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The microbiology of beef carcasses and primals during chilling and commercial storage

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ABSTRACT

The primary objective of this study was to characterise (microbiology and physical parameters) beef carcasses and primals during chilled storage. A minor aim was to compare observed growth of key spoilage bacteria on carcasses with that predicted by ComBase and the Food Safety Spoilage Predictor (FSSP). Total viable count (TVC), total *Enterobacteriacae* count (TEC), *Pseudomonas* spp., lactic acid bacteria (LAB), *Brochothrix thermosphacta* and *Clostridium* spp. were monitored on beef carcasses (n = 30) and primals (n = 105) during chilled storage using EC Decision 2001/471/EC and ISO sampling/laboratory procedures. The surface and/or core temperature, pH and water activity (a_w) were also recorded. *Clostridium* (1.89 $\log_{10}$ cfu/cm^2) and *Pseudomonas* spp. (2.12 $\log_{10}$ cfu/cm^2) were initially the most prevalent bacteria on carcasses and primals, respectively. The shortest mean generation time (G) was observed on carcasses with *Br. thermosphacta* (20.3 h) and on primals with LAB (G = 68.8 h) and *Clostridium* spp. (G = 67 h). Over the course of the experiment the surface temperature decreased from 37 °C to 0 °C, pH from 7.07 to 5.65 and a_w from 0.97 to 0.93 The observed *Pseudomonas* spp. and *Br. thermosphacta* growth was more or less within the range of predictions of Combase. In contrast, the FSSP completely overestimated the growth of LAB. This study contributes to the very limited microbiological data on beef carcasses and primals during chilling.

1. Introduction

Beef carcasses must be chilled immediately after slaughter and dressing to ensure quality and safety. Current legislation in the European Union, Regulation EC 853/2004 (Regulation, 2004), requires that beef carcasses be immediately chilled after *post-mortem* inspection to ensure a core temperature of not more than 7 °C in the case of meat and not more than 3 °C for offal. However, the European Food Safety Authority (EFSA) recently suggested that different carcass time-temperature combinations could be applied without increasing the growth of pathogenic bacteria and thereby the risk to the consumer (EFSA, 2014a,b). In response the European Commission are reviewing EC 853/2004 with a view to allowing greater flexibility.

In commercial beef plants, carcasses are placed in the chilling unit immediately after slaughter where they usually remain for 48–72 h before being moved to the boning hall. Although beef carcass chilling temperature data is scarce, one study reported average core and surface temperatures of 37.2 °C and 22.1 °C, respectively, immediately after slaughter thereafter decreasing to 9.2 °C and 2.9 °C after 24 h chilling and 3.3 °C and 2 °C after 48 h (EFSA, 2014a). In the boning hall the carcasses are cut into primals and sub-primals which are vacuum packaged and the meat allowed to mature for 3–6 weeks. Deboning normally occurs at temperatures not exceeding 12 °C and vacuum packed meat products are stored between 0 and 4 °C for periods of up to 6 weeks (EFSA, 2014a). The biochemical processes and structural changes that occur in beef during the first 24 h *post-mortem* are critical in determining quality and palatability. Thus, 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, 2014a).

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Meat is a nutrient rich environment that supports bacterial growth. Temperature, pH and a_w affect the growth rate and meat spoilage, which normally occurs when the bacterial count reaches approximately 10^{7-8} $\log_{10}$ cfu/cm^{-2} (Jones, 2004; Nychas et al., 2008). Spoilage is usually characterised by discolouration, strong off-odours and/or slime production. Although it is known that the pH of muscle is about 7.0 at slaughter thereafter decreasing to approximately 5.3–5.8, data on the pH of the carcass surface is lacking as is information on a_w (EFSA, 2014a). Under the aerobic low temperature conditions encountered in beef carcass chill rooms, the spoilage consortium of bacteria is usually dominated by *Pseudomonas* spp. (Stanbridge and Davies, 1998; Koutsoumanis et al., 2006), including slime and off-odour producing *Ps. fragi*, *Ps. fluorescens* and *Ps. lundensis*. *Enterobacteriaceae*, especially cold-tolerant species such as *Hafnia alvei*, *Serratia liquefaciens* and *Pantoea agglomerans*, may also contribute to spoilage especially if there is temperature abuse (Nychas et al., 2008). Other bacteria such as lactic acid bacteria (LAB) and *Brochothrix thermosphacta* are oxygen tolerant but are not major contributors to the spoilage of carcasses. However, carcass contamination with these bacteria is important as once deboned, the primals are stored in anaerobic vacuum packs, where they become the predominant spoilage organisms (Russo, 2006; Ray, 2008; Hernández-Macedo et al., 2011). Chilled meat stored under anaerobic conditions may also be spoiled by a range of psychrotolerant/psychrophilic *Clostridium* spp. (Moschonas et al., 2010; Yang et al., 2010; Bolton et al., 2015).

