Alternative Methods for Assessment of Boar Taint

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Summary The Danish Meat Research Institute has developed two methods for determination of the dominant boar taint compound skatole in pig back fat. A high capacity automated spectrophotometric procedure (MORTENSEN and SØRENSEN, 1984) and a gradient HPLC method (HANSEN-MØLLER, 1992). The latter method is in addition capable of determining a number of precursors and metabolites of skatole, and recent comparative studies of the results obtained with the alternative methods have indicated that the automated equipment analyzes primarily for skatole. This may seem surprising, as the spectrophotometric procedure is optimized against a sensoric panel by tuning the colour development time rather than on skatole. However, skatole standards are conveniently used in calibrating the procedure, hence the expression "skatole equivalents" for the results obtained.

A high correlation (r = 0.973) between "skatole equivalent" as determined by the automated equipment and content of skatole as found by HPLC consequently exists. As the methods utilize different analytical principles this correlation indicates that the results are accurate.

Introduction It is a well-known fact that uncastrated male pigs grow faster on less feed compared to castrated ones. In addition, the former yield a higher content of lean meat in the carcass, and they are generally healthier. Consequently, the economic incentive in avoiding castration is strong; so is, of course, the animal welfare aspect.

On the other hand, meat from a small percentage of uncastrated males exhibits an unpleasant odour when heated and this so called boar taint has till now prevented large scale production of "entire males". Danish experience gained during the last 10 years has documented that the occurrence of tainted carcasses can be kept at or below 5%, which makes production of entire males with subsequent outsorting of odorous carcasses attractive.

The Danish Meat Research Institute has developed a high capacity automated analytical procedure based on spectrophotometric detection of the dominant boar taint compound skatole in fat. Actually, it was never postulated that the colourimetric procedure used analyzed exclusively for skatole, hence the analytical results were always given as "skatole equivalents". Compounds related to skatole are also known to form transient, coloured products with the reagent used, but more concern was placed in obtaining high correlation between the analytical result and a trained sensoric panel than on elucidating the chemistry taking place during analysis. The colourimetric procedure is conveniently calibrated with pure skatole solutions, however.

Such equipments are presently being installed in Danish slaughterlines. As doubts concerning the accuracy and specificity of the method have been raised, alternative procedures based on HPLC-analysis were recently developed.

The HPLC methods are targeted on the detection of chemical compounds containing indolic structures. Amongst these, only three are found in significant amounts in samples of fat from entire males: The essential amino acid tryptophan and its metabolites skatole (3-methylindole) and indole. Tryptophan is a constituent of a wide variety of foods and it does not contribute to boar taint.

Both skatole and indole may be suspected to contribute to the odour; however, skatole is known to be the more offensive to the human nose between the two. Interestingly, the HPLC results have indicated that the automated equipment analyzes primarily for skatole. Moreover, a high correlation (r = 0.973) between "skatole equivalent" as determined by the automated equipment and content of skatole as found by HPLC exists. As the methods utilize different analytical principles this correlation indicates that the results are accurate.

Materials and Methods **Chemicals**: All indolic compounds were purchased from Sigma (St. Louis, MO, USA). Dimethylamino-benzaldehyde (DMAB) and TRIS were obtained from Fluka (Buchs, Switzerland). Demineralised water for HPLC was treated in a Milli-Q Plus Water Purification System from Millipore (Bedford, MA, USA). Acetonitrile (MeCN) was of HPLC grade obtained from Romil (Loughborough, Leics, UK). Acetone was obtained from BDH (Pool, UK). All other chemicals were of analytical grade.

Back fat samples: Back fat samples used for comparison of the methods were selected at the local abattoir based on a screening of skatole content of the carcass by means of the fully automated method.

The HPLC method: The method is a gradient HPLC method for separation of 13 different indolic compounds and quantitative determination of 7 of these. The 13 indolic compounds are indole, 3-indolepropionic acid, 3-indoleethanol, 3-indoleacetic acid, 3-indolylacetonitrile, skatole, tryptophan, 3-indolecarboxylic acid, 3-indolemethanol, 2-indolecarboxylic acid (internal standard), 3-indolebutyric acid, 2-methylindole (internal standard), and tryptamin, of which the first 7 can be assayed quantitatively. A solid phase sample preparation is used for the analysis

of the indolic compounds in pig back fat. The indolic compounds are extracted with a mixture of acetone/TRIS–HCl buffer and lipid/fatty acid are removed from the extract by trapping them on a Bond–Elute C_{18} column. Fluorescence (Ex./Em. 280/340 nm) is used for selectiv detection. The method is described in detail elsewhere (HANSEN–MØLLER, 1992).

