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Volatile organic compounds and *Photobacterium phosphoreum* associated with spoilage of modified-atmosphere-packaged raw pork

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ABSTRACT

Accumulation of volatile organic compounds was monitored in association with sensory quality, bacterial concentrations and culture-independent microbial community analyses in raw pork loin and pork collar during storage under high-oxygen modified atmosphere at +4 °C. Of the 48 volatile compounds detected in the pork samples, the levels of acetoin, diacetyl and 3-methyl-1-butanol had the highest correlations with the sensory scores and bacterial concentrations. These compounds accumulated in all of the four monitored lots of non-sterile pork but not in the sterilized pork during chilled storage. According to the culture-dependent and culture-independent characterization of bacterial communities, *Brochothrix thermosphacta*, lactic acid bacteria (*Carnobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Weissella*) and *Photobacterium* spp. predominated in pork samples. *Photobacterium* spp., typically not associated with spoilage of meat, were detected also in 8 of the 11 retail packages of pork investigated subsequently. Eleven isolates from the pork samples were shown to belong to *Photobacterium phosphoreum* by phenotypic tests and sequencing of the 16S rRNA and *gyrB* gene fragments. Off-odors in pork samples with high proportion of *Photobacterium* spp. were associated with accumulation of acetoin, diacetyl and 3-methyl-1-butanol in meat, but these compounds did not explain all the off-odors reported in sensory analyses.

1. Introduction

Raw pork sold at retail level in Europe is commonly packaged under high-oxygen modified atmosphere containing 60%–80% of O_2 and 20%–40% of CO_2. Oxygen is added to maintain the red color of meat whereas CO_2 suppresses microbial growth. Despite the effect of CO_2, shelf-life of modified atmosphere packaged (MAP) raw meat is commonly limited by bacterial spoilage. Lactic acid bacteria (LAB) and/or *Brochothrix thermosphacta* tolerate high concentrations CO_2 and typically predominate in MAP meat stored at refrigeration temperatures (Samelis, 2006).

Meat processing industry and food safety authorities monitor the quality of MAP raw pork by sensory and microbiological analysis. Both approaches have limitations. Sensory analysis can be subjective and it is difficult to assign meaningful sensory criteria differentiating acceptable meat from non-acceptable. The traditional microbiological methods provide only retrospective information and correlate poorly with the remaining sensory shelf-life of fresh meat stored under modified atmosphere, mainly because bacterial levels can remain at their maximum levels for irregular time periods before the sensory changes occur (Dainty, 1996, Leisner et al., 1995, Skandamis and Nychas, 2002).

Bacterial metabolism changes the chemical composition of meat. Formation of a number of chemical compounds has been monitored in meat to find compounds that could be used as a quality index of fresh meat instead of microbial and sensory analyses (Argyri et al., 2011, Dainty, 1996, Ercolini et al., 2011). However, no single compound suitable for this purpose has been identified. Accumulation of for example diacetyl, acetoin, acetic acid and D-lactic acid has been observed during chilled storage of fresh pork or beef in MAP or in vacuum (Casaburi et al., 2011, Dainty, 1996, De Pablo et al., 1989, Ercolini et al., 2011, Ordonez et al., 1991, Tsigarida and Nychas, 2001). Nevertheless, the accumulation of these compounds in fresh pork during storage has not correlated with sensory changes. Furthermore, the association of volatile compounds with the composition of bacterial communities in raw pork is of interest because most of the previous studies have been dealing with accumulation of volatile compounds in beef.

This study aimed to identify the volatile organic compounds (VOCs) that accumulate in raw high-oxygen MAP raw pork during storage at +4 °C. VOC levels were correlated with sensory scores and bacterial levels in order to identify the VOCs most suitable for spoilage indicators of raw pork stored chilled under elevated concentrations of CO_2 and O_2. Predominant bacteria in meat were identified by culture-dependent and culture-independent methods to evaluate whether community composition affected VOC profiles.

