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Evaluation of volatile organic compound release in modified atmosphere-packaged minced raw pork in relation to shelf-life

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ABSTRACT

Volatile organic compounds (VOCs) released from fresh meat during storage have the potential to be reliable parameters for monitoring shelf-life and/or spoilage using simple sensors. The VOC release profiles in raw, minced pork packaged under modified atmosphere (70% O_2, 30% CO_2) stored at 4 °C were measured on days 0, 3, 6, and 12 of storage by proton transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS). Additional sensory and solid-phase microextraction gas chromatography mass spectrometry/olfactometry (SPME-GC–MS/O) analyses of sample subsets allowed for a comparison of VOC profile changes with changes in sensory impressions and aided compound identification. Alcohols, aldehydes, ketones and acids were observed to change over time, either increasing continuously or decreasing after reaching maximum concentrations early to midway during storage. Marked differences were observed in the concentrations of several compounds between the fresh (day 0) and aged samples (day 12). Sensory analyses indicated strong increases in buttery and greasy odours, moderate increases in cheesy, vomit-like, sweaty and sour-cream-like odours, and decreases in fruity odours. The number of lactic acid bacteria (LAB), *Enterobacteriaceae*, pseudomonads, psychrotrophs, mesophiles, yeasts and moulds increased throughout storage. Percentage wise, LAB dominated the microbial population at each sampling point and pH decreased during storage. Values of thiobarbituric acid reactive substances (TBARS) indicated marginal lipid oxidation during the first week of storage. The present study provides useful information on the development of characteristic volatile spoilage compounds and offers insights for the potential future implementation of specific sensors to monitor shelf-life.

1. Introduction

It is estimated that approximately one-third of all edible food currently produced for human consumption is wasted or otherwise lost along the supply chain, which equates to about 1.3 billion metric tonnes or ~95–115 kg per capita per year in North America and Europe (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011). Food wastage in the European Union exceeds 89 million tonnes per annum (179 kg per capita per year) and is expected to reach up to 126 million tonnes by 2020 (a 40% increase) if no action is taken (Secondi, Principato, & Laureti, 2015). The increasing worldwide trend towards a meat-based diet, of which pork currently accounts for approximately 40% of red meat consumed, will lead to a greater demand on meat resources as the global population rises (Revell, 2015).

Consumers demand high food quality in terms of odour, flavour, composition, texture and appearance. When consumers perceive that a product has deteriorated it is often discarded even if it poses no risk to health; odour is a key indicator for consumers to make such decisions. Detailed knowledge on the sensory cues that lead consumers to reject a product offers opportunities to develop methods and/or strategies to monitor food quality or to extend product shelf-life, thereby helping to mitigate food waste and economic loss. In meat, an increased production of odorous sulphur derivatives, ammonia, acetone, methane, and organic acid-based components are the major volatile organic compounds (VOCs) impacting on quality and indicating the deterioration of a packaged product (Lovestead & Bruno, 2010). A greater knowledge of the relationships between packaging regimes, storage conditions, contaminating microorganisms, and VOC generation – what the consumer smells – may help to establish strategies to extend the shelf-life of packaged meat products.

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Previous studies have shown that specific VOCs in packaged meats are associated with the enzymatic degradation of proteins and fats by exogenous enzymes of bacterial origin (Balamatsia, Patsias, Kontominas, & Savvaidis, 2007). Spoilage microorganisms in refrigerated meats included *Pseudomonas*, lactic acid bacteria (LAB) and *Enterobacteriaceae,* all of which typically metabolise components such as sugars and amino acids into volatile and non-volatile compounds (Nychas, Marshall, Sofos, Doyle, & Beuchat, 2007). The type and rate of spoilage is governed by many factors, notably the storage environment of the meat. Fresh meat is often sold in modified atmosphere packaging (MAP) that contains CO_2 to extend its shelf-life by inhibiting the growth of the obligate aerobic pseudomonads and shifting the microflora towards gram-positive facultative anaerobic bacteria such as LAB (Doulgeraki, Ercolini, Villani, & Nychas, 2012). MAP currently in use for fresh, red meat typically contains 70–80% oxygen to enhance myoglobin oxygenation and thereby retain the red colour of the meat, which is a major freshness indicator for consumers. Oxygen also inhibits the growth of anaerobes, some of which can be pathogenic (Doulgeraki et al., 2012). Identifying and characterising VOCs released from meat may provide key information on the sensory and microbiological quality of packaged products. Furthermore, a qualitative assessment of VOCs can potentially be used for the identification of specific microorganisms such as *Enterobacteriaceae, Pseudomonas* and LAB in packaged meats (Nieminen, Dalgaard, & Björkroth, 2016).

The present study aimed to establish an analytical method that allows for continuous, on-line monitoring of VOCs in the headspace of meat stored under modified atmosphere at chilled conditions over an extended period. The analytical approach utilised proton transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS) to monitor and quantify VOCs released from fresh, raw minced pork stored under modified atmosphere during spoilage. These analyses were complemented with solid-phase microextraction gas chromatography mass spectrometry/olfactometry (SPME-GC–MS/O) to confirm compound identifications. Sensory, microbial, pH and lipid oxidation analyses provided supporting data to understand the observed VOC profiles.

