G:18

Volatile fatty acids detected in vacuum-packed beef during storage at chill temperatures

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Introduction

Prolonged storage of normal pH (5.5-5.8) beef in vacuum packs at chill temperatures (0-5°C) produces odours described as sour, acid, cheesy. Short chain, volatile fatty acids are thought to be responsible and several, including acetic, propionic, n-butyric, isobutyric and isovaleric acids, have been detected in such meat (Sutherland et al., 1976; Dainty et al., 1979). Higher levels of the acids were present in high pH (6.3-6.7) beef (Dainty et al., 1979) but their smell was masked by the putrid odours characteristic of spoiled high pH beef (e.g. Bem et al., 1976). Fatty acids are typical end-products of the energy metabolism of many bacteria including the major types found on vacuum packed meat i.e. lactic acid bacteria, Brochothrix thermosphacta and Enterobacteriaceae. While these organisms may therefore be the source of the acids in the meat, other routes of formation, for example, via meat enzymes, are possible. In this paper we present results to assess the relative importance of these two possibilities and to explain the higher levels of fatty acids detected in high pH meat.

Materials and Methods

The bacteria were isolated from vacuum packed meat and maintained on Plate Count Agar (Oxoid M235) + 1% (w/v) NaCl (PCA + 1). Washed cell suspensions of cultures grown overnight in All Purpose Tryptone (APT: Evans & Niven 1951) were used to inoculate the meat. M. longissimus dorsi (LD) with pH_u values of 5.6 (range 5.5-5.7) and 6.5 (range 6.3-6.7) were removed from two different bull carcasses 5d post slaughter and sterile slices approximately 1 cm thick inoculated (Dainty & Hibbard 1980). Samples were packed in Synthene, oxygen permeability 25$cm^3m^{-2}d^{-1}$ atm O_2^{-1} at 20°C, 90% RH, and at each sampling time bacteria were enumerated on PCA + 1 for total viable count; on Streptomycin Thallous Acetate Agar (STAA: Gardner 1966) for B. thermosphacta; and on de Man, Rogosa & Sharpe Agar (MRS: de Man et al., 1962) for lactic acid bacteria. Fatty acids were extracted into alkaline zinc sulphate, concentrated and after acidification, extracted into ether for analysis by g.l.c. (Dainty & Hibbard 1980). The effect of pH and glucose concentration on fatty acid formation was determined in APT at 20°C.

Results

In extracts of fresh, normal and high pH LD muscles only acetic and n-butyric acids were detected. Both were present in greater quantities in stored, uninoculated samples despite the absence of microbial growth (Tables 1 and 2). The inoculated samples developed microbial flora dominated by organisms indistinguishable from the inocula except that the lactic acid bacterium was outgrown by organisms assumed to be B. thermosphacta on the high pH meat. In general they con tained more acetic acid than the stored, uninoculated samples but the amounts of n-butyric acid were similar. Isobutyric and isovaleric acids were only detected in B. thermosphacta-inoculated samples, while propionic acid was not present in measurable amounts in any sample.

In liquid medium there was a positive correlation between the amounts of fatty acids produced and increasing pH for both B. thermosphacta and the lactic acid bacterium, and a negative correlation between the amounts of acids and increasing glucose concentration (Table 3). The lactic acid bacterium produced far more acetic acid than B. thermosphacta and the latter more of the branched chain acids.

Discussion

The concentrations of acetic and n-butyric acids in the initial samples of normal (5.7) and high (6.5) pH LD muscles were of the same order as those recorded by Shank et al., (1962) for beef and by Krylova & Bazarova (1971) for beef and pork. The source of the acids is not known but they may be microbial products absorbed from the rumen or they may be derived from acetyl-CoA accumulating as a result of incomplete oxidation of carbohydrate and/or long chain fatty acids by meat tissue enzymes (Shank et al., 1962; Hagenfeldt & Wahren 1971; Knowles et al., 1974). Clearly a mechanism such as the latter is needed to explain the increased levels of both acids in the stored, uninoculated samples. What proportion of the acetic acid exists as such within the meat samples is unclear since acetyl-CoA would be hydrolysed to the acid during the extraction procedure. Extraction at pH values near 6.0 followed by specific assays for the acid and the CoA derivative would provide an answer.

