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Determination of indolic compounds in pig back fat by solid-phase extraction and gradient high-performance liquid chromatography with special emphasis on the boar taint compound skatole

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ABSTRACT

A gradient high-performance liquid chromatographic method for the separation of thirteen indolic compounds and the determination of seven of them was developed. The indolic compounds include indole and skatole, both of which are involved in the boar taint. A solid-phase extraction method for sample preparation for the determination of the indolic compounds in pig back fat is described. The indolic compounds are extracted with acetone–Tris buffer, and lipids and fatty acids are trapped on a Bond-Elut C_{18} column. Fluorescence (excitation and emission at 280 and 340 nm, respectively) is used for selective detection. The detection limit for indole and skatole is 15 $\mu g\ kg^{-1}$ back fat. The sample preparation procedure is simple and the method is sensitive and reproducible. The method was compared with a spectrophotometric method for the determination of skatole in back fat.

INTRODUCTION

In many countries, castration of male pigs has been practised for centuries in order to avoid the occurrence of male odour (boar taint) in meat from 5–10% of the animals. The male odour is only a problem when meat or meat products are heated by the consumer prior to consumption. However, the advantages of entire male, *i.e.* uncastrated, pig production compared with hog production are large; the animal welfare aspect is important, male pigs grow faster, they suffer less illness and the meat is leaner with a better dietary composition of fatty acids.

The compounds responsible for the boar taint are known to be skatole [1–3] and the pheromone 5α-androst-16-en-3-one [4]. Other steroids that have also been associated with the boar taint are 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol [5–8].

Several methods for the determination of skatole have been described; these include UV spectrophotometric, high-performance liquid chromatographic (HPLC), thin-layer chromatographic (TLC) and gas chromatographic (GC) procedures. For the determination of skatole in gastrointestinal content, TLC [9], HPLC with UV detection [10] and GC [11] methods have been described. However, the sample preparation procedures used are not suitable for the determination of skatole in adipose tissue.

Different chromatographic methods for the determination of skatole in back fat using normal-phase [12] and reversed-phase HPLC [13,14] or GC [15,16] have been described. The critical step in the determination of skatole in adipose tissue is the sample preparation procedure, because skatole is a very lipophilic compound and the chromatographic system is disturbed or destroyed by fat. Sample preparation procedures used include liquid–liquid extraction [16], steam distillation [3] and solid-phase extraction in the normal-phase [12,15] and reversed-phase modes [14].

A routine UV method involves extraction of fat samples with Tris–acetone followed by measurement of the absorbance at 580 nm after derivatiza-

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tion with 4-dimethylamino benzaldehyde [17]. This method, which when fully automated has a capacity of 200 samples per hour, is currently used for analysing large numbers of carcasses on slaughterhouse lines in order to sort out carcasses with unacceptable male odour. The method, however, is believed to be unspecific, because of interference from other indolic compounds. Nevertheless, there is an excellent correlation between the results of this method and the judgement by a trained taste panel [18].

The factors causing the occurrence of male odour are mainly unknown. In order to elucidate the mechanisms behind the boar taint problem, investigations are in progress to study the physiological, microbiological, hereditary and practical problems responsible for boar taint.

In order to have a general method for the determination of skatole and indole and also several other indolic compounds which may be precursors or, alternatively, metabolites of skatole, two gradient HPLC methods were developed. For the determination of indolic compounds in back fat samples, a solid-phase extraction procedure for the removal of fat from the samples was developed. The solid-phase extraction is not used in the traditional manner, *i.e.*, concentration of compounds followed by selective elution of interfering compounds and finally elution of the compounds of interest. In the proposed method, solid-phase extraction is used for trapping lipids and fatty acids while the indolic compounds pass through the column. The extract is analysed directly, which makes sample preparation simple and fast. The method developed is compared with the spectrophotometric method for the determination of skatole described by Mortensen and Sørensen [17].

EXPERIMENTAL

Chemicals

Indole, 3-indoleacetic acid (IAA), 3-indolyacetonitrile (IACN), 3-indolebutyric acid, 2-indolecarboxylic acid (2-ICA), 3-indolecarboxylic acid (3-ICA), 3-indoleethanol (IEtOH), 3-indolemethanol (IMeOH), 2-methylindole (2-MID), skatole (3-methylindole), tryptamine and tryptophan (THY) were obtained from Sigma (St. Louis, MO, USA). Demineralized water was treated in a Milli-Q Plus water purification system from Millipore (Bedford, MA, USA). Methanol, acetone, acetonitrile (ACN) and tetrahydrofuran (THF) were of HPLC grade from Romi (Loughborough, UK). All other chemicals were of analytical-reagent grade.

