Relationship Between the Content of Skatole and Indole in Backfat and Lean Pork Obtained by a New HPLC Method.

M. GIBIS*, M. DEHNHARD** and A. FISCHER*

*Institut für Lebensmitteltechnologie, **Institut für Tierhaltung und Tierzüchtung, Universität Hohenheim, W - 7000 Stuttgart 70, Germany

SUMMARY:

This paper presents a fast and specific method of high-performance liquid chromatography (HPLC) with fluorimetric detection for the determination of indole and skatole (3-methylindole) in backfat and lean meat of pigs. The detection limit for the two substances is approximately 0.005 µg/g. The average recovery rate of added skatole and indole is 104 % and 106 % in backfat and lean meat respectively. The skatole content in the lean meat of boars established in this investigation exhibits a clearly linear significant correlation ($r = 0.96$; $P \leq 0.001$) with the content in backfat. A lower significant coefficient of correlation ($r = 0.76$; $P \leq 0.001$) was obtained between the concentrations of indole in lean meat and backfat.

INTRODUCTION:

Despite the in some cases considerable variations in odour and taste of the meat, boar fattening is pursued in a number of countries on account of the economic advantages, because in comparison with castrates boars utilize diet better and have a better meat-fat ratio (WALSTRA et al., 1970). In the Federal Republic of Germany, a carcass with even only a moderate variation in odour and taste is judged inferior according to Anlage 1, Kap. IV Nr. 4.1 Fleischhygiene-Verordnung (30.10.1986, BGBl. I, p. 1678) . This sexual or boar taint is caused by the steroid 5α-androst-16-en-3-one (PATTERSON, 1968), which is formed in the testes and accumulates in fat due to its lipophile characteristics. The other substances that are held responsible for variations in the odour and taste of pork are the two compounds skatole and, to a lesser degree, indole (VOLD, 1970; WALSTRA and MAARSE, 1970; HANSON et al., 1980a; LUNDSTRÖM et al., 1984), which are formed by the microbial degradation of tryptophan in the colon (YOSHIRA, 1979; WILKINS, 1990). The formation of skatole and indole is not directly related to sex; however, boars have higher skatole contents than either castrates or gilts (HANSON et al., 1980b).

A variety of methods have been described for determining skatole and indole in the backfat of pigs. The GC methods generally require time-consuming cleaning procedures (HANSON et al., 1980b; PELERAN and BORIES, 1985; PORTER et al., 1989). A colorimetric routine method (MORTENSEN and SORENSEN, 1984) is based on the spectrophotometric determination of a compound that is formed by the reaction of skatole and 4-dimethylaminobenzaldehyde. This method allows large numbers