PASTORAL-FLAVOUR DETECTION IN BEEF FAT USING SPME-GCMS

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Background

Some less desirable flavours have been reported in beef from forage-feeding, described as 'grassy' or 'gamey' (Larick et al., 1987). Maruri and Larick (1992) found six diterpenoids compounds in fat volatiles, phyt-1-ene, phytane, neophytadiene, phyt-2-ene, phytadiene and dihydrophytol, and showed these comprised 55.7% of the total volatiles from fat of pasture-fed steers. Bendall (2001) studied aroma compounds of milk, and showed phytol, phyt-1-ene and phyt-2-ene were more easily detected by panellists than neophytadiene and -diene compounds. The aroma of phyt-1-ene and phyt-2-ene were described as 'grassy', 'cardboard', or 'hay'. These volatiles as a component of pastoral flavour are important for consumer acceptance. However, their analysis requires a large amount of sample, and a costly module like purge-and-trap systems for concentrating volatiles.

Objectives

The solid phase micro extraction (SPME) fibre has been widely used because it is simple to use.. The aim of the present study was to establish the method for the sampling of head-space by SPME fibre and the analysis of pastoral-flavour by GCMS, and to clarify the kinetics of change in diterpenoids during a dry-lot period after grazing.

Materials and methods

Animals and sample preparation

Fourteen Japanese Shorthorn steers (14 months of age) were fed on pasture between May and September. After these five months, the steers were finished in a dry lot (concentrate diet 1.6 % of body weight/day, *ad libitum* access to rice straw), and serially slaughtered between 0 day to 154 days. Three steers which were fed on dry lot only were designated controls. Back fat samples were excised from carcasses two days after slaughter. The fat was minced and heated with an equal volume of distilled water at 100 ºC for 15min. This mixture was centrifuged at 3000 rpm for 5 minutes. An aliquot of rendered fat was removed into a glass vial and BHT was added as an internal standard and ant-oxidant (final concentration 0.5 mg/g of fat). Vials were purged with N_2 and stored with oxygen absorber in an aluminium bag at -80 ºC until analysed.

Flavour sampling and GCMS analysis

Fat samples were melted at 60 ºC, and 0.25g was placed in a glass crimp vial (10mL) with a glass fibre filter at the bottom. The vial was brought to 60 ºC for 5 min, after which the SPME fibre were inserted into the headspace and exposed for 30 minutes (static sampling). For selecting the right fibre, four types were purchased from Supelco (Bellefonte, PA), CAR/PDMS, DVB/CAR/PDMS, PDMS/DVB and CW/PDMS and were screened using the day 0 fat sample. The GC oven was held at –10 ºC for one minute after injection and rapidly increased to 40 ºC, held for one minute, and then increased to 290 ºC at 5 ºC /minute. Volatiles were analysed by MS at 70ev and a scanning range of m/z 29 to m/z 350.

Sensory evaluation

A trained sensory panel of 14 members was used. A triangle test was employed for the detection of the flavour difference between the fat from the pasture-fed animals at different dry lot periods, and the fat from the animals reared at the dry lot only.

Results and discussion

Optimization of the extraction by SPME

The day 0 fat sample was used to optimize the extraction of volatiles. The results of analyses are shown in figure 1 as chromatograms. Peaks were identified by mass spectrometry according to Maruri and Larick (1992). Phyt-1-ene, phytane, phytol, and phyt-2-ene were determined in all SPME-fibre extractions. The

CW/PDMS fibre showed the highst sensitivity for these volatile compounds, but lowest for the lower-boiling compounds. The CAR/PDMS fibre showed the higher sensitivity for lower and higher boiling compounds, but phytane was not clearly separated because of interfering peaks. The PDMS/DVB and the DVB/CAR/PDMS fibre were moderately useful for low and high boiling compounds. Although these data indicated that any SPME fibre could be used to study changes in phyt-1-ene, phytane, phytol, and phyt-2-ene, the DVB/CAR/PDMS fibre was selected for the present study.

Figure 2-a, for a DVB/CAR/PDMS fibre, shows the relationship between sampling temperature and extracted diterpenoids during the 20-minute exposure. Higher temperatures were more favourable. Figure 2-b shows the effect of holding time on diterpenoids extraction at 60 ºC, which indicates that the holding time of 40 minutes or more began to exceed the capacity of the absorption. Therefore, the sampling conditions were set to 30 minutes at 60 ºC with a DVB/CAR/PDMS fibre.

Changes of pastoral flavour and sensory analysis

Figure 3 shows the changes of pastoral flavour (phyt-1-ene, phytane, phytol and phyt-2-ene) during dry lot feeding after grazing. The headspace concentration of these compounds decreased with time of dry lot feeding. Among these compounds, phyt-1-ene was the most dominant in GC traces, and showed the highest negative correlation with time in the dry lot period, expressed by the equation of $Y= 12.455e^{-0.0135x}$ ($R^2= 0.90$). The concentration of phyt-1ene, relative value to BHT as internal standard, decreased from about 12 ppm to about one ppm during five months of dry lot feeding. The dry lot-only sample showed 0.10 ppm of phyt-1-ene.

