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In vitro and *in situ* growth characteristics and behaviour of spoilage organisms associated with anaerobically stored cooked meat products

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ABSTRACT

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Aims: Understanding spoilage caused by different types of spoilage organisms, associated with vacuum-packaged sliced cooked meat products (CMP).

Methods and Results: First, strains were characterized in a broth at 7°C under anaerobic conditions to compare their growth rate, acidifying character and metabolite production under conditions simulating refrigerated vacuum-packaged conditions. *Brochotrix thermosphacta* grew faster than the lactic acid bacteria (LAB). Within the group of the LAB, all strains grew fast except *Leuconostoc mesenteroides* subsp. *dextranicum* and *Leuconostoc carnosum.* Secondly, the organisms were inoculated on a model cooked ham to better understand the relationship between spoilage, microbial growth, pH, metabolite production and accompanying sensory changes. Most rapidly growing strains were *Leuc. mesenteroides* subsp. *mesenteroides* followed by *B. thermosphacta*, while *Leuc. mesenteroides* subsp. *dextranicum* and *Leuc. carnosum* grew very slowly compared with the other LAB. *Brochotrix thermosphacta* caused sensory deviations at a lower cell number compared with the LAB. The related pH changes, metabolite production and sensory perception are presented.

Conclusions: In this pure culture study, *B. thermosphacta* and *Leuc. mesenteroides* subsp. *mesenteroides* had the highest potential to cause rapid spoilage on CMP.

Significance and Impact of the Study: A systematic study on the behaviour of spoilage organisms on a model cooked ham to establish the relationship between microbial growth, pH, metabolite formation and organoleptic deviations.

Keywords: *Brochotrix thermosphacta*, cooked meat products, lactic acid bacteria, sensory evaluation, spoilage, vacuum packaging.

INTRODUCTION

Cooked cured meat products are economically important refrigerated products including products such as pâté, cooked ham, emulsion-style sausages (e.g. frankfurters, luncheon meat) and cooked poultry products. A considerable part of these meat products are sliced and prepackaged commodities in vacuum or gas atmosphere to be sold with sell-by dates at 7°C varying from 3–4 weeks, for e.g. cooked cured ham (Stekelenburg and Kant-Muermans 2001) to 6 weeks, for e.g. pâté. As meat products are heated to a temperature of 65–75°C, most vegetative cells are killed and postheat treatment recontamination determines the shelf life (Borch *et al.* 1996). Product handling after cooking plus slicing prior to packaging recontaminates the cooked cured meat products with *ca* 0·5–2 log CFU g^{-1} of total bacteria,

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mainly lactic acid bacteria (LAB) (Holley 1997; Samelis *et al.* 2000). When stored under anaerobic conditions and under refrigeration, psychrotrophic LAB are most often responsible for spoilage of cooked cured meat products because of their tolerance to micro-aerophilic or anaerobic atmospheres (Korkeala and Mäkelä 1989; von Holey *et al.* 1991). The lactic acid flora of vacuum or MA-packaged cooked meats consists mainly of homofermentative *Lactobacillus* spp., predominantly *Lactobacillus sakei* and *Lactobacillus curvatus* (Korkeala and Mäkelä 1989; von Holey *et al.* 1991; Devlieghere *et al.* 1998; Samelis *et al.* 2000). In addition to these, obligate heterofermentative lactobacilli, e.g. *Lactobacillus brevis* and *Leuconostoc* spp. (von Holey *et al.* 1991; Björkroth *et al.* 1998; Samelis *et al.* 1998), followed by other species such as *Weisella* spp. (Samelis *et al.* 2000) and *Carnobacterium* spp. (Borch and Molin 1989) have been found to cause spoilage. Homofermentative LAB ferment glucose exclusively to lactic acid, while heterofermentative LAB ferment hexoses to lactic acid, acetic acid and/or ethanol and carbon dioxide (Stiles and Holzapfel 1997). However, glucose limitation, for example during anaerobic growth of homofermentative *Lactobacillus* spp. on a meat surface, can induce a metabolic switch from homo- to heterolactic fermentation (Borch *et al.* 1991). The metabolic activity of LAB results in spoilage appearing as sour, off-flavours, off-odours, milky exudates, slime production, swelling of the package through gas production and discolouration such as greening (Holzapfel 1998; Samelis *et al.* 2000). *Brochotrix thermosphacta* may also form a dominant part of the spoilage flora depending on the film permeability and the residual oxygen obtained through the vacuum process (Borch *et al.* 1996; Samelis *et al.* 2000). The spoilage pattern of *B. thermosphacta* associated with anaerobic metabolism is slower developing than that associated with aerobic growth (Pin *et al.* 2002). *Brochotrix thermosphacta* is homofermentative and produces L(+)-lactic acid from glucose, but under glucose limitation, small amounts of other metabolites are detected (Stiles and Holzapfel 1997). The main metabolites resulting from consumption of glucose under anaerobic conditions are lactic acid and ethanol, but no acetoïn and only small or no amounts of short-chain fatty acids (Pin *et al.* 2002). *Brochotrix thermosphacta* is not competitive under anaerobic conditions and it is rapidly outgrown by lactobacilli (especially *Lact. sakei* and *Lact. curvatus*) in refrigerated vacuum-packaged meat products (Stiles and Holzapfel 1997).

