BACTERIAL OFF-ODOURS IN VACUUM-PACKAGED LAMB

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SUMMARY

Studies with vacuum-packaged (VP) lamb showed sporadic development of off-odours which limited the storage life to less than half that of VP beef. The source of the off-odours was investigated using boned out lamb stored for 5 weeks in VP at 4°C. Beef striploins were used to provide a control microflora for comparison. The main microbiological difference appeared to be in the proportion of Enterobacteriaceae (abbreviated to enteros) present after storage. Lactic acid bacteria (LAB) comprised 98% of the microflora in beef samples but some lamb samples had about 70% LAB and 10% enteros. Odours from VP lamb were analysed and sulphur-based compounds typical of those produced by enteros were seen. Sterile lamb was inoculated with 80 pure cultures taken from the spoiled VP lamb and stored in VP. Sensory analysis of the packs confirmed that enteros were the dominant producers of off-odours. The principal species present were Serratia liquefaciens and Enterobacter aerogenes. Detailed studies on these organisms revealed an unusual tolerance of CO_2, for Gram-negative bacteria, at low temperatures and this property may have allowed their successful competition with the LAB.

Introduction

The rearing of sheep is of great financial importance to Northern Ireland (NI) with a population of over 2 500 000 animals reported annually since 1990. However currently exports are largely of live animals or chilled carcasses with few added value products being produced. Vacuum packaging of beef has helped add value locally to this commodity hence it was decided to study the effects of VP on local lamb since some companies who had conducted brief trials with VP lamb reported odour problems and consequent short shelf-life. Earlier work on VP beef (Madden and Moss 1984) showed properly packed meat of good quality could be stored for three months at 1°C with no adverse effects. Accordingly beef was selected as a 'control' meat for comparison with the lamb.

Materials and Methods

Beef striploins (Longissimus dorsi) were obtained less than 48h post-kill as were boned-out lamb necks. The meats were transported to the laboratory under refrigeration and vacuum packed in high barrier pouches as previously described (Madden and Moss 1984) within 4h. To ensure the climax community was present samples were stored for up to 5 weeks at 4°C. All experiments were conducted with duplicate samples and all were repeated at least twice. Microbiological analyses for total aerobic counts (TVC) and total anaerobic counts (TAC) were performed as previously described (Madden and Moss 1984) and Enterobacteriaceae were enumerated using overlaid pour plates of violet red bile agar with glucose (VRBG) incubated at 30°C for 48h. All final samples plated out for TAC (5 weeks incubation) had 20 colonies selected at random to be subsequently purified, identified, and used in off-odour production trials. Identification used the procedures described by Board et al. (1992).

Sterile lamb was produced using a ^{60}Co Chi-irradiation facility on site. A 'model meat' system was developed using brain heart infusion with yeast extract (BHIYE) (Baird and Patterson 1982) soldified with 1% agar to compare with meat and reduce sample-sample variation. After autoclaving (121°C, 15 min) and cooling the medium was poured into Petri dishes (approximately 30ml/dish). When cool the solid discs of medium were transferred to pouches for inoculation, evacuation and sealing. For gas backflushed packs a Multivac A300/42 vacuum packaging unit (Sepp Haggenmüller ICG, Wolfertschwenden, Germany) was used. This

machine removes air from the chamber then allows the selected gas, CO_2 in this case, to enter the chamber until a set pressure (expressed as a percentage of atmospheric) is reached. The bag is then sealed as normal.

Headspace analysis used a purge and trap system followed by separation on a capilliary gas chromatograph then mass spectrometry analysis (GCMS) on a Kratos MS25RFA. Growth temperature profiles were produced using a Toya gradient incubator over the range 0-40°C. Cultures were inoculated into BHIYE broth and nephelometry performed daily over a 2-week period.

