5:17 Time course of volatile compound formation during microbial growth on beef stored at +5°C in air

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Introduction

Volatile end products of microbial growth on meat stored in air at chill temperatures are major components of the "off-odours" which signal the end of shelf-life. To date, inoculation of sterile meat with single strains of the numerically most important types of aerobic spoilage bacteria i.e. Pseudomonas spp and Brochothrix thermosphacta has been the experimental method of choice in attempts to establish the chemical identity of spoilage compounds (Dainty & Hibbard 1980; Stanley et al. 1981; Dainty et al. 1984).

While such studies provide a source of readily reproducible data, direct extrapolation of the findings to naturally contaminated samples supporting a mixed flora could be misleading. For example, relatively few, and therefore not necessarily representative, strains have been studied; numerically minor elements of the flora may contribute to spoilage processes; and interactions between organisms and/or their end products could produce a different pattern of volatiles to that derived from simple combinations of the pure culture results.

We have therefore identified the volatiles produced during storage of naturally contaminated beef bought at retail. The time course of appearance of the volatiles was also established by semiquantitative determinations of the individual compounds at regular sampling intervals throughout the storage period, together with the associated microbiological and sensory changes. Such data is essential in any attempt to evaluate the potential usefulness of chemical changes as indicators of microbial quality and/or product acceptability.

Materials and methods

MEAT

On 2 separate occasions, stewing or braising steak was obtained from local shops, trimmed of fat, cut into cubes c. 3cm^3 and mixed. Replicate 50g samples were placed in sterile 250ml conical flasks fitted with modified Dreschel heads (Dainty et al. 1984) and stored at +5°C.

VOLATILES

After storage, 5ml of headspace gases were removed with a gas tight syringe and analysed for volatile sulphur compounds by g.l.c. using a flame photometric detector. Quantitation was by peak height measurements.

Remaining headspace gases were entrained onto Porapak Q porous polymer in a steam of O_2-free N_2 and thermally desorbed for analysis by combined capillary g.c./m.s. (Mottram et al. 1982). Quantitation was by peak area measurement of characteristic ions.

ODOUR ASSESSMENT

Odours in flasks kept specifically for the purpose and in those used for volatile analyses were described independently by the authors.

BACTERIOLOGICAL ANALYSIS

After volatile entrainment 100ml of maintenance medium (MM) containing (g/l distilled water) peptone (Oxoid L37) 1.0, NaCl 8.5, was added to each flask and shaken vigorously for 1 min. Decimal dilutions were prepared in MM and appropriate dilutions used for the following counts: Total viable counts (TVC) on plate count agar (Oxoid CM326 + 1% (w/v) NaCl; PCA+1); Pseudomonas spp on cephaloridine fucidin cetrimide agar (CFC; Mead & Adams 1977); Brochothrix thermosphacta on streptomycin sulphate thallous acetate actidione agar (STAA; Gardner 1966); all incubated in air at 20°C for 3d. Presumptive Enterobacteriaceae were counted on pour plates of violet red bile agar (VRBG; ISO Document 5512) incubated under H_2 at 30°C for 1d.

Results

In the first experiment, selective media counts showed the flora to be dominated by virtually equal numbers of Broc. thermosphacta and Pseudomonas spp. The latter gradually outgrew Broc. thermosphacta until by the sixth day of storage they represented some 70% of the flora (Table 1). In the second experiment, initial numbers of Broc. thermosphacta were only 10-20% of those of Pseudomonas spp and declined to around 5% by the seventh day of storage (Table 2). Enterobacteriaceae strains remained a small percentage of the flora (up to 1%) throughout both experiments, although actual numbers exceeded 10^7/g.

Odour development during storage followed a similar pattern in the two experiments. Fresh, meaty odours at purchase were followed by non-fresh, but inoffensive, odours described as dairy, fatty, etc. as total bacterial numbers approached 10^7/g and then sequentially by fruity, sweet and finally putrid odours as microbial numbers continued to increase.