The large amounts of acetic acid in extracts of inoculated samples strongly suggests that the bacteria are responsible and this is consistent with their known metabolic pathways (Wood 1961). The acid produced in this way would obviously be in the form of the free acid or the anion in the meat. Although none of the bacteria produced n-butyric acid, a microbial contribution to the high levels sometimes detected in natural spoilage (Dainty et al., 1979) should not be ruled out until more strains have been examined. A non microbial source of the branched-chain acids can be discounted since they were only found in high pH meat samples inoculated with B. thermosphacta, an observation consistent with the stimulatory effects of high pH and low glucose concentration on fatty acid formation demonstrated in laboratory media. The higher levels of acetic acid in the samples compared to the normal pH ones can be similarly explained. Unfortunately the lactic acid bacterium which showed the same stimulatory effects in laboratory medium, was outgrown on the high pH meat. However, strains of lactic acid bacteria dominate the natural spoilage flora of vacuum packed high pH beef so it seems probable that they contribute to the high levels of fatty acids and particularly acetic acid, as will the Gram negative bacteria which also grew to high numbers (Table 2). Of the latter only S. liquefaciens grew on normal pH vacuum packaged meat when it also produced significant amounts of acetic acid, but it is not normally a significant component of the natural mixed flora.

Although each of the major types of bacteria typically found on vacuum packed meat can produce one or more fatty acids during storage, only the production of isobutyric and isovaleric acids can be attributed solely to microbial activity on meat substrates. As yet unidentified mechanisms are responsible for the formation of at least part of the other acids.

TABLE 1

Concentrations of short chain fatty acids in fresh and stored vacuum packaged samples of beef M. longissimus dorsi

Sample	Storage time at 1°C (weeks)	Count on PCA+1 (log_{10} no/g meat)	Count on STAA (log_{10} no/g meat)	Count on MRS (log_{10} no/g meat)	Acetic acid (µmol/100g meat)	Propionic acid (µmol/100g meat)	n-butyric acid (µmol/100g meat)	Isobutyric acid (µmol/100g meat)	Isovaleric acid (µmol/100g meat)
(a) Normal pH 5.6*									
Initial	0	< 2.0	< 2.0	< 2.0	72	0	5.7	0	0
Uninoculated	8	< 2.0	< 2.0	< 2.0	113	t	11.4	0	0
Lactic acid bacterium		7.3	4.6	7.3	158	0	14.8	0	0
Serratia liquefaciens		9.7	< 2.0	< 2.0	397	t	12.5	0	0
Brochothrix thermosphacta		6.8	6.9	< 2.0	177	0	14.9	0	0
(b) High pH 6.5**									
Initial	0	< 2.0	< 2.0	< 2.0	148	0	5.7	0	0
Uninoculated	8	< 2.0	< 2.0	< 2.0	160	0	11.4	0	0
Lactic acid bacterium		8.7	8.7	< 5.0	235	0	9.1	0	0
Serratia liquefaciens		9.8	< 2.0	< 2.0	452	t	12.5	0	0
Alteromonas putrefaciens		8.3	< 2.0	< 2.0	550	t	10.2	0	0
Aeromonas sp.		9.9	6.0	6.5	338	0	6.8	0	0
Brochothrix thermosphacta		8.5	8.6	< 2.0	273	0	13.6	3.4	2.9

* Average pH (range 5.5 - 5.7)

** Average pH (range = 6.3-6.7)

Isovaleric acid = 3 methyl-1-butyric + 2-methyl-1-butyric acids

TABLE 2

Fatty acid formation during anaerobic growth of Brochothrix thermosphacta and a lactic acid bacterium

Initial pH	Glucose added (%, w/v)	Brochothrix thermosphacta C420 Cell dry weight (mg/ml)	Brochothrix thermosphacta C420 Acetic (µmol/mg dry wt)	Brochothrix thermosphacta C420 Isobutyric acids (µmol/mg dry wt)	Brochothrix thermosphacta C420 Isovaleric (µmol/mg dry wt)	Lactic acid bacterium A232 Cell dry weight (mg/ml)	Lactic acid bacterium A232 Acetic (µmol/mg dry wt)	Lactic acid bacterium A232 Isobutyric acids (µmol/mg dry wt)	Lactic acid bacterium A232 Isovaleric (µmol/mg dry wt)
(a) pH effect									
5.5	0.2	0.43	3.2	0	0.2	0.49	24	0	0
6.0	0.2	0.72	3.0	0	0.4	0.58	26	0	0.1
6.5	0.2	0.68	3.6	t	0.6	0.63	27	0	0.6
7.0	0.2	0.67	5.3	0.1	1.4	0.58	30	0	1.1
(b) effect of glucose concentration									
6.0	0	0.15	6.3	1.2	6.9	0.24	65	0	1.5
6.0	0.06	0.31	5.0	0.5	1.7	0.30	52	0	1.3
6.0	0.20	0.66	2.3	0.1	0.2	0.56	26	0	0.1
6.0	1.00	0.53	0	0	0	0.83	22	0	0

Cultures were grown for three weeks at 20°C in APT. t = trace amount.
Isovaleric acid = 3-methyl-1-butyric + 2-methyl-1-butyric acids.

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