The study presented here has been performed in the framework of a screening for protective cultures demonstrating antagonism towards spoilage organisms associated with cooked cured meat products (Devlieghere *et al.* 2004; Vermeiren *et al.* 2004). An in-depth study of the growth characteristics of and product formation through heterofermentative LAB and *B. thermosphacta* has been set up. Their growth was characterized in a liquid broth at 7°C under anaerobic conditions to compare the spoilage organisms concerning their growth rate, acidifying character and metabolite production under conditions not causing glucose depletion. Further, the spoilage organisms were inoculated on a model cooked ham product to (i) establish whether the model cooked ham supported growth of the spoiling strains, (ii) characterize the spoilage phenomena and (iii) establish the relationship between microbial growth, pH, metabolite production and sensory changes.

MATERIALS AND METHODS

Bacterial strains and preparation of inoculum

Nine spoilage organisms, typically associated with vacuum-packaged cooked cured meat products, were chosen for this study and presented in Table 1. Stock cultures of the strains were maintained on de Man Rogosa Sharpe agar (MRS; Oxoid) agar or tryptone soya agar (TSA; Oxoid) slants at 7°C and revived by transferring a loop inoculum into 5 ml MRS (Oxoid) broth or brain–heart infusion (BHI; Oxoid) broth followed by incubation at 30°C for 24 h.

Growth, acidification profile and metabolite production in a liquid broth under anaerobic refrigerated conditions

The aim of this experiment was to characterize the nine spoilage organisms and to compare their growth characteristics, acidifying character and metabolite production profile in a broth at 7°C under anaerobic conditions at a pH and salt concentration occurring in cooked meat products. Therefore, strains were inoculated at 10^5 CFU ml^{-1} in an adapted BHI broth (pH 6·2 and 3% of NaCl). Growth of the cultures was followed during storage under an atmosphere of 100% N_2 at 7°C to simulate refrigerated vacuum-packaged conditions. The adapted BHI broth consisted of BHI (37 g l^{-1}) supplemented with 4 g l^{-1} yeast extract (Oxoid), 18 g l^{-1} D-(+)-glucose (Sigma), 1 ml l^{-1} Tween 80 (Sigma), 0·2 g l^{-1} $MgSO_4.7H_2O$ (Sigma) and 0·04 g l^{-1} $MnSO_4{\cdot}H_2O$ (Sigma). Additional NaCl was added to obtain a level of 3% of NaCl. The broth was not formulated to simulate cooked ham as the broth does not contain nitrite, contains a higher amount of glucose and has another buffering capacity than cooked ham. This experiment should be seen as a prescreening of the strains for the further ham model experiments. Twice a day, samples of 5 ml were taken to determine cell number and pH. Cell numbers were determined by plating on MRS agar by using a Spiral

Table 1 Overview of strains used in this study

Strain	Code	Origin (Reference)	Obtained from
Leuconostoc mesenteroides subsp. *mesenteroides*	LM2	Fermented olives	LMG 6893
Leuconostoc mesenteroides subsp. *dextranicum*	LM3	Not reported	LMG 6908
Leuconostoc mesenteroides subsp. *mesenteroides*	LM4	Vacuum-packaged smoked turkey fillet	LFMFP 666
Lactobacillus sakei subsp. *carnosum* GERT 15	LS2	Cooked ham (Devlieghere *et al.* 1998)	LFMFP 217
Leuconostoc citreum	LC1	Not reported	LMG 9824
Leuconostoc carnosum	LC2	Vacuum-packaged beef	LMG 11498
Weisella viridiscens	WV1	Frankfurters	LMG 13093
Brochotrix thermosphacta	BT1	Cooked ham	LFMFP 230
Brochotrix thermosphacta	BT2	Vacuum-packaged turkey fillet	LFMFP 227

LMG, Laboratory Microbiology Gent (Gent, Belgium); LFMFP, Laboratory of Food Microbiology and Food Preservation (Gent, Belgium).

Plater (Model D; Spiral Systems Inc., Cincinnati, OH, USA) and pH measurements were carried out by using a pH electrode (type 763; Knickn, Berlin, Germany). At the end of each growth experiment, when the pH was changing not more than 0·01 pH units in 24 h, a sample was taken for the determination of the concentration of glucose and metabolites (lactic acid, acetic acid, propionic acid and ethanol). This determination was performed by using a high-performance liquid chromatograph, isocratically with a cation exchange column (Aminex HPX-87H; Bio-Rad Laboratories, Nazareth, Belgium) at a flow rate of 0·6 ml min^{-1} of 5 mmol l^{-1} H_2SO_4 at 35°C and a run time of 25 min. The HPLC equipment consisted of a pump (type 307; Gilson, Den Haag, the Netherlands), an injector (Rheodyne 9096, Bensheim, Germany) with a 20-μl loop and a refractive index detector (type 132; Gilson). Growth curves were modelled by the model of Baranyi and Roberts (1994) and pH curves by a modified Gompertz equation used by Linton *et al.* (1995) for modelling survival curves of *Listeria monocytogenes* and adjusted (Vermeiren *et al.* 2004) into:

$$A \times \exp\left\{-\exp\left[\frac{\mathrm{AR} \times 2{\cdot}718281828 \times (S-t)}{A}+1\right]\right\} - A \times \exp\left[-\exp\left(\frac{\mathrm{AR} \times 2{\cdot}718281828 \times S}{A}+1\right)\right]$$

where A is the tail of the sigmoid curve or final pH; t is the time (h); S is the shoulder of the sigmoid curve (h), AR is the acidification rate (h^{-1}) or slope of the linear part of the sigmoid curve.