Various volatile compounds were also detected in defined sequence in the two experiments although there were marked quantitative differences for some of them e.g. acetoin (Tables 1 and 2). Maximum concentrations of this compound were attained after 3 days, further storage producing relatively small changes except for a marked fall in concentration between the penultimate and final samples in the second experiment (Table 2). In the first experiment the concentrations of two other compounds, viz. 3-methyl-1-butanol and diacetyl, the latter a close metabolic relative of acetoin, followed similar patterns of production. Neither was detected at any stage of storage in the second experiment.

Ethyl esters of short chain (C_2 - C_6) fatty acids were first detected after 3 to 4 days of storage, their appearance being rapidly followed by fruity, sweet odours. Total ester concentration, with ethyl acetate as a major component in both experiments, increased with further storage. Esters of 2 other even-numbered acids were common to both experiments while esters of 2 odd-numbered aids were present in only one of the experiments (Table 1).

Only towards the end of storage were sulphur containing compounds detected, dimethylsulphide alone in experiment 1, but together with the corresponding thiol and disulphide in experiment 2.

Amongst the hydrocarbons, 1-undecene was produced in small amounts and on a similar time scale to the esters in both experiments. However three others, toluene and 2 dimethylbenzenes, were present in the initial samples and showed a tendency to decline in concentration with storage, although the pattern of change was not consistent.

Discussion

The absence of storage-associated concentration increases, despite substantial microbial growth, is good evidence of a non-microbial origin for that compound. With the exception of 1-undecene, this was the case for all the hydrocarbons detected in the present study thus confirming earlier findings on volatile compound formation during storage of sterile and pure culture inoculated meat samples (Pittard et al. 1982; Dainty et al. 1984). Both groups concluded that 1-undecene was a product of the growth of Pseudomonas spp and the results of Dainty et al. (1984) suggested that it had potential value as a spoilage indicator. The relatively small amounts detected in the present study, and its appearance at a late stage of storage, throw doubt on that suggestion.

All the other compounds have previously been shown to be by-products of common spoilage organisms grown in pure culture on meat; esters and sulphur compounds of Pseudomonas spp (Dainty et al. 1984); acetoin, diacetyl and 3-methyl-1-butanol of Broc. thermosphacta (Dainty & Hibbard 1980; Stanley et al. 1981). Data from the present study of naturally contaminated meat are consistent with these particular sources. For example, more of the suspected end products of Broc. thermosphacta metabolism were detected, and in greater amounts in the first experiment, when the numbers of this organism relative to Pseudomonas spp were greater. Furthermore, the earlier appearance of Broc. thermosphacta's end products is consistent with glucose being the preferred growth substrate for all the common spoilage bacteria (Gill 1976). Thus, acetoin and diacetyl are major end products of the energy yielding metabolism of glucose by Broc. thermosphacta (Dainty & Hibbard 1980; Stanley et al. 1981), while carbon dioxide is the expected end product of the complete oxidation of organic compounds carried out by Pseudomonas spp. Not until glucose was nearing depletion at the surface of the meat would the latter organisms begin to metabolize amino acids with the resulting formation of compounds such as esters, sulphur compounds etc.

Hence the particular order of appearance of volatile compounds found in the present study could reasonably be expected to hold when Broc. thermosphacta is a co-dominant element of the flora, a situation sometimes reported for meats stored under gas permeable membranes. Under the more common condition of a pseudomonad dominated flora, esters would be expected to be amongst the earliest detectable products and therefore of greatest potential use as indicators of such growth. This is because pure culture experiments suggest ester rather than sulphur compound formation (Dainty et al. 1984) to be a more widespread property of those Pseudomonas strains which typically predominate on meat stored chilled in air, i.e. the cluster 2 strains of Shaw & Latty (1982).

