

in Vacuum Packaged Meat



Control of Blown Pack Spoilage in Vacuum Packaged Meat

Editor-in-Chief:

Professor Gerard Downey PhD DSc

Authors:

Declan J. Bolton BSc PhD

Galatios Moschonas BSc

Professor James J. Sheridan MA MSc PhD

Ashtown Food Research Centre, Ashtown, Dublin 15

ISBN: 1-84170-555-1

Project RMIS number: 5417

October 2009

Report number: 101



Table of Contents

	<i>page number</i>
1. Summary	1
2. Introduction	2
3. Development of methodologies to isolate and detect <i>Clostridium</i> species from vacuum packaged beef and other sources	3
4. Examination of the presence of psychrotrophic <i>Clostridium</i> species on beef animals, meat samples and in the abattoir and boning hall environments	3
5. Determination of the ability of <i>Clostridium</i> species isolated from various sources to cause spoilage of vacuum packaged beef	6
6. Control of blown pack spoilage	8
7. Conclusions	9
8. Recommendations to Industry	10
9. References	11

Summary

Blown pack spoilage (BPS) represents a significant commercial loss to Irish meat processors. This research discovered that the organisms causing BPS are ubiquitous in the abattoir environment, making eradication very difficult. The risk of BPS is best managed through a process of regular treatment of plant and equipment with a sporicidal agent such as peroxyacetic acid, good hygiene to minimise carcass contamination and removal of the heat shrinkage stage during vacuum packaging as this activates the spores and reduces the time to spoilage.

Introduction

In the late 1960s, primal meat cuts were first vacuum packaged (VP); in the 1980s, the technology was used for retail cuts. Centralised butchering and vacuum packaging of primal joints of beef is widely practised in the meat industry in Ireland. With strict adherence to good manufacturing practices and maintenance of high standards of hygiene and refrigeration, the use of packaging materials which are highly impermeable to oxygen and carbon dioxide, and the exclusion of muscles with a pH of >6.0, a shelf-life of 10 weeks or more can readily be attained (Sheridan and Sherington, 1982). However, in 1989 a type of VP spoilage later referred to as 'blown pack spoilage' (BPS) emerged in the USA (Kalchayanand *et al.*, 1989). This was followed by incidences in the UK (Dainty *et al.*, 1989), New Zealand (1996) and Ireland in 2000. Each spoilage event occurred in correctly chilled batches (0 to 2°C) after 2 to 4 weeks and was characterised by the production of large volumes of gas, a putrid smell (H₂S) and a metallic sheen on the meat. Meat spoiled in this way has no commercial value and blown pack spoilage represents a considerable loss to meat processors in monetary terms (although the exact costs of BPS associated losses is commercially sensitive information and BPS events range from a few spoiled packs to the loss of a whole batch, based on data obtained from several processors we estimate the average cost per BPS event to be €375,000). Furthermore, a major incident may result in failure to meet a customer order thus jeopardising future contracts.

While initial reports implicated *Clostridium estertheticum* as the agent of spoilage, other psychrotrophic (low temperature) *Clostridium* species have also been confirmed as the causative agents of "Blown Pack" spoilage of vacuum packed chilled meats (Lawson *et al.*, 1994; Broda *et al.*, 1996, 1999, 2000). However, *C. estertheticum* and *C. gasigenes* are the main BPS *Clostridial* spp. in Ireland. This type of spoilage is not restricted to beef and has also occurred in vacuum packaged lamb in Ireland, deer and cooked meat products, particularly cooked pork and turkey meat (Lawson *et al.*, 1994 and Kalinowski & Tompkin, 1999).

In 2005, Teagasc commenced a FIRM funded project '*Control of blown pack spoilage in vacuum packaged meat*' (04/R&D/TN/251) which had five component tasks; [1] development of methodologies to detect and isolate BPS *Clostridia*; [2] a survey of Irish beef abattoirs for BPS *Clostridia*; [3] an assessment of the ability of isolated *Clostridia* to cause BPS; [4] an investigation of the role of heat shrinkage of VP films in BPS *Clostridial* spore activation and [5] dissemination. Outcomes of this research are discussed in this report.

Development of methodologies to isolate and detect *Clostridium* species from vacuum packaged beef and other sources.