Back fat samples

Back fat samples for validation of the method were obtained from a local butcher. Prior to use, they were tested for content of indolic compounds to ensure a low content of skatole. Back fat samples used in the developmental stages were selected at the local abattoir based on a screening of the skatole content of the carcasses by means of the method described by Mortensen and Sørensen [17].

Conditioning of solid-phase extraction columns

A Bond-Elut column (C_{18}, 500 mg, 6 ml, from Analytichem, Harbor City, CA, USA) was conditioned prior to use by passing two 5-ml aliquots of methanol, acetone and acetone/0.1 *M* Tris–HCl buffer (pH 7.5)–0.1 *M* sodium sulphite (acetone–Tris) (75:25:1, v/v/v) through the column by means of a Vac Elute SPS 24 (Analytichem). Prior to application of tissue homogenate, the column was cooled by slowly passing 5 ml of ice-cold acetone–Tris through the column.

High-performance liquid chromatography

HPLC system 1 was a Hitachi system from Merck (Darmstadt, Germany) consisting of a Model 655 A-40 autosampler, a Model L-6200 gradient pump, a Model F-1000 fluorescence detector and Model D-6000 HPLC manager software installed in a Compaq Deskpro 386s PC.

The column was a LiChrospher RP-Select B (5 μm) (250 mm × 4 mm I.D.) fitted with a 4-mm RP-Select B precolumn (LiChroCART system, Merck). The mobile phases consisted of (A) ACN–50 m*M* potassium phosphate buffer (pH 6.0) (5:95, v/v) and (B) ACN–water (90:10, v/v) with the following gradient profile: 0–16.0 min, 0–80% B; 16.0–16.1 min, 80–100% B; 16.1–18.0 min, 100% B; 18.0–18.5 min, 100–0% B; 18.5–21.0 min, 0% B. The flow-rate was 1.2 ml min^{-1}.

HPLC system 2 was an LC-Analyst system from Perkin-Elmer (Norwalk, CT, USA) consisting of a Model 620 four-solvent pump with continuous helium degassing, an ISS 200 autosampler fitted with a 150-μl loop, an LC-235 diode-array detector, an

LC-240 luminescence detector, a DEC station 316 SX PC, an Analyst software kit, a PESOS software kit and an ML HPLC column oven (Mikrolab, Aarhus, Denmark). A PEEK back-pressure regulator on 40 p.s.si. (Upchurch Scientific, Oak Harbor, WA, USA) was installed after the detectors.

The column was a Superspher RP-8 (4 μm) (120 mm × 4 mm I.D.) operated at 40°C, fitted with a 4-mm RP-8 precolumn LiChroCART system, Merck). The composition of the mobile phases was (A) methanol–acetic acid–water H_2O (5:2:93, v/v/v); (B) THF–water (90:10, v/v) with the following gradient profile: equilibration, 5 min 15% B; 0–11.5 min, 15–47% B; 11.5–11.7 min, 47–70% B; 11.7–13.9 min, 70% B; 13.9–14.2 min, 70–15% B; 14.2–15.2 min, 15% B. The flow-rate was 1.2 ml min^{-1}.

The principle of detection in both systems was fluorescence with excitation at 285 nm and emission at 340 nm. In all assays 40 μl of the sample were injected. 2-MID was used as an internal standard for the determination of indole and skatole and 2-ICA for the determination of THY, IAA, IACN, IPA and IEtOH. The internal standards were added to the samples prior to sample pretreatment to a final concentration of 0.1 mg l^{-1}.

The linearity of the calibration graphs based on peak area was investigated in the range 0.0001– 5.0 mg l^{-1}.

Stability of the indolic compounds

The stability of skatole, indole, IAA, IACN, IEtOH, IPA, 2-MID, 2-ICA and THY was determined in the following different solutions at ambient temperature. (1) 2.5% trichloroacetic acid; (2) acetone–0.1 *M* Tris–HCl buffer (pH 7.5)–0.1 *M* sodium sulphite (75:25:1; v/v/v); and (3) acetone–0.1 *M* Tris–HCl buffer (pH 7.5) (75:25; v/v).

Sample pretreatment

A 5.00-ml volume of acetone–Tris and 50 μl of an internal standard solution containing 10 mg l^{-1} of 2-MID and 2-ICA were added to 2.50 g of back fat. After homogenization by means of a Kinematica (Littau, Switzerland) Polytron PT 3000 fitted with a 20-mm aggregate, the samples were sonicated for 5 min and cooled in an ice-bath for 15 min. The ice-cold solution was then passed through an activated and chilled Bond-Elut column. The column was washed by passing two 1.0-ml portions of ice-cold acetone–Tris through it. The eluate was diluted with three parts of water and analysed.