2. Materials and methods

2.1. Packaging and storage

Pork collars were purchased from a local butchery in Freising, Germany, transported in cold storage to the laboratories and immediately processed. Three batches of pork were purchased for the three independent trials and the analytical experiments of each trial were conducted in triplicate (n = 9). The pork collars were prepared by first removing the outer layer (~1 cm thickness) of the fresh cuts under sterile conditions using a sterile knife. The remaining meat was minced in a meat grinder (MEW 713-H82, MADO, Dornham, Germany) and formed by hand into meatballs (~ 20 g each). A total of 120 meatballs were prepared in this manner, allocated as four sets (four storage periods) of three meatballs each (triplicate analysis) for PTR-TOF-MS, SPME-GC–MS/O, microbial, pH and lipid oxidation analyses, respectively (totalling 60 meatballs), plus the samples for sensory analyses comprising four sets (four storage periods) of three meatballs (three independent trials) in five replicates (five trays of each sample for distribution amongst the panel) (totalling 60 meatballs). All prepared samples were immediately placed in a freezer at −18 °C and stored overnight. The following day they were thawed for 10 h at 4 °C before preparing them for packaging and storage under modified atmosphere; this initial step was introduced so that all samples – including the fresh reference samples that underwent no further storage – were subjected to a freeze-thaw cycle prior to analysis in order to remove this variable as an influencing factor. Each meatball was packaged individually in a polypropylene (PP) tray using a commercial packaging system (Multivac A 300; Sepp Haggenmüller KG, Wolfertschwenden, Germany). Trays were filled with modified atmosphere comprising 70% O_2 and 30% CO_2 and were then covered and sealed with a high-barrier film (of 90 µm thickness) that exhibited an oxygen transmission rate of 1 $cm^3 m^{-2}$ 24 h^{-1} atm^{-1} at 23 °C and 75% relative humidity. The use of a gas mixture comprising 70% O_2 (rather than N_2) and 30% CO_2 was chosen to (*i*) maintain a high potential for myoglobin to oxygenate, therefore to retain a stable bright red colour (bloom), which is a major freshness indicator for consumers, and (*ii*) to slow growth of spoilage microorganisms (Devine & Dikeman, 2014); this composition is typical of commercial MAP on the market in Germany. The MAP meatballs were stored at 4 °C for up to 12 days in an approximate sample to gas volume ratio of 1:10 (v/v). The 12-day storage period was chosen to cover the typical shelf-life of MAP minced pork, which is up to 10 days (Lambert, Smith, & Dodds, 1991). Upon reaching the target storage time, individually packed meatballs were removed; one each for microbiological, PTR-TOF-MS, and SPME-GC–MS/O analyses and five packs for sensory evaluation. The samples from two extra packs were removed, then vacuum-packed and stored at −80 °C for subsequent pH and lipid oxidation analyses in the following week. All measurements were conducted in three independent experiments and the mean values (n = 9) and the standard errors of the means (± SEM) were calculated and are reported. The experimental design is depicted in Fig. 1.

2.2. Microbial assessment

Dominant bacteria in the meat samples were identified by culture-dependent methods to evaluate whether the community composition affected the VOC profiles. On each sampling day (0, 3, 6 and 12 days of storage), each meatball (~20 g) was transferred aseptically into a sterile 500 g stomacher bag (Interscience, France) in which 180 mL of sterile Ringer's solution (Merck, Darmstadt, Germany) was added. The mixture was homogenised for 60 s in a Stomacher blender (Stomacher 400 Circulator, Seward, Worthing, West Sussex, UK). A decimal dilution series from 10^{-1} to 10^{-5} was prepared to determine the microbial profile of the samples using standard procedures for total mesophilic bacteria (37 °C, 48 h) on plate count and potato dextrose agars (PCA and PDA, respectively; Merck, Darmstadt, Germany) at 32 °C for 7 days for yeasts and moulds (Speck, 1992). Psychrotrophic aerobic bacteria were cultured on PCA using the pour plate technique with plates incubated at 6.5 °C for 10 days according to the ISO standards for microbial analysis of food. As psychrotrophs were cultivated at this temperature, the mesophile count was conducted at 37 °C rather than the standard APC at 25 °C, which could include both mesophiles and psychrotrophs.

Cultivation of LAB was made on de Man–Rogosa–Sharpe agar (MRS, Merck, Darmstadt, Germany). MRS plates were placed in an anaerobic atmosphere created by a commercial atmosphere generation system (AN0035 Anaerogen Atmosphere Generating Systems, Oxoid, Basingstoke, Hampshire, UK) at 25 °C for five days. *Pseudomonas* were quantified by spread plating on supplemented cetrimide fucidin cephaloridine (CFC) agar (Oxoid CM0559 with supplement SR103, Oxoid, Basingstoke, Hampshire, UK) and incubated at 25 °C for two days. *Enterobacteriaceae* were measured by the pour plating method using Violet Red Bile Glucose agar (VRBG, Merck, Darmstadt, Germany) and incubated at 25 °C for two days. All microbial counts are expressed as $\log_{10}$ CFU g^{-1} pork; the mean values (n = 9) and the standard errors of the means (± SEM) were calculated and are reported here.

2.3. pH measurement

To determine the pH of the samples, 4 g of each sample was individually blended with 20 mL deionised and micro-filtered water for 10 min using a magnetic stirrer. The pH of the resultant suspension was measured with a probe and pH meter (Sentix 91 and WTW 197-S pH meter, Xylem, Rye Brook, NY, USA). Triplicate measurements were carried out in three independent experiments (n = 9).

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