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An investigation of the effect of rapid slurry chilling on blown pack spoilage of vacuum packaged beef primals

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Running heading: Rapid slurry chilling to control blown pack spoilage

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Significance and Impact of the study: This study adds to our growing understanding of blown pack spoilage of vacuum packaged beef primals and suggests that rapid chilling of vacuum packaged beef primals is not a control option for the beef industry. The results suggest that neither eliminating the heat shrinkage step or rapid chilling of vacuum packaged beef, retard the time to blown pack spoilage.

Abstract

The aim of this study was to investigate if rapid slurry chilling would retard or prevent blown pack spoilage (BPS) of vacuum packaged beef primals. Beef primals were inoculated with *Clostridium estertheticum* subspp. *estertheticum* (DSMZ 8809), *C. estertheticum* subspp. *laramenise* (DSMZ 14864) and *C. gasigenes* (DSMZ 12272) and vacuum packaged with and without heat shrinkage (90°C for 3 seconds). These packs were then subject to immediate chilling in an ice slurry or using conventional blast chilling systems and stored at 2°C for up to 100 days. The onset and progress of BPS was monitored using the following scale; 0 - no gas bubbles in drip; 1 - gas bubbles in drip; 2 - loss of vacuum; 3 - "blown"; 4 - presence of sufficient gas inside the packs to produce pack distension and 5 - tightly stretched, "overblown" packs/ packs leaking. Rapid slurry chilling (as compared to conventional chilling) did not significantly affect ($P > 0.05$) the time to the onset or progress of BPS. It was therefore concluded that rapid chilling of vacuum packaged beef primals, using an ice slurry system, may not be used as a control intervention to prevent or retard blown pack spoilage.

Keywords: Beef, spoilage, blown pack spoilage, *Clostridium estertheticum*, *Clostridium gasigenes*, rapid chilling.

Introduction

Blown pack spoilage (BPS) occurs in correctly chilled vacuum packaged beef when psychrophilic *Clostridium* spp., such as *Clostridium estertheticum* and *Clostridium gasigenes*, grow and produce large volumes of gas, predominantly carbon dioxide. Meat spoiled in this way, which may also have a metallic sheen and putrid smell (Dainty et al.1989, has no commercial value and BPS is thus a major issue for the beef industry (Moschonas et al. 2009).

Controlling blown pack spoilage is difficult. These *Clostridium* spores are widespread in the beef abattoir environment (Moschonas et al. 2009) and an initial contamination level as low as 10 spores per cm^2 is sufficient to cause spoilage in vacuum packaged primals after 2-4 weeks at 0 – 2°C (Moschonas et al. 2011). Within the EU, carcass treatments (with the exception of lactic acid (2% v/v) which is not effective against bacterial spores), are not permitted. Thus current BPS control is reliant on the application of effective prerequisite programme (PRP) activities including actions to prevent cross-contamination from bovine hides and the environment and storage at low temperatures (-1.5°C) (Silva et al. 2012). Cleaning and disinfection procedures often include treatment with peroxyacetic acid (2%, v/v), a sporicidal agent that kills *Clostridium* spores if applied at the correct dilution. However, this is highly corrosive causing pitting on stainless steel surfaces.

Previous research established that heat shrinkage (typically 80°-90°C for 2-3 seconds), applied immediately after primals are vacuum packaged to remove pack corners (that could otherwise get caught in conveyor belts) and reduce drip loss during subsequent chilled

storage, activates BPS spores resulting in a reduced time to spoilage (Bell et al. 2001; Moschonas et al. 2011). Other factors such as the speed at which the surface temperature of the vacuum packaged primal is reduced post heat shrinkage may also influence the onset of blown pack spoilage. However, to the best of our knowledge, the influence of parameters such as beef primal chilling rate has not been investigated. A better understanding of the interaction between chilling temperature and blown pack spoilage could contribute to a reduced occurrence and protection against the large economic losses faced by the beef sector (Adam et al. 2010; Adam et al. 2013). The objective of this study was therefore to investigate the effect of rapid chilling (using an ice slurry system) on the time to blown pack spoilage of beef primals inoculated with *C. estertheticum* and *C. gasigenes*, vacuum packaged (with and without heat shrinkage) and stored at 2°C.