Gravimetric determination of extractable matter removed by solid-phase extraction

A 10.0-g amount of back fat was homogenized in 200.0 ml of acetone–Tris, cooled on ice and filtered. First, three 20.0-ml aliquots of the filtrate were evaporated to dryness. Next, three 20.0-ml aliquots of the filtrate were passed through activated Bond-Elut columns and were subsequently evaporated to dryness. Finally, three 20.0-ml acetone Tris aliquots were evaporated to dryness; all were further dried for 1 h at 110°C. The residues from evaporation were analysed by weighing.

Effect of sonication on recovery

The recoveries from samples spiked with 0.2 mg/kg of indole, IAA, THY and skatole were determined after sonication for 0, 5, or 30 min prior to solid-phase extraction.

Validation

The recovery and the intra- and inter-assay variability of the sample preparation procedure were determined by spiking three different back fat samples with a low content of indolic compounds at three different levels (see Table I).

Application

Samples (137) of back fat from uncastrated male pigs were analysed in duplicate using HPLC system 1. The same samples were also analysed by a spectrophotometric method [17,19] and the results were compared.

RESULTS AND DISCUSSION

Selectivity of the chromatographic system

As part of the boar taint project, it was decided to develop at least one general-purpose HPLC system for the determination of indolic compounds which could be precursors to skatole or indole formed in the gastrointestinal tract by various bacteria (THY, IAA, IEtOH, IPA and tryptamine) [20]. Other possible metabolites of skatole (IMeOH and 3-ICA) were included. IAA may be a precursor to skatole, as heating causes decarboxylation above 160°C. It was intended to use 2-ICA and 2-MID as internal

TABLE I

SPIKING LEVELS OF INDOLIC COMPOUNDS ADDED TO SAMPLES ORIGINALLY CONTAINING LOW AMOUNTS OF INDOLE AND SKATOLE IN ORDER TO DETERMINE RECOVERIES AND WITHIN-DAY AND BETWEEN-DAY COEFFICIENTS OF VARIATION

Level	Concentration added (mg kg^{-1})						
	THY	IAA	IRO	IEtOH	IACN	Indole	Skatole
1	0.5	0.02	0.02	0.02	0.02	0.05	0.05
2	2.0	0.08	0.08	0.08	0.08	0.2	0.2
3	5.0	0.2	0.2	0.2	0.2	0.5	0.5

standards. A LiChroCART Select B column was selected for development of one of the systems because of poor peak shape of tryptamine on most other stationary phases investigated. The good selectivity of the system is shown in Fig. 1. The total time of analysis is 25 min for thirteen indolic compounds.

Inspecting the chromatogram of standards in Fig. 1a, a total of fourteen peaks can be counted. This is due to the fact that IMeOH was found to be highly unstable under the conditions applied. After preparation of a fresh solution of IMeOH in methanol–water (1:1, v/v) and repeated analysis as a function of time, it was discovered that the original peak slowly split into two with the emerging peak appearing later in the chromatogram. The identity of the compound in this peak is not known.

In order to develop a faster alternative system with a selectivity different from the acetonitrile system, a system using a Superspher RP-8 column (4 μm) and THF, methanol and acetic acid in the mobile phase was developed. From Fig. 2 it is seen that the selectivity of the latter system is excellent and, just as important, different from the first. This system cannot be used for the determination of tryptamine and IMeOH because of the poor peak shapes of these compounds. Efforts to decrease the time of analysis by increasing the content of organic modifiers under the initial conditions were not successful, because k' for THY remained constant when increasing the content of THF in the mobile phase (Fig. 3).

Principle of detection

Both UV and fluorescence detection were investigated. Fluorescence detection was selected because of the better selectivity in the detection of indolic

