Influence of Anaerobes on Volatiles Accumulating in Vacuum Packaged Beef

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SUMMARY: Vacuum packaged (VP) beef held at chill temperatures shows a microflora dominated by lactic acid bacteria (LAB) which can cause sour spoilage during prolonged storage. Souring occurs weeks after maximum numbers of LAB have been reached hence simple microbial enumeration is of little use in assessing remaining shelf life. Gas-liquid chromatography (GLC) was used to study the levels of simple carbohydrate volatiles in the exudate of VP beef but the expected pattern of accumulation was not found. Volatile concentrations rose, then fell. Analysis of the microbial populations on the meat surface, and the barrier bag surface, showed Pseudomonas spp preferentially colonised the bag whilst anaerobes, Enterobacteriaceae and Brochothrix thermosphacta were equally distributed between the two surfaces. Studies with pure cultures in a model meat system showed that compounds produced by LAB could subsequently be metabolised by pseudomonads hence explaining the patterns seen in VP beef and rendering volatile concentrations unsuitable for the estimation of shelf life.

INTRODUCTION: Vacuum packaging (VP) of beef for chilled storage is a process of increasing popularity (Jay 1986). The process is usually applied to wholesale cuts i.e. primals, although smaller pieces can be successfully stored for shorter times (Madden and Moss 1987). Normally spoilage occurs due to the activity of lactic acid bacteria (LAB) causing souring (Siedeman et al. 1976, Sutherland et al. 1975). However as souring of VP beef occurs some time after numbers of LAB have reached their maximum the prediction of the remaining shelf life of VP beef from microbial numbers is not posssible. With aerobic spoilage the relationship between the numbers of the dominant spoilage flora and shelf life is well understood (Jay,1986). Thus enumeration of bacteria can be used to directly assesss quality and predict shelf life.

It was therefore decided to study the exudate of VP beef with the aim of searching for compounds which accumulated during the growth of LAB. It was proposed that certain compounds found might thus serve as indices of LAB activity and hence allow a rapid estimation of the remaining shelf life of VP beef.

MATERIALS AND METHODS: Striploins (Longissimus dorsi) from steers (approximately 2 years old) were collected 24h post-kill and transported to the laboratory for immediate use. Slices weighing about 1kg were packed in bags (25x50cm) of normal commercial quality as previously described (Madden and Moss,1987). Beef was stored for 10 weeks at $1^{o}C$ and model meat for up to 8 weeks at $4^{o}C$. Bags were sealed close to the meat to minimise the surface area available for gas exchange. Enumeration of microorganisms used Oxoid media; total viable count (TVC) on nutrient agar ($22^{o}C$,3d), pseudomonads on cephalosporidine, fucidin, cetrimide agar ($22^{o}C$,3d), total anaerobic count (TAC) on brain-heart infusion agar supplemented with 3g/l yeast extract (BHIYE)($22^{o}C$,3d), Brochothrix thermosphacta on STAA medium (Gardner 1964), and yeasts and

moulds on malt extract agar plus chloramphenicol (50mg/l)(22°C,3d). Anaerobes were cultured in a Forma Scientific Model 1024 anaerobic cabinet (Marietta, Ohio, U.S.A.) fitted with a cooler to maintain 22°C, and with gas mix 85% nitrogen, 10% hydrogen, 5% carbon dioxide. Gas-liquid chromatography used a Shimadzu GC-9A chromatograph (Dyson Instruments,Houghton-le-Spring,Tyne and Wear, G.B.) fitted with a 2m by 2mm I.D. glass column packed with Chromosorb 101, the carrier gas was nitrogen (60ml/min) and the column was isothermal (180°C). A flame ionisation detector was used and concentrations calculated using a Shimadzu CR3A integrator. Ethanol and acetic acid concentrations in model meat studies were determined using Boehringer test kits.

