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Microbial growth, communities and sensory characteristics of vacuum and modified atmosphere packaged lamb shoulders

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ABSTRACT

Packaging fresh lamb in a vacuum (VAC) versus a 100% CO_2 modified atmosphere (MAP) may influence product shelf-life and the bacterial communities. While VAC is a common packing method and 100% CO_2 MAP is used in some countries, there is little information about how these different techniques affect the growth of spoilage bacteria and sensory attributes of lamb. The aim of this study was to assess changes in microbiological and organoleptic properties, and determine differences in microbial communities by terminal restriction fragment length polymorphism (TRFLP) and 454 pyrosequencing, in bone-in (BI) and bone-out (BO) MAP- and VAC-packed lamb shoulders stored at −0.3 °C over 12 wk. VAC and MAP lamb shoulders were acceptable in sensory test scores over 12 wk of storage at −0.3 °C, despite total viable count (TVC) and lactic acid bacteria (LAB) levels increasing to 8 $\log_{10}$ CFU/cm^2 for VAC lamb and 4–6 $\log_{10}$ CFU/cm^2 for MAP lamb. Similar to the sensory results, there were no significant differences in microbial communities between BI and BO product. However, types of bacteria were different between VAC and MAP packaging. Specifically, while VAC shoulder became dominated by *Carnobacterium* spp. in the middle of the storage period, the MAP shoulder microbial population remained similar from the start until later storage times.

1. Introduction

Most studies on vacuum (VAC)-packed meat have used beef cuts. By comparison, there is little information on the hygienic quality of sheep meat (Phillips et al., 2001). However, there are some early investigations of microbial communities on Australian and New Zealand sheep meat and in abattoirs (Gill and Penney, 1985; Phillips et al., 2006, 2001; Sumner et al., 2003; Vanderlinde et al., 1999). A recent microbiological survey on fresh sheep meat in Australian abattoirs found, in general, Total Viable Count (TVC) have shown a downward trend with counts approximately 2–2.8 log CFU/g in the most recent survey (Phillips et al., 2013). Beef generally has a uniform surface, being largely tissue from a single muscle, and a low pH (5.5–5.8). In contrast, lamb meat tends to have a higher pH (5.6–6.8) and a heterogeneous surface, as much of the cuts are adipose rather than muscle tissue (Gill and Penney, 1985).

Not much is known about the diversity of microbial community dynamics on lamb meat, as microbiological testing has depended on culture-based methods with limited ability to assess the total microbial community. In recent years, advances in molecular techniques, such as 16S rRNA gene-based terminal restriction fragment length polymorphism (TRFLP) and 454 pyrosequencing provide a culture-independent means to improve microbial community analysis. TRFLP provides a generalised appraisal of the diversity of bacterial communities by assessing phylogenetic differences of fragment lengths in a given sample (Liu et al., 1997) and has been used in a variety of food such as meat (Nieminen et al., 2011) fermented beverages (Bokulich and Mills, 2012) and salmon (Powell and Tamplin, 2012). TRFLP has the advantage of been able to detect differences in a large number of samples and the data produced can be analysed using conventional multivariate statistical software. 454 Pyrosequencing can also give a phylogenetic assessment of microbes in a complex community such as meat, for example, mince (Nieminen et al., 2011), chicken meat (Nieminen et al., 2012) and beef (Ercolini et al., 2011; Ercolini, 2013). Furthermore, combining these techniques with environmental and/or biochemical measurements can reveal the relationships between microbial community, metabolism and how this affects meat sensory perception and spoilage (Ercolini et al., 2011).

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Australian lamb meat exports in 2011–2012 were worth $1.094 million, hence, a product with a long shelf-life could provide some market advantage (Anon., 2012). Previous research by the authors on boneless lamb shoulders indicated that consumer acceptability remained high for product that had been VAC-packed and stored for up to 78 d (Holds et al., 2010; Kiermeier et al., 2009). There has also been a study of Australian beef cuts that demonstrated long shelf-life associated with aerobic plate (APC) and lactic acid bacteria (LAB) counts (Small et al., 2012). However, the growth curves for total viable counts (TVC) and LAB have not been quantified for lamb products. In addition, there is no information on the microbial ecology of these products. Furthermore, there is only anecdotal evidence that bone-in product often has a shorter shelf-life than the corresponding boneless product. Hence, these variables were included in this study.

The aim of this study was to assess changes in the microbiological (TVC and LAB) and determine differences in microbial communities by TRFLP and 454 pyrosequencing, in bone-in (BI) and bone-out (BO), modified atmosphere packaged (MAP) and VAC-packed lamb shoulders stored at −0.3 °C over 12 wk.