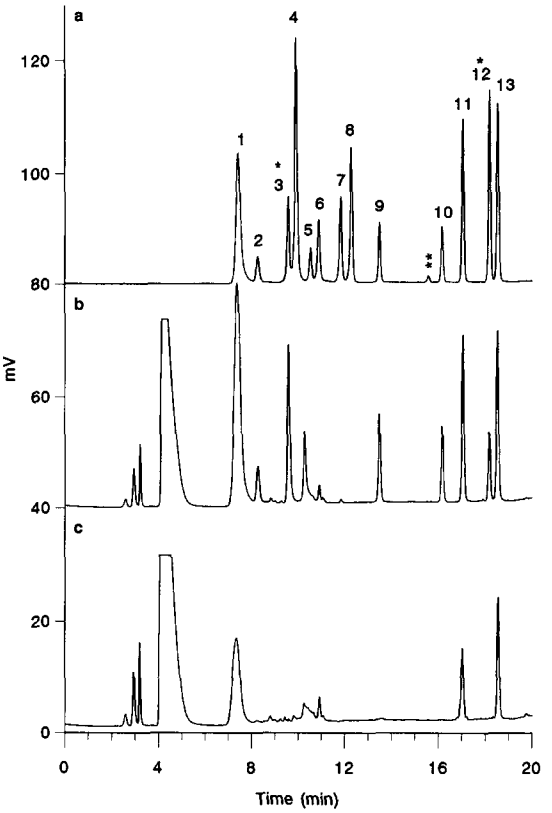

Fig. 1. Selectivity of the chromatographic system 1. Peaks: 1 = tryptophan; 2 = indoleacetic acid; 3 = 2-indolecarboxylic acid; 4 = 3-indolecarboxylic acid; 5 = tryptamine; 6 = 3-indolepropionic acid; 7 = 3-indolemethanol; 8 = 3-indolebutyric acid; 9 = 3-indoleethanol; 10 = 3-indoleacetonitrile; 11 = indole; 12 = 2-methylindole; 13 = skatole; * = internal standards; ** = rearrangement product of 3-indolemethanol. Column: LiChroCART Slect B (5 μm; 250 mm × 4 mm I.D.). The mobile phases consisted of (A) ACN–50 m*M* potassium phosphate buffer (pH 6.0) (5:95, v/v) and (B) ACN–water (90:10, v/v) with a gradient profile from 0 to 80% B in 16.0 min. Flow-rate: 1.2 ml min^{-1}. (c) Back fat sample with a content of 0.12 and 0.19 μg g^{-1} of indole and skatole, respectively; (b) same sample spiked with nine different indolic compounds; (a) standard solution containing 0.5 μg ml^{-1}.

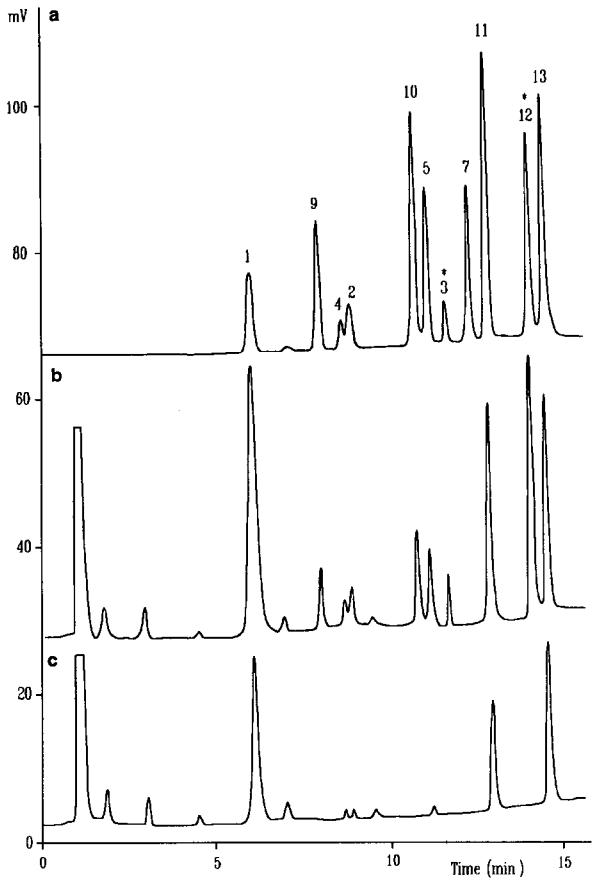

Fig. 2. Selectivity in chromatographic system 2. Identity of the compounds as in Fig. 1. Column: Superspher RP-8 (4 μm, 120 mm × 4 mm I.D.), operated at 40°C. The mobile phases consisted of (A) methanol–acetic acid–water (5:2:93, v/v/v) and (B) THF–water (90:10, v/v) with a gradient profile from 15 to 47% B in 11.5 min. Flow-rate: 1.2 ml min^{-1}. (c) Back fat sample with a content of 0.16 and 0.19 μg g^{-1} of indole and skatole, respectively; (b) same sample spiked with eleven different indolic compounds; (a) standard solution containing 0.5 μg ml^{-1}.

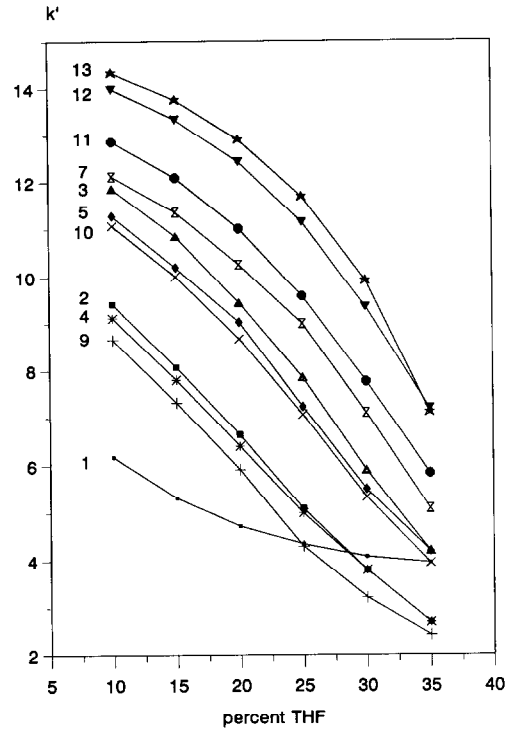

Fig. 3. Values of k' for the different indolic compounds as a function of the concentration of THF in the initial mobile phase of the gradient system (system 2). Identity of compounds as in Fig. 1.

compounds and an at least ten times better sensitivity.