Results and Discussion

Neither the method of chilling (rapid v conventional),heat shrinkage treatment or combination thereof, significantly ($P > 0.05$) affected the time to the onset of spoilage (score 2) for any of the BPS *Clostridium* spp. tested (Table 1). A similar result was obtained with the time to full spoilage (score 5) except for packs inoculated with *C. estertheticum* (DSMZ 8809) which spoiled significantly ($p < 0.05$) faster when heat treated as compared to no heat treatment.

Rapid slurry chilling reduced the surface temperature of heat treated vacuum packaged primals from a mean of 21.2°C to 2 ± 0.5°C within 8 minutes, a full 83 minutes faster than using conventional chilling (Figure 1). The corresponding times to reduce the surface temperature from 21.2°C to 0°C were 76 and 174 minutes for rapid and conventional chilling, respectively. To the best of our knowledge this is the first time the effect of chilling method of vacuum packaged primals has been investigated and hence there are no similar studies

with which to compare our findings. However, the lack of any effect of chilling rate may be due to the psychrophilic nature of these organisms, suggesting that once germinated, low temperature may not be used as an efficient control strategy.

There are other studies on the effect of heat shrinkage on the time to the onset of BPS. Silva et al. (2012), for example, also reported that heat shrinkage did not reduce the time to the onset of BPS. In contrast other research has demonstrated significantly quicker spoilage after heat shrinkage. Bell et al. (2001) reported that post-packaging heat treatments had a significant effect ($P < 0.001$) on the time to spoilage by *Clostridium estertheticum* NCIMB 12511 and BPS isolates in their culture collection, with the time to gas production decreasing with increased temperature of treatment (70°C for 15s < 80°C for 5s < 90°C for 3s). Moschonas et al. (2011), using the same cultures as in our experiment, made a similar finding. The apparently contradictory findings in the various studies may be attributed to the ratio of spores to vegetative cells (Silva et al. 2001). Moreover, the presence of other spore germination triggers, such as L-lactate, L-valine or L-noraline may further complicate interpretation of the results from different studies (Adam et al. 2011).

In conclusion, rapid chilling of vacuum packaged beef primals using an ice slurry system is effective at reducing the surface temperature of beef primals but, if contaminated with BPS *Clostridium* spp., will not prevent or retard the time to onset of blown pack spoilage.

Materials and Methods

Microorganisms

Reference strains *Clostridium estertheticum* subsp. *estertheticum* (DSMZ 8809), *C. estertheticum* subsp. *laramiense* (DSMZ 14864) and *C. gasigenes* (DSMZ 12272) were

purchased as freeze dried cultures from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunchweig, Germany). Each strain was revived under anaerobic conditions in 10 ml pre-reduced Peptone Yeast Extract Glucose Starch (PYGS) broth (Lund et al., 1990), incubated at 4°C for 3 weeks. The purity of each strain was confirmed using culture based and molecular (PCR) techniques. By culture-based methods, 0.1 ml aliquots were plated on Columbia Blood Agar (CBA) (Oxoid, Basingstoke, UK) supplemented with 5% defibrinated horse blood (Cruinn Diagnostics, Dublin, Ireland) and incubated at 4°C for 3 weeks. Colony morphology was checked against the following criteria; round with often coarsely granulated margins, smooth, slightly raised, cream-white to greyish and semi-transparent to opaque colonies that are non-haemolytic for *C. estertheticum* subsp. *estertheticum* and β-haemolytic for *C. estertheticum* subsp. *laramiense*; grey-white and opaque, circular, raised, convex, shiny and smooth with β-haemolytic activity for *C. gasigenes*. Moreover, all strains were examined under the microscope and confirmed to be Gram-positive rods (Moschonas et al. 2009).

DNA Isolation

The purity of each culture was also tested using molecular methods. For DNA isolation, 4-5 colonies were removed from the CBA plates using a sterile loop and suspended in 1 ml of phosphate buffered saline (PBS; Oxoid) and centrifuged at 5000g for 10 minutes. 1 ml of phosphate buffered saline (PBS; Oxoid) was then used to wash the pellet. The sample was vortexed for 15 s and then centrifuged again. Genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Ltd, Crawley, UK) according to the manufacturer's recommended protocol for DNA extraction from Gram positive bacteria.

PCR detection

The extracted DNA was analysed by PCR using 16SF and 16SER primers for the detection of 16s rRNA gene fragments of *C. estertheticum* and 16SDBF and 16DBR primers for the detection of 16s rRNA gene fragments of *C. gasigenes* (Broda *et al*., 2009). All primers were purchased from MWG Biotech, Martinsried, Germany.