Counts on the bag surface were made by first swabbing an external area with 95% ethanol in sterile airflow cabinet. Once dry, an area of 16cm^2 was excised using a sterile template and scalpel. The excised film was placed in 90ml peptone saline diluent and mixed for 30s using an Ultra Turrax TP-18/80 blender (Janke+Kunkel, D7813 Staufen, Germany) to suspend attached microbes. The meat surface was sampled by the method of Williams (1967), using a tube of 10cm^2 area, after the bag had been removed and the exudate recovered. One ml of exudate was acidified with 5ul of 5M HCl then centrifuged at 10 000g (ave) for 10min. The supernatant was recovered and the centrifugation repeated. Two microlitres were injected onto the GC column. For model meat work BHIYE (15ml) was poured into plates then, when set, the agar discs were vacuum packed in 25 by 15cm pouches (40ml/m^2/d O_2 transmission, as with the bags above).

RESULTS AND DISCUSSION: To meet the aim of a simple analytical test on VP beef indicating the extent of LAB activity analyses were based on an the use of an isothermal gas chromatograph and exudate with no chemical modification other than acidification. Pre-columns were used to trap non volatile materials. Lactic acid could not be used to assess growth due to high endogenous levels in the meat, as well as its natural variability from animal to animal (Gill and Newton 1981). However GC analyses of C_2-C_6 compounds showed an unexpected pattern of rising concentrations followed by decreases, Fig1. Thus the volatiles studied were apparently being utilised and research turned to elucidating the fate of these compounds. More sophisticated GLC analyses failed to detect higher molecular weight compounds hence fermentations, such as those shown by Clostridium kluyveri (Gottschalk 1986) did not appear to occur. Thus oxidation was investigated.

Regressing $\log_{10}$(counts/cm^2) on the bag against those on the meat showed that coliforms, and B. thermosphacta all colonised the two surfaces equally i.e. the slope was not significantly different from 1. All regressions had $p < 0.001$. However for the pseudomonad count on the meat surface =1.41+0.6(bag surface counts). Thus pseudomonads preferentially colonise the bag surface, presumably to be where the oxygen concentration was highest, since these organisms are obligate aerobes. To allow comparative studies models for VP beef were studied and BHIYE discs selected. These were rendered anaerobic by overnight storage in the anaerobic cabinet. Fig2 shows the effect of added glucose; 0, 5, 10g/l, on lactate production by a L. isolate and a pseudomonad in VP BHIYE. From such studies, and a comparison with published values for glucose in meats (Gill 1982, McVeigh and Tarrant 1981) no additional glucose was

Fig1 Volatile production in VP beef

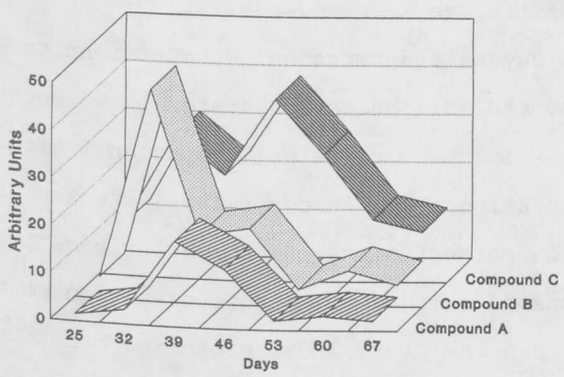
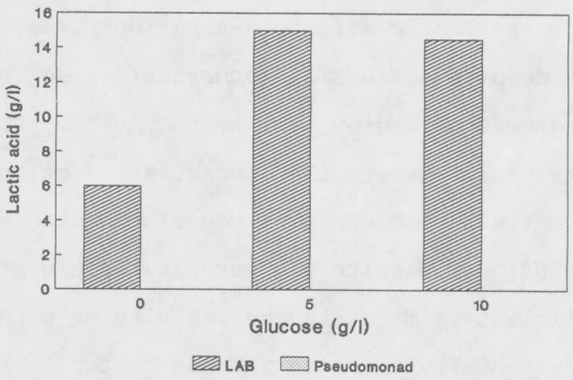


Fig2 Lactate production in VP BHIYE



deemed neccessary in this model system.

