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Culture-dependent and culture-independent assessment of spoilage community growth on VP lamb meat from packaging to past end of shelf-life

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ABSTRACT

Packaging and storage temperature are important factors that influence the shelf-life of vacuum packed (VP) meat. In this study the shelf-life of VP bone-in lamb hind shanks stored at 8 °C and −1.2 °C was determined in parallel to analyses of starting and eventual spoilage bacterial communities via Illumina MiSeq based 16S rRNA amplicon sequencing. The mean total viable counts (TVC) and lactic acid bacterial viable counts (LAB) were observed to increase to log 7.5 CFU/cm^2 and 7 CFU/cm^2 after 6 and 42 days at 8 °C and −1.2 °C and stayed stable until shelf-life termination after 13 and 124 days, respectively. The sequence data showed initial communities were patchily distributed and were mainly derived from skin microbiome taxa likely prevalent within the abattoir. A broad diversity of VP meat associated specific spoilage organisms (SSO) were comparatively abundant in this initial population. Overtime meat spoilage communities developed a distinctive and stable microbiome. At −1.2 °C SSO included mainly *Carnobacterium*, *Yersinia* and *Clostridium* spp. while at 8 °C SSO expanded to include *Hafnia*, *Lactococcus*, *Providencia* spp. Growth curves inferred from the sequence data after taking into account rRNA copy number suggested that SSO growth rates were consistent with overall growth rates determined from TVC and LAB data and are predictable.

1. Introduction

Food spoilage is considered to be any change in food quality that makes it undesirable for human consumption (Hayes, 1998). Spoilage is normally associated with obvious changes in product appearance, odour and taste. Since food spoilage is the combination of microbial and biochemical activities (Huis in't Veld, 1996) controlling microbial growth and maintaining product stability is required to ensure consistent shelf-life and product quality (Eustace et al., 2014). During slaughtering and handling lamb meat is contaminated with a broad category of microorganisms (Doulgeraki et al., 2012; Garcia-Lopez et al., 1998); only small fraction of these microbes will grow sufficiently to cause overt deterioration during distribution and storage. Estimates of meat wastage within/from Oceania at the packaging/processing and subsequent distribution level is at 5 and 4 percent, respectively, though for red meat this is likely lower since the data incorporates poultry meat, which has a short shelf-life (FAO, 2011).

The meat industry produces meat products with shelf-life ranging from a few days (ground meat) to more than one year (frozen meat) (Eustace et al., 2014). Meat is very susceptible to microbial growth since it has high water activity and high concentrations of readily available nutrients. Combined environmental conditions including temperature, pH and gaseous atmosphere dictate the growth rates and yields of specific spoilage organisms (SSO) with temperature being instrumental in determining the length of shelf-life (Mills et al., 2014; Nychas et al., 2008). Good temperature control of meat during distribution substantially improves shelf-life since it constrains the division rates of microbes present that are able to grow under the given packaging conditions. Vacuum (VP) or modified atmosphere packaging using plastic bags with low O_2 permeability effectively extends the shelf-life of refrigerated fresh meat by inhibiting the growth of aerobic psychrotolerant SSO (Gill and Penney, 1985). The combination of negligible O_2 levels, elevated CO_2, meat pH, chill storage, and potential antimicrobial activities of microbes that grow under VP conditions (Zhang et al., 2015) synergistically interact to dictate meat stability. Minimizing contamination and maintaining low

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temperature of VP meat is considered key to reduce the incidence of blown pack spoilage caused by excessive CO_2 production by SSO (Moschonas et al., 2011).

