GAS PRODUCTION BY PSYCHROTOLERANT CLOSTRIDIA ASSOCIATED WITH 'BLOWN PACK' SPOILAGE OF VACUUM PACKAGED BEEF

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Abstract – 'Blown pack' spoilage (BPS) is characterized by copious accumulation of gas in packs stored at chiller temperatures. The aim of this study was to determine whether 11 recently isolated species of psychrotolerant clostridia were able to cause blowing, i.e. swelling of vacuum packs of beef; and whether the pH of the meat had an effect on time of onset of BPS. Suspension of each species was inoculated to sterile beef steaks of three pH ranges; normal, pH 5.4-5.6; intermediate pH, 5.7-5.9; and high pH, > 6. All steaks were vacuum packaged and stored at 2 °C. Change of pack volumes during storage was monitored by water displacement. With high pH meat, none of the clostridial cultures caused pack swelling. With intermediate pH meat, the three packs that had been inoculated with *Clostridium estertheticum* showed swelling, starting from 14 d of storage, with a linear rate of increase of 5.7 ml/d; and one of the three packs that had been inoculated with *C. frigoris* showed moderate swelling after 37 d of storage. With normal pH meat only packs that had been inoculated with *C. estertheticum* showed significant swelling starting after 14 d of storage with a linear rate of increase of 6.5 ml/d.

Key Words – Meat pH, chiller temperature, blown packs.

I. INTRODUCTION

Blown pack spoilage (BPS) of vacuum packaged beef, lamb and venison is characterized by gross swelling of the packs at early times during storage at chiller temperatures [1, 2, 3]. This form of spoilage was originally attributed to a psychrotolerant, strictly anaerobic, spore forming organism, *Clostridium estertheticum*; but subsequently several other psychrotolerant *Clostridium* species have been isolated from or identified as being present in blown vacuum packs of meat [4]. It has generally been assumed that all these clostridia can cause BPS. However, only *C. estertheticum* has been shown to produce gas in the large amounts needed to cause gross swelling of packs. Therefore, for better understanding of how the various psychrotrophic clostridia may be involved in BPS, cultures of the organism were inoculated into vacuum packs of beef steaks of various pH, and the volumes of the packs during storage at 2 °C were monitored.

II. MATERIALS AND METHODS

Cultures

Clostridium algidixylanolyticum (BAA-156), *C. bowmanii* (BAA-581), *C. frigidicarnis* (BAA-154), *C. frigoris* (BAA-579), *C. lacusfryxellense* (BAA-580), *C. psychrophilum* (BAA-582) were obtained from the American Type Culture Collection (ATCC). *C. algoriphilum* (DSM 16153), *C. gasigenes* (DSM 12272), *C. tagluense* (DSM 17763), and *C. vincentii* (DSM 10228) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). All bacterial strains were revived and maintained in reinforced clostridial medium (RCM) that was prepared anaerobically in Hungate tubes. *C. psychrophilum*, *C. frigoris* and *C. algoriphilum* were cultivated at 4 °C, because their optimal growth temperatures are between 4-7 °C and the other 8 strains were cultivated at 10 °C. Inocula were prepared from log phase cultures grown at 4 °C or 10 °C to attain an absorbance at 600 nm of about 0.5. A ten-fold dilution was made by adding 1 ml of culture to 9 ml of prereduced RCM.

Preparation of steaks

Fresh inside round primal cuts of normal (5.4-5.6), intermediate (5.7-5.9) or high (6.1-6.4) pH from a local beef packing plant were each immersed in boiling water for 2 min to reduce the numbers of bacteria on their surfaces. Each pasteurized primal

was placed on a sterile tray and the ends were cut off. The primal was cut into steaks each 2 cm thick, with major surfaces measuring approximately 10x10 cm^2. For meat of each pH group, 36 steaks were prepared. Each steak was placed in a vacuum pouch made of an ethylene-vinyl alcohol copolymer/nylon laminate with an oxygen transmission rate of 63 cc/m2/24h/atm at 23 °C and 0% r.h. (3.0 mil standard vacuum pouch; West Coast FoodPak Systems Ltd, Vancouver, British Columbia, Canada). The vacuum pouch was evacuated and sealed to leave 10 cm between the steak and the seal. All pouches containing steaks were stored overnight in a cooler that was operated at 2 °C, to allow residual oxygen to be removed by the muscle tissue.

Inoculation and packaging of steaks

On the second day, the seal was cut from the bag and a 1 ml portion of an inoculum or 1 ml of 0.1% wt/vol peptone water was injected by inserting the needle of a gas tight syringe down the edge of the bag. The meat within the pouch was massaged for 5 s to distribute the inoculum over the surface of the meat without detachment of the film from the meat surface. Then, the pouch was evacuated to remove any air that had entered the part of the bag that did not contain meat, and the pack was sealed to leave < 3 cm between the steak and seal. The time between cutting a pack for inoculation operation and resealing it was < 1 min. Each culture or peptone water was inoculated onto three steaks of each pH range. All packs were stored at 2 °C for 8 weeks.

Determination of pack volumes and analysis of samples

The volumes of packs were determined by water displacement, as previously described [5], before they were placed in storage, after storage for 14 days, and subsequently at intervals of 3-4 days up to 8 weeks. All packs were opened at the end of incubation and exudates were withdrawn for determination of the pH.

III. RESULTS AND DISCUSSION

The initial volumes of packs were mostly 300 +/- 50 ml. The volumes determined at different times for packs containing meat that had not been inoculated with clostridia differed by up to 15 ml without any upward trend. For all packs containing high pH meat that had been inoculated with clostridia, the maximum increase in volumes was ≤17 ml (Figure 1; A).