Linearity, detection and quantification limit

The detection and quantification limits were determined for the different indolic compounds (Table II). The detection limits (signal-to-noise ratio = 3) were determined in standard solutions. The quantification limits were defined as ten times the detection limit unless authentic samples showed less sensitivity. The quantification limit of THY could not be verified because it proved impossible to find a sample with low content of THY. For IPA the detection limit is fairly high, as a system peak close to the IPA peak makes the quantification of IPA difficult at low concentrations. A quantification limit of 15 μg kg^{-1} for skatole is satisfactory when the determinations are used for elucidations of relationships between smell or taste and content. The threshold for sensing skatole is 0.2 mg kg^{-1} [18].

Indolic compounds identified in pig back fat

During the development of the chromatographic systems, some back fat samples were analysed for their content of various indolic compounds in order to verify that the selectivity of the method was sufficient for determination of THY, IAA, indole and skatole. During these investigations the presence of IMeOH, IPA, IACN and IEtOH was discovered. Their presence was corroborated by spiking with

TABLE II

DETECTION AND QUANTIFICATION LIMITS FOR THE DIFFERENT INDOLIC COMPOUNDS IN HPLC SYSTEM 1

The detection limits (signal-to-noise ratio = 3) were determined using standard solutions. The quantification limits were defined as ten times the detection limit unless authentic samples showed less sensitivity.

Parameter	THY	IAA	IPA	IEtOH	IACN	Indole	Skatole
Detection limit ($\mu g\ kg^{-1}$)	4.0	2.0	0.8	0.4	0.4	0.2	0.2
Quantification limit ($\mu g\ kg^{-1}$)	40	20	100	15	15	15	15
Linearity ($\mu g\ kg^{-1}$)	10–9000	4–1500	8–1500	4–1500	4–1500	2–1500	2–1500

known amounts of standards in the two systems; for IMeOH, however, only in system 1, owing to the poor chromatographic properties of IMeOH in the acidic system 2. In order to determine if the presence of IPA, IACN and IEtOH is general for back fat, the method was validated for the determination of these compounds. IMeOH was not included in the validation owing to its instability.

Choice of internal standards

During development of the chromatographic systems, several not naturally occurring indolic compounds were considered as possible internal standards in the systems. Using HPLC system 2 and 2-ICA as internal standard, the coefficients of variation (C.V.s) for the determination of THY, IAA, skatole and indole were 0.5, 1.2, 10.4 and 11.6%, respectively, whereas the C.V.s when using 2-MID were 11.0, 11.2, 0.8 and 1.7%, respectively. Hence 2-ICA is suitable as internal standard for the more polar idolic compounds such as THY and IAA, whereas 2-MID is suitable for the more lipophilic indolic compounds such as indole and skatole. This is in good agreement with general practice for the selection of internal standards.

Stability of the indolic compounds

A solution of skatole in acetone–Tris solutions, with and without addition of sodium sulphite, is stable for at least 60 h (less than 2% decrease in peak area). However, in a 2.5% trichloroacetic acid solution skatole is stable for only 10 h (less than 2% decrease in peak area). The pattern is similar for the other indolic compounds. The stability of IMeOH was not tested separately. As a consequence of observations made during the development of the chromatographic system, it was discovered that a standard solution of IMeOH in methanol–water (1:1, v/v) is highly unstable. During a few hours the peak split into two different peaks (Fig. 1).

Conditioning of the columns

Chilling of the solid-phase extraction columns prior to application of tissue homogenate and also the application of cold homogenate were found to be essential. If the homogenate was at ambient temperature the lipids would melt, with the risk of liquid lipids passing through the columns, and consequently lipids would later be injected on to the analytical column.

Effect of sonication on recovery

The duration of the sonication had no significant effect on the recovery, but sonication for 5 min was maintained in order to ensure that equilibrium of the internal standards between the fat particles and the solvent was achieved.

Removal of fat by solid-phase extraction

Several procedures for removal of fat from the samples were investigated. A very simple procedure using microwave melting followed by extraction of the melted fat with methanol [21] was initially investigated. The method was not adopted due to a rapid increase in the column back-pressure and difficulties with proper assessment of the recovery of this procedure. An obvious sample preparation procedure for removal of fat would be solid-phase extraction using normal-phase conditions as in the method described by García-Regueiro *et al.* [12], who used Florisil columns. The method was tried but was unsatisfactory due to problems with some compounds (IAA, indole and skatole) subliming away during evaporation of the sample solutions.

