MASS SPECTROMETRY-BASED SENSOR ANALYSIS OF RAW MEAT FOR THE AUTHENTICATION OF ANIMAL FEEDING

V. Vasta*[1,2], J. Ratel[1] and E. Engel*[1]

[1] Institut National de la Recherche Agronomique, UR Qualité des Produits Animaux, Equipe Typicité Aromatique et Authentification, 63122 Saint-Genès-Champanelle, France. [2] Dottorato di Ricerca in Produzioni Foraggere Mediterranee, Università di Palermo, Viale delle Scienze 13, 90128 Palermo, Italy. Email: vvasta@unict.it

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Introduction

The study of volatile compounds (VC) in ruminant meat is of great interest because of the role that volatiles have in meat flavour (Rousset-Akrim, *et al.*, 1997). Most of the studies on meat VC have been performed on heated meat fat (Young *et al.*, 1997, Priolo *et al.*, 2004) or on cooked meat comprising muscle external fat (Elmore *et al.*, 1997) in order to reproduce consumers' eating conditions. Apart from the sensorial aspect, consumers demand healthier food and they prefer meat from pasture-fed than meat from stall-fed animals for its "green" image (Grunert *et al.*, 2004). It has been shown that VC in ruminant fat can be used as markers of the animal feeding system, as their occurrence is affected by diet (Vasta and Priolo, 2006). Although the muscle is the portion usually eaten by consumers, to date no studies are available on the use of muscle VC as tracers of animal feeding background probably because the low percentage of the intramuscular fat in which volatiles are accumulated and the presence of water which reduces VC extraction yield (Canac-Arlega *et al.*, 2000). The aim of the present study was to investigate raw muscle volatile profile for the authentication of animal feeding system by DHSE-Mass Spectrometry (MS). Firstly, extraction operative conditions were set up by using a DHSE-Gas Chromatography (GC)-MS device: different extraction temperatures (trial 1) and sample sizes (trial 2) were tested in order to reduce the formation of heat-induced compounds and to minimise the release of water during the analysis. Once the best analytical conditions were determined, a third trial was undertaken to assess the application of the DHSE-MS analysis of raw muscle to the authentication of animal diet (pasture *vs* concentrate) (trial 3).