The PCR mix consisted of 25 µl of Taq PCR Master Mix (Qiagen, Ltd, Crawley, UK), 0.5 µl of each primer, 19 µl molecular-grade water and 5 µl template DNA. The final reaction volume was 50 µl. Amplifications were performed in a Gradient Cycler DNA Engine (MJ Research, Waltham, MA). After initial denaturation for 3 min at 93°C, target DNA was amplified in 30 cycles. Each cycle consisted of denaturation for 1 min at 92°C, annealing for 1 min at 55°C and extension for 2 min at 72°C. The final extension was for 3 min at 72°C. A 7µl aliquot of the PCR products was examined by electrophoresis on a 2.0% (w/v) agrose gel containing 0.5mg ml^{-1} Sybr safe at 90V for 1.5 h and visualised on an ultraviolet trans illuminator. A 100 bp DNA ladder (Promega, Southampton, UK) was used to determine PCR product size.

Preparation of the spore inocula

Spore concentrates were prepared by transferring 5 ml of exponentially growing culture to 100 ml of pre-reduced PYGS broth (Lund *et al*., 1990) and incubating at 4°C for a minimum of 3 months. Prior to inoculation, all media was pre-reduced in an anaerobic cabinet for 24 hours (Don Whitley Scientific Ltd, Shipley, UK) under an atmosphere of 100 % carbon dioxide at 20°C. Spores were harvested using the method described by Moschonas *et al.* (2010). Briefly, spore suspensions were recovered by centrifugation (7500g, 4°C, 10 min) and washed with saline (0.85% NaCl) (Sigma Aldrich, Ireland). This was repeated 3 times. The washed spore suspension was then sonicated (40 kHz for 15 min) in an ultrasonic

waterbath at room temperature (VWR International, USA) and centrifuged / washed as described above (three sonification / centrifugation/ wash cycles). The spore suspension was then suspended in 10 ml saline and stored at -20°C. Final spore numbers were estimated by preparing serial dilutions of the spore suspensions in saline after heat treatment at 60°C for 10 min and plating out 0.1 ml aliquots on CBA supplemented with 5% defibrinated horse blood, incubated anaerobically for 3 weeks at 4°C.

Preparation of meat samples, inoculation and packing

Biceps Femoris muscles were purchased from a local beef slaughter plant and transported to the Industry Development Unit (IDU) at the Teagasc Food Research Centre for sample preparation. Primal samples of approximately 1cm x 1 cm x 100cm were prepared. All samples were placed in refrigerated storage for transport to the laboratory for spore inoculation. Exactly 60 samples were prepared, with 20 being inoculated with each of *C. estertheticum* (DSMZ 8809), *C. estertheticum* (DSMZ 14864) and *C. gasigenes* (DSMZ 12272). Serial dilutions of each BPS *Clostridium* strain were prepared in saline and beef pieces were spread inoculated with 0.1 ml serial dilutions of the prepared inocula to a final mean concentration of 10^3 $\log_{10}$ cfu cm^{-2}. The inoculated meat samples were allowed to dry for 30 mins at room temperature in a laminar flow unit to allow for cell adhesion. Samples were then placed in individual vacuum pack bags (BB3055X, Cryovac, Sealed Air Ltd, St Neots, UK) containing a hydrogen sulphide strip (Sigma Aldrich, Ireland) and vacuum packed using the AK VP-580 vacuum packer (Asgaard Packaging, Rathcoole, Dublin).

Within each sample set, 5 samples were randomly assigned to each of the following treatment combinations; [1] heat shrinkage (90°C for 3s) and conventional chilling; [2] heat shrinkage and rapid (slurry) chilling; [3] no heat shrinkage and conventional chilling and [4] no heat

shrinkage and rapid chilling. All samples were then stored at 2°C in a commercial beef abattoir chilling unit for 100 days.

Rapid Slurry Chilling

The slurry for rapid chilling was prepared from fresh water and ice (60% water 40% ice w/w). A cooler box was used to store the ice slurry. The ice was 3-5 mm and frequent agitation of the mixture was applied to aid rapid heat transfer. Samples were rapidly chilled for 48 hours following vacuum packaging and heat shrinkage. They were then placed in a conventional chiller operating at 2°C for the remainder of the experiment. The temperature of the slurry was monitored using a Testo T-175 data logger (Eurolec Instrumentation Ltd.).