A range of media and agars were prepared and tested for their ability to facilitate the culture and isolation of *Clostridium* species from meat and other samples. These included Reinforced *Clostridial* agar (RCM), skim milk powder, sodium thioglycollate, Peptone Yeast Extract Glucose Starch broth (PYGS), Columbia Blood Agar (CBA) and Cooked Meat Medium (CMM). All media were inoculated with a type strain of *Clostridium estertheticum* obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), GmbH, Germany. The type strain grew successfully on all media at 8°C except the CMM. Growth on this medium was not supported because the reducing agent cystine hydrochloride, which is an essential agent involved in the pre-reduction of media for anaerobic growth, was not added. In the development of anaerobic media, oxygen scavengers in sachets were assessed in liquid media in place of cystine hydrochloride. The results were inconclusive and the manufacturers advised against their use in this way.

Examination of the presence of psychrotrophic *Clostridium* species on beef animals, meat samples and in the abattoir and boning hall environments.

Meat plants were chosen in four locations in the north, south, east and west of the country in order to obtain a representative sample of the animals and plants processing beef in Ireland. Each plant was visited once per month over the course of 1 year and 30 samples were taken at each visit. These included walls and floor (lairage) swabs and an examination of the hooves taken after the first or second legging operations. Hide samples were also taken by cutting pieces from the flanks of animals just prior to hide removal while faeces were removed from the animal bung on the evisceration table. Surfaces in the bleeding area, the wall at hide removal and the surface of the evisceration table were also swabbed. In the boning hall, the cutting table, meat conveyor belts and vacuum packing machines were tested. The soil outside in the immediate environment of each plant was examined as were the trucks delivering animals to the plants. The latter were swabbed at the tail gate area where faeces had dried and become embedded in the wooden surfaces. Finally, meat samples from different plants throughout the country were routinely examined for the presence of gas blowing organisms detected using a PCR method. Four hundred and twenty eight (428) presumptive psychrophilic *Clostridia* were obtained; these were confirmed and speciated using PCR (Table 1). The results for *C. estertheticum* and *C. gasigenes* are shown in Table 2. To the best of our knowledge, this is the first study of its kind and hence there

are no other similar studies with which to compare this work. However, several authors have suggested that the majority of spoilage organisms in vacuum packaged meats originate in the exogenous environment of the animal (Ercolani, 1997; Del Torre *et al.*, 2004).

Table 1. Species of *Clostridia* isolated in the abattoir survey and their ability to produce gas

<i>Isolate</i>	<i># of isolates</i>	<i># of gas producers in broth</i>
<i>Cl. estertheticum</i>	17	13
<i>Cl. gasigenes</i>	315	253
<i>Clostridium algidixylanolyticum</i>	14	3
<i>Clostridium beijerinckii</i>	1	0
<i>Clostridium botulinum</i> type E	1	1
<i>Clostridium botulinum</i> type F	1	0
<i>Clostridium glycolicum</i>	1	1
<i>Clostridium sp.</i> CYP11	1	1
<i>Clostridium sp.</i> CYP7	3	2
<i>Clostridium sp.</i> V13	1	1
<i>Clostridium sulfatireducens</i>	1	1
<i>Clostridium xylanolyticum</i>	1	0
Swine effluent bacterium CHNDP10	1	1
Swine effluent bacterium CHNDP4	1	1
Uncultured bacterium	11	8
Uncultured <i>Clostridium</i>	56	28
<i>Vagococcus sp.</i>	1	0
Failed	1	0
	428	314

Table 2. The presence (% positive) of psychrotrophic Clostridium species on surfaces in beef abattoirs and boning halls, on hide, hooves, transport vehicles, in faeces and soil and on meat.

<i>Sites tested</i>	<i>Cl. estertheticum</i>	<i>Cl. gasigenes</i>
Lairage	29.2	35.4
Bleeding area	16.7	37.5
Hide removal	31.3	37.5
Evisceration table	4.2	6.3
Hooves	16.7	20.8
Tables (BH)	6.3	8.3
Conveyor belt (BH)	-	2.1
Vac Pack Machine	-	2.1
Hides	13.3	18.8
Faeces	17.9	25.4
Soil	14.6	10.4
Transportation	22.9	12.5
Carcasses	-	2.1

Determination of the ability of *Clostridium* species isolated from various sources to cause spoilage of chilled vacuum packed beef.