2. Materials and methods

2.1. Sample collection

Lamb shoulders were either BI or BO and sourced from a commercial abattoir in Victoria, Australia. Shoulders were boned as required and packed on the morning of 2 March 2011 from a single mob of animals slaughtered on 28 February 2011. Shoulders were individually vacuum-packed or modified atmosphere packed in packs of four in Protite Ultra Shrink Bags (O_2 trans of 18.6 cc/m^2/24 h at 23 °C, 0% relative humidity). MAP was done using a ratio of 1:1.5 of meat to CO_2 (pers. comm. I. Eustace). MAP shoulders were also individually wrapped in moisture-absorbing paper. To allow for weekly sampling of four shoulders over the 85-day storage trial, a total of 48 shoulders were ordered per product type. Meat samples were collected for microbiological testing from product prior to packing. Product was shipped to the South Australia Research and Development Institute (SARDI) Food Safety and Innovation laboratories by a commercial carrier using refrigerated transport.

2.2. Product storage and temperature monitoring

Four data loggers were placed inside four separate boxes of product prior to shipping to SARDI laboratories. The product arrived at SARDI on a shipping pallet and the boxes with loggers were located at different points throughout the pallet. Boxes were removed from the pallet and spread throughout a walk-in cool room set at −0.5 °C to allow for quick cooling. For long-term storage, 12 data loggers set to record every 20 min were placed in different boxes of product to monitor temperature throughout the trial. At each sampling time, one logger was removed when product was sampled for microbiological analyses.

2.3. Sampling

One surface slice sample was collected aseptically from each of four BI and four BO shoulders prior to VAC or MAP packaging. Microbiological evaluation of the various product types was conducted on a weekly basis for a total of 85 days. Because not all product requested from the company was delivered, or suitable for the storage trial, a reduced sampling scheme (Table 1) was developed, including modifications that were necessary throughout the trial when packs were found to not be intact. Sampling of VAC product was undertaken on shoulders that had been individually packed, while for MAP packs the four samples were collected from the four shoulders packed together (one per shoulder).

Table 1
Sampling schedule indicating the number of replicates sampled at each time point for sensory analysis, TRFLP and pyrosequencing.

Age (days)	Boning status	Packaging	Replicates	TRFLP	Pyrosequencing
0	BI	Fresh	4	4	2
0	BO	Fresh	4	4	2
7	BI	MAP	4		
7	BI	VAC	4		
7	BO	VAC	2		
14	BI	MAP	4	4	2
14	BO	MAP	4	4	2
14	BI	VAC	4	4	2
14	BO	VAC	3	3	2
21	BI	MAP	4		
21	BO	MAP	4		
21	BI	VAC	4		
21	BO	VAC	2		
28	BO	MAP	4	4	
28	BI	MAP	4	4	
28	BO	VAC	2	2	
28	BI	VAC	4	4	
35	BI	MAP	4		
35	BO	MAP	4		
35	BI	VAC	4		
35	BO	VAC	3		
41	BI	MAP	4		
41	BO	MAP	4		
41	BI	VAC	4	4	2
41	BO	VAC	3	3	2
49	BI	MAP	4	4	2
49	BO	MAP	4	4	2
49	BI	VAC	4		
49	BO	VAC	3		
56	BI	MAP	4		
56	BO	MAP	4	4	
56	BI	VAC	4	4	
56	BO	VAC	2	2	
63	BI	MAP	4	4	
63	BO	MAP	4		
63	BI	VAC	4		
63	BO	VAC	2		
70	BO	MAP	4	4	
70	BI	VAC	4	4	
70	BO	VAC	2	2	
77	BI	VAC	4		
77	BO	VAC	2		
77	BI	MAP	4	4	2
84	BO	MAP	4	4	2
84	BI	VAC	4	4	2
84	BO	VAC	2	2	2
			164	**90**	**28**

2.4. pH

The pH of the uncut (freshly exposed surface) and cut surface (immediately after taking a sample) were measured using a Hanna pH electrode (HI1413B, Hanna Instruments Ltd Bedfordshire, UK). The calibration of the pH probe was checked prior to every sampling session and approximately twice during each sampling session. Differences in the average pH over time between the packaging type and boning state, and their interaction were assessed using a two-way analysis of variance.

2.5. Microbiological analyses

Excision samples were aseptically taken from each of four BI and BO lamb shoulders immediately prior to packing at the abattoir. Fat and lean surfaces were excised to a depth of approximately 2–