Temperature Analysis

Testo–T175 data loggers were used to monitor temperature. The surface temperature of the vacuum packs was monitored for both chilling treatments during heat shrinkage and storage. A white seal (Eurolabs) was taped onto the outside of an uninoculated vacuum pack using multipurpose tape. The thermocouple was then pushed through the seal and placed into the surface of the meat. Multipurpose tape was used to seal around the thermocouple to prevent water entering the pack.

Monitoring vacuum packs

Packs were visually examined every 4 days over the 100 day trial for the presence of gas and scored against the following criteria described by Boerema *et al.*, (2007): Score 0; no gas bubbles in drip. Score 1; gas bubbles in drip. Score 2; loss of vacuum. Score 3; "blown". Score 4; presence of sufficient gas inside the packs to produce pack distension. Score 5; tightly stretched, "overblown" packs/ packs leaking.

Statistical Analysis

The time to onset of gas production was defined as the day the packs were given a score of 2 (loss of vacuum) as suggested by Moschonas *et al.,* (2011). Statistical analysis on each gas distension score was performed using Genstat version 14.1 (VSN International Ltd, Hemel Hempstead, UK). All individual and pooled data failed the normality tests (Anderson Darling, Cramér-Von Mises and Watson) and was analysed using non-parametric methods (ANOVA, Mann-Whitney U and Kruskal-Wallis).

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Conflict of interest

No conflict of interest was declared.

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Table 1. Average time (days) to the onset (score 2) and full (score 5) blown pack spoilage for beef primal samples inoculated with *C. estertheticum subspp. estertheticum* (DSMZ 8809), *C. estertheticum subspp. laramenise* (DSMZ 14864) and *C. gasigenes* (DSMZ 12272), with and without a heat shrinkage treatment and subject to conventional or rapid chilling.

BPS Organism	Heat Shrinkage	Chilling Method	Initial (Score 2)	Full (Score 5)
			Time (days)	
C. estertheticum (DSMZ 8809)	Yes	Conventional	26 ± 7^{AA}	77± 12 + ^{AB}
	Yes	Rapid	30 ± 9 ^{AA}	62± 3 + ^{AB}
	No	Conventional	39 ± 11 ^{AA}	100 + ^{AA}
	No	Rapid	38 ± 6 ^{AA}	100 + ^{AA}
C. estertheticum (DSMZ 14864)	Yes	Conventional	35 ± 2 ^{AA}	100 + ^{AA}
	Yes	Rapid	37 ± 6 ^{AA}	100 + ^{AA}
	No	Conventional	41 ± 5 ^{AA}	100 + ^{AA}
	No	Rapid	38 ± 2 ^{AA}	100 + ^{AA}
C. gasigenes (DSMZ 12272)	Yes	Conventional	48 ± 5 ^{AA}	100 + ^{AA}
	Yes	Rapid	40 ± 5 ^{AA}	100+ ^{AA}
	No	Conventional	44 ± 5 ^{AA}	100 + ^{AA}
	No	Rapid	48 ± 3 ^{AA}	100 + ^{AA}

Different letters indicate significant difference ($P < 0.05$). The first letter indicated statistical comparison between chilling methods while the second covers heat versus no heat shrinkage.

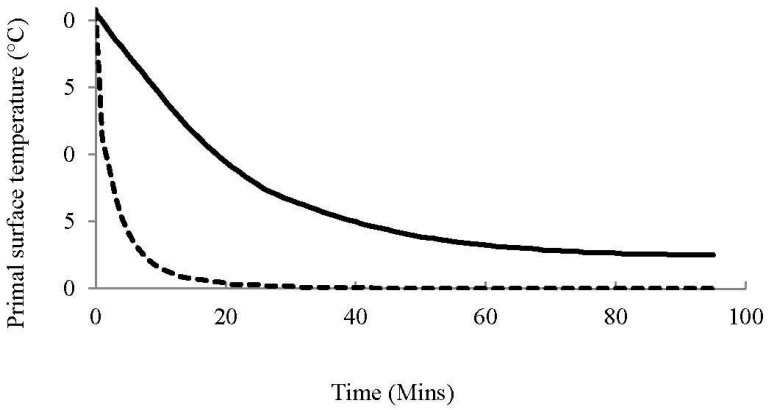

Figure 1. Surface temperature of vacuum packed beef after heat shrink at 90°C for 3 seconds during conventional chilling (solid black line) and rapid chilling in ice slurry composed of 40% ice and 60% water (black dotted line).