\%$ $N_2 > 30\%$ $CO_2 > 100\%$ $N_2$ (Borch and Nerbrink, 1989).

The growth of lactic acid bacteria is favoured in atmospheres of $CO_2$ plus $N_2$, while the growth of *Enterobacteriaceae*, *B. thermosphacta* and yeast is restricted (Blickstad and Molin, 1983b; Simard et al., 1983; Borch and Nerbrink, 1989). However, the growth rate of some lactic acid bacteria is reduced in $CO_2$-atmospheres compared to aerobic (Blickstad and Molin, 1984), which may explain the prolonged shelf life.

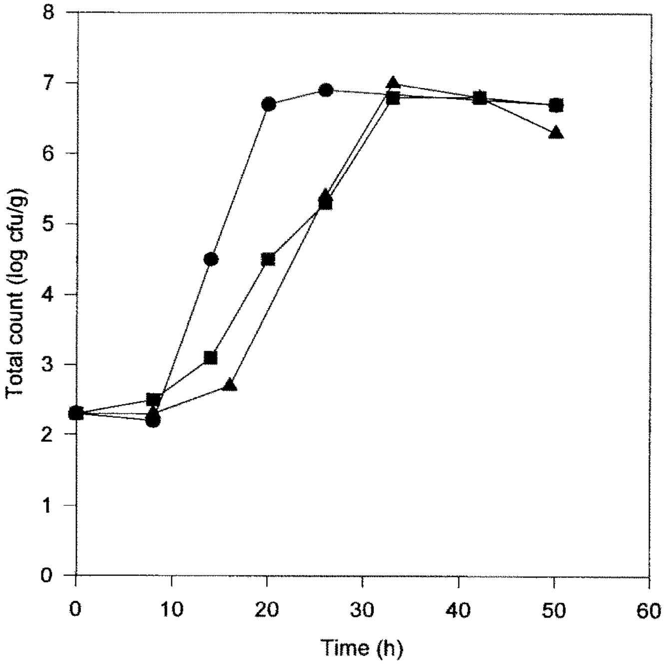

Fig. 2. Total count on emulsion sausage stored at 4°C in ●, vacuum; ■, $N_2$; ▲, 50% $N_2$ + 50% $CO_2$.

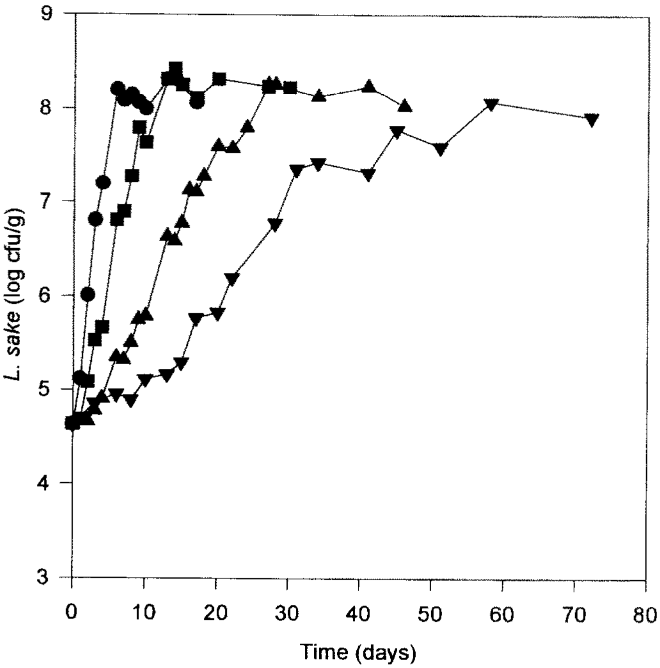

Fig. 3. Growth of *L. sake* in MA-packaged (30% $CO_2$:70% $N_2$) Bologna-type sausage at ●, 15°C; ■, 7°C; ▲, 2.4°C and ▼, 0.6°C.

The lactic flora of vacuum or MA-packaged cooked, cured meat products consists of *Lactobacillus* spp., predominantly *L. sake* and *L. curvatus* (Dykes and von Holy, 1994). *Leuconostoc* spp. such as *Leuconostoc gelidium*, *Leuconostoc carnosum* and *Leuconostoc mesenteroides sensu stricto*-group are also frequently found to dominate (Schillinger and Lücke, 1987; Korkeala and Mäkelä, 1989; Dykes et al., 1994). Furthermore, *Weissella* [*Lactobacillus*] *viridescens*, *Carnobacterium divergens*, *C. maltaromicus* [*piscicola*] and *B. thermosphacta* have been isolated (Egan et al., 1980; Gardner, 1983; Collins et al., 1987).

