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Characterization of volatile metabolites associated with confinement odour during the shelf-life of vacuum packed lamb meat under different storage conditions

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ABSTRACT

Confinement odour was investigated. Volatiles were extracted directly from the pack, using solid phase microextraction and analysed by gas chromatography–mass spectrometry. Sensory evaluation and microbiological analysis of the meat surface were also performed. Commercial samples of vacuum packed lamb legs (n = 85), from two meat processing plants, were kept for 7 weeks at −1.5 °C then at different regimes of temperature (−1.5 to +4 °C) until 11, 12 or 13 weeks. Persistent odour was observed in 66% of samples, confinement odour in 24% and no odour in 11%. Volatiles associated with confinement odour (3-methyl-butanal, 3-hydroxy-2-butanone and sulphur dioxide) corresponded with end/sub products of glucose fermentation and catabolism of amino acids by bacteria (all bacteria naturally found in meat and do not represent a risk to health). Confinement odour could indicate a stage at which the environment for bacteria growth is becoming favourable for the production of volatiles with strong odours that are noticed by the consumer.

1. Introduction

The shelf-life of meat comprises the period of acceptability by the consumer, the end of which is marked by the deterioration of one or several noticeable attributes. Extension of shelf-life has been successfully achieved by several strategies including the combination of strict temperature control and packaging system. For example, the storage lifetime for vacuum packed lamb held at −1.5 °C has been estimated to be up to 70 days (Mills et al., 2014). Most vacuum packed lamb products have a heterogeneous surface, comprising of a mixture of adipose and muscle tissue, which can result in a high pH allowing the growth of bacteria with high potential to cause spoilage (Kiermeier et al., 2013). As a result of vacuum packaging and ongoing respiration of the meat tissue, the oxygen present in atmosphere of the meat is depleted and CO_2 is released, preventing the growth of obligate aerobic spoilage bacteria and leading to a slow growth of CO_2 tolerant bacteria. It is proposed that a succession of different bacterial populations is expected to develop during storage (Mills et al., 2014). At −1.5 °C, this is usually dominated by lactic acid bacteria (LAB), unless sufficient numbers of spoilage bacteria are present to sustain a population. As a result of this bacterial growth the vacuum packed lamb product will reach the end of shelf-life marked by quality faults such as discoloration, off-flavours and odours. The presence of significant populations of spoilage bacteria (notably psychrotrophic *Clostridium* spp., Enterobacteriaceae, *Brochothrix thermosphacta* and *Shewanella putrefaciens*) will bring about earlier loss of shelf-life through off-flavours and odours, with or without pack distortions and discolouration (Mills et al., 2014).

Meat stored in a vacuum packs may display unusual odours that disappear just after it is opened. This phenomenon is known as confinement odour (Johnson, 1991). While it does not occur in every pack, it can be easily mistaken as spoilage, even though the meat is microbiologically safe and wholesome to eat. The nature and origin of confinement odour in lamb meat is not yet fully understood. While several studies have been undertaken to characterize and understand the production of volatile organic compounds (VOCs) by spoilage causing bacteria, a limited number of them focus on vacuum packed lamb (Casaburi et al., 2014). We propose that the presence of confinement odour does not necessarily indicate spoilage and the VOCs associated with it might not be directly linked to spoilage. Therefore, this study investigates the association of confinement odour with measures of bacterial growth and VOCs found inside vacuum packed chilled lamb to provide a better understanding of the causes of confinement odour and impact if any on shelf-life.

To investigate VOCs associated with confinement odour it is necessary to detect the VOCs inside the vacuum pack before it is open.

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Headspace static–solid phase microextraction (HS–SPME) is a sampling technique that fits this purpose (Kataoka et al., 2000). HS–SPME has previously been applied to meat and meat products (Jeleń et al., 2012) but most studies have been performed on samples placed in a sterile vial (Ercolini et al., 2011; Estévez et al., 2003; Garcia-Esteban et al., 2004; Ruiz et al., 1998; Vasta et al., 2011) and only a few studies have attempted to performed it in an intact vacuum pack (Bhattacharjee et al., 2011; Casaburi et al., 2011). Jackson et al. (1992) described the evaluation of VOCs in vacuum pack but using a different procedure for sampling VOCs, based on dynamic headspace samples collection. The SPME fibre is an apparatus coated with an extracting phase where the volatile compounds are concentrated onto. The type of fibre coating plays an important role on the performance of the SPME, as it controls the amount and type of compounds that are collected from the sample within a period of exposition time. Also important is the length of time the fibre is exposed to volatiles. The aim of this study was to investigate the nature of confinement odour by sampling VOCs inside vacuum packaged chilled lamb packs before they were opened. Initially, we investigated the conditions for application of HS–SPME required for collection of volatiles from inside of the intact vacuum pack. We then performed the evaluation of commercial samples stored at three different storage periods under different temperature profiles to stimulate different levels of bacterial growth. These conditions were selected to mimic those used for the transport of vacuum packed lamb to distant markets and also to include variation below and above these standard conditions.

2. Material and methods

2.1. Evaluation of HS–SPME for sampling volatiles in the vacuum packed lamb

2.1.1. Sample collection

Lamb legs with shank on were obtained from a commercial abattoir. The legs (n = 9) were packed under CO_2 atmosphere in one bag. The expected shelf-life for these samples at −1.5 °C is 14 weeks (98 days). The samples were kept at −1.5 °C for 90 days after which they were opened, kept on a sterilised table for 60 min at 10 °C (to allow CO_2 to be expelled) then re-packed into individual bags and transferred to a chiller at 2 °C. Samples were divided in two sets, one set packed (A600 Barrier bag, CRYOVAC, Sealed Air, Hamilton New Zealand) in bag size 1 (300 × 390 mm) and the second in bag size 2 (400 × 595 mm). The two different bag sizes were used to allow two different gas volumes to be pumped into the bag, as explained in more detail in the Section (2.1.2).