The colourimetric method: 5.00 ml acetone/TRIS–HCL buffer is added to approx. 500 mg of back fat in a polypropylene beaker. After homogenization with a specially designed homogenization device and chilling to approx. 4° C the homogenate is filtered through a sintered glass filter. To 1.00 ml of the filtrate 1.42 ml of DMAB reagent is added. (DMAB reagent is prepared by dissolving 10 g of DMAB in 100 ml of a mixture of ethanol/75% sulphuric acid (60:40; v/v), previously degassed by applying a vacuum of 15 mm Hg to the solution for 3 min.) After 180 s at ambient temperature 2 ml of the solution is transferred to the 30 mm flowcell of a Lambda II spectrophotometer (Perkin Elmer, Überlingen, Germany), and the spectrum from 460–730 nm is recorded. The absorption at 580 nm is used for quantitation by comparison with a known skatole standard curve. The method is fully automated with a capacity of 200 samples/standards per hour. Experimental details may be found in MORTENSEN and SØRENSEN, 1984.

Results and Discussion Figure 1 shows the absorption spectra of a back fat sample containing 0.25 ppm "skatole equivalents" and "blank". The characteristics of the skatole–DMAB spectrum are clearly visible and well above the noise level. The actual concentration of analyte in solution is 25 ppb, as the pretreatment causes a 10–fold dilution.

In Figure 2 an example of a determination of the content of the indolic compounds in a back fat sample containing 0.12 and 0.19 µg/g indole and skatole, respectively, is shown (A) as well as a chromatogram of a standard solution (B).

In a comparative study of the results obtained by the two alternative methods 137 back fat samples were analyzed both by the spectrophotometric method and the HPLC procedure. The samples were not selected randomly, but on the basis of "skatole equivalents". The this set of samples can not be regarded as representing the general level of skatole (and indole) in Danish domestic pigs. The average level of "skatole equivalents" in Danish male pigs is currently 0.09 ppm (n > 1.000.000).

In Figure 3 the two sets of results are plotted against each other, **i.e.** "skatole equivalents" **versus** HPLC skatole. A rather high correlation between the results is obtained, r = 0.973. The equation of the regression line is Y = 1.0743X – 0.0671. Thus the colourimetric method tends to overestimate the content of skatole, when the concentration is low, compared to the HPLC method. This is probably due to the unspecificity of the colourimetric 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 (ANON, 1990). It was not possible to obtain a better correlation using all the results. Reducing the interval surveyed to samples with "skatole equivalents" in the range 0.08 – 0.48, the correlation (r = 0.911) between HPLC skatole and "skatole equivalents" can be marginally improved (r = 0.914) by adding a 25% contribution from indole to the HPLC skatole results. Thus there is a slight possibility that the "skatole equivalent" contains a contribution from indole; until further experiments are carried out, however, this is far from conclusive. Table 1 summarizes the results along with results reported by other authors. The results are not directly comparable, due to differences in the way the samples were selected. The Spanish samples (GARCíA–REGUEIRO and DIAZ, 1989) were selected on basis of the presence of boar taint (subjective judgement). The samples used in this study were selected on basis of "skatole equivalents" as determined by the automated method, see above. Among the Danish samples was found the highest content of skatole, while the highest concentration of indole was reported for the German results (GIBIS et al, 1991).

Conclusion Both skatole and indole may be suspected to contribute to the odour; however, skatole is known to be the more offensive to the human nose between the two. Interestingly, the HPLC results have indicated that the automated equipment, which is optimized against a sensoric panel by tuning the colour development time, analyzes for skatole only. Moreover, a high correlation (r = 0.973) between "skatole equivalent" as determined by the automated equipment and content of skatole as found by HPLC exists. As the methods utilize different analytical principles this correlation indicates that the results are accurate.

When producing uncastrated male pigs it is important to have methods capable of determining the concentration of "skatole equivalents" in the carcasses. One such method could be a HPLC procedure. Development is currently progressing in making an isocratic fast semiautomated HPLC method for determination of indole and skatole with a capacity of approximately 400 samples per day.

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

Table 1: Comparison of the concentrations of indole and skatole from the literature. The methods used are HPLC (HANSEN–MØLLER, 1992; GARCíA and DIAZ, 1989; GIBIS et al, 1991) and GC (PORTER et al 1989).