### *3.1.2. Temperature*

On vacuum or MA-packaged meat products the dominance of lactic acid bacteria is unaltered by the refrigeration temperature used, but the growth rate is affected. Inoculation studies with lactic acid bacteria on vacuum-packaged Bologna-type sausage demonstrated that with a decrease in temperature from 7°C to 2°C, the growth of lactic acid bacteria was retarded almost two-fold; from 7°C to 0°C about four-fold (Fig. 3; Muermans et al., in press). Thus, for meat products the storage temperature is an important factor influencing the shelf-life.

### 3.1.3. Product composition

Intrinsic factors that contribute to the microbial stability of cooked, cured meat products are mainly salt content, water activity ($a_w$), pH and nitrite concentration (Grant et al., 1988). The concentration of NaCl is 3–5% calculated on the water content, pH values are 6.0–6.5, water activity values 0.96–0.99, and the residual nitrite level is below 100 $\mu$g/g.

Cooked meat products may, in addition to sodium chloride, also contain other humectants such as phosphates and sodium lactate. These humectants will result in a decrease of the $a_w$. The addition of 4% sodium chloride in the water phase, will decrease the $a_w$ value from 0.99 to about 0.97. Salt-sensitive microorganisms, such as *Pseudomonas* spp. and *Enterobacteriaceae*, will not grow at these reduced water activity values, and the microflora developing will shift to more salt tolerant microorganisms such as lactic acid bacteria and yeasts (Blickstad and Molin, 1983a). The growth of yeasts is furthermore greatly affected by the gaseous atmosphere, being restricted in an anaerobic atmosphere. The growth rate and the lag phase of lactic acid bacteria are influenced by reduced $a_w$ values. For example, a decrease in $a_w$ value from 0.98 to 0.96 in a Bologna-type sausage resulted in a three-fold increase in the lag time and a two-fold decrease in the growth rate of the lactic acid bacteria that dominated the bacterial flora (Kant-Muermans, unpublished results).

The initial pH-value of cooked meat products will not restrict microbial growth. The pH may, however, decrease from pH 6.0–6.5 to pH 5.0–5.3 during storage, due to the activity of lactic acid bacteria (Fig. 4; Kant-Muermans, unpublished results; Dykes et al., 1991). Such a decrease will restrict the growth of *B. thermosphacta*, but not of *Lactobacillus* spp. (Blickstad, 1983). Figure 4 also shows that product formulation, e.g. the addition of liver, affects the decrease in pH, and the growth rate.

The pink colour of cooked, cured meat products is the result of the addition of nitrite and/or nitrate prior to heating, and the subsequent formation of nitrosohaemochrome (Gardner, 1983). Nitrite has an inhibitory action on the the growth of several micro-organisms, such as *Enterobacteriaceae* and *B. thermosphacta*, but not on lactic acid bacteria (Nielsen, 1983).

## 3.2. Bacteria associated with spoilage of meat products

Lactic acid bacteria are the major bacterial group associated with the spoilage of refrigerated vacuum- or MA-packaged cooked, cured meat products (Blickstad and Molin, 1983b; Shaw and Harding, 1989; von Holy et al., 1991). At the time of spoilage some products contain a 'pure' culture of only one species, while in others a mixture of *Lactobacillus* spp. and *Leuconostoc* spp. was found (Kant-Muermans, unpublished results). The great diversity of bacteria isolated from spoiled meat products is confirmed by several studies (Korkeala and Mäkelä, 1989; von Holy et al., 1991; Yang and Ray, 1994). The genus/species of lactic acid bacteria responsible for spoilage, depend on the product composition (product-related flora) as well as the manufacturing site (house-related flora; Korkeala and Mäkelä, 1989).

Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime production and decrease in pH.

### *3.2.1. Off-odours and off-flavours*

Off-flavours in vacuum- or MA-packaged cooked meat products are typically described as sour and acid (Gardner, 1983). The dominating bacteria, lactic acid bacteria, produce acids such as lactic acid, acetic acid and formic acid; the levels depending on genus species and growth conditions (Borch and Molin, 1988; Borch and Molin, 1989; Borch et al., 1991). Meat products stored aerobically or vacuum-packaged using a film with a relatively high permeability to oxygen may, in addition to sour and acid flavours, develop a slightly sweet, cheesy obnoxious odour. This is also found in meat products that have initially been stored anaerobically and subsequent to opening the package in an aerobic atmosphere. An aerobic atmosphere induces the formation of acetoin in *B. thermosphacta*, *Lactobacillus* spp. and *Carnobacterium* spp. (Borch and Molin, 1989).