In this way estimations for generation time (h), lag phase (h), acidification rate (AR) (h^{-1}), time to acidification starting from 10^6 CFU ml^{-1} (t_{ac-6}) (h) and depth of acidification could be made.

Behaviour on a model cooked ham product

A model cooked ham product was manufactured on a semi-industrial scale at Dera Food Technology N.V. (Bornem, Belgium) with the following recipe: 80% of pork meat, 20% of water, 18 g kg^{-1} nitrited salt (containing 0·6% of nitrite), 5 g kg^{-1} Deraphos C107 (potassium and sodium-, di-, tri- and polyphosphates) (Dera Food Technology N.V., Bornem, Belgium) and 0·5 g kg^{-1} Na-ascorbate. After boning and defatting, hams were cut in pieces of ±10 cm to 10 cm to 10 cm. To ensure homogeneity of the meat surface, these pieces were homogenized and further minced to 20 mm and finally cuttered in a vacuum bowl cutter (Kilia, Neumünster, Germany) together with the nitrited salt and other ingredients. The cutter mixture was filled in a cook-in-casing to a final diameter of 100 mm and tempered for minimum 2 h at 4°C before pasteurization occurred at 75°C to a core temperature of 70°C in a cooking chamber (Kerres, Sulzbach Murr, Germany) during 2 h and 45 min. After cooling at 4°C, cooked ham sausages were sliced with a nonautomatic slicer (Omas, Oggiona S. Stefano, Italy) in slices of 2 mm thickness (±20 g/slice). In packages of 25 slices/package the product was quick-frozen in a blast freezer (Friginox-Le Froid Professionnel; Frispeed SR-range, Villevaliers, France) at −40°C to a core temperature of −10°C to avoid formation of large ice crystals and finally further stored in a freezing room at −18°C. When an experiment started, the necessary amount of product was transferred from the freezer to a cooler at −3°C for 48 h and later at 4°C for 24 h. The chemical characteristics of the cooked ham were as follows: 24·60 ± 0·63% of dry matter, 2·68 ± 0·02% of NaCl (on aqueous phase), pH of 6·06 ± 0·06 and a water activity of 0·9831 ± 0·001.

The model cooked ham product was inoculated with the nine spoilage organisms at a level of 10^4–10^5 CFU g^{-1} in three consecutive experiments. Each experiment consisted of four series: one blank series of noninoculated ham and three series of ham inoculated with one of the nine strains. Each series was performed in triplicate. The inoculum was subcultured twice (24 h, 30°C) in 5 ml MRS (for LAB) or BHI (for *B. thermosphacta*) broth. To reach an inoculation level of 10^4–10^5 CFU g^{-1}, 200 μl of the appropriate dilution of the 24 h culture was divided over and spread on the

surface of eight slices (110 g per eight slices) of cooked ham. After inoculation, slices were vacuum-packaged (eight slices per package) and stored at 7 ± 1°C in a ventilated refrigerator. Packaging was performed using a Multivac A300/42 (Hagenmüller, Wolfertschwenden, Germany) gas packaging machine in a high barrier film (NX90; Euralpak, Wommelgem, Belgium) of 90 μm thickness with an oxygen transmission rate of 5·2 ml m^{-2} 24 h^{-1} atm^{-1} at 23°C and 85% of relative humidity.

At day 0, 2, 6, 9, 13, 20, 27, 34 and 41 of the storage period, cooked ham samples were analysed for growth of the inoculated strain, pH and concentration of metabolites. Furthermore, the sensory characteristics were evaluated.

For the microbial analyses, a sample of 15 g of ham was taken aseptically and a decimal dilution series in maximum recovery diluent [8·5 g l^{-1} NaCl (VWR International, Amsterdam, the Netherlands) and 1 g l^{-1} peptone (Oxoid)] was prepared to plate the appropriate dilutions on MRS agar (aerobic incubation at 22°C for 3–5 days) and M5 agar (anaerobic incubation at 30°C for 2 days) to determine the level of LAB. The M5 agar differentiates between homo- and heterofermentative LAB (Zuniga *et al.* 1993). The blank series was also plated on respectively plate count agar (PCA; Oxoid) (aerobic incubation at 22°C for 3–5 days), reinforced clostridial Agar (RCA; Oxoid) (anaerobic incubation at 37°C for 3–5 days) and yeast glucose chloramphenicol agar (YGC; Bio-Rad) (aerobic incubation at 22°C for 3–5 days) to determine respectively total aerobic psychrotrophic count, total anaerobic count and number of yeasts and moulds. In case of inoculation with *B. thermosphacta*, STAA agar (Oxoid) (aerobic incubation for 2–3 days at 22°C) supplemented with STAA (streptomycin sulphate, thallous acetate, actidione, agar) selective supplement (Oxoid) was used.

pH measurements and HPLC analyses were performed as described earlier. Before HPLC analysis, meat samples were subjected to an extraction procedure: a 10-g sample was homogenized with 50 ml of distilled water, 5 ml of Carrez I (0·407 mol l^{-1} $K_4Fe^{II}(CN)_6$) (Sigma) and 5 ml of Carrez II (0·814 mol l^{-1} ZnSO4) (Merck, Amsterdam, the Netherlands) and finally filled up to 100 ml with distilled water. The deproteinized mixture was filtered through a 125 mm diameter filter (Schleicher & Schuell, Dassel, Germany) and filtered again through a HPLC syringe 0·2 μm filter (Alltech, Capelle aan den ljssel, the Netherlands) before injection.

