Meat Science 93 (2013) 310–315

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Reduction of spoilage of chilled vacuum-packed lamb by psychrotolerant clostridia

Katharine H. Adam [a,*], Steve H. Flint [b], Gale Brightwell [a]

[a] Food and Bio-Based Products, AgResearch, Ruakura, Private Bag 3115, Hamilton 3240, New Zealand
[b] Food, Nutrition & Human Health, Massey University, Private Bag 11222, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 10 May 2012
Received in revised form 31 August 2012
Accepted 12 September 2012

Keywords:
Blown pack spoilage
Clostridium estertheticum
Vacuum-pack
Psychrotolerant
Lamb

ABSTRACT

Methods for the reduction of spoilage, of lamb, by psychrotolerant clostridia were investigated including exposure to air, hot and cold water spray washing and tyndallisation. Initially vegetative cells of psychrotolerant clostridia associated with spoilage of chilled vacuum-packed meat were exposed to aerobic cooked meat medium at room temperature (21 °C) to determine how long they remained viable. Survival of strains varied from 2 h to 3 days. Vegetative cells of *Clostridium estertheticum* subsp. *estertheticum* survived 7 days at 10 °C with little reduction in viable numbers. This ruled out exposure to air as a practical method for reducing spoilage. Trials were also carried out on chilled vacuum-packed lamb inoculated with spores of *Cl. estertheticum* subsp. *estertheticum*. The time until inoculated packs reached the loss of vacuum stage varied from 38 to 53 days. Hot and cold water washing extended the shelf life by 12 to 13 days in comparison to untreated packs.

1. Introduction

A range of psychrotolerant clostridia have been identified as causing spoilage of chilled, vacuum-packed red meat (beef, lamb and venison). The most common is *Clostridium estertheticum* which causes blown pack spoilage. Typically a pack suffering from blown pack spoilage contains copious quantities of drip and gas, exhibits a highly unpleasant odour on opening and spoiled meat is discoloured and excessively tender (Dainty, Edwards, & Hibbard, 1989; Kalchayanand, Ray, Field, & Johnson, 1989). *Clostridium gasigenes* has also been identified as a causative agent of blown pack spoilage (Broda, Saul, Lawson, Bell, & Musgrave, 2000). *Clostridium algidicarnis*, *Clostridium algidixylanolyticum* and *Clostridium frigidicarnis* have been identified as causing surface spoilage of chilled vacuum-packed red meat (Adam, Flint, & Brightwell, 2010). Psychrotolerant clostridia are obligate anaerobes and able to grow at low temperatures, in the case of *Cl. estertheticum*, down to −1.5 °C, the optimal temperature for shipping chilled red meat (Spring et al., 2003). Unlike other meat spoilage bacteria such as Enterobacteriaceae, clostridia grow well in the anaerobic environment inherent in vacuum packs. Clostridial spoilage of optimally chilled vacuum-packed red meat develops over several weeks (Clemens, Adam, & Brightwell, 2010). This is of particular concern when shipping product over long distances such as from New Zealand to Europe. Recently fuel saving measures, have resulted in the introduction of shipping at reduced speed termed super slow steaming. As a result shipping times have been extended considerably, reducing the guaranteed shelf life, at market, by several days and potentially increasing the risk of spoilage.

Currently the only method for preventing spoilage by clostridia is through the use of best practices in meat processing plants, such as preventing the outside of the pelt rolling back on to the carcass after making the opening cut and avoiding spatter of mud and faeces on to the carcass during hide removal. This study is part of a larger project that ultimately aims to develop a method of reducing red meat spoilage by cold tolerant clostridia involving a two-step process. First the spores would be treated to induce germination then inactivated by a second mitigation step. A previous study identified a germinant system in *Cl. frigidicarnis* consisting of L-lactate and L-valine or L-norvaline. L-lactate and L-valine are known to be present on the surface of red meat (Adam, Brunt, Brightwell, Flint, & Peck, 2011). The present study focuses on the second step, inactivating germinated spores.

