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

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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 nor rapid chilling of vacuum-packaged beef retard the time to blown pack spoilage.

Keywords
beef, blown pack spoilage, Clostridium estertheticum, Clostridium gasigenes, rapid chilling, spoilage.

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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 subsp. estertheticum (DSMZ 8809), C. estertheticum subsp. laramense (DSMZ 14864) and C. gasigenes (DSMZ 12272), and vacuum-packaged with and without heat shrinkage (90°C for 3 s). These packs were then subjected 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.

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. Clostridium spores are widespread in the beef abattoir environment (Moschonas et al. 2009) and an initial contamination level as low as 10 spores per cm² 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.
Rapid slurry chilling to control blown pack spoilage

R. Reid et al.

Previous research established that heat shrinkage (typically 80–90°C for 2–3 s), 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, 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 \textit{C. estertheticum} and \textit{C. gasigenes}, vacuum packaged (with and without heat shrinkage) and stored at 2°C.

Results and discussion

Neither the method of chilling (rapid vs 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 \textit{Clostridium} spp. tested (Table 1). A similar result was obtained with the time to full spoilage (score 5) except for packs inoculated with \textit{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 to 2 ± 0.5°C within 8 min, a full 83 min faster than using conventional chilling (Fig. 1). The corresponding times to reduce the surface temperature from 21.2 to 0°C were 76 and 174 min 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 onset time of BPS. In contrast, other research has demonstrated significantly quicker spoilage after heat shrinkage. Bell et al. (2003) reported that post-packaging heat treatments had a significant effect (\(P < 0.001\)) on the time to spoilage by \textit{Clostridium estertheticum NCIMB 12511} and BPS isolates in their culture collection, with the time to gas production decreasing with increased temperature (70°C for 15 s <80°C for 5 s <90°C for 3 s). 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. 2012). Moreover, the presence of other spore

<table>
<thead>
<tr>
<th>BPS organism</th>
<th>Heat shrinkage</th>
<th>Chilling method</th>
<th>Initial (Score 2)</th>
<th>Full (Score 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{C. estertheticum} (DSMZ 8809)</td>
<td>Yes</td>
<td>Conventional</td>
<td>26 ± 7AA</td>
<td>77 ± 12+AA</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Rapid</td>
<td>30 ± 9AA</td>
<td>62 ± 3+AA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Conventional</td>
<td>39 ± 11AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Rapid</td>
<td>38 ± 6AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td>\textit{C. estertheticum} (DSMZ 14864)</td>
<td>Yes</td>
<td>Conventional</td>
<td>35 ± 2AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Rapid</td>
<td>37 ± 6AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Conventional</td>
<td>41 ± 5AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Rapid</td>
<td>38 ± 2AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td>\textit{C. gasigenes} (DSMZ 12272)</td>
<td>Yes</td>
<td>Conventional</td>
<td>48 ± 5AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Rapid</td>
<td>40 ± 5AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Conventional</td>
<td>44 ± 5AA</td>
<td>100 +AA</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Rapid</td>
<td>48 ± 3AA</td>
<td>100 +AA</td>
</tr>
</tbody>
</table>

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.
germination triggers, such as L-lactate, L-valine or L-norvaline, may further complicate interpretation of the results from different studies (Adam et al. 2011).

In conclusion, rapid chilling of vacuum-packaged beef primal surface temperature of vacuum-packed beef after heat shrink at 90°C for 3 s during conventional chilling (solid black line) and rapid chilling in ice slurry composed of 40% ice and 60% water (black dotted line).

**Materials and methods**

**Micro-organisms**

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, Braunschweig, 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 nonhaemolytic 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 min. 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. 2003). 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 7 μl aliquot of the PCR products was examined by electrophoresis on a 2.0% (w/v) agarose gel containing 0.5 mg 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 cultures 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 were pre-reduced in an anaerobic cabinet for 24 h (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 three times. The washed spore suspension was then sonicated (40 kHz for 15 min) in an ultrasonic waterbath at room temperature.
temperature (VWR International, Blanchardstown, Dublin, Ireland) 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, inoculated 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 1 cm × 1 cm × 100 cm 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. gasgenes* (DSMZ 12272). Serial dilutions of each BPS *Clostridium* strain were prepared in saline and beef pieces were spread inoculated with 0.1 ml aliquots on CBA supplemented with 5% defibrinated horse blood, inoculated anaerobically for 3 weeks at 4°C.

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 h 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., Dundalk, County Louth, Ireland).

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, Crawley, UK) 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 ver. 14.1 (VSN International Ltd, Hemel Hempstead, UK). All individual and pooled data failed the normality tests (Anderson Darling, Cram–Von Mises and Watson) and was analysed using nonparametric methods (ANOVA, Mann–Whitney U and Kruskal–Wallis).

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Conflict of Interest

No conflict of interest was declared.

References