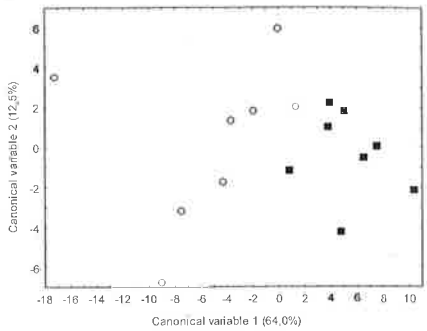
Materials and Methods

Trials 1 and 2. The muscle *semimembranosus* was excised from the carcass of an adult cattle after slaughter and was stored at 4°C for 6 days. Meat was trimmed of any visible fat, cut into small pieces, wrapped in aluminium foil, vacuum packed and stored at -80°C. Meat was defrosted overnight at 4°C and finely ground. For trial 1 fifteen tablets of ground meat were made, each of them weighing 50g (± 2.5g), while for trial 2 ground meat was shaped into four series of five tablets, weighing 50g (± 2.5g), 25g (± 1.25g), 12.5g (± 0.6g) and 6.25g (± 0.3g) respectively. Tablets were immersed into liquid nitrogen for 2 min, wrapped in aluminium foil, vacuum packed and stored at -80°C. Twenty-four hours before analysis, samples were stored at 4°C. *Trial 3.* The right *semimembranosus* muscles were excised from the carcasses of eighteen adult lambs: nine lambs were allowed to graze for 85 days (H= herbage group), while the other nine lambs were confined in stall being offered commercial concentrate for the same period (C= concentrate group). Muscles were handled as described above and from each muscle tablets weighing 6.25g were prepared. *Extraction and Analysis of Volatile Compounds.* Raw meat volatile compounds were extracted and analysed by DHSE-GC-MS (GC: Agilent 5890; MS: 5973N). After defrosting, tablets were spread and cut in small squares to increase the surface of meat samples. Meat was put into a glass cartridge partially filled with glass wool. The meat samples were purged with He as carrier gas (60mL min^{-1}) for 30 min. In trial 1 three different purge temperatures were tested (35°C, 60°C and 90°C) while for trial 2 and 3 purge temperature was set at 35°C. After the purge, a dry-purge step was held for 5 min. Volatiles were trapped using a Tenax A column (Tekmar 3100, Cincinnati, USA); Operative parameters were set as follows: preheat: 15 min at purge temperature; desorption: 10 min at 180°C; cyofocusing at -150°C; desorption into the GC column (Supelco, DB5, 60mx0.32mmx1μm) at 225°C for 2 min; splitless injection; oven temperature: 40°C for 5 min, increasing up to 230°C at 3°C min^{-1}, 230°C for 5 min; GC-MS transfer line temperature: 280°C; electronic impact ionization energy: 70 eV; scanned mass range: 33-230 amu. The identification of volatiles was achieved by comparing mass spectral data with those of the 270 Wiley library (Hewlett Packard Co.) and by comparing the experimental retention indices (KI) with those reported in published databases (Kondjoyan and Berdagué, 1996). The area of each peak was integrated by using ChemStation software (Hewlett Packard Co.). For trial 3, the global spectral were obtained from the sum of the mass spectra generated by the DHSE-GC-MS device. DHSE-GC-MS data were analysed by one way ANOVA of peak area according to the model : peak area = extraction temperature (trial 1), or sample size (trial 2). Similarly, one way ANOVA was processed on the abundance of the DHSE-MS fingerprint fragment according to the model : abundance = feeding system (trial 3). When significant differences were observed, ($P<0.05$), mean values were compared by Newman-Keuls multiple comparison tests. Principal component analysis (PCA) was processed on the DHSE-MS fingerprint to assess the potential of meat VC to discriminate between animal diet .

Results and Discussion

Trial 1: A number of the 222, 214 and 213 peaks respectively were quantified using the extraction temperatures of 90°C, 60°C and 35°C. Among these compounds, 140 were affected ($p < 0.05$) by the extraction temperature. Eighty-eight of them were identified, including 19 alcohols, 16 ketones, 12 aldheydes, 8 aromatic hydrocarbons, 11 alkanes, 7 heterocyclic compounds, 3 sulphuric compounds, 7 halogens and 5 terpenes. Eighty-four identified VC were present at significantly higher amounts in the samples extracted at 90°C compared to those purged at 35 °C or 60 °C ($p < 0.05$) including some potential diet tracers like the 1-alcohols, the 1-alken-3-ols, 2- and 3-ketones or 3-aldehydes. However, the main qualitative information remained exploitable at 35°C and, according to confidence interval values, the quantification of VC was more reproducible. Consequently, the purge temperature was set at 35°C for the following trials. ***Trial 2***: Among the 80 VC that were quantified, 18 were detected at a higher amount ($p < 0.05$) in the samples weighing 6.25g compared to the 50g samples. Probably, the small sample size has decreased the amount of water released from the matrix reducing its interference on VC absorption on the Tenax trap. The analysis of the 6.25g meat samples has the additional advantage of being less destructive and this sample size was selected for trial 3. ***Trial 3***: Thiry-four mass fragments were found to discriminate meat samples according to animal diet ($p < 0.05$). The PCA first map of the DHSE-MS fingerprint (Figure 1) was useful in resolving animals fed different regimes (H or C) demonstrating that muscle volatile compounds allow us to discriminate animal feeding background. Additionally, data of stall-fed lambs showed greater dispersion compared to pasture-fed lambs.

Figure 1: PCA of DHSE-MS fingerprint. Red and blue squares refer to grass- and concentrate-fed lambs.



Conclusions

A soft headspace method was set up to extract the volatile compounds released from raw lean muscle. It was found that animal diet (grass or concentrate feeding) affects the appearance of volatiles in lamb muscle. The analysis of the fingerprint of muscle VC allows us to discriminate between meat samples of animals raised at pasture or in stall, thus representing a useful and rapid tool for the authentication of the animal feeding system. The analysis of muscle VC by DHSE-GC-MS could also allow the study of the metabolic origin of molecular markers of animal feeding system.

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