Being strictly anaerobic is a defining feature of the genus *Clostridium* (Stackbrandt & Rainey, 1997). Conceivably the simplest method of inactivating germinated cells would be through exposure to air. The tolerance of vegetative cells of clostridia to oxygen has been studied in the context of culturing methods. *Clostridium* sp. isolates were shown to survive on deoxygenated Columbia Blood Agar (CBA) plates for 72 h under aerobic conditions (Tally, Stewart, Sutter, and Rosenblatt, 1975). If red meat spoilage clostridia exhibit similar tolerance to aerobic conditions exposure to air will not be a practical option for their inactivation.

Other interventions that may be effective against spores and or vegetative psychrotolerant clostridia include hot water wash (HWW), and tyndallisation. In meat processing plants HWW is usually applied to whole carcass following slaughter, removal of the head, hooves and pelt and prior to boning out which may be done within an hour of

* Corresponding author. Tel.: +64 7 838 5372, +64 21 054 4890 (mobile).
E-mail address: katharine.adam.1@uni.massey.ac.nz (K.H. Adam).

0309-1740/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2012.09.011

slaughter (hot boning) or following overnight chilling (cold boning) (Waylan & Kastner, 2004). Ideally in HWW hot water (80 °C) is applied evenly to the whole carcass. Application time is limited to approximately 10 s to prevent permanent discolouration of the meat (Castillo, Lucia, Goodson, Savell, & Acuff, 1998). HWW has been shown to be effective in reducing *Escherichia coli* 0157:H7 and *Salmonella typhimurium* but has not been specifically investigated for use against spores of cold tolerant clostridia (Castillo et al., 1998). Exposure of bacterial spores to sublethal heat has been shown to activate spores resulting in faster and higher levels of germination in a population (Yi & Setlow, 2010). Heat shrinking has been shown to reduce the time till spoilage by *Cl. estertheticum* probably as a result of increasing in the speed of germination (Bell, Moorhead, & Broda, 2001). Potentially the heat from hot water washing could activate spores. We compared the effect of HWW with cold water wash (CWW) on meat inoculated with spores prior to vacuum packaging to determine if this was the case. Chemical wash interventions are also used to reduce microbial loading. A peroxyacetic acid based wash has been shown to be effective in delaying the onset of spoilage by *Cl. estertheticum* spores (Boerema, Broda, Penney, & Brightwell, 2007) however Europe does not accept red meat that has undergone chemical treatment. Tyndallisation involves the exposure of cells to nutrients which causes them to enter an actively growing vegetative state. Actively growing vegetative cells have reduced resistance in comparison to spores. The cells are then exposed to heat. The process is repeated three times to ensure the maximum number of spores are killed. The timing and temperature of treatments depends on the target organisms and the product being treated. It is important that fresh meat retain its colour and texture following tyndallisation limiting the possible exposure time.

This study aims to determine if the shelf life, of chilled lamb, inoculated with *Cl. estertheticum* can be increased by CWW, HWW, tyndallisation or inducing spores to germinate then exposing them to oxygen.

2. Materials and methods

2.1. Strains

Cl. algidicarnis (NCFB 2931^T) was obtained from NCFB, the National Collection of Food Bacteria (Reading, UK. CSIRO). *Cl. algidixylanolyticum* (DSM 12273^T), *Cl. estertheticum* subsp. *estertheticum* (DSM 8809^T), *Cl. frigidicarnis* (DSM 12271^T), and *Cl. gasigenes* DSM (12272^T) were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), *Cl. estertheticum* subsp. *laramiense* (ATCC 51254^T, formerly *Cl. laramiense*) was obtained from ATCC (American Type Culture Collection, Manassas, VI, USA) and LA1 was isolated locally from spoiled vacuum-packed beef. Cultures were maintained under strict anaerobic conditions on CBA (Oxoid CM331, Oxoid Ltd., Basingstoke, England) plates containing 5% defibrillated sheep's blood.