### *3.2.2. Discolouration*

Bacteria producing $H_2O_2$ may cause a green discolouration through the oxidation of nitrosohaemochrome to choleomyoglobin, frequently seen as green spots. Expo-

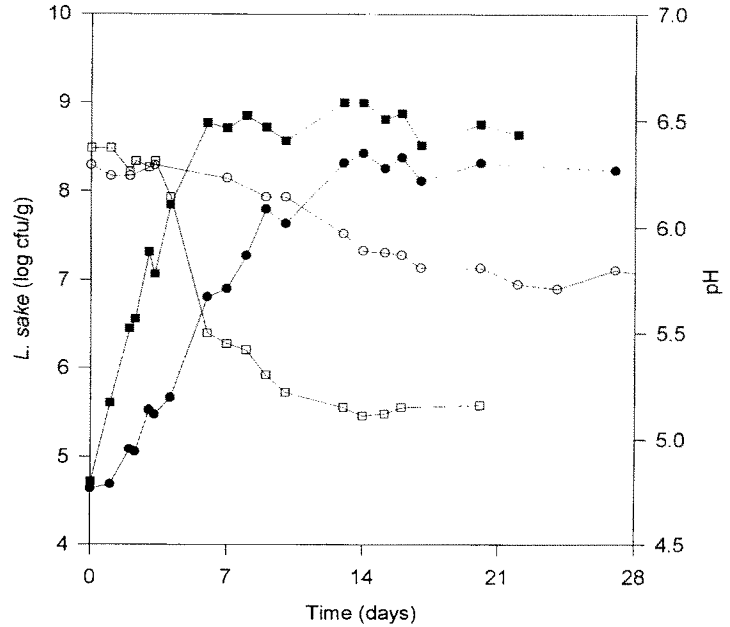

Fig. 4. Growth (filled symbols) and pH curves (open symbols) of *Lactobacillus sake* in MA packaged (30% $CO_2$/70%$N_2$) Bologna-type sausage containing 0% (○) and 15% (■) liver at a storage temperature of 7°C.

sure to air is necessary for the formation of $H_2O_2$. The bacterial greening in the centre of meat products is caused by bacteria surviving the cooking process which after exposure to air start to produce $H_2O_2$. Due to a high heat resistance, *W. viridescens* has been demonstrated to survive regular heat processing in sausage processing, being able to survive for more than 40 minutes at 68°C (Niven et al., 1954; Borch et al., 1988). The surface greening is caused by bacteria which contaminate the product after cooking. Homofermentative *Lactobacillus* spp., heterofermentative *Lactobacillus* spp., *Leuconostoc* spp. and *C. divergens* are able to form $H_2O_2$ (Borch and Molin, 1989). Other bacteria that have been associated with greening are *Enterococcus* spp. and *Pediococcus* spp. (Grant et al., 1988).

### *3.2.3. Gas formation*

Accumulation of gas ($CO_2$) is often associated with the growth of *Leuconostoc* spp., such as *Ln. mesenteroides*, *Ln. carnosum* and *Ln. amelibiosum* (Mäkelä et al., 1992; Yang and Ray, 1994; Dykes et al., 1994).

### *3.2.4. Slime*

Ropy slime formation associated with vacuum-packed, cooked meat products is caused by homofermentative *Lactobacillus* spp. and *Leuconostoc* spp. (Korkeala et al., 1988; von Holy et al., 1991; Dykes et al., 1994). *Lactobacillus sake*, *Ln. amelibiosum*, *Ln. carnosum*, *Ln. gelidum*, *Ln. mesenteroides* subsp. *dextranicum* and *Ln. mesenteroides* subsp. *mesenteroides* have been associated with slime formation on meat products. Ropy slime-producing *L. sake* and *Ln. amelibiosum* were recovered from the processing rooms at meat plants where the meat products were handled after heat processing (Mäkelä et al., 1992). Furthermore, slime formation is an early indication of spoilage, often observed before the sell-by date (Korkeala et al., 1988). The formation of ropy slime does not require that the meat product contains sucrose.