Despite many years of research on meat microbiology, there is still very little published research on the fate of general bacterial populations (TVC and TEC) and even less on key spoilage bacteria (*Pseudomonas* spp., lactic acid bacteria (LAB), *Brochothrix thermosphacta* and *Clostridium* spp. on beef carcasses and subsequent beef cuts during chilled storage (EFSA, 2014a). As new, more distant markets open for European beef processors, especially in the United States of America and China, a more fundamental understanding of bacterial growth during carcass chilling and primal storage is required if shelf life is to be estimated and extended thus allowing access to these markets.

Predictive models are important tools in predicting the growth of spoilage bacteria thus facilitating the optimisation of chilling regimes that maximise the shelf life of beef products. Combase Predictor in the online ComBase tool provides predictions for two of the main beef spoilage bacteria, *Pseudomonas* spp. and *Br. thermosphacta* (Baranyi and Tamplin, 2004). The Food Spoilage and Safety Predictor software was developed to predict the growth of spoilage and pathogenic microorganisms in a range of foods (FSSP, 2014) but LAB are the only beef spoilage bacteria covered by this software.

The main objective of this study was therefore to microbiologically characterise beef carcasses and primals during the first 2 stages in the beef chill chain (carcass chilling and primal chilled storage) and to obtain physical data during this process (surface and/or core temperatures, pH and a_w measurements). A minor objective was to use these parameters to predict and compare (to that observed) the growth of *Pseudomonas* spp. and *Br. thermosphacta* (using ComBase) and lactic acid bacteria (LAB) (using FSSP) on beef carcasses.

2. Materials and methods

2.1. Carcass chilling

2.1.1. Temperature analysis

On 3 separate occasions, 10 carcasses were randomly selected immediately after entry into the chill room in a commercial beef slaughter plant (n = 30). The ambient temperature as well as the surface and core temperature of 2 of the carcasses were monitored (every 10 min for 96 h) using Testo-T175 (Eurolec Instrumentation LTD) data loggers. The relative humidity in the chiller was also recorded (every 5 min) using an Easylog data logger (Lascar).

2.1.2. Surface pH measurements

At times t = 0, 24, 48, 72 and 96 h, the pH of a lean area (brisket) and fat tissue (rump) was obtained on 5 different carcasses using a SENTEK P-17 surface electrode (Lennox). The electrode was calibrated with pH 4, 7 and 10 standards immediately before use.

2.1.3. Surface a_w measurements

The surface a_w was recorded by excising an area of 5 cm^2 of lean (brisket) and fat (rump) tissue from each of the 10 carcasses at times t = 0, 24, 48, 72 and 96 using a 25 mm cork borer (VWR), sterile scalpel and forceps. 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), sealed and immediately transported back to the laboratory. The water activity values (a_w) of each sample was then measured using an Aqualab model CX-2 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).

2.1.4. Carcass sampling and microbial analysis

The 30 carcasses were sampled at time t = 0, 24, 48, 72 and 96 h using the sampling procedure described in EC Decision 2001/471/EC and using the sampling method of Lasta et al. (1992). Briefly a sterile cellulose acetate sponge (10 × 10 cm) was pre-soaked in 10 ml MRD (Technical Service Consultants Ltd.) 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 (100 cm^2) delineated using a sterile template. Alternative sides of the same sponge were used for 2 sites. After swabbing the loaded sponge was withdrawn into the reverted bag and the 2 sponges (4 sites on a single carcass) pooled. As per EC Decision 2001/471/EC, the neck, brisket, flank and rump were sampled on each carcass.

In the laboratory, 100 ml of MRD (0.1% peptone, 0.85% NaCl: MRD; Oxoid, Basingstoke, Hampshire, England) was added to each pair of pooled swabs and pulsified for 30 s (Pulsifier, Microgen Bioproducts), serial dilutions prepared in MRD and plated in duplicate. Mesophilic total viable counts (TVCm) were enumerated using standard plate count agar (SPCA; Oxoid) and incubated at 30 °C for 72 h. Psychrophilic TVC (TVCp) were enumerated using SPCA and incubated at 6.5 °C for 10 days. Total *Enterobacteriaceae* Counts (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. Lactic acid bacteria (LAB) were enumerated using de Man Rogosa agar (MRS, Oxoid) (pH 6.2) at 30 °C for 72 h. *Br. thermospachata* was plated onto Streptomycin-thallous acetate-actidione agar base (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 was incubated anaerobically at 30 °C for 72 h using AnaeroGen sachets (Oxoid) and an Anerojar (Biomerieux). As RCA is non-selective for *Clostridium* spp., their presence was confirmed by real time PCR detection using the method developed by Song et al. (2004).