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Note

Gas chromatographic determination and mass spectrometric confirmation of traces of indole and 3-methylindole (skatole) in pig back fat

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Pigs used for meat production are castrated to prevent boar taint. Carcasses from uncastrated pigs have a more or less pronounced "off-odour", and procedures for identifying such carcasses must be developed. The correlation coefficient between the androstenone (5-androst-16-en-3-one) content and sensory evaluation of the intensity of taint has been reported to vary between 0.41 and 0.76 (review from Bonneau[1]), but this does not exclude the possible contribution from other compounds. Both indole and skatole (3-methylindole) are strong-smelling substances resulting from tryptophan metabolism by the gut microflora. Skatole has been identified in fat from boars[2,3]. Hanson *et al.*[4] established the presence of indole and skatole in fat tissue from boars, but also in tissues from castrates and gilts, the latter enhancing the sensory impression of boar "off-odour" to a higher degree than could be explained by the actual androstenone concentrations. However, these assays of indole and skatole in fat, carried out using a method based on steam distillation followed by determination by gas chromatography (GC) with flame ionization detection[5], were not reliable due to low and variable extraction yields, allowing only rough estimates of these indolic substances. Moreover, the very time-consuming analysis and need for large fat samples due to the limited sensitivity of the detection precluded the use of that method for routine determinations.

We have developed a new method based on selective solvent extraction of indole and skatole from the fatty tissues, followed by gas–liquid chromatographic (GLC) separation and sensitive detection using a nitrogen/phosphorus-selective detector. Gas chromatographic–mass spectrometric (GC–MS) confirmation of the identity of these naturally occurring substances at trace levels (0.025 ppm) has also been carried out.

EXPERIMENTAL

Instrumentation

A Girdel Model 3000 gas chromatograph (Giravions Dorand, Suresnes, France) equipped with a nitrogen-specific thermoionic detector was used. The coiled glass column (1.5 m × 2 mm I.D.) was packed with 3% SP 2250 on Chromosorb W (80–100 mesh). The gas flow-rates were: hydrogen, 15 ml/min; air, 300 ml/min and nitrogen (carrier gas), 30 ml/min. The temperatures were: 300°C (detector) and 250°C

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(injection port); 110°C isothermal for 2 min, then increase at 5°C/min to 220°C (column).

GC–MS analyses were performed using a Model 5992B quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA, U.S.A.) with an HP 5700 A series gas chromatograph fitted with a 12.5 m × 0.2 mm I.D. OV-1 capillary column interface. The operating conditions were: injection port, 250°C; oven programmed from 95 to 140°C at 5°C/min following initial 1-min hold at 95°C; helium (carrier gas) flow-rate 0.8 ml/min. An Hewlett-Packard magnetic tape software program (07992-10015, Peakfinder for Normalized Spectra) was used to calibrate and autotune the mass spectrometer in the electron impact mode at 70 eV. The GC–MS system was operated in selected ion monitoring (SIM) mode. Using the Hewlett-Packard 05991-10006 SIM tape program, six masses were selected in a cycling time of 0.1 sec.

Materials

Solvents and chemicals. 2,2,4-Trimethylpentane, *n*-pentane and methanol were analytical grade. 4-Methyl-1,3-dioxolan-2-one (propylene carbonate) purum was obtained from Fluka (Buchs, Switzerland). Indole and 3-methylindole standards were obtained from Fluka. *n*-Tridecylcyclohexane from K and K (Plainview, NY, U.S.A.) was used as a 40% solution in isooctane.

Fat sampling and fortification. The back fat of 20 boars grown in pathogen-free conditions and fed a corn–soya meal diet was sampled from fresh carcasses upon slaughter (100 kg) and kept frozen (−30°C) until analysis. Samples that did not exhibit measurable indole nor skatole responses using our method were considered as "controls", and were used for the fortification procedure as follows.

"Control" fat aliquots (100 g) were weighed into 250-ml screw-cap glass bottles and placed in an oven at 100°C. Samples of vegetable oil (10 ml) were spiked with 100 µl benzene solutions containing 1 and 2.5 mg/ml each of indole and skatole. After careful mixing, 1 ml of each oil sample was then added to the melted fat samples. The caps were quickly and tightly replaced to avoid any loss of the volatile indolic substances, and the bottles were vigorously shaken by hand, then left to stand until cool. Aliquots (10 g) of the solidified fats, spiked with 0.1 and 0.25 ppm of indole and skatole, were subjected to the analytical procedure.