2.2. Cultures

Vials of peptone, yeast, glucose, starch medium (PYGS) (Peck, Fairbairn, & Lund, 1992) were inoculated with a single colony of the strains described in Section 2.1 and incubated anaerobically until late growth phase to produce vegetative cultures. *Cl. estertheticum* subsp. *estertheticum* and *Cl. estertheticum* subsp. *laramiense* were incubated at 10 °C for 7 to 15 days and *Cl. algidicarnis*, *Cl. algidixylanolyticum*, *Cl. frigidicarnis*, LA1 and *Cl. gasigenes* were incubated at 25 °C for 20 to 36 h. Spores of *Cl. frigidicarnis* and *Cl. estertheticum* subsp. *estertheticum* were prepared as described in Adam et al. (2011) and Stringer, Webb, and Peck (2009). Germinated *Cl. frigidicarnis* spores were prepared by exposing spores to PYGS for 150 min at 25 °C. This resulted in 80 to 90% of the spore population becoming phase dark. For the purpose of this work germinated spores are defined as cells that are phase dark but have not fully elongated and are not able to divide. All culturing was carried out under strictly anaerobic conditions.

2.3. Exposure to air in vitro

Prior to exposure to air, cultures were diluted to approximately 10^6 colony forming units (CFU) ml^{-1} in deoxygenated physiological saline. One ml aliquots were then transferred into vials containing nine ml aerobic, cooked meat medium (CMM, Acumedia, Fort Richard, Auckland, New Zealand) supernatant, prior to incubation at room temperature (21 °C) or 10 °C. During the course of the experiment, sub-samples were taken for enumeration. The time between samplings increased with the duration of the experiment. All cultures and germinated spores of *Cl. frigidicarnis* were exposed to air at 21 °C, *Cl. estertheticum* subsp. *estertheticum* and *Cl. frigidicarnis* were also exposed at 10 °C and the effect of exposure of *Cl. estertheticum* subsp. *estertheticum* to 21 °C under anaerobic conditions was tested.

2.4. Ethanol treatment

Samples containing germinated spores were ethanol treated to determine the portion of viable dormant spores using a method adapted from Broda, De Lacy, and Bell (1998). Deoxygenated absolute ethanol was added to sub-samples of cultures at a final concentration of 50% and incubated for 60 min at 15 °C prior to enumeration.

2.5. Enumeration of in vitro samples

Sub-samples were enumerated on deoxygenated CBA plates containing 5% defibrillated sheep's blood. *Cl. estertheticum* subsp. *estertheticum* and *Cl. estertheticum* subsp. *laramiense* plates were incubated anaerobically at 10 °C for 21 days, *Cl. algidicarnis*, *Cl. algidixylanolyticum*, *Cl. frigidicarnis*, LA1 and *Cl. gasigenes* plates were incubated anaerobically at 25 °C for 48 to 72 h. The assay has a detection limit of 10 CFU ml^{-1}.

2.6. Lamb

A fresh lamb carcass was obtained from a local lamb processing plant (in the North Island of New Zealand) within half an hour of slaughter. The carcass was fully boned out and cut into 40 steaks with a surface area of approximately 100 cm^2, in a cool room (10 °C). Individual steaks contained multiple muscle groups.

2.7. Spoilage of lamb by Cl. estertheticum

HWW and tyndallisation were investigated as methods of reducing spoilage by spores of *Cl. estertheticum* subsp. *estertheticum*. Individual lamb steaks were randomly assigned to eight treatments with five replicates of each treatment and packed into 70 μm barrier bags (Cryovac, New Zealand). The treatments were: A) uninoculated control; B) inoculated control; C) inoculated and CWWed; D) inoculated and HWWed; E) inoculated, vacuum packed, stored at −1.5 °C for 3 days then exposed to air; F) inoculated, vacuum packed, stored at −1.5 °C for 3 days then HWWed; G) tyndallised; and H) tyndallised, vacuum packed, stored at −1.5 °C for 3 days and HWWed. The initial inoculum was 1 ml containing approximately 1 CFU ml^{-1} spores and was enumerated on deoxygenated CBA plates. Germination of *Cl. estertheticum* subsp. *estertheticum* spores is poor on CBA, the best growth medium available. A cell density of 1 CFU ml^{-1} on CBA equates to ~100 spores ml^{-1} as determined by haemocytometer count. All barrier bags were vacuum packed using a Securepak 10 Controlled Atmosphere Packaging Machine prior to storage (Securefresh Pacific, Auckland, New Zealand). Tyndallisation consisted of heat shrinking by immersion in 78 °C water for 4 to 5 s then chilling packs in iced water for 10 min. This was repeated three times. The