Cooked ham samples were evaluated by a trained sensory panel (nine persons) using a scoring method. Attributes were odour, acid odour, rot odour, taste, acid taste, general appearance, slimy appearance and colour. Attribute scales varied from 1 to 9 with 1 being very good, 5 the limit of acceptability and 9 very bad. A score above 5 indicated the sample being unacceptable. Finally, the panel was asked to evaluate the fitness for human consumption. If five or more of the nine persons considered a sample unfit, the sensorial quality was considered as to be rejected.

RESULTS

Growth, acidification profile and metabolite production in a liquid broth under anaerobic refrigerated conditions

For the nine spoilage organisms, growth curves and pH curves were obtained. By using the model of Baranyi and Roberts (1994) an estimation for the growth rate (h^{-1}) and lag phase (h) was calculated. The pH-model resulted in a shoulder S (h), an acidification rate AR (h^{-1}) and a tail A or final pH, used to calculate the acidification depth, being the difference between the initial pH (6·2) and the estimated final pH. To obtain a parameter indicating the time before acidification in the medium starts which is independent from the initial cell number or inoculum (varying between 10^5–10^6 CFU ml^{-1}), the shoulder obtained from the pH-model was corrected for the time necessary to reach 10^6 CFU ml^{-1} starting from the initial count. In this way, the time to acidification starting from 10^6 CFU ml^{-1} (t_{ac-6}) was calculated. The most important model parameters are summarized in Table 2. Figure 1 shows the levels of lactic acid, acetic acid, propionic acid, ethanol and the level of glucose respectively produced and consumed at the end of the growth experiment. The pattern of metabolite production reflects the homo- or heterofermentative character of the tested strains.

Behaviour on a model cooked ham product

The noninoculated cooked ham had an initial microbial contamination with LAB, enumerated on M5 agar, of 1–4·2 × 10^2 CFU g^{-1}. Aerobic count and LAB count were of the same magnitude and no yeasts or moulds were detected. Near the end of the storage period, the level of endogenous LAB increased up to 10^4–10^5 CFU g^{-1}. The initial microbial load was very low compared with the inoculation level of 10^4–10^5 CFU g^{-1}. Furthermore, dominance of the inoculated strains over the background flora was confirmed by identical API-profiles of the inoculum and isolates (results not shown). Figure 2 shows the growth of the tested strains, as enumerated on M5 agar for LAB or STAA for *B. thermosphacta*, on the model cooked ham product.

Most strains reached a maximal population of *ca* 10^7–10^8 CFU g^{-1} on the cooked ham. The most rapidly growing organisms were both *Leuc. mesenteroides* strains LM2 and LM4, followed by both *B. thermosphacta* strains BT1 and BT2. Strains LS2 and WV1 had a comparable but slower

Table 2 Model parameters of the growth and acidification experiment for the nine spoilage organisms in adapted BHI broth at 7°C under anaerobic conditions

Strain	Lag phase (h)	Generation time (h)	Time to acidification t_{ac-6} (h)*	Acidification rate (h^{-1})	Depth of acidification†
LS2	40·88 ± 4·11	3·70 ± 0·28	21·10 ± 2·87	0·033 ± 0·001	2·16 ± 0·02
LM2	33·21 ± 3·77	3·77 ± 0·37	46·39 ± 3·56	0·021 ± 0·000	1·77 ± 0·01
LM3	354·19 ± 32·55	10·90 ± 6·08	48·46 ± 4·29	0·011 ± 0·000	1·91 ± 0·04
LM4	40·44 ± 2·41	4·53 ± 0·33	49·52 ± 8·08	0·029 ± 0·001	2·10 ± 0·01
LC1	32·62 ± 4·75	5·82 ± 0·48	43·24 ± 1·61	0·024 ± 0·001	2·00 ± 0·01
LC2	52·29 ± 12·22	10·78 ± 1·05	76·93 ± 2·99	0·014 ± 0·000	1·90 ± 0·07
WV1	46·67 ± 1·09	5·61 ± 0·30	67·96 ± 1·35	0·022 ± 0·001	1·94 ± 0·01
BT1	11·98 ± 0·03	2·00 ± 0·16	25·53 ± 1·25	0·034 ± 0·001	1·63 ± 0·04
BT2	9·19 ± 1·15	2·39 ± 0·28	29·08 ± 0·52	0·037 ± 0·001	1·66 ± 0·01

*Starting from 10^6 CFU ml^{-1}.
†Difference between initial pH (6·2) and estimated final pH.

growth than LM2, LM4, BT1 and BT2. Strains LC1, LC2 and LM3 grew very slowly on the cooked ham and reached a level of *ca* 10^7 CFU g^{-1} only after 27 days for LC1 and after 41 days for LM3 and LC2. Strain LM3 was identified as *Leuc. mesenteroides* subsp. *dextranicum* whereas LM2 and LM4 were identified as *Leuc. mesenteroides* subsp. *mesenteroides*.