Procedure

Ten grams of fat were weighed into a 50-ml screw-cap centrifuge tube, and 10 ml propylene carbonate added. The tube was placed into a shaking water-bath set at 75°C for 15 min, then centrifuged at 1600 *g* for 10 min. A 2-ml volume of the propylene carbonate phase was transferred to a 10-ml conical centrifuge tube. After addition of 2 ml isooctane, the tube was shaken for 1 min then centrifuged at 1600 *g* for 2 min. A 1-ml volume of the lower phase was transferred to a 125-ml PTFE-lined screw-cap bottle with 60 ml saline (12% sodium chloride), 30 ml *n*-pentane and 0.2 ml *n*-tridecylcyclohexane as trapping solvent. After shaking vigorously , for 3 min, the contents were transferred to a 125-ml separating funnel. The aqueous phase was discarded, and the organic phase placed into a 125-ml glass bottle containing 5 g sodium sulphate. After careful stirring, the pentane phase was filtered over glass wool, and the bottle and funnel rinsed with 5 ml pentane. The filtrate was placed into a conical flask, and the solvent was evaporated under vacuum at 40°C. The residual

n-tridecylcyclohexane phase was diluted in 0.3 ml isooctane pre-saturated with methanol–distilled water (95:5). A 0.2-ml volume of the same mixture was added, then after Vortex stirring for 1 min, the methanol phase was transferred to a 1-ml PTFE-stoppered conical flask.

The sample was ready for analysis, but could be kept at 4°C for 1 week. A 3-μl volume was injected into the gas chromatograph for quantitation.

GC–MS confirmation

GC–MS was used for the identification of naturally occurring indoles in pig fat samples. It was performed on 3 μl of the purified extract injected into the system using a Ross injector. The following ions were monitored: m/z 131, 130, 117, 103, 90 and 89, based on the mass spectra of the compounds indole and skatole.

RESULTS AND DISCUSSION

GC determination

Under the chromatographic conditions described, indole and skatole were well separated, with retention times of 6.2 and 7.9 min respectively (Fig. 1). The response

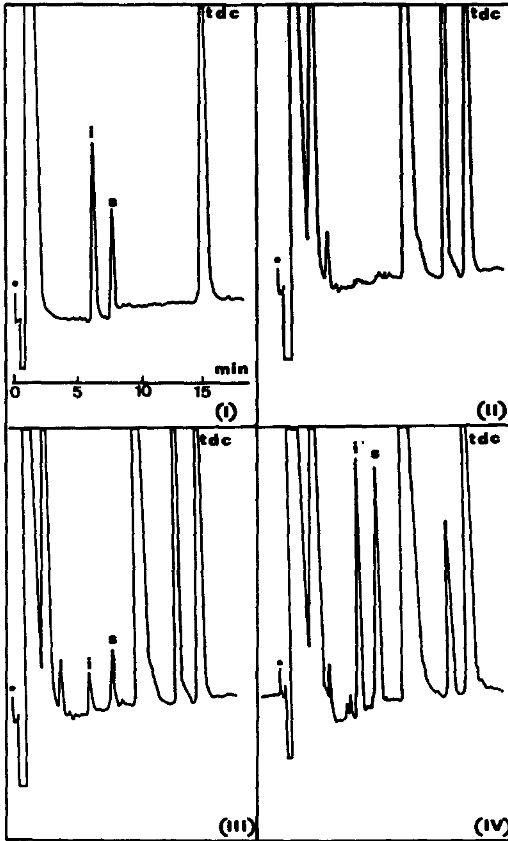

Fig. 1. Gas–liquid chromatograms of standard indole and skatole, and sample of boar back fat. I, Standard indole (i) and skatole (s), 1.5 ng of each injected; II, control pig back fat extract; III and IV, extract from pig back fat containing indole substances. tdc = Trapping hydrocarbon, n-tridecylcyclohexane.

of the detector was linear over the range 0 to 10 ng for each of the two substances. The specific response was 1.17 times higher for skatole than for indole.

The extraction and clean-up procedures were designed to eliminate tissue interferences during fat analysis. As is seen in Fig. 1, no interfering peak occurred in the neighbourhood of these retention times. Average recoveries of indole and skatole from pig back fat fortified at the 0.1 ppm level were 62 ± 5.5 and 83 ± 6.7% respectively (six analyses). At the 0.25 ppm level the corresponding values were 79.8 ± 6.4 and 100 ± 2%. The lower response observed with indole is related to its greater volatility and therefore less efficient trapping by the *n*-tridecylcyclohexane phase. To take into account this incomplete recovery, one spiked sample was included in every series of analyses.

At the 0.025 ppm level, the peak height was about four times the average baseline fluctuation. This value was chosen as the detection limit for routine GC analysis. It should be noted that comparable sensitivities have been reached for the analysis of skatole in plasma and rumen fluid, using a similar nitrogen/phosphorus-specific detector[6]. However, more advanced nitrogen-selective flame ionization detectors allow the detection of 10–100 pg biogenic amines[7] or substances containing nitro groups[8]. They offer new perspectives for the development of a micro-method for 1-g fat samples, and therefore for the analysis of these indolic substances in biopsies.

GC–MS confirmatory assays

GC–MS analysis performed with standard indole and skatole yielded the electron impact mass spectra of both compounds. Table I summarizes the fragmentation data and subsequent analysis, according to the pattern already described for indole-3-acetic acid[9]. The fragments at *m/z* 117, 90, 89, 130, 131 and 103 were selected for simultaneous single ion monitoring of both compounds.