2.1.2. Volatile collection

After 27 days at 2 °C samples were analysed. On the day of analysis each sample was taken out of the chiller (2 °C) and left for 1 h at room temperature, after which N_2 was pumped into it using a digital mass flow controller (part number MC-5SLPM-D, Alicat, USA). The set of samples in bag size 1 (n = 5) were filled with 500 mL of N_2 and those in the bag size 2 (n = 4) were filled with 1000 mL of N_2. Each bag was sampled with 3 SPME fibres and for 2 exposition periods (i.e. 15 min and 30 min). The three fibre types investigated were: 1) CAR-PDMS-DVB (Supelco, Bellefont, PA, 50/30 μm 57298-U); 2) CAR-PDMS (Supelco, Bellefont, PA, 75 μm, 57343-U); and 3) PDMS-DVB (Supelco, Bellefont, PA, 65 μm, 57310-U). These are mixed coating fibres, containing polydimethylsiloxane (PDMS), divinylbenzene (DVB) and carboxen (CAR). The fibres were conditioned following supplier recommendations. The order of fibre exposition was done randomly. An internal standard was added (5 μL, 0.96 mg/mL of 1,2-dichloro benzene in methanol, Sigma Aldrich) after filling the bag with N_2. The vacuum bag containing gas (N_2) and internal standard were equilibrated for 30 min and then the SPME fibre was exposed for 15 or 30 min at room temperature (20 °C) using a septum (15 mm, PBI Dansensor from FF instrumentation, Christchurch—NZ) glued to the bag. However, the internal standard used for the volatile compounds analyses did not show consistent results across the samples (mean-peak area = 762,604, sd = 269,374, cv = 35%) and could not be used to normalize the compounds areas. After adsorption, the fibre was removed from the bag and immediately inserted into the GC–MS injector (Shimadzu QP2010 GC–MS). The injector was set at 250 °C and splitless mode. The SPME desorption time was 1 min, and the fibre was maintained at injector for 14 min to clean and avoid contamination between samples. Helium was used as carrier gas with a flow rate at linear velocity of 43 cm/s. Volatiles were separated using a Restek RTx 5sil MS column (30 m × 0.25 mm × μm). The GC oven temperature was 0 °C (this temperature was achieved using a cryogenic valve and liquid CO_2), held for 2 min, then raised to 200 °C at 6 °C/min and to 290 °C at 30 °C/min and then held for 2 min with a total acquisition time of 37 min. The GC–MS interface was set to 250 °C. The mass spectra acquisition was performed in electron impact (70 eV), in the full scan mode from m/z 43 to 500. Compound identification was performed by comparison with the mass spectra of the NIST8 mass spectral library, by comparison with linear retention indices calculated using commercial standards of hydrocarbons (n-paraffin mix C5 to C8 and n-paraffin mix C10–16 from Aldrich) with those described in the database of the National Institute of Standards and Technology. Table 2 presents information about identified compounds. In the following are the retention time in min., ions monitored and retention index calculated (respectively in brackets) for those compounds not presented in Table 2: 2-methyl-propanal (6.278, 72, 558); 2,3-butanedione (7.387, 43, 592); hexene (7.648, 86, 600); 2-pentanone (10.927, 86, 700); pentanal (11.243, 44, 709); heptane (11.357, 71, 713); 3-hydroxy-2-butanone (11.708, 45, 730); 3-methyl-2-hexanol (14.55, 45, 836); 2-octanol (17.845, 45, 959); ethyl acetate (8.518, 43, 626); 3-methyl-hexane (10.438, 71, 685); methyl butanoate (12.21, 74, 749); 3-methyl-1-pentanol (14.458, 69, 833); butanoic acid (15.007, 73, 853); 4-methyl-1-pentanol (16.172, 69, 897); 2-methyl-butanoic acid, (16.772, 74, 919); methyl 4-methyl-pentanoate (17.885, 74, 961); decanol (24.785, 70, 1255); ethyl hexanoate (20.138, 88, 1076); butyl heptanoate (25.502, 99, 1280); pentanoic acid (19.963, 60, 952); heptanoic acid (20.555, 60, 1096); octanoic acid (22.252, 60, 1178); 3-methyl-1-butanol (13.812, 70, 809). The identification of sulphur dioxide (SO_2) was confirmed by analyses of commercial SO_2 gas (BOC, Hamilton).

Sampling for aerobic bacteria counts (APCs) was performed after each sample was evaluated following protocol described in Sections 2.2.4.1 and 2.2.4.2.

2.1.3. Data analysis

2.1.3.1. Data collection. All GC–MS data files were converted from QGD format (Shimadzu proprietary data file format) to netCDF (network Common Data Format) to allow the data to be interpreted outside the Shimadzu LabSolutions software. Relevant data was then extracted from the netCDF file using 'xcms' package from R (R Core Team, 2012).

2.1.3.2. Exploratory analysis. The peak area in the selected ion chromatograms were organized in a matrix, where each row of the matrix corresponded to a sample and each column to a volatile compound. The exploratory analysis was performed to identify similarity among compounds and how they were related to different HS–SPME conditions (exposition time, fibre type and head space volume). Prior to the analysis the matrix was auto-scaled (also known as z-transformation applied to each column), where each column was subtracted by the mean of its values and divided by the standard deviation of its values (Hendriks et al., 2011). With this pre-processing each column had a mean value equal to zero and was expressed in units of standard deviation, i.e. all columns were in the same scale having similar importance in the analysis. Hierarchical cluster analysis (HCA) with complete linkage as clustering method using Euclidean distance was applied across