The pH of the cooked ham was *ca* 6·05 ± 0·02 and, for most strains, decreased near the end of the storage period (Fig. 3). Whereas the pH of the noninoculated ham and ham inoculated with LC1, LC2 and LM3 did not decrease significantly with regard to the initial pH-value, significant differences with regard to the initial pH value were observed, for LS2 on day 41, for LM2 from day 13 onwards, for LM4 from day 20 onwards and for BT1 and BT2 on day 27. The total pH decrease was, however, very limited and varied between 0·1 and 0·2 pH units. This can easily be understood taking into account the small level of glucose (0·10 ± 0·02%) initially present in the cooked ham.

Near the end of the storage period, the glucose level of the cooked ham decreased and most rapidly for the fast growing strains. For LM2 and LM4, no glucose was found anymore in the ham from the 20th day onwards. The noninoculated ham and the ham with the slow-growing strains LM3 and LC2 showed no significant decrease in glucose level compared with the initial glucose level. A significant decrease in glucose with regard to the initial glucose concentration was observed for LM2, LM4 and BT1 from day 20 onwards and for LS2 from day 41 onwards.

For none of the strains, a significant lactic acid production could be observed. Some small amounts of acetic acid, propionic acid and ethanol were detected for some strains near the end of the storage period. However, these levels were accompanied by large 95% confidence intervals and consequently the production was not always found to be statistically significant. A slightly increasing but nevertheless not statistically significant trend in acetic acid concentration as a function of time was noticed for LM2 from day 13

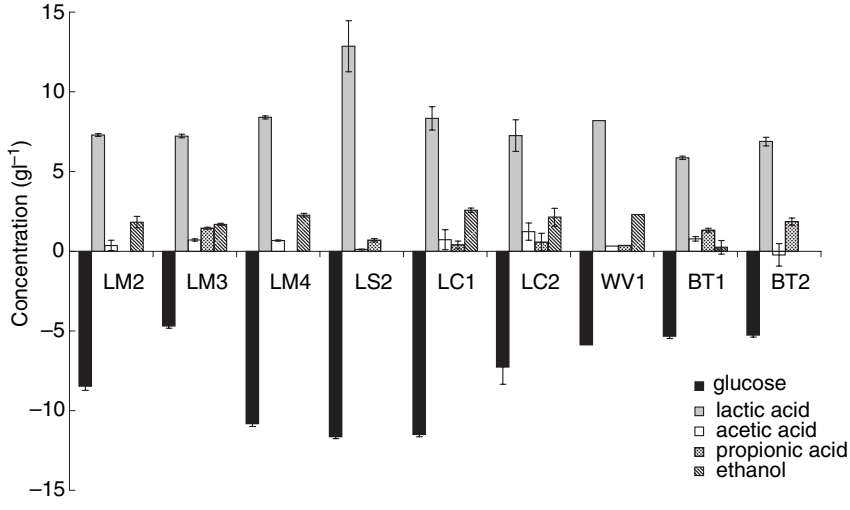

Fig. 1 Production of metabolites and consumption of glucose at the end of the growth of the nine spoilage organisms in adapted BHI broth at 7°C under anaerobic conditions

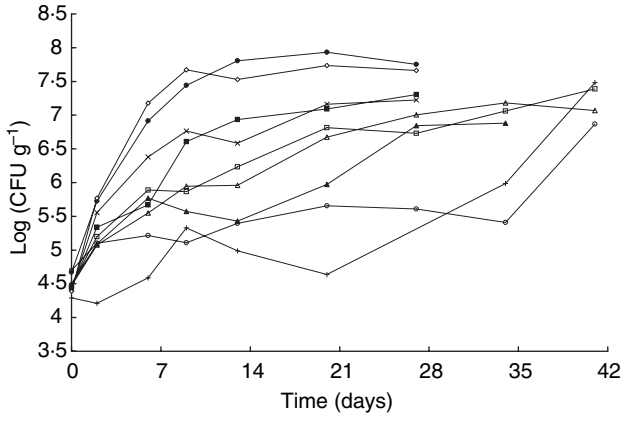

Fig. 2 Growth of the nine spoilage organisms on the vacuum-packaged model cooked ham product during storage at 7°C. Strains: (□) LS2, (▲) LC1, (△) WV1, (●) LM2, (◇) LM4, (◯) LC2, (+) LM3, (■) BT1 and (×) BT2

