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Comparison of hot *versus* cold boning of beef carcasses on bacterial growth and the risk of blown pack spoilage

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ABSTRACT

Primals were prepared from beef *Longissimus thoracis et lumborum* (LTL), *psoas major* (PM), *quadriceps femoris* (QF) and *semitendinosus* (S) muscles from cold and hot boned carcasses, vacuum-packaged and stored for 42 or 100 days at 2 °C and 7 °C. Storage temperature, carcass or primal surface temperature, pH and a_w were monitored. Samples were taken periodically and tested for total viable count mesophilic (TVCm), TVC psychrophilic (TVCp), total Enterobacteriaceae count (TEC), presumptive *Pseudomonas* spp., lactic acid bacteria (LAB), *Clostridium* spp. and *Brochothrix thermosphacta*. A fifth muscle, *biceps femoris* (BF), was used to examine the impact of hot boning on blown pack spoilage (BPS). Primal counts increased to 6–7 $\log_{10}$ cfu cm^{-2} after 6 weeks. Significantly ($P < 0.05$) higher TEC, *Pseudomonas* spp. and *Br. thermosphacta* counts were observed on cold *versus* hot boned primals. In contrast, significantly ($P < 0.05$) higher TVC, LAB and *Clostridium* spp. concentrations were obtained on hot boned beef. Moreover, BPS pack distension/bursting occurred considerably sooner in hot boned product.

1. Introduction

European Regulation EC 853/2004 requires that beef carcasses be immediately chilled after *post-mortem* inspection to ensure a core temperature of not >7 °C in the case of meat and not >3 °C for offal. However, with the approval of the competent authority, carcasses may be deboned while still warm. This process, often referred to as 'hot' or 'warm boning', has several advantages over traditional cold boning including reduced energy inputs, chiller space and drip loss (Cuthbertson, 1980; Devine & Dikeman, 2014; Pisula & Tyburcy, 1996; Williams, 1978). Bowater estimated that warm/hot boning would increase yield by 4% and a beef plant processing 500 cattle a day for 250 days *per annum* would save €2.7 million (Bowater, 2001). Furthermore, it has been reported that hot boning requires less effort and strain-induced injuries in workers are reduced (Adam, 2012; Fung, Kastner, Hunt, Dikeman, & Kropf, 1980; Herbert & Smith, 1980; Van Laack & Smulders, 1989)

Despite these advantages, warm/hot boning is practised in relatively few countries (Australia, Sweden, South Africa and New Zealand) because meat may be tougher, darker and of different shape to cold boned cuts, all of which are less acceptable to customers. Shelf-life may also be reduced as bacteria grow rapidly at the temperatures encountered in hot boned vacuum packaged beef (Sheridan & Sherington, 1982; Spooncer, 1993; Yang, Balamurugan, & Gill, 2011). Although blown pack spoilage (BPS) occurs at correctly chilled temperatures, the higher temperature encountered in hot boned meat may also promote the growth of *Clostridium estertheticum* and *Clostridium gasigenes* leading to BPS with affected meat having no commercial value (Moschonas, Bolton, Sheridan, & McDowell, 2010).

In European beef plants, carcasses are placed in chilling units immediately after slaughter where they usually remain for 48–72 h before being moved to the boning hall. In the boning hall, the carcasses are cut into primals and sub-primals which may then be vacuum packaged and the meat allowed to mature for 3 to 6 weeks. The biochemical processes and structural changes that occur in beef during the first 24 hours *post-mortem* are critical in determining quality and palatability and the temperature profiles used typically ensure the core temperature does not decrease below 10 °C in the first 10 h to prevent cold shortening (EFSA, 2014).