Normally solid-phase extraction systems are used for the concentration of compounds by retaining them on the column, followed by elution of interfering compounds with a suitable eluent and finally elution of the compounds of interest. In the present method the compounds of interest are not retained by the column, but the interfering fat is retained by the stationary C_{18} phase.

The effectiveness of the procedure in removing fat from the samples was assessed gravimetrically. The average residue of evaporation of pure extract was 96.8 ± 1.8 mg, that of evaporation of extract after passage through a Bond-Elut column was 91.5 ± 0.50 mg and that of evaporation of acetone–Tris was 86.5 ± 2.2 mg. Hence the solid-phase extraction procedure is capable of removing 5.3 mg, corresponding to 51% of the extractable matter. The rest of the extractable compounds can pass through the analytical column, a fact demonstrated by no significant increase in column back-pressure and no change in column selectivity during the *ca.* 1000 sample analyses to date.

The eluent/extraction medium contains 75% acetone, which makes it a very strong eluent compared with the initital conditions of the HPLC systems; the samples have to be diluted with three parts of water in order not to disturb the peak shape of the first-eluting peaks.

Validation

It was not possible to find back fat samples for validation that did not contain any of the indolic compounds. THY, indole and skatole were always present. As regards to indole and skatole, however, it was easy to find samples with a low content.

Only HPLC system 1 was validated. The recovery of the sample preparation procedure was assessed by spiking samples with a low content of indolic compounds with known amounts of the same indolic compounds. The levels used for spiking (Table I) were chosen after an investigation of the content of indolic compounds in about twenty back fat samples. The recovery was calculated from the difference between the average value recorded during 5 days for spiked an unspiked samples.

The average recoveries for skatole and indole are 92% and 117%, respectively, when using 2-MID as internal standard. The recoveries of the two compounds are different but stable. An explanation for this difference may be that the extraction is not 100% partly because the samples are only extracted once and the three compounds have different lipophilicities, a fact which also is reflected in the order of elution in the chromatograms. For the other compounds (except IPA) the recoveries are stable only they vary slightly at level 1 (IAA, IACN and IEtOH) because this level is close to the quantification limit.

The within-day ($n=7$) and between-day ($n=5$) C.V.s for the different indolic compounds are given in Table III. For THY, indole and skatole the C.V.s are acceptable at all levels used for the validation. For the other four indolic compounds the C.V.s are fairly high at the lowest concentration because the concentration is just above the quantification limit of the system. The C.V. for IPA is generally high owing to problems with a system peak close to the IPA peak.

Hence HPLC system 1 can be used for the determination of THY, indole and skatole at all the levels investigated. The method can be used for the determination of concentrations of IAA, IEtOH and IACN higher than level 1, and for IPA at concentrations higher than level 2.

Application

Table IV summarizes the concentrations of the different indolic compounds determined using HPLC system 1. Only THY is present in all the samples whereas at least trace levels of skatole and indole are found in all samples. IPA, IEtOH and IACN are not present in significant amounts.

On comparing the content of skatole determined by HPLC and the spectrophotometric method (Fig. 4), a good correlation ($r = 0.973$) between the two methods is seen. The equation of the regression line is $y = 1.0743x - 0.0671$ (y = HPLC result; x = spectrophotometric result). Hence the spectrophotometric method tends to overestimate the content of skatole, when the concentration is low, compared with the HPLC method. This is probably due to the unspecificity of the spectrophotometric method. In order to establish whether some of the other indolic compounds determined by the HPLC method contribute to the results from the spectrophotometric method, the results were compared by means of the multivariate analysis program Unscrambler [22] (Camo, Trondheim, Norway). It was not pos-

TABLE III

RECOVERIES AND INTRA- AND INTER-ASSAY VARIABILITIES DETERMINED BY SPIKING THREE SAMPLES, A, B AND C, WHICH ORIGINALLY CONTAINED LOW AMOUNTS OF INDOLE AND SKATOLE WITH KNOWN AMOUNTS OF THE INDOLIC COMPOUNDS

The levels used for spiking are given in Table I. The recovery was calculated from the difference between the average recorded during 5 days for spiked and unspiked samples.