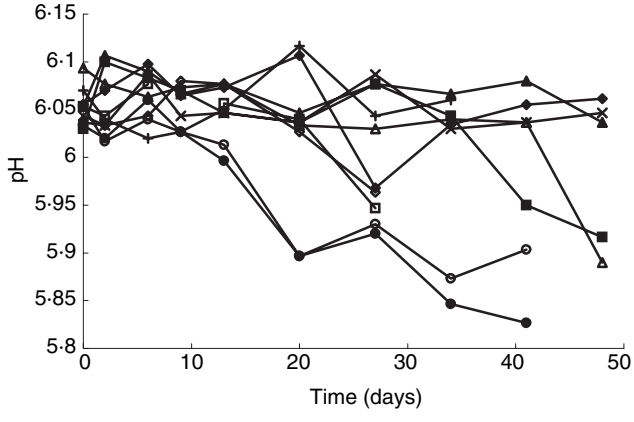

Fig. 3 pH evolution of the vacuum-packaged model cooked ham inoculated with the nine spoilage organisms during storage at 7°C. Strains: (■) LS2, (▲) LC1, (△) WV1, (●) LM2, (◯) LM4, (×) LC2, (◇) BT1, (□) BT2, (♦) blank and (+) LM3

onwards and for LM4 from day 9 onwards. Concerning ethanol production, a similar trend was observed for LM2, LM4 and BT2 from day 13 onwards and related to propionic acid production, for LS2 and LC1 from day 41 onwards and for LM4 and LC2 from day 13 onwards. Especially the growth of strains LM2 and LM4 resulted in the production of these heterofermentative end products.

Table 3 gives the day of rejection and the mean score of the sensory panel for the different sensory attributes at the day of rejection or in case of no rejection on the last day of the storage period. The day of rejection is related to the growth rate of the strains. Ham with the fast growing strains BT1, BT2, LM2 and LM4 was rejected early in the storage period, while ham containing the slow growing strains was acceptable almost until the end of the storage period. Rejection was based mainly on the attributes odour, taste and acid taste. Sensory deviations could not always be described as acid but for some strains (LM3) a more fermented and rotten taste/odour was observed. Highest scores were given for LC1 and LM3 indicating that these organisms caused the most intensive spoilage, although they were growing rather slow. For all LAB, scores for general appearance, slime production and colour never reached a value higher than 3·50, 2·78 and 2·78, respectively, indicating that inoculation with the strains did not influence the colour and general appearance of the cooked ham in a negative way and that the strains did not produce slime on the surface of the product.

DISCUSSION

Growth, acidification profile and metabolite production in a liquid broth under anaerobic refrigerated conditions

All spoilage organisms were able to grow in the adapted BHI broth at 7°C under anaerobic conditions, but large differences in growth characteristics could be observed. The most rapidly growing strains were both *B. thermosphacta* strains followed by both *Leuc. mesenteroides* subsp. *mesenteroides* strains. The *Leuc. carnosum* and *Leuc. mesenteroides* subsp. *dextranicum* strains grew slowly. The acidification rate was related to the growth rate as the fastest growing strains were most rapidly acidifying the medium. However, the acidification depth was more related to the pattern of metabolite production as the homofermentative LS2 acidified the medium to a greater extent than all the heterofermentative LAB. *Brochotrix* spp. acidified the medium to a lesser extent compared with the LAB as these strains also produced lower levels of lactic acid than the LAB did. All strains converted the glucose into lactic acid as the major end product, while the heterofermentative LAB and both *B. thermosphacta* strains also produced other metabolites such as ethanol and acetic acid.

Few studies have investigated the behaviour of several spoilage causing LAB and *B. thermosphacta*, as has been performed in this experiment. Blickstad and Molin (1984) investigated the growth and end product formation in fermenter cultures of *B. thermosphacta*, *Weisella viridiscens* and a homofermentative *Lactobacillus* spp. in different gaseous atmospheres, including N_2 and 5%CO_2 + 95%N_2. However, results are difficult to compare as experiments were performed at 25°C. In agreement with our results, all test strains produced under anaerobic conditions mainly lactic acid and *W. viridiscens* also produced ethanol while *B. thermosphacta* produced small amounts of ethanol. Blickstad (1983) did investigate the same three organisms as in the study of Blickstad and Molin (1984) but under

Table 3 Scores for the different sensory attributes at the day of rejection or in case of no rejection at the 48th day of the storage experiment

Strain	Day of rejection	Odour	Acid odour	Rot odour	Taste	Acid taste	Slime	Colour	General appearance	% Yes*
Blank 1	34	6·00	5·11	4·51	6·11	5·11	2·78	2·78	3·22	22·2
LS2	34	5·11	3·67	3·29	6·00	5·11	1·67	2·67	2·89	22·2
LC1	34	7·22	5·33	4·71	7·67	6·22	2·33	2·78	3·44	0·0
WV1	34	5·89	4·78	4·37	5·67	5·11	2·78	4·22	4·78	22·2
Blank 2	41	2·38	1·75	1·63	3·50	3·00	1·75	2·38	2·38	62·50
LC2	>41	4·13	2·25	2·13	4·50	3·38	2·00	2·25	2·63	50·0
LM2	20	6·33	5·56	4·56	5·67	5·22	2·22	2·56	2·89	11·1
LM4	20	5·22	5·22	3·78	4·67	4·56	2·67	2·56	3·11	44·4
Blank 3	41	6·43	3·57	4·86	7·14	5·86	1·86	2·29	2·57	14·3
LM3	41	7·57	4·43	6·29	8·14	5·86	2·00	2·14	2·43	0·0
BT1	13	5·75	4·75	4·75	6·5	6·13	2·50	2·75	3·38	37·5
BT2	13	6·25	4·75	4·63	5·63	5·13	2·13	2·63	3·50	25·0

*% of the panel members willing to consume the product.

anaerobic conditions at pH 6·3 and 8°C in a complex medium with 2% of glucose and no nitrite, thus very similar to the conditions of this experiment. The results for *W. viridiscens* correspond very well. Blickstad (1983) found for *W. viridiscens* a growth rate of 0·06 h^{-1}, while in our study a very similar growth rate of 0·054 ± 0·003 h^{-1} was noticed and main metabolites were lactic acid and ethanol in both studies. Growth rates for *B. thermosphacta* do not correspond that well, as both *B. thermosphacta* strains grew very fast in our study, while Blickstad (1983) found a growth rate comparable with that of *W. viridiscens.* This might be attributed to inter-strain variations within the *Brochotrix* species.