Beef cuts have been used in many studies of VP red meat spoilage (Hernández-Macedo et al., 2012; Silva et al., 2012; Yang et al., 2014; Jääskeläinen et al., 2016) but less information is available for the spoilage of VP lamb meat, which typically has a higher pH and more adipose tissue (Mills et al., 2014). The storage life for chilled VP lamb meat is shorter than VP beef, approximately 12 weeks at −0.3 °C (Kiermeier et al., 2013). According to Gill (2004) low pH meat normally has high concentration of undissociated lactic acid that is able to inhibit bacterial growth under anaerobic conditions. Whereas, high pH favours bacteria with greater spoilage potential to grow and some at relatively higher rate (Mills et al., 2014). Speedy spoilage and shorter shelf-life of dark, firm dry VP beef is often attributed to its high pH (pH 6), favouring the growth of the members of *Enterobacteriaceae* family (Newton and Gill, 1980). The research described here determined the spoilage dynamics of bacteria on VP lamb meat at temperatures that represent the limits of commercially feasible chilling (~-1 °C) and an elevated temperature (8 °C) that is indicative of failure of effective refrigeration within a supply chain. The lamb cut examined here included bone-in hind shank, which can be considered to potentially have a more variable shelf-life than bone-less primals due to uneven bone sections potentially causing problems with VP bag sealing as well as potentially greater microbial loads due to being located at the rear of the carcass. The main questions that we wanted to answer in the study included: i) what particular microbial species predominate on VP lamb meat under different temperatures and how variable is this association; ii) how do initial bacterial populations on meat surfaces derived from exposure to the abattoir environment associate with resultant spoilage; and iii) determine how growth dynamics of SSO vary from the packaging step to product spoilage and how this relates back to the initial bacterial colonisation process in the abattoir. An attempt was made to estimate absolute bacterial numerical data based on the Illumina sequence information and from that estimate growth rates for individual SSO on the VP samples analysed. For this process we relied on total viable counts (TVC) data using the assumption it was close to the total bacterial population, however primarily the purpose was to determine if growth rates of SSO were comparable to growth rates derived from the TVC data. This approach aligns with the primary research approach to analyse microbial growth dynamics up to and past the end of product shelf-life starting with initial communities established on fresh raw meat within the abattoir. Bacterial communities were determined using 16S rRNA gene amplicon sequencing with the Illumina MiSeq platform, providing a deep analysis of the community especially in the initial samples allowing assessment of multiple taxa across logarithmic scales of abundance. These analyses were correlated to product quality changes investigated using total viable counts and sensory analysis (using an informal panel) allowing demarcation of the end of shelf-life. A number of 16S rRNA gene based techniques such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP), species specific PCR and real time PCR have been applied in meat for monitoring bacterial communities (Brightwell et al., 2009; Jiang et al., 2011; Kaur et al., 2017; Pennacchia et al., 2009). Although being culture independent thereby avoiding problems associated with selective cultivation, the resolution of microbial community structure provided by these techniques is still limited and some are semiquantative. High throughput 16S rRNA gene amplicon sequencing is an established tool in food studies providing comprehensive insight into bacterial community diversity and dynamics during storage, contaminants source tracking and identification of core spoilage bacteria (Chaillou et al., 2015; Ercolini et al., 2011; Kergourlay et al., 2015; Stellato et al., 2016). Illumina MiSeq, a high throughput sequencing approach allows rapid identification of a wider number of species and provide quantitative estimation of abundance of different taxa based on the number of reads of a particular 16S rRNA gene sequence in studied samples (Doulgeraki et al., 2012; Ercolini, 2013).

The results obtained indicate that there is a strong connection between microbial communities that are stochastically formed on the meat surface during processing and the final spoilage community. Dynamics of bacterial growth of the primary spoilage taxa could be assessed with reasonable accuracy from the sequence data by utilizing the TVC data with clear observations possible between spoilage bacteria growth in relation to progressive product spoilage.

2. Material and methods

2.1. Sample collection and storage

The meat cut studied consisted of bone-in hind shank that did not undergo any form of treatment before the experiment. Hind shanks were cut and vacuum packaged within a local export abattoir from a single mob of animals slaughtered on the previous day. Hind shanks were individually vacuum packaged in Thermasorb bone-in barrier bag E86 (O_2 transmission rate of <10 cc/m^2/24 h at 23 °C, 85% relative humidity). A total of 74 fresh VP lamb meat samples were obtained with 30 samples stored at 8 °C while 39 samples were stored at −1.2 °C. The five samples collected at the point of packaging (time 0) were common for both storage temperatures.

2.2. Microbiological and sensory analyses

For each time point of sampling 5 replicates (except at last three time points of storage at −1.2 °C where the numbers of replicates were either 3 or 4) were analysed. For −1.2 °C storage samples were subsequently tested after 42, 70, 77, 84, 93, 98, 115, 124 and 140 days, while for 8 °C samples, sampling was done at 7, 10, 13, 16, 19 and 21 days of storage. For the sensory assessment, both vacuum package and seal/shrink used a score of 0 (no vacuum/seal - leaker) to 8 (complete vacuum/good seal - tight package adhesion), confinement odour was assessed on an 8-point categorical hedonic scale (0 = off-odour, spoiled to 8 = no odour, normal meaty odour), and colour was determined by visually anchored scale (0 = other colour e.g. green colouration; 2 = very poor bloom, grey colouration; 4 = poor bloom, some greyness; 6 = bloom, light red colouration; 8 = bloom, red colouration) (Small et al., 2012). Any score ≤4 was considered as commercially unacceptable. Persistent odour and colour bloom were assessed after 30 min of pack opening. The colour and odour were observed twice i.e. on opening the pack and after 30 min, because they were the parameters that showed the most noticeable change in sensory quality in the experiments. In addition, the meat colour was compared against meat colour chips (AUS MEAT, 2005). The meat surface pH was measured using a digital pH meter (ORION model 250A). The whole meat cut surface (hind shank) area including both lean and fat surfaces was calculated in order to allow for determination of colonies forming unit per unit area of meat. After opening, the pack individual hind shanks were transferred to sterile bags and were massaged in 100 mL of sterile 0.1% peptone water for 2 min. Serial dilutions were prepared in sterile 0.1% peptone water and spread plated. Total viable counts were enumerated using tryptone soya agar (TSA) (Oxoid, Australia) plates, which were incubated at 20 °C for 5 days. For lactic acid bacteria (LAB) de Man-Rogosa-Sharpe (MRS)