Compound	Level	Sample	Assay conc.: mean ± S.D. ($n=7$) (mg kg^{-1})	Recovery (%)	Coefficient of variation		Compound	Assay conc.: mean ± S.D. ($n=7$) (mg kg^{-1})	Recovery (%)	Coefficient of variation	
					Within day ($n=7$)	Between days ($n=5$)				Within day ($n=7$)	Between days ($n=5$)
Skatole	0	A	0.009 ± 0.001	–	7.8	12	Indole	0.009 ± 0.001	–	10.4	12.0
		B	0.021 ± 0.003	–	15.3	21.9		0.008 ± 0.003	–	46.9	10.7
		C	0.052 ± 0.004		8.1	10.3		0.006 ± 0.002	–	27.2	31.7
	1	A	0.054 ± 0.004	93.2	7.5	5.2		0.069 ± 0.004	119.3	5.3	6.3
		B	0.072 ± 0.003	95.9	4.4	8.6		0.069 ± 0.005	117.5	7.0	3.3
		C	0.106 ± 0.002	93.0	1.9	6.1		0.064 ± 0.002	113.9	3.1	3.6
	2	A	0.189 ± 0.004	90.6	2.2	2.0		0.234 ± 0.006	117.4	2.9	4.3
		B	0.211 ± 0.008	92.6	4.0	4.9		0.258 ± 0.011	118.8	4.2	5.0
		C	0.238 ± 0.008	90.7	3.7	6.0		0.228 ± 0.008	116.5	3.5	6.1
	3	A	0.452 ± 0.016	88.5	3.6	2.7		0.598 ± 0.038	117.5	6.4	2.7
		B	0.513 ± 0.022	92.8	4.5	5.5		0.671 ± 0.054	118.9	8.1	8.1
		C	0.514 ± 0.009	90.1	1.7	4.5		0.551 ± 0.016	113.5	2.9	5.5
IAA	0	A	–	–			IPA	–	–		
		B	–	–				–	–		
		C	–	–				–	–		
	1	A	0.015 ± 0.002	76.3	14.9	24.3		0.038 ± 0.003	132.3	7.8	50.4
		B	0.063 ± 0.011	75.0	18.8	21.9		0.025 ± 0.005	122.5	22.9	50.8
		C	0.021 ± 0.001	90.0	5.4	20.0		0.069 ± 0.006	214.0	8.8	28.8
	2	A	0.065 ± 0.003	83.8	5.6	6.8		0.085 ± 0.004	114.8	5.7	19.1
		B	0.052 ± 0.010	83.4	19.8	17.3		0.122 ± 0.045	100.0	37.3	15.4
		C	0.081 ± 0.004	93.8	5.2	4.2		0.126 ± 0.014	88.0	11.2	25.6
		B	0.170 –	85.0	–	5.8		0.217 ± 0.011	86.4	4.7	14.7
		C	0.202 ± 0.002	89.7	1.1	9.2		0.219 ± 0.001	94.0	0.5	16.6

IEtOH	0	A	–	–			IACN	–	–		
		B	–	–				–	–		
		C	–	–				–	–		
	1	A	0.025 ± 0.06	107.0	24.3	10.9		0.016 ± 0.001	86.9	9.3	6.4
		B	0.027 ± 0.003	104.1	12.8	15.8		0.021 ± 0.002	93.4	8.9	11.4
		C	0.021 ± 0.001	95.0	5.4	12.5		0.019 ± 0.001	90.8	6.0	13.5
	2	A	0.084 ± 0.007	97.5	9.1	5.2		0.068 ± 0.001	84.3	2.7	4.5
		B	0.091 ± 0.018	99.7	19.8	8.1		0.072 ± 0.008	87.6	11.4	5.0
		C	0.079 ± 0.004	94.7	5.2	7.1		0.074 ± 0.014	87.0	18.9	10.7
	3	A	0.183 ± 0.010	93.2	5.7	3.5		0.166 ± 0.009	83.2	5.8	6.1
		B	0.217 ± 0.015	97.2	7.0	7.7		0.165 ± 0.018	84.9	11.3	7.5
		C	0.203 ± 0.007	94.6	3.5	8.9		0.193 ± 0.007	85.5	3.6	12.1
THY	0	A	2.49 ± 0.03	–	1.2	4.5					
		B	2.93 ± 0.21	–	7.2	5.6					
		C	2.31 ± 0.22	–	9.6	2.8					
	1	A	3.01 ± 0.17	91.3	2.3	5.7					
		B	3.34 ± 0.07	94.8	5.3	9.0					
		C	2.73 ± 0.07	81.3	2.7	3.8					
	2	A	4.25 ± 0.07	92.3	1.8	3.7					
		B	4.89 ± 0.04	90.1	7.0	2.8					
		C	4.29 ± 0.03	92.0	0.8	4.6					
	3	A	7.23 ± 0.14	94.4	1.9	2.0					
		B	8.52 ± 0.43	95.8	5.1	4.3					
		C	7.24 ± 0.08	95.2	1.2	1.8					

TABLE IV

SUMMARY OF THE CONCENTRATIONS OF THE DIFFERENT INDOLIC COMPOUNDS DETERMINED BY MEANS OF HPLC SYSTEM 1

The samples used were not selected randomly, but based on the skatole content determined by means of a spectrophotometric method [17]. Hence the samples cannot be regarded as representative for the general level of skatole in Danish domestic pigs. The average level of skatole in Danish male pigs is currently 0.10 ppm ($n > 100\,000$).