Meat spoilage is usually characterised by discolouration, strong off-odours and/or slime production which occurs when the bacterial count reaches approximately 10^{7-8} $\log_{10}$ cfu cm^{-2} (Jones, 2004; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Carcasses are chilled aerobically and the spoilage microflora are usully dominated by *Pseudomonas* spp. (Stanbridge & Davies, 1998; Koutsoumanis, Stamatiou, Skandamis, & Nychas, 2006), including slime and off-odour producing *P. fragi*, *P. fluorescens* and *P. lundensis*. Enterobacteriaceae, especially cold-tolerant species such as *Hafnia alvei*, *Serratia liquefaciens* and *Pantoea agglomerans*, may also contribute to spoilage especially if

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there is temperature abuse (Nychas et al., 2008) but other bacteria such as oxygen tolerant lactic acid bacteria (LAB) and *Brochotrix thermosphacta* are not major contributors to the spoilage of carcasses under aerobic conditions. However, when beef primals are stored anaerobically in vacuum packs, LAB and *Br. thermosphacta* become the predominant spoilage organisms. Spoilage by the latter is associated with a cheesy or dairy odour. Under anaerobic conditions blown pack spoilage caused by the germination and growth of a range of psychrotolerant/psychrophilic *Clostridium* spp., often associated with a sulphite odour, may also occur (Moschonas et al., 2010; Xianqin Yang, Gill, & Balamurugan, 2010; Bolton, Carroll, & Walsh, 2015). Despite many years of research, there is still very little data on the fate of general bacterial populations, total viable counts (TVC) and total Enterobacteriacae counts (TEC), and even less on key spoilage bacteria (*Pseudomonas* spp., LAB, *Br. thermosphacta* and *Clostridium* spp. on beef primals during chilled storage (EFSA, 2014). Moreover, data is required on the influence of cold *versus* hot/warm boning on the growth of spoilage bacteria and the risk of blown pack spoilage to allow the beef industry to make informed decisions regarding the application of this technology. The aims of this study were therefore [1] to provide data on the levels and growth of indicator (TVC and TEC) and key spoilage bacteria (*Pseudomonas* spp., LAB, *Clostridium* spp. and *Br. thermosphacta*) on vacuum packaged beef primals and [2] to investigate if warm/hot boning resulted in higher growth rates and an increased risk of blown pack spoilage.

2. Materials and methods

2.1. Monitoring growth of spoilage bacteria

2.1.1. Sample preparation

On three separate occasions, two Charolais Cross heifers ($n = 6$) were slaughtered in the Meat Industry Development Unit (IDU) in Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland. After evisceration, each heifer was centrally split into left and right sides ($n = 12$). On the right side, the *Longissimus thoracis et lumborum* (LTL, *psoas major* (PM), *quadriceps femoris* (QF) and *semitendinosus* (S) *muscles* were hot boned (HB) out within 90 min of slaughter and Pi-vaced (Pi-Vac Elasto-pack system, Nofima) to prevent cold shortening. These primals were then placed in a commercial chiller at 0 °C for 24 h. The left side was treated as the conventional side and was placed in a commercial chiller (air flow: 2.0 m/s) for 48 h (10 °C for 10 h followed by 0 °C for the remaining 38 h in line with Irish industry practice). After 48 h the same four muscles as above were boned out and prepared.

All muscles were cut to form $10 \times 10 \times 1$ cm samples. Samples were then vacuum packaged using BB3055X vacuum bags (17 cm^3/m^2 O_2, 24 h at 23 °C, 0% relative humidity; 17 cm^3/m^2 O_2, 24 h at 23 °C, 100% relative humidity; 50 cm^3/m^2 CO_2, 24 h at 23 °C, 0% relative humidity) (CryoVac, Sealed Air Ltd., Dublin, Ireland) and heat shrunk at 82 °C for 2–3 s as per industry practice. Samples were then stored at 2 °C and 7 °C and tested immediately ($t = 0$) and every second week for 6 weeks for TVC mesophilic (m), TVC psychrophilic (p), TEC, presumptive *Pseudomonas* spp., LAB, *Clostridium* spp. and *Br. thermosphacta.*

2.1.2. Microbial analysis

Beef primals were sampled by swabbing an area of 100 cm^2 using a sterile cellulose acetate sponge (10×10 cm), pre-soaked in 10 ml Maximum Recovery Diluent (MRD; Technical Service Consultants Ltd., Heywood, Lancashire, UK) in a sterile bag. Samples were obtained by inverting the bag to expose the sterile sponge and rubbing the sponge 5 times horizontally and 5 times vertically over the target area delineated using a sterile template.