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2. Materials and methods

2.1. Bulk samples of pork

Two sets of pork samples, referred to as bulk samples of pork later on, were examined at different time points during their storage. The first set consisted of fresh pork loins and pork collars collected from a meat processing plant in Denmark within 48 h of slaughter (Collar P, Loin P). The second set, originating from another manufacturer, was purchased from a wholesaler in Denmark and consisted of vacuum-packaged pork loin and pork collar with a remaining commercial shelf-life of eight days (Collar W, Loin W). Both sets of meat samples were stored in ice for 20–24 h after purchase before packaging at DTU Food. Meat was cubed (ca. 3 cm) and packaged in portions of 100 g in a 117 +/− 6 μm packaging film (NEN 40 HOB/LLPDE 75, Amcore Flexibles, Horsens, Denmark) with low gas permeability of 3.9 g/m^2/day for water vapor, 0.45 ± 0.15 cm^3/m^2/day/atm for O_2 and 1.8 ± 0.6 cm^3/m^2/day/atm for CO_2. This film consisted of polyamide, ethylenevinyl, polyamide, polyurethane adhesive and polyethylene layers. The packages were filled with an atmosphere containing 60% of O_2 and 22 to 25% of CO_2, with the remaining gas being N_2, and stored at +4 ± 1 °C at least until considered spoiled by sensory evaluation (see Section 2.8.). The storage times before sensory spoilage occurred were 14 days for Loin P and Collar P and 8 days for Loin W and Collar W. At appropriate intervals, three parallel packages were taken for sensory analysis and three packages for microbiological and chemical analyses at the same sampling occasion. Altogether 15 to 24 packages were analyzed per set of bulk samples (e.g. Collar P). Storage temperatures of the pork samples were monitored throughout the storage trials by data loggers (TinytagPlus, Gemini Data Loggers Ltd., Chichester, UK). The composition of the headspace gas was measured at regular intervals during storage using a Combi Check 9800-1 gas analyzer (PBI, Dansensor, Ringsted, Denmark).

2.2. Sterilized samples of pork

Sterilized pork samples were used as controls for the VOC analyses of bulk samples of pork described above. To prepare sterilized meat, fresh pork loin and collar obtained from the same lot as Loin P and Collar P was cut to >400 g pieces and fried in rapeseed oil for 30 s at 200 °C. The fried surface was cut off aseptically and the remaining meat was sliced to 2 cm thick slices and packaged and stored in the laboratory as described above for the bulk samples of pork.

2.3. Retail samples of pork

To evaluate whether the results obtained from the bulk samples of pork (see Section 2.1) were similar to those obtained from packaged retail pork, nine high-oxygen modified atmosphere and two vacuumed packages of raw pork (Samples F1–F11, Table 1) were purchased from local supermarkets in Denmark and stored unopened in the laboratory at +4 ± 1 °C until the time of microbiological, chemical and sensory analyses. Vacuumed meat was purchased to include meat from an additional processor to the experiment.

2.4. Enumeration of bacteria by culturing

Twenty five grams of each meat sample was homogenized with 225 ml of peptone saline solution (0.85% NaCl and 0.1% peptone in distilled water) for 1 min in a stomacher 400 (Seward Medical, London, UK). Values for meat pH were measured from the meat homogenates with an Autocal pH meter (Radiometer, Copenhagen, Denmark).

The concentrations of aerobic bacteria in meat samples were determined by spread plating meat homogenate on standard plate count (PC) agar (Oxoid CM0463, Basingstoke, UK). The PC plates were

Table 1
Retail samples of pork.

Sample	Cut	Package	Manufacturer	Viable count, log CFU/g						pH	Class 3 sensory evaluations[c]	Headspace $CO_2/O_2/N_2$%
				P. phosphoreum[a]	Psychrotrophic bacteria[b]	Aerobic bacteria[b]	Lactic acid bacteria[b]	*Brochothrix thermosphacta*[b]	*Pseudomonas* spp.[b]	*Enterobacteriaceae*[b]			
F1	Loin slices	MAP	A	<3.5	5.5	5.7	5.2	<4	3.3	<2	5.6	0/6	24/68/8
F2	Ham cubes	MAP	B	4.5	5.3	5.7	5.9	4.6	4.3	2.7	5.8	0/6	25/66/9
F3	Loin slices	MAP	B	<3.5	3.5	4.3	4.3	<4	<3	<2	5.5	0/5	27/63/10
F4	Collar slices	MAP	B	7.6	7.2	6.5	6.6	5.1	4.3	ND	6.2	1/4	36/55/9
F5	Loin slices	MAP	C	3.6	6.7	6.2	7.0	5.8	3.6	ND	5.6	0/4	22/72/6
F6	Loin	Vacuum	D	5.8	7.2	7.3	7.3	4.2	3.5	ND	5.6	0/4	ND
F7	Collar	Vacuum	D	6.4	7.4	7.0	7.5	5.3	6.0	ND	6.4	0/4	ND
F8	Minced (with 25% beef)	MAP	B	4.6	8.1	8.4	8.4	8.0	5.0	ND	5.6	2/8	ND
F9	Minced	MAP	A	<3.5	7.4	7.7	8.1	7.5	4.2	<3	5.7	0/6	ND
F10	Collar slices	MAP	B	6.5	6.7	7.4	7.7	5.9	5.4	<3	6.0	1/6	ND
F11	Ham slices	MAP	B	3.5	5.3	7.1	7.2	<5	<3	<3	5.5	0/6	ND

ND not determined.
[a] Selective conductance method.
[b] Plate count.
[c] Evaluated as spoiled.