The time course of voltile compound appearance correlated closely with that of the perceived odours, which were initially described as dairy, buttery, cheesey, then sweet, fruity and finally putrid. It therefore seems reasonable to conclude that amongst the compounds identified were those responsible for the odours. Fruity odours and ester formation have been linked (Castell & Greenough 1959; McGugan 1980; Dainty et al. 1984) while the odours of diacetyl and 3-methyl-1-butanol have been described as dairy, buttery (McGugan 1980). And, amongst the many different types of compound associated with the term putrid are sulphur compounds (McGugan 1980). Dimethylsulphide and dimelthyldisulphide were major components of the headspace volatiles associated with a putrid off-condition of minced beef (Stutz 1978) and the author suggested their use, together with two ketones, as indicators of microbial growth. Their late appearance in our present study suggest they have less potential than esters.

Clearly any discrepancies such as that just noted, need to be clarified before any general conclusions regarding the use of particular compounds as indicators of microbial growth/acceptability can be made. While they may simply reflect analytical methodology, they could result from inevitable inter-sample flora differences, in which case analysis of a range of compounds will be necessary.

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Table 1. Changes in microbial numbers, odours and volatile compounds during storage of naturally contaminated stewing steak at 5°C in air

	Day 0	Day 3	Day 4	Day 5	Day 6
Organisms	Counts (log_{10}no./g)				
Total viable organisms	6.4	9.1	9.4	9.9	9.9
Pseudomonas spp	5.9	8.9	9.3	9.6	9.8
Brochothrix thermosphacta	5.8	8.6	9.0	9.1	9.1
Enterobacteriaceae	2.8	6.5	7.4	7.8	7.9
Volatile compounds (a) non-sulphur containing	Characteristic mass spectrum ion (no.x10^{-3})				
Diacetyl	2	199	89	40	48
3-methyl-1-butanol	0.1	25	28	33	33
Acetoin	0.8	257	278	264	257
Ethyl acetate	-	-	0.3	14	15
Ethyl propionate	-	-	-	t	2
Ethyl butanoate	-	-	0.2	3	0.9
Ethyl isovalerate	-	-	0.3	3	10
Ethyl hexanoate	-	0.2	2	3	4
Toluene	172	19	31	53	25
1,2-dimethylbenzene	10	6	4	3	20
1,4-dimethylbenzene	26	22	20	12	30
1-undecene	-	t	0.2	0.4	1
1,4-undecadiene	-	-	-	t	t
(b) sulphur containing	Peak height from g.c. chromatogram				
Dimethylsulphide	-	-	-	0.7	110
	Odour descriptions				
	Meaty Fresh	Creamy Dairy Cheesey	Creamy Dairy Cheesey Sweet	Sweet Fruity	Sweet Fruity Putrid

Each value is the mean of 2 replicates except day 0 when single sample was analysed;
-, not detected; t, < 0.1.

Table 2. Changes in microbial numbers, odours and volatile compounds during storage of naturally contaminated chuck steak at 5°C in air.

	Day 0	Day 2	Day 3	Day 4	Day 7
Organisms:	Counts (log_{10}no./g)				
Total viable organisms	5.4	7.9	9.1	9.7	10.2
Pseudomonas spp	4.9	7.8	9.6	9.6	10.1
Brochothrix thermosphacta	4.3	7.0	8.2	8.7	8.9
Enterobacteriaceae	2.7	4.6	6.2	6.4	7.7
Volatile compounds:					
(a) non-sulphur containing	Characteristic mass spectrum ion (no.x10^{-3})				
Acetoin	7	13	37	35	0.1
Ethyl acetate	-	t	5	12	21
Ethyl butanoate	-	-	0.2	1	0.2
Ethyl hexanoate	-	-	t	0.9	1
Toluene	183	5	5	17	0.4
1,2-dimethylbenzene	13	0.9	1	5	0.1
1,4-dimethylbenzene	36	3	1	2	0.9
1-undecene	-	-	0.2	0.4	0.1
(b) sulphur containing	Peak height from g.c. chromatogram				
Methanethiol	-	-	-	-	190
Dimethylsulphide	-	-	-	-	10880
Dimethyldisulphide	-	-	-	-	135
	Odour descriptions				
	Meaty Fresh	Bland	Fatty Dairy	Sweet	Sweet Putrid

Each value is the mean of 2 replicates except days 0 and 7 when single samples were analysed.