Flora analyses of 20 LAB isolates from VP beef using 60 phenotypic characters showed 5 to be Lactobacillus casei sbsp pseudoplantarum whilst 10 corresponded to Cluster 1 (non-aciduric streptobacteria) of Shaw and Harding (1984). The remaining isolates did not cluster with known species. However the majority of isolates were heterofermentative, producing ethanol or acetic acid (compound C, Fig1) as well as lactic acid. Inoculating the VP BHIYE with isolated LAB showed the simple pattern of product accumulation initially expected in VP. Hence the pseudomonad flora was investigated as a possible 'sink'. The ability of pseudomonads to utilise ethanol or lactic acid as carbon sources in vacuum packages was studied. Since pseudomonads selectively use glucose before lactic acid (Gill and Newton, 1980), and analyses

Fig3 Substrates used by pseudomonads in VP model meat

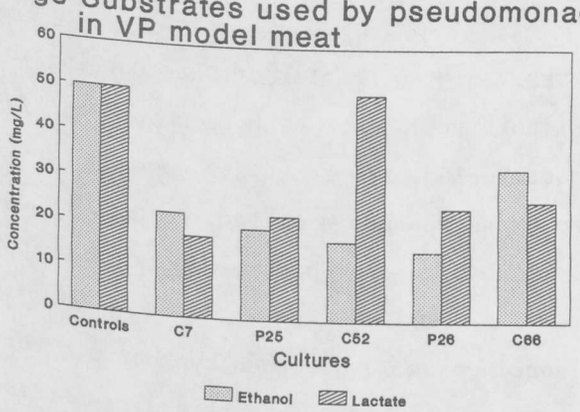
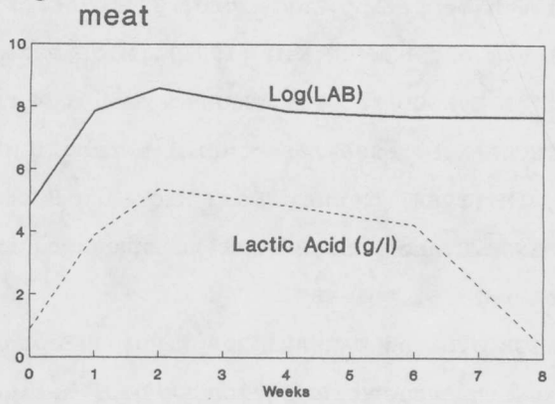


Fig4 Pseudomonad+LAB in VP model meat



showed 1-1.2g/l glucose in BHIYE, nutrient agar (NA) was used in this series of experiments. NA plus either lactate or ethanol (50mg/l) was rendered anaerobic and inoculated with single isolates then analysed after 56d (4°C). Concentrations fell, Fig3, confirming that pseudomonads could utilise these carbon sources. Susequently all packs were checked for culture purity and to ensure that all of the organisms used were obligate aerobes that therefore the concentration reductions must have been due to oxidation.

A final series of trials at 4°C used VP BHIYE inoculated with one LAB and one pseudomonad and Fig4 shows typical results. After opening the bag its entire contents were macerated and the counts are expressed g^{-1} of BHIYE. Pseudomonas counts were not significantly different from LAB counts after 2 weeks and are omitted for clarity. However it is clear that in the VP

model meat system the pseudomonads can utilise the fermentation end products of the LAB an hence the lactic acid concentration rises, then falls, as seen in Fig1.

Thus despite the use of commercial grade VP bags oxygen transmission is sufficient to allo pseudomonads to grow, mainly on the film surface, and utilise carbohydrates in VP beef ex udate. This renders the concentration of volatiles in the exudate useless as an indicator LAB activity and explains the fluctuating concentrations seen in our initial studies.

CONCLUSIONS: Despite the very low levels of oxgyen permeating vacuum packaging bags it is sufficient to maintain the activity of populations of obligately aerobic pseudomonads whic preferentially colonise the bag surface, rather than the meat. These organisms can thus oxidise LAB fermentation end products, presumably after simple sugars have been utilised b the dominant LAB population. Hence volatile compounds produced by the anaerobic population cannot serve as indicators of LAB activity and be linked to shelf life. Also enumeration o aerobes on the meat surface can cause a marked underestimate of the total population as th majority of the organisms will be present on the bag surface. Therefore estimates of aerob activity in VP meats must consider the bag surface as well as the meat.

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