Behaviour on a model cooked ham product

On the model cooked ham, the most rapidly growing strains were both *Leuc. mesenteroides* subsp. *mesenteroides* strains followed by both *B. thermosphacta* strains. This is in contradiction with the broth experiment where the *B. thermosphacta* strains grew more rapidly than the *Leuc. mesenteroides* strains. As inoculum levels were similar, it might be that the model cooked ham substrate is better supporting the growth of *Leuc. mesenteroides* strains than of *B. thermosphacta* strains. A possible explanation might be the presence of residual nitrite in the cooked ham product, while this compound was not present in the adapted BHI broth. It is known for some time that *B. thermosphacta* is more sensitive towards nitrite than LAB are (Gardner 1981). Other differences between the broth and the model-cooked ham are the different level of glucose and the difference in buffering capacity. Furthermore, surface growth is difficult to compare with growth in a liquid broth. *Lactobacillus sakei* has been identified as the specific spoilage organism of cooked meat products but on this model cooked ham it did not grow the fastest in comparison with other spoilage causing LAB. It has to be mentioned that this study was a pure culture study and is not showing which strain would grow the fastest when inoculating, for e.g. *Lact. sakei* and *Leuc. mesenteroides* together on the cooked ham. Similar to the broth experiment, *Leuc. carnosum* and *Leuc. mesenteroides* subsp. *dextranicum* strains grew slowly on the cooked ham.

Growth of the spoilage organisms resulted in a limited pH decrease and no significant lactic acid production could be observed. This can be understood taking into account the initial levels of glucose (0·10 ± 0·02%) and lactic acid (0·89 ± 0·15%). The latter level corresponds to the level of 0·76% lactate, mentioned by Stekelenburg and Kant-Muermans (2001), being a possible level naturally present in meat. As LAB can form maximum two moles of lactic acid by conversion of one mole of glucose, the maximum expected amount of lactic acid produced out of ±0·1% glucose is *ca* 0·1% of lactic acid, depending of the heterofermentative character of the strain. This level is very low compared with the initial level of lactic acid of 0·89% and taking into account a mean 95% confidence interval of ±0·2% on the lactic acid concentrations, no significant lactic acid production can be found. The small amount of lactic acid, which could be produced, is responsible for the limited decrease in pH. About 0·1% lactic acid is, from our experience, needed to create a pH drop of ±0·1 pH units in cooked ham.

Table 4 summarizes the number of LAB or *Brochotrix* spp., as enumerated on M5 agar or STAA, the pH and the concentration of some typical metabolites of the cooked ham at the day of rejection or in case of no rejection at the last day of the storage experiment.

Samples were considered unfit for human consumption after the LAB count had reached levels of 6·9–8·2 $\log_{10}$

Table 4 Summary of the cell number (log_{10} CFU g^{-1}), pH and concentration (g kg^{-1} of ham) of glucose and metabolites of the cooked ham at the day of rejection or in case of no rejection at the last day of the storage experiment

Strain	Rejected on day	Cell number	pH	Glucose	Lactic acid	Acetic acid	Propionic acid	Ethanol
BL1	34	5·0 ± 1·0	6·07 ± 0·08	0·22 ± 0·38	12·3 ± 3·8	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
LS2	34	7·1 ± 0·1	6·04 ± 0·01	0·91 ± 0·11	9·5 ± 0·9	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
LC1	34	6·9 ± 0·2	6·07 ± 0·02	0·90 ± 0·07	8·7 ± 2·3	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
WV1	34	7·2 ± 0·1	6·04 ± 0·02	0·55 ± 0·32	9·0 ± 2·5	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
BL2	41	3·2 ± 1·2	6·00 ± 0·01	0·82 ± 0·11	8·2 ± 1·7	1·76 ± 3·05	0·00 ± 0·00	0·00 ± 0·00
LC2	>41	6·9 ± 1·0	6·04 ± 0·06	0·67 ± 0·58	14·7 ± 6·2	0·00 ± 0·00	0·92 ± 1·59	0·00 ± 0·00
LM2	20	7·9 ± 0·4	5·90 ± 0·01	0·00 ± 0·00	9·9 ± 0·7	0·97 ± 0·90	0·00 ± 0·00	1·16 ± 1·09
LM4	20	8·2 ± 0·1	5·90 ± 0·03	0·00 ± 0·00	10·3 ± 0·6	0·42 ± 0·73	0·50 ± 0·86	1·63 ± 1·43
BL3	41	5·4 ± 0·7	6·01 ± 0·04	0·71 ± 0·23	8·3 ± 1·7	0·44 ± 0·76	0·00 ± 0·00	1·30 ± 2·25
LM3	41	7·5 ± 0·2	5·92 ± 0·09	0·00 ± 0·00	9·4 ± 7·5	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00
BT1	13	6·9 ± 0·3	6·08 ± 0·01	1·14 ± 0·11	8·58 ± 0·80	0·05 ± 0·09	0·00 ± 0·00	0·00 ± 0·00
BT2	13	6·6 ± 0·3	6·06 ± 0·02	1·10 ± 0·21	12·4 ± 3·9	0·00 ± 0·00	0·00 ± 0·00	0·94 ± 0·87

BL, blank.