	Concentration (mg kg^{-1})					
	THY	IAA	IEtOH	Indole	Skatole	Skatole[a]
Highest	8.85	0.038	0.028	0.302	1.71	1.49
Lowest	1.28	<0.02	<0.015	<0.015	<0.015	0.05
Average	3.51	0.003	0	0.035	0.25	0.30

[a] Determined by means of the spectrophotometric method described by Mortensen and Sørensen [17].

sible to obtain a better correlation, hence it may be argued that none of the other indolic compounds contributes to the results obtained by the spectrophotometric method.

The samples used for the appplication were not randomly selected, but on the basis of the skatole content determined by means of the spectrophotometric method. Some of the samples were used for an investigation of the relationship between the content of indolic compounds and the taste or smell. Hence the samples cannot be regarded as representative of the general level of skatole and indole in Danish domestic pigs. The average level of skatole in male pigs is currently 0.10 ppm ($n > 100\,000$).

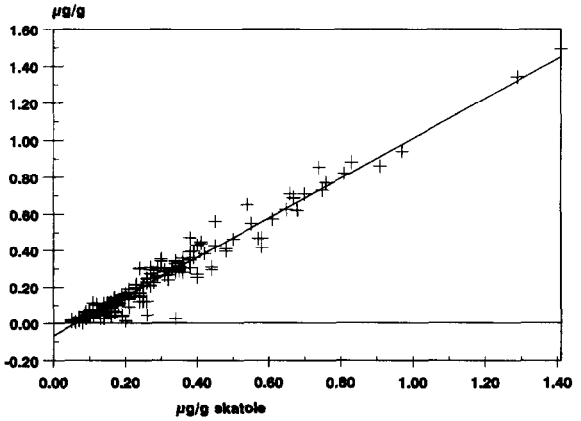

Fig. 4. Comparison of skatole determination in 137 pig back fat samples by use of the spectrophotometric method described by Mortensen and Sørensen [17] (y-axis) and the proposed HPLC method (x-axis). The correlation between the methods is 0.973 and the regression equation is $y = 1.0743x - 0.0671$.

Table V summarizes the present results and those reported by other workers. The results are not directly comparable, owing to differences in the way the samples were selected. The Spanish samples [13] were selected on the basis of the presence of boar taint (subjective judgement). The samples used in this study were selected on the basis of skatole determined by a spectrophotometric method [17]. Among the Danish samples was found the highest content of skatole, whereas the highest concentration of indole was reported in the German results [14].

CONCLUSIONS

As part of a project on studying the boar taint problem, a gradient HPLC method for the separation of thirteen indolic compounds was developed. The method has been validated for determination of seven indolic compounds in pig back fat. Only four of these compounds were found in significant amounts in 137 back fat samples analysed. The skatole concentrations obtained by the method were compared with those given by a spectrophotometric method for the determination of skatole; the correlation between the methods was excellent ($r = 0.973$).

When producing uncastrated male pigs, it is important to have a method capable of determining the concentration of skatole in the carcass. This method could be a HPLC procedure. The gradient

TABLE V

COMPARISON OF THE CONCENTRATIONS OF INDOLE AND SKATOLE OBTAINED BY THE PRESENT METHOD AND OTHER PUBLISHED METHODS

The other methods used were HPLC [13,14] and GC [15].

	Present method			García-Regueiro and Diaz [13]			Gibis *et al.* [14]			Porter *et al.* [15]	
	Indole (mg kg^{-1})	Skatole (mg kg^{-1})	Skatole/ indole	Indole (mg kg^{-1})	Skatole (mg kg^{-1})	Skatole/ indole	Indole (mg kg^{-1})	Skatole (mg kg^{-1})	Skatole/ indole	Indole (mg kg^{-1})	Skatole (mg kg^{-1})
Highest	0.302	1.71	15.4	0.16	0.186	3.2	0.602	0.901	4.0	0.057	0.177
Lowest	<0.015	<0.015	0.13	0	0	0.4	0.013	0.023	0.4	0.012	0.019
Average	0.035	0.25	3.7	0.084	0.101	1.4	0.151	0.201	1.8	0.029	0.046
n	137	137	137	15	15	15	20	20	20	14	14

methods described here are by no means rapid and they would not be suitable for sorting carcasses in an abattoir. Currently an isocratic, rapid, semi-automatic HPCL method is being developed for the determination of indole and skatole with a capacity of *ca.* 400 samples per day.

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