## **4. Analysis of spoilage**

### *4.1. Bacterial indicators*

The maximum level of bacteria reached during refrigerated storage of meat is $10^7$–$10^9$ cfu/cm$^2$, and of meat products about $10^7$–$10^8$ cfu/g. The correlation between bacterial numbers, in particular lactic acid bacteria, and sensorial spoilage is imprecise, which makes it difficult to use bacterial levels as an estimate of spoilage. Korkeala et al. (1987) concluded that the probability that $10^7$ *Lactobacillus* spp./g cooked meat product would cause overt spoilage is about 10%. The times between reaching bacterial counts of $10^7$ cfu/g, and that of evident spoilage, were 19 and 30 days at 4°C and 2°C, respectively (Korkeala et al., 1989). A similar situation is also valid for vacuum-packaged beef (Fig. 1a). At 4°C off odours occurred one week after achieving a count of $10^7$ cfu/cm$^2$. However, at − 1.5°C off-odours were pronounced as early as four weeks before a count of $10^7$ cfu/cm$^2$ (Blixt and Borch,

Table 3
Odours from bacteria associated with spoilage, inoculated on sterilized beef and stored in vacuum packs at 4°C for 30 days

| Bacterial strain | Odours, normal pH | Odours, high pH |
|---|---|---|
| *Brochothrix thermosphacta* | Butter, slightly yeast | Yeast, sweaty feet |
| *Carnobacterium divergens* | Butter, acid | Acid, slightly sulphurous |
| *Lactobacillus* sp. | Butter, acid, putrid, plastic | Butter, acid, plastic |
| *Lactobacillus* sp. | Sulphurous, acid | Sulphurous |
| *Lactobacillus* sp. | Butter, sulphurous | Sulphurous |
| *Lactobacillus sake* | Sulphurous, butter | Sulphurous |
| *Lactococcus raffinolactis* | Butter, slightly fresh | Butter, fresh |
| *Leuconostoc* sp. SMRICC 219 | Butter, fresh, ethanol | Butter, fresh, sweet dissolvent |
| *Leuconostoc* sp. | Acid, very fresh, sour milk | Acid, fresh |
| *Serratia liquefaciens* | Slightly butter, plastic, slightly acid | Slightly sulphurous, acid, fruity |
| *Serratia liquefaciens* | Acid, meat | Acid, fresh, slightly sweet |

unpublished results). Off-odours formed before achieved maximum bacterial count are also reported for pork stored at − 1.5°C in 100% $CO_2$ (McMullen and Stiles, 1994).

Instead of using the total count of bacteria as a spoilage indicator, the growth of specific spoilage bacteria could be analysed. This approach requires the frequent presence of a few specific spoilage bacteria. However, based on present knowledge, at least for anaerobically stored meat, these bacteria have not yet been identified. Sterilized beef inoculated with bacteria previously associated with meat spoilage, developed a range of odours during storage in vacuum at 4°C (Table 3; Blixt and Borch, unpublished results). However, a typical vacuum off-odour (dense, sour and slightly putrid) was not identified.

On the contrary, sterilized beef inoculated with *H. alvei* together with a mixture of lactic acid bacteria isolated from spoiled vacuum-packed beef, gave rise to the typical vacuum off-odour after 8 weeks in a vacuum-pack at 4°C. However, if solely the mixture of lactic acid bacteria was inoculated, the spoilage odour was sulphurous (Blixt and Borch, unpublished results). *L. sake*, known to produce $H_2S$, was isolated on the spoiled beef inoculated with solely lactic acid bacteria, while this strain was not isolated on the beef co-inoculated with *H. alvei*. This indicates that the off-odour emanates from interactions between several bacteria.

### 4.2. Chemical indicators

As an alternative to bacterial determinations, D-lactate, acetoin, tyramine, pH-value and headspace gas composition have been suggested as chemical indicators of bacterial spoilage in meat and meat products (Korkeala et al., 1987; De Pablo et al., 1989; Yano et al., 1995). The use of such spoilage indicators is, however, dependent on product composition. The occurrence of slime and the decrease in pH in meat products, will depend on the presence of fermentable carbohydrates. For example, a drop in pH from 6.3 to 5.6 was observed in Bologna-type sausage, while in liver sausage, the pH droped to 5.0 (Fig. 4; Kant-Muermans, unpublished results). Furthermore, in the study by Borch and Agerhem (1992) it was concluded that the type and amount of bacterial end-products formed were dependent on the type of bacteria growing on the meat. D-lactate and acetate indicated high numbers of a *Lactobacillus* sp., while D-lactate and ethanol indicated high numbers of a *Leuconostoc* sp.

It may be postulated that several chemical indicators will be needed in order to estimate the degree of spoilage. Differences in concentrations of single compounds of the headspace from unspoiled and spoiled meat have been revealed by comparing the GC-MS spectra of the volatile compounds (Edwards and Dainty, 1987). Use of an electronic nose to analyse headspace volatiles also shows promise. Differences in the patterns of output from the instrument have been used to distinguish between ground pork and beef meat and, furthermore, predict the time of storage (Winquist et al., 1993). The advantages of the electronic nose are that it can be made small and portable, and rapid on-line analysis may be performed.

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