CFU g^{-1}, corresponding to the findings of Korkeala *et al.* (1987) on cooked ring sausages. The highest maximum cell concentration was reached by LM2 and LM4. Cooked ham inoculated with *Brochotrix* strains was rejected when the cell number was *ca* 6·6–6·9 log_{10} CFU g^{-1}. The noninoculated cooked ham series were rejected at very low cell numbers, varying from 3·2 to 5·4 log_{10} CFU g^{-1}. Also cooked ham samples inoculated with strains LS2, LC1 and WV1 were rejected at rather low cell numbers of 7·1, 6·9 and 7·2 log_{10} CFU g^{-1} respectively. It might be that other types of spoilage, e.g. chemical spoilage was occurring after these prolonged storage explaining why these samples were rejected at a rather low level of LAB. At the moment of rejection, pH-values were for all strains, except for the three *Leuc. mesenteroides* strains, not significantly lower than the initial pH-value as glucose concentrations at that moment were also not significantly lowered. For the three *Leuc. mesenteroides* strains, pH-values at the day of rejection were lowered to a value of *ca* 5·90 and at that moment no glucose was present anymore in the ham. The main conclusion which can be drawn from the pattern of metabolite production at the day of rejection is that mainly *Leuc. mesenteroides* subsp. *mesenteroides* strains produced the typical heterofermentative end products. Cooked ham containing *B. thermosphacta* was rejected on day 13 and from that day on, ethanol was produced for BT2 but not for BT1. It is possible that the sensory deviations caused by *B. thermosphacta* were caused by other metabolites or volatiles that were not analysed in this study.

Egan *et al.* (1980) performed a comparable study on vacuum-packaged sliced cooked luncheon meat at 5°C. The tested LAB were a mix of four homofermentative and a mix of four heterofermentative LAB, but the identity was not further specified. Furthermore, the study of Egan *et al.* (1980) was less extended than our study as pH and metabolite production were not examined. In agreement with our study, Egan *et al.* (1980) concluded that *B. thermosphacta* caused rapid spoilage, that homofermentative LAB caused spoilage much more slowly and that heterofermentative LAB were intermediate in their effect. Another study to compare with is the study of Borch and Agerhem (1992), investigating the chemical, microbial and sensory changes during anaerobic cold (4°C) storage of raw beef inoculated with a homofermentative *Lactobacillus* spp. or a *Leuconostoc* spp. However, care must be taken when comparing as the food products of study differ strongly. In their study, both species reached a maximal population of 10^7 CFU cm^{-2} at the same moment, i.e. after 2 weeks whereas in our study, two *Leuconostoc* strains grew faster and a third one grew slower than the tested *Lactobacillus* strain. Borch and Agerhem (1992) also observed a drastic decrease in glucose concentration on beef slices inoculated with *Leuconostoc*, while the change in glucose concentration was less drastic for *Lactobacillus*. Table 4 reveals that our results correspond to these findings.

Because of the small initial level of glucose in our test product, being however a relevant level for CMP, and as a consequence limited pH decrease and lactic acid production, a poor correlation between pH, lactic acid concentration and cell number was observed. Cooked meat products can contain higher concentrations of glucose and then correlation between these parameters is more obvious, for e.g. in the studies of Korkeala *et al.* (1987, 1989, 1990). In our study, rejection of cooked ham samples inoculated with *Leuc. mesenteroides* subsp. *mesenteroides* was related to the production of the heterofermentative end products, mainly acetic acid and ethanol. However, a relation between formation of one of these metabolites and rejection by the

sensory panel was not found for all cooked ham series. In the case of *Leuc. mesenteroides* subsp. *mesenteroides*, glucose was depleted. Dainty (1996) noticed that when glucose becomes depleted other substrates begin to be metabolized. These include lactate, amino acids and creatine under aerobic storage and lactate and arginin during storage in vacuum or MAP. Amino acid catabolism produces a number of compounds including ammonia, aldehydes, phenols, indol and alcohols, all being volatile flavours (Borch *et al.* 1991).

In conclusion, the results of this pure culture study show that within a group of nine spoilage organisms, typically associated with anaerobically packaged cooked sliced meat products, *B. thermosphacta* and *Leuc. mesenteroides* subsp. *mesenteroides* seem to have the highest potential to cause rapid spoilage. This was confirmed in both, broth experiments and an inoculation study with a model cooked ham product. *Lactobacillus sakei*, identified as the specific spoilage organism of these types of meat products, was not the most rapidly growing organism on the model cooked ham. It has to be stressed that this study was a pure culture study and it is not showing which strain would grow the fastest in a real life situation when different types of spoiling LAB are present together on the cooked ham.

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