	HANSEN–MØLLER, 1992			GARCíA–REGUEIRO & DIAZ, 1989			GIBIS et al, 1991			PORTER et al, 1989	
	Indole (mg/kg)	Skatole (mg/kg)	Skatole/indole	Indole (mg/kg)	Skatole (mg/kg)	Skatole/indole	Indole (mg/kg)	Skatole (mg/kg)	Skatole/indole	Indole (mg/kg)	Skatole (mg/kg)
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

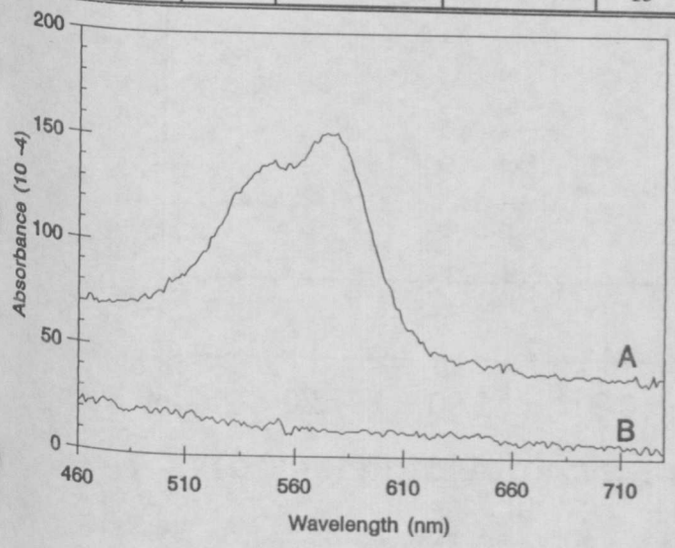

Fig.1 Spectrum of a back fat sample containing 0,25 µg/g "skatole equivalents" (A) and a "blank" spectrum (B).