TABLE I

70-MeV ELECTRON IMPACT MS FRAGMENTATION PATTERN OF INDOLE AND SKATOLE

Indole			*Skatole*		
m/z	*Abundance*	*Fragment*	*m/z*	*Abundance*	*Fragment*
117	100	$[M]^+$	131	55	$[M]^+$
90	46	$[C_6H_5\text{–}CH_2]^+$	130	100	[quinolinium-type bicyclic ring with NH]$^+$
89	36	?	103	10	$[C_6H_5\text{–}CH{=}CH_2]^+$

Chemical confirmation of the substances preliminarily identified as indole and skatole on the basis of their GC retention times was carried out on two samples of pig back fat. One contained 0.15 and 0.225 ppm indole and skatole according to the

procedure described, *i.e.*, significant quantities. The other contained 0.027 and 0.058 ppm respectively, *i.e.*, very low concentrations. The results are shown in Fig. 2. The first sample showed intense and well shaped peaks for all the monitored ions. The retention times and abundances of the main fragments were similar to those of the standard substances, confirming the identity of the two compounds. The second sample exhibited weaker but significant ion current peaks and consistent abundances for the major ion. The presence and identity of the two indolic substances was thus demonstrated.

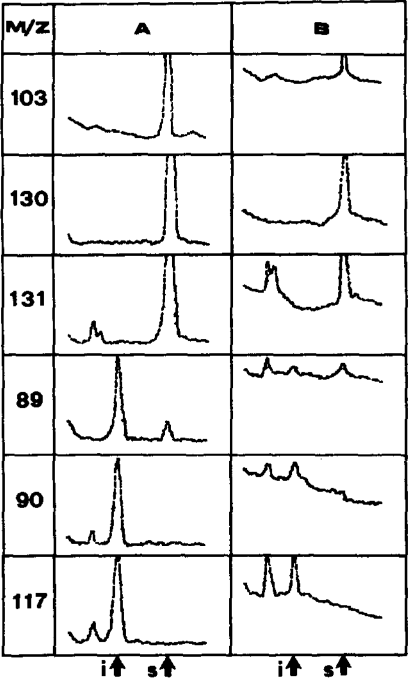

Fig. 2. GC–MS single ion monitoring of indolic substances in extracts from boar back fat. A, Sample with significant indole and skatole concentrations (0.15 and 0.225 ppm respectively); B, sample with very low levels (0.027 and 0.058 ppm respectively). Retention times: indole (i), 2.0 min, skatole (s), 3.4 min.

Analysis in fat samples

Table II reports the results of the analysis of indole and skatole in the back fat of experimental animals. Only one sample was in the 0.15–0.25 ppm range, while the others exhibited very low or undetectable levels.

In conclusion, the method described permits routine monitoring of indole and skatole, substances involved in the organoleptic defects of boar meat. It offers a great improvement in extraction recovery, reproducibility and sensitivity compared with existing analytical methods. Attempts are now being performed to adapt the procedure to the micro scale.

TABLE II

INDOLE AND SKATOLE LEVELS (ppm) MEASURED IN BACK FAT OF BOARS

ND = not detectable.

Animal No.	*Indole*	*Skatole*	*Animal No.*	*Indole*	*Skatole*
1	ND	ND	11	ND	ND
2	0.028	ND	12	ND	ND
3	ND	ND	13	0.026	ND
4	ND	ND	14	ND	ND
5	ND	ND	15	0.150	0.225
6	0.043	0.10	16	0.038	ND
7	ND	ND	17	ND	ND
8	ND	ND	18	0.034	ND
9	ND	0.130	19	ND	0.031
10	ND	ND	20	ND	ND

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REFERENCES

1 M. Bonneau, *Livest. Prod. Sci.*, 9 (1982) 687–705.
2 E. Vold, *Meld. Nor. Landbrukshoegsk.*, 49 (1970) 1–25.
3 P. Walstra and H. Maarse, *I.V.O.-Rapport C-147 and Rapport No. 2, Researchgroep Vlees en Vleeswaren T.N.O.*, Zeist, 1970.
4 K.-E. Hanson, K. Lundstrom, S. Ejelkner-Modij and J. Personn, *Swedish J. Agr. Res.*, 10 (1970) 167–173.
5 H. Maarse, P. C. Moerman and P. Walstra, *I.V.O.-Rapport C-180 and Rapport No. 3, Researchgroep Vlees en Vleeswaren T.N.O.*, Zeist, 1972.
6 G. Norheim and J. Rygge, *J. Chromatogr.*, 154 (1978) 291–294.
7 M. Mumrtaz, N. Narasimhachari and R. O. Friedel, *Anal. Biochem.*, 126 (1982) 365–373.
8 C. M. White, A. Robbat and R. M. Hoes, *Anal. Chem.*, 56 (1984) 232–236.
9 E. Epstein and J. D. Cohen, *J. Chromatogr.*, 209 (1981) 413–420.