Volumes of 100 ml of MRD (0.1% peptone, 0.85% NaCl: Oxoid, Basingstoke, Hampshire, England) were added and samples pulsified for 30 s (Pulsifier, Microgen Bioproducts) before preparation of serial dilutions in MRD and plating in duplicate onto the appropriate agar. TVCm were enumerated using Standard Plate Count agar (SPCA; Oxoid) and incubated at 30 °C for 72 h. TVCp were enumerated using SPCA and incubated at 6.5 °C for 10 days. TEC were obtained on Violet Red Bile Glucose agar (VRBGA; Oxoid) and incubated at 37 °C for 24 h. *Pseudomonas* spp. were enumerated on *Pseudomonas* Base agar (Oxoid) which contained Cetrimide Fucidin Cephalosporin (CFC) selective supplement (Oxoid) and were incubated at 30 °C for 48 h. LAB were enumerated using de Man, Rogosa and Sharpe agar (MRS, Oxoid) at 30 °C for 72 h. *Br. thermospachata* was enumerated by plating onto Streptomycin-thallous acetate-actidione agar (STAA; Oxoid) containing STAA selective supplement (Oxoid) which was incubated at 23 °C ± 2 °C for 48 h. Reinforced Clostridial agar (RCA; Sigma Aldrich) was used for the enumeration of *Clostridium* spp. and plates were incubated anaerobically at 30 °C for 72 h using AnaeroGen sachets (Oxoid) and an Anaerojar (Biomerieux).

2.1.3. Temperature analysis

The core and surface temperature of the hot boned LTL muscle (24 h chill at 0 °C) and the conventionally treated carcasses (10 °C for 10 h followed by 0 °C for the remaining 38 h) were monitored (every 10 min for 96 h) using Testo-T175 (Eurolec Instrumentation LTD) data loggers. For the conventionally treated carcass temperature measurements, one thermocouple was inserted into the *semitendinosus* muscle to measure the core temperature and the other was inserted just under the surface of the same muscle to record the surface temperature. For the hot boned LTL muscle, white seal (Eurolabs) was taped onto the outside of the Pi-Vac pack using multipurpose tape. The thermocouple was then pushed through the seal and placed either into the core or just onto the surface of the sample. Multipurpose tape was used to seal the thermocouple. The core, surface and ambient (air) temperatures of the samples were also monitored over the 6 week storage period at 2 °C and 7 °C.

2.1.4. Surface pH measurements

The surface pH of all samples was also monitored at 0, 1, 2, 3, 4, 5 and 6 weeks using a Sentek P-17 surface electrode (Lennox, Ireland), calibrated with pH 4, 7 and 10 standards immediately before use.

2.1.5. Surface water availability (a_w) measurements

Water availability of sample surfaces was recorded by excising an area of 5 cm^2 using a 25 mm cork borer (VWR), sterile scalpel and forceps. The surface a_w values of all samples were also monitored at 0, 1, 2, 3, 4, 5 and 6 weeks. Before sampling, the cork borer and forceps were flame sterilised using 70% ethanol. Each sample was placed in a sterile plastic Aqualab cup (Labcell, Basingstoke, England). The water activity values (a_w) of each sample were then measured using an Aqualab Lite model water activity meter (Labcell), calibrated before use using a saturated solution of sodium chloride (NaCl, $a_w = 0.984 \pm 0.003$ at 20 °C).

2.2. Monitoring growth of blown pack spoilage Clostridium spp.

2.2.1. Preparation of blown pack spoilage Clostridium spp.

Reference strains *Clostridium estertheticum* subsp. *estertheticum* (DSMZ 8809^T), *C. estertheticum* subsp. *laramiense* (DSMZ 14864^T) and *C. gasigenes* (DSMZ 12272^T) 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) and incubated for 3 weeks at 4 °C. The purity of each strain was confirmed by plating out 0.1 ml aliquots on Columbia Blood Agar (CBA; Oxoid, Basingstoke, UK) supplemented with 5% defibrinated horse blood (Cruinn Diognostics, Dublin, Ireland) and incubated anaerobically at 4 °C for 3 weeks. Colony morphology was identified according to Moschonas, Bolton, Sheridan, and McDowell (2009); grey-white and opaque,