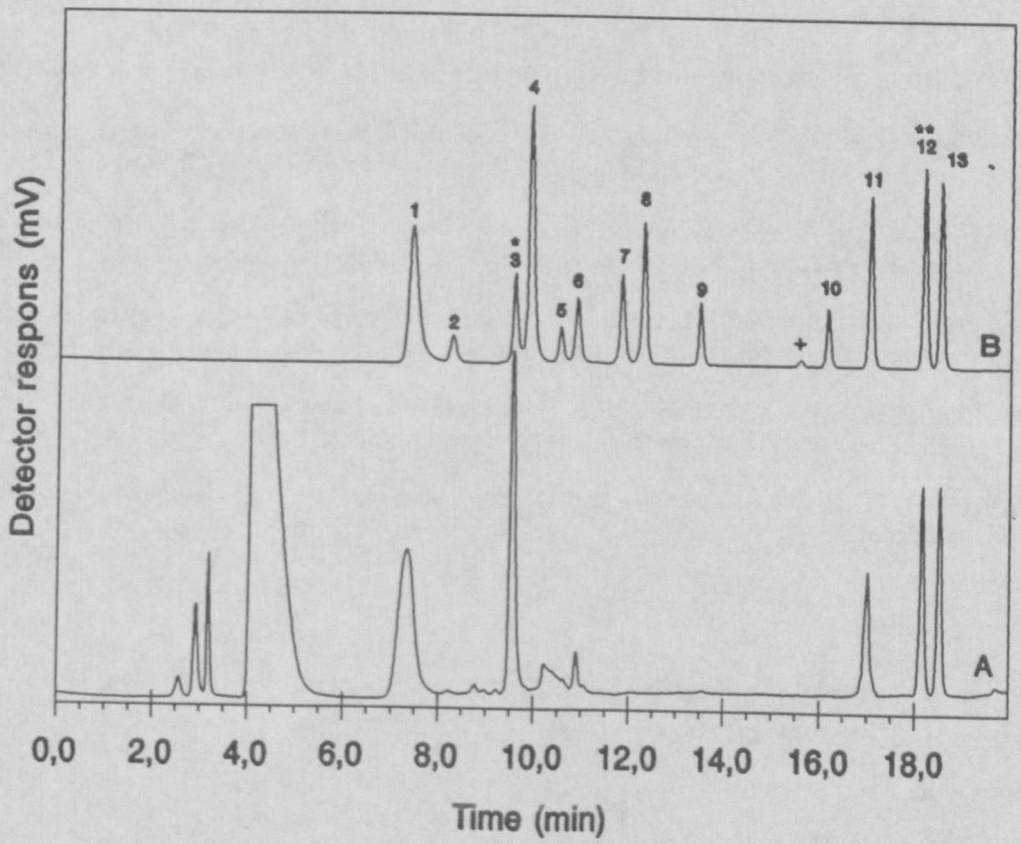

Fig. 2 Selectivity of the chromatographic system.
1: Tryptophan, 2: 3–Indoleacetic acid, 3: 2–indolecarboxylic acid, 4: 3–Indolecarboxylic acid, 5: Tryptamin, 6: 3–Indolepropionic acid, 7: 3–Indolemethanol, 8: 3–Indolebutyric acid, 9: 3–Indole–ethanol, 10: 3–Indoleacetonitrile, 11: Indole, 12: 2–Methylindole, 13: Skatole, *: Internal standards and **: Rearrangement product of 3–indolemethanol.Column: Licro–Chart Select B. (5 µm; 250 mm x 4 mm I.D)
The mobile phases consisted of I: MeCN/50 mM potassium phos–phate buffer pH 6.0 (5:95; v/v); II: MeCN/H_2O (90:10; v/v). Gradient profile: 0–80% B in 16.0 min. Flow rate: 1.2 ml/min.

A: Back fat sample with a content of 0.12 and 0.19 $\mu g g^{-1}$ indole and skatole, respectively.

B: Standard solution containing 0.5 $\mu g ml^{-1}$.

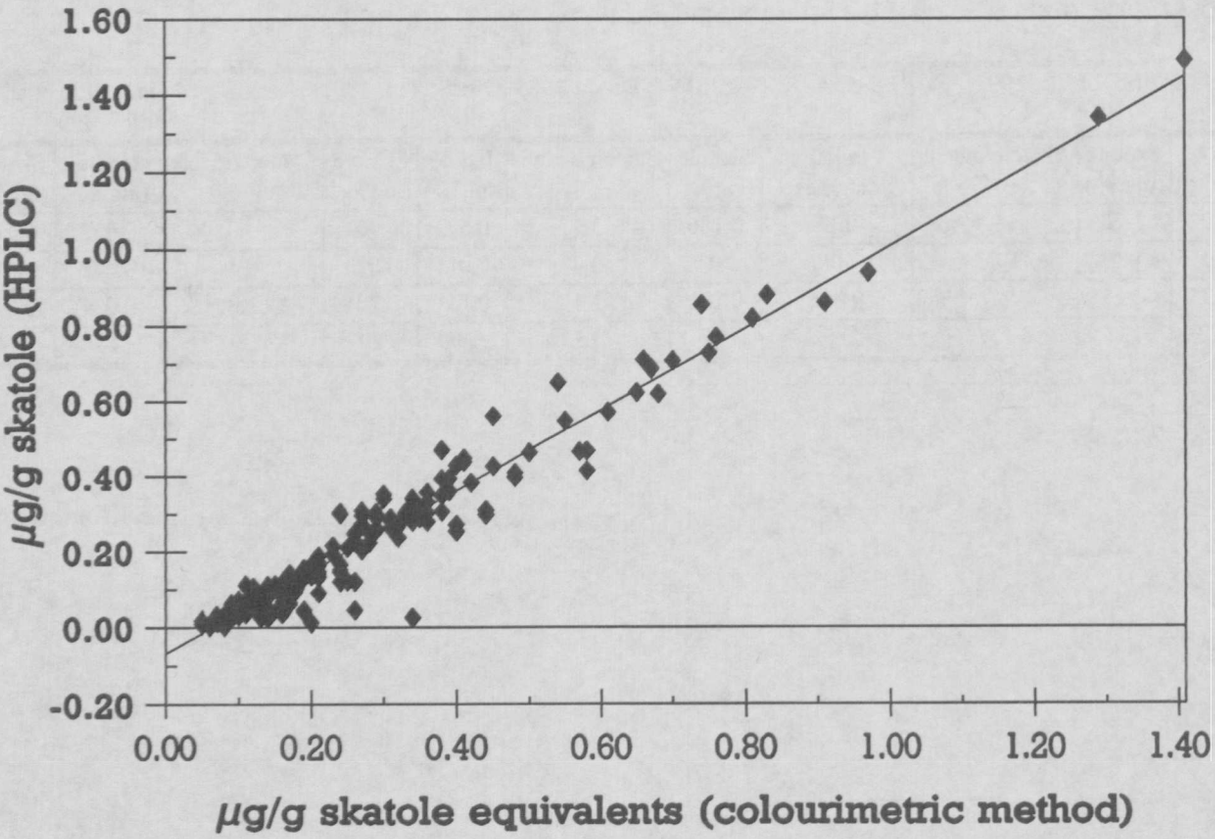

Fig. 3 Comparison of skatole determination in 137 pig back fat samples by use of the automated method and the HPLC method The correlati between the methods is 0.973 while the equation of regression is y = 1.0373 x −0.0671.

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