Sensory evaluation revealed that the dry lot feeding over five months (154 days) completely eliminated the flavour. At that point, the concentration of phty-1-ene was below 0.98 ppm (Table 1). Young et al. (1999) reported that 4-methylphenol and indoles were among the compounds causing of pastoral odour and flavour in ruminant fat. These compounds were not detected with the present SPME method (Braggins et al. found already in 1999 that branch-chain fatty acids require dynamic SPME for detection). Phyt-1-ene, however, could be analysed quantitatively, and had a high negative correlation with the length of the dry lot period. These results indicate that phyt-1-ene may be used as a marker of pastoral flavour.

Conclusions

These results showed that SPME-GCMS technique is useful for rapid detection of pastoral flavour, for which phyt-1-ene could be used as a marker. The recognition of this kind of flavour is difficult if the concentration of phyt-1-ene is below one ppm (relative concentration to BHT in fat). Five months of dry lot feeding after grazing eliminated the flavour.

The authors wish to express their sincere appreciation to Dr. T. Braggins (Hill Laboratories, New Zealand) and Dr. O.Young (Auckland University of Technology, New Zealand) for their helpful comments.

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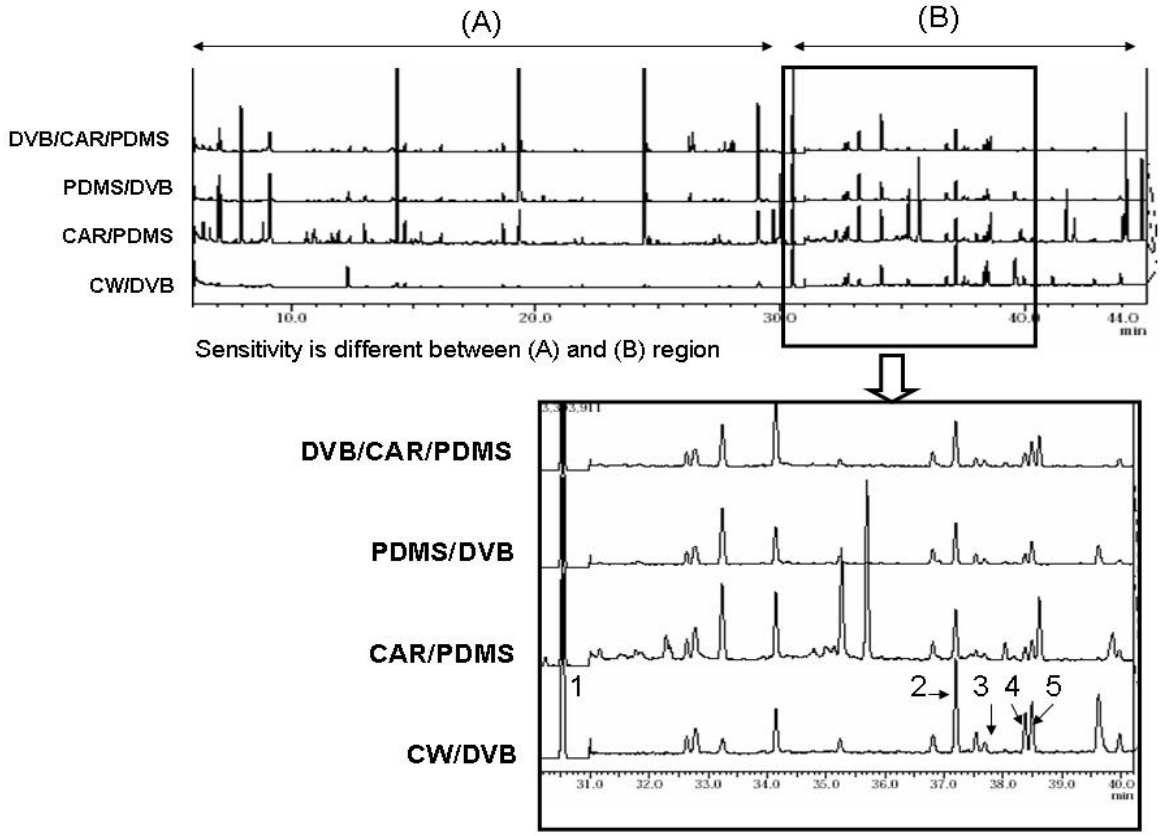
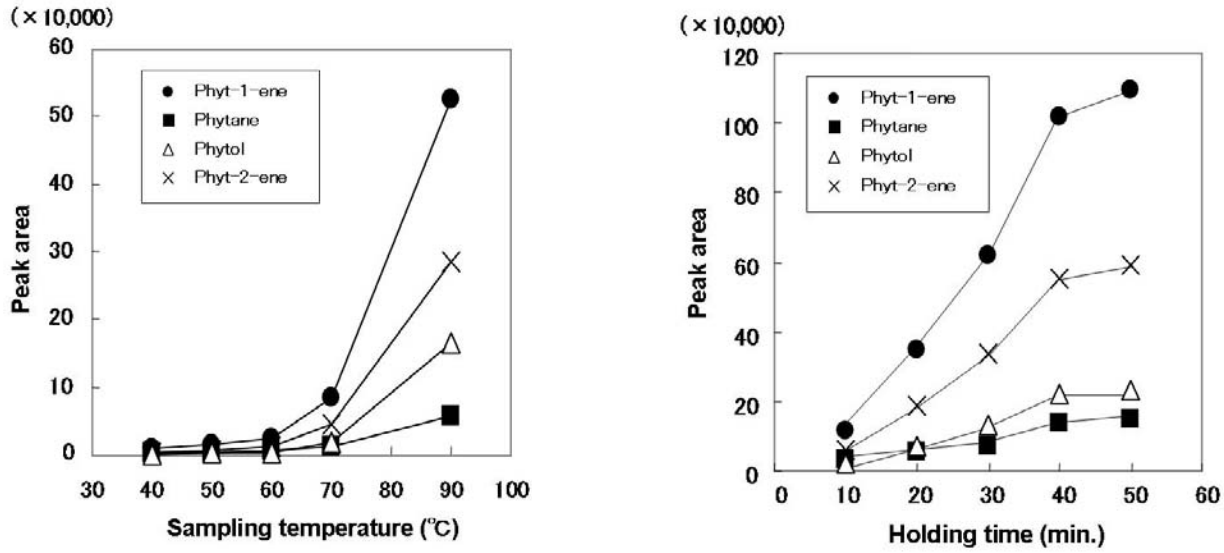