The 428 isolates were tested for the ability to produce gas using a broth system (PYGS broth) and Durham tubes; 314 out of the 428 were gas producers. From these, 11 were selected for further trials (see Tables 3 & 4) on vacuum packages of beef and lamb. Briefly, each meat species was inoculated with each organism at 1 spore/cm², 10 spores/cm², 100 spores/cm² and 1000 spores/cm². Spore inoculation was per cm² instead of per gram as contamination and spoilage is surface area related and is not affected by the weight of the meat. The inoculated meat samples were packed in Cryovac vacuum bags, the vacuum pulled, the bags sealed and shrunk in waterbaths at 90°C for 3 seconds before storage in the Meat Industry Development Unit (MIDU) at AFRC at -1.5°C, 1°C and 4°C. When each pack spoiled, the gaseous composition of the headspace was determined using semi-quantitative gas chromatographic (GC) analysis. The onset of gas production was influenced by both the spore concentration and storage temperature. This is consistent with results reported by Jensen *et al.* (1987). At the lower spore concentrations, strains of *C. botulinum* type E and *C. sp V13* did not produce gas at -1.5°C or +1°C. At the higher spore concentrations and 4°C, spoilage occurred after 60 days. This reflected an overall trend as, in general, BPS only occurred at 4°C and at the higher spore concentrations. *C. estertheticum* was an exception to this, causing BPS at -1.5°C at the highest spore concentration within 44 days. Boerema *et al.* (2007) reported a similar finding with this organism. The minimum and maximum time (days) to spoilage are shown in Tables 3 (beef) and 4 (lamb).

Table 3. Minimum and maximum times to spoilage of vacuum packed beef.

<i>Isolate</i>	<i>Time to spoilage (days)</i>	
	<i>Minimum</i>	<i>Maximum</i>
<i>Cl. estertheticum</i> 1	14+/-3	52+/-7
<i>Cl. estertheticum</i> 2	23+/-4	63+/-6
<i>Cl. gasigenes</i> 1	35+/-6	100 days +
<i>Cl. gasigenes</i> 2	36+/-3	100 days +
<i>Clostridium botulinum</i> E	61+/-23	100 days +
<i>Clostridium sulfatireducens</i>	70+/-23	100 days +
<i>Bacteroides propionificaciens</i>	100 days +	100 days +
<i>Clostridium</i> sp. V13	54+/-12	100 days +
<i>Clostridium</i> sp. CYP7	39+/-5	100 days +
Uncultured <i>Clostridium</i> 1	100 days +	100 days +
Uncultured <i>Clostridium</i> 2	100 days +	100 days +

Table 4. Minimum and maximum times to spoilage of vacuum packed lamb.

<i>Isolate</i>	<i>Time to spoilage (days)</i>	
	<i>Minimum</i>	<i>Maximum</i>
<i>Cl. estertheticum</i> 1	27+/-10	57+/-1
<i>Cl. estertheticum</i> 2	16+/-4	38+/-3
<i>Cl. gasigenes</i> 1	36+/-4	100 days +
<i>Cl. gasigenes</i> 2	24+/-5	100 days +
<i>Clostridium botulinum</i> E	39+/-5	100 days +
<i>Clostridium sulfatireducens</i>	39+/-5	100 days +
<i>Bacteroides propionificaciens</i>	36+/-7	100 days +
<i>Clostridium</i> sp. V13	48+/-8	100 days +
<i>Clostridium</i> sp. CYP7	56+/-12	100 days +
Uncultured <i>Clostridium</i> 1	40+/-5	100 days +
Uncultured <i>Clostridium</i> 2	44+/-8	100 days +

Control of blown pack spoilage

Research was undertaken to test the hypothesis that heat shrinkage of the vacuum packs (typically performed at 70°C for 10 seconds or 90°C for 3 seconds) activates spores of *Clostridia* thereby promoting blown pack spoilage. Briefly, using the same organisms as above, beef and lamb samples were inoculated with 1000 spores/cm², vacuum packaged and then shrunk at 50°C for 15 seconds, 70°C for 10 seconds or 90°C for 3 seconds. Non-shrunk packs were used as controls. All packs were stored at -1.5°C, 1°C and 4°C and, when each pack spoiled, the gaseous composition of the headspace was determined using semi-quantitative GC analysis.

In general, the absence of shrinkage or the lower the shrinkage temperature the longer the time observed before BPS. In the absence of any shrinkage treatment and storage at -1.5°, there was no BPS with the exception of *Cl. estertheticum* which caused BPS after 67 days. Without shrinkage and storage at 1°C, *Cl. estertheticum* caused BPS after 32 days. With the 50°C-15 seconds, 70°C-10 seconds and 90°C-3 seconds treatments, *Cl. estertheticum* caused BPS after 30 days, 28 days and 18 days respectively at 1°C. The shelf-life extensions obtained without heat shrinkage treatment are shown in Table 6.