For packs containing intermediate pH meat that had been inoculated with *C. estertheticum*, volumes had increased (>30 ml) at 17 d of storage and the rate of increase from 17 d onward was linear (R^2=0.92; Y=5.68x - 48.31 where Y=volume, x=days of storage). The three packs containing intermediate pH meat that had been inoculated with *C. frigoris* each behaved differently. There was no increase in the volume of one pack; one pack had increase in volume at 17 d (31 ml), but the increase stopped at 25 d (56 ml); the third pack had increased in volumes at 37 d of storage and the rate of increase from 37 d onward was linear (R^2=0.97; Y= 8.36x - 257.37). None of the other clostridia caused significant increase in volumes for packs containing intermediate pH meat (Figure 1; B).

For packs containing normal pH meat that had been inoculated with *C. estertheticum*, volumes had increased (>50 ml) at 17 d of storage and the rate of increase from 17 d onward was linear (R^2=0.89; Y=6.5x - 51.85 where Y=volume, x=days of storage). The maximum change in volume for the packs containing normal pH meat that had been inoculated with other clostridia was 8 ml, which was less than the change in volumes of controls.

pH values increased by 0.94 and 0.72 units, respectively, for exudates from the packs containing normal pH and intermediate pH meat that had been inoculated with *C. estertheticum*. pH values increased by 0.31 units for exudates from the packs containing intermediate pH meat that had been inoculated with *C. frigoris*. The change in pH was ≤ 0.18 and 0.15 for exudates from the packs that had been inoculated with other clostridia and the packs that had not been inoculated with clostridia, respectively.

Previous work has shown that *C. estertheticum* requires glucose for growth on meat; but when glucose is exhausted the organism ferments lactic acid, without growth but with the production of large volumes of gas and increase of the meat pH

[5]. Even so, if the organism does not attain numbers about 6 log cfu/cm^2 the amount of gas produced is insufficient to cause pack swelling during usual storage times for vacuum packaged meat and the meat pH is not noticeably affected [6]. The absence of swelling of any packs of high pH meat was probably due to the meat being deficient in glucose, so *C. estertheticum* did not reach numbers sufficient to produce pack swelling. The same may be true for the other clostridia, as all are closely related to *C. estertheticum* [6] and so may be similarly dependent on glucose to support their growth on meat. However, *C. frigoris* was the only other organism able to cause swelling of packs containing meat of lower pH, and to raise the pH of the meat. Its failure to cause swelling of packs of normal pH meat was probably due to the low PH being outside the range for growth of *C. frigoris.*

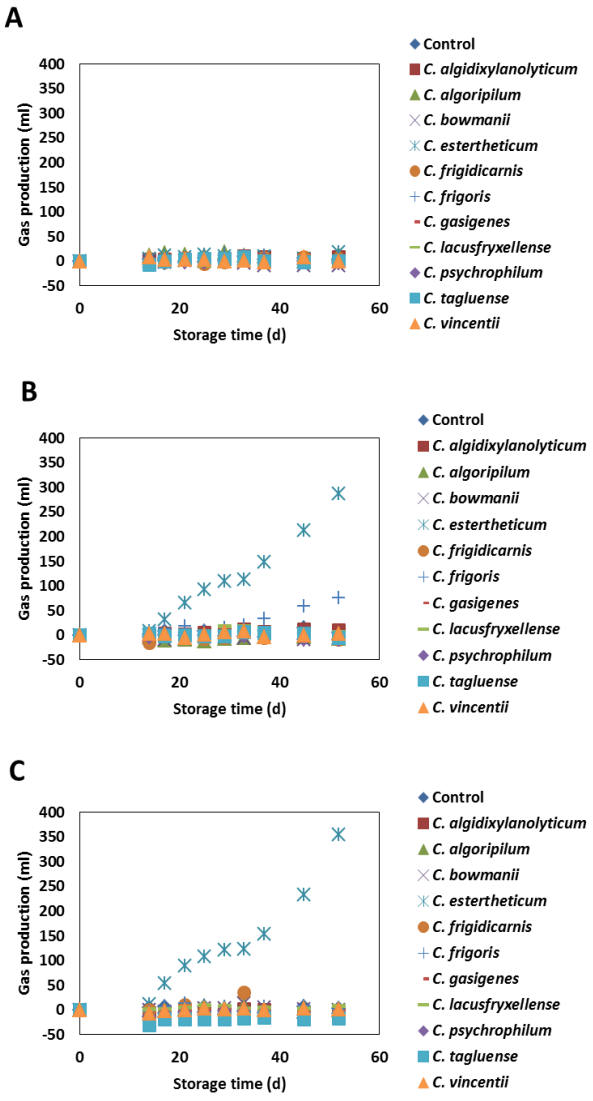

Figure 1. Changes during storage at 2 °C in the volumes of vacuum packs containing meat with high pH (A), borderline pH (B) and normal pH (C) that were inoculated with different strains of psychrotolerant clostridia or 0.1% of peptone water.

IV. CONCLUSION

C. estertheticum was apparently the only one of the tested organisms that was able to produce sufficient gas to cause pack swelling at early times, and *C. frigoris* was the only other organism to cause swelling at later times. Thus it appears that the early onset blown pack spoilage of vacuum packaged chilled meats, which is an increasing cause of commercial concern is due to *C. estertheticum. C. frigoris* and other gas producing organisms, including *Enterobacteriaceae* may cause pack swelling at later times; and those and other organisms may play a role in the off-odours and discolouration of meat in blown packs. However, what roles the other psychrotolerant clostridia may play in spoilage of vacuum packaged meat remains to be determined.

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