Results and discussion

The basic properties of both beef and lamb in terms of pH and Aw showed no difference between the samples hence no meat from stressed animals was present (Tarrant 1981), and no carcasses had experience excessive drying in the chill. However during incubation in VP at 4°C some lamb showed much higher entero counts than did the beef . The possible causes of high entero counts in VP meats include high initial contamination, leaking packs and temperature abuse. All of these possibilities were investigated and none were present. Initial microbial loadings were acceptable and rigorous examination of packs, including gas chromatography of gas bubbles, showed no evidence of leaks. All of the incubators used were subject to logging of the air and pack temperatures and all variation was within acceptable limits with packs generally remaining at 4____°C.

The most conclusive evidence against physical problems came from the results of the microbial analyses of the beef packs, which were mixed with the lamb packs, where the normal LAB flora was dominant. Whilst the reasons for the proliferation of the enteros were not elucidated the effects of their presence were apparent from the odour of the lamb packs which was unpleasant and contrasted with the normal faint 'cheesy' odour noted in the beef packs.

A panel familiar with meat microbiology assessed the odour from packs of sterilised lamb inoculated with pure cultures picked from the TAC plates of the final samples of the lamb. From 80 samples of those giving an unpleasant odour 93% were found to be Gram negative bacteria. Characterisation showed the dominant species were Serratia liquefaciens and Enterobacter aerogenes. Hence isolates of these two were selected for detailed study.

In parallel with this work the volatiles had been subjected to GCMS but these analyses were hampered by a lack of standards for comparison with the profiles obtained. However the following compounds were detected; methane thiol, hydrogen sulphide, dimethyl sulphide and trimethyl sulphide. Thus the unpleasant nature of the off odours is unsurprising, and such compounds have been reported where the meat spoilage flora contains a significant level of enteros (Freeman et al. 1976, Patterson and Gibbs 1977). Since the GCMS analyses could not readily be made quantitative work concentrated on the two dominant odour-producers.

The temperature profiles of S. liquefaciens and E. aerogenes were studied in detail with 40 isolates of each on BHIYE broth being investigated. Typical profiles are shown on Fig 1. Both organisms were capable of growth in VP at 1oC on both the model meat and sterilised lamb. Thus whilst E. aerogenes had a mean optimum growth temperature of 35.5°C and was hence a mesophile it still was able to grow under refrigeration conditions. S. liquefaciens, however had an optimum growth temperature of 28.5°C and was hence a psychrotroph.

Enterobacter spp. have been found to grow at low temperatures in anaerobic conditions (Newton and Gill 1978) but at 5°C or below Lactobacillus spp. grew significantly faster. Rosset (1982) postulated that the dominance of the LAB was due to the low temperature, low oxygen content and increase in CO_2 levels. Normally Gram negative bacteria are susceptible to increases in CO_2 levels (Clark and Takacs, 1980), such as take place in VP during storage. Since only the level of CO_2 had not been investigated the growth of the two spoilage species under different levels of CO_2 was studied at 0°C and 4°C.

Under these conditions of storage virtually no growth was seen with 10 reference strains of Gram negative bacteria isolated from VP beef. However both S. liquefaciens (Fig 2) and E. aerogenes grew showing an unusual resistance to elevated levels of CO_2 . That of S. liquefaciens was especially marked with the organism leaving lag phase after 35d even at 0°C. E. aerogenes was less resistant but this may reflect its higher optimal growth temperature. However given the lack of growth shown by the reference organisms it appears that the ability of these two species to grow to significant levels in VP lamb is related to their ability to tolerate higher levels of CO_2 than the rest of the Gram negative population.

Conclusion

Lamb stored in vacuum packs may develop off-odours which limit its storage life. Detailed microbiological analyses showed that Gram-negative bacteria were probably responsible for these odours with E.aerogenes and

S. liquefaciens the dominant species. These species can grow well in anaerobic conditions at 4°C and below and possess an unusually high, for Gram negative bacteria, tolerance of elevated levels of CO_2. These properties, especially the latter, probably enable these organisms to avoid the normal dominance by lactic acid bacteria and cause organoleptic problems. However artificially raising the level of CO2 may ensure the problem does not arise.

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