Figure 1. Chromatograms from different SPME fibers.
Four kinds of SPME fiber were tested for detection of components responsible for pastoral flavor, ie., phyt-1-ene (2), phytane (3), phytol (4) and phyt-2-ene (5) were determined. Peak No.1 is BHT used as an internal STD.



(2-a) **(2-b)**

Figure 2. Effects of sampling temperature (2-a), and holding time (2-b) on extraction volume.

Sampling temperature was varied while the holding time remained 20 minutes (2-a), then the holding time was varied while the temperature remained at 60 °C.

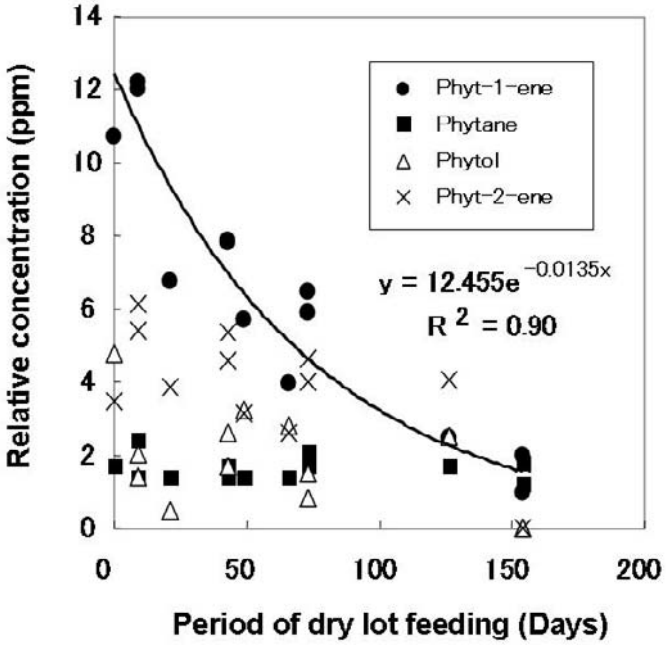


Figure 3. Effects of the dry lot feeding after grazing on flavour compounds

Phyt-1-ene showed the highest correlation to the period of dry lot feeding after grazing.

Table 1. Result of sensory evaluation (triangle test)

Dry lot period [a)]	Phyt-1-ene [b)]	Correct Answer	Significance [C)]
9 days	12.19 ppm	71 %	**
43 days	7.89 ppm	79 %	***
73 days	5.87 ppm	71 %	**
154 days	0.98 ppm	36 %	N.S.

Triangular test was performed between samples from dry lot after grazing and dry lot only (0.10 ppm of phyt-1-ene). a) Steers were fed concentrate (1.6 % of body weight/day) and given *ad libitum* access to rice straw during dry lot period after grazing. b) Phyt-1-ene concentration was defined as a relative value to BHT in fat. c) ***; $p<0.001$. **; $p<0.01$. N.S; not significant