Table 6. Shelf-life extension achieved in vacuum packaged beef and lamb as a result of removing the VP heat treatment (average for all temperatures)

Isolate	Shelf-life extension (days)	
	Beef	Lamb
<i>Cl. estertheticum</i> 1	42	43
<i>Cl. estertheticum</i> 2	57	38
<i>Cl. gasigenes</i> 1	31	20
<i>Cl. gasigenes</i> 2	20	22
<i>Clostridium botulinum</i> E	15	10
<i>Clostridium sulfatireducens</i>	20	20
<i>Bacteroides propionificaciens</i>	9	7
<i>Clostridium</i> sp. V13	5	5
<i>Clostridium</i> sp. CYP7	5	7
Uncultured <i>Clostridium</i> 1	37	33
Uncultured <i>Clostridium</i> 2	22	20

Conclusions

It was concluded that blown pack spoilage *Clostridia* are widespread in the abattoir environment. The resultant risk is best managed through an effective prerequisite programme including GHP, storage at as low a chilled temperature as possible and avoidance of the heat shrinkage step during vacuum packaging.

Recommendations to industry

The following recommendations are made to the Irish meat industry based on our research:

- Keep contamination levels to a minimum by implementing GHP in the abattoir and boning hall. This should include regular sanitation of plant and equipment with a sporicidal agent such as peroxyacetic acid. Surfaces in direct contact with meat should be dry before treatment and spray cleansed with these agents.
- Vacuum packed meat should be chilled at as low a temperature as possible. The higher the storage temperature, the quicker blown pack spoilage occurs.
- Heat shrinkage of vacuum packs activates spores and significantly reduces the time to BPS. If possible, heat shrinkage of vacuum packs should be avoided.

References

- Boerema, J.A., Broda, D.M., Penney, N. and Brightwell, G.** 2007. Influence of peroxyacetic acid-based carcass rinse on the onset of “blown pack” spoilage in artificially inoculated vacuum-packed chilled beef. *Journal of Food Protection*, **70**, 1434-1439.
- Broda, D.M., DeLacy, K.M., Bell, R.G. and Cook, R. L.** 1996. Psychrotrophic *Clostridium* spp. associated with “blown pack” spoilage of chilled vacuum-packed red meats and dog rolls in gas-impermeable plastic casings. *International Journal of Food Microbiology*, **29**, 335-352.
- Broda, D.M., Lawson, P.A., Bell, R.G. and Musgrave, D. R.** 1999. *Clostridium* frigidicarnis sp. nov., a psychrotolerant bacterium associated with “blown pack” spoilage of vacuum packed meats. *International Journal of Systematic and Evolutionary Microbiology*, **49**, 1539-1550.
- Broda, D.M., Saul, D.J., Lawson, P.A., Bell, R.G. and Musgrave, D. R.** 2000. *Clostridium* gasigenes sp. nov., a psychrophile causing spoilage of vacuum-packed meat. *International Journal of Systematic and Evolutionary Microbiology*, **50**, 107-118.
- Dainty, R.H., Edward, R.A. and Hibbard, C.M.** 1989. Spoilage of vacuum packed beef by a *Clostridium* sp. *Journal of the Science of Food and Agriculture*, **49**, 473-486.
- Del Torre, M., Stecchini, M.L., Braconnier, A. and Peck, M.W.** 2004. Prevalence of *Clostridium* species and behaviour of *Clostridium* botulinum in gnocchi, a REPFED of Italian origin. *International Journal of Food Microbiology*, **96**, 115-131.
- Ercolani, G.L.** 1997. Note: Occurrence and persistence of culturable *clostridial* spores on the leaves of horticultural plants. *Journal of Applied Microbiology*, **82**, 137-140.
- Jensen, M.J., Genigiorgis, C. and Lindroth, S.** 1987. Probability of growth of *Clostridium* botulinum as affected by strain, cell and serologic type, inoculum size and temperature and time of incubation in a model broth system. *Journal of Food Safety*, **8**, 109-126.
- Kalinowski, R.M. and Tompkin, B.R.** 1999. Psychrotrophic *Clostridia* causing spoilage in cooked meat products. *Journal of Food Protection*, **62**, 766-772.

References

- Lawson, P.A., Dainty, R.H., Kristiansen, N., Berg, J. and Collins, M.D.** 1994. Characterisation of a psychrotrophic *Clostridium* causing spoilage in vacuum packed cooked pork: Description of *Clostridium algidicarnis* sp. *Letters in Applied Microbiology*, **19**, 153-157.
- Kalchayanand, N., Ray, B. and Johnson, M.C.** 1989. Spoilage of vacuum packed refrigerated beef by *Clostridium*. *Journal of Food Protection*, **52**, 424-426.
- Sheridan, J.J. and Sherington, J.** 1982. The microbiology of hot and conventionally deboned vacuum packaged beef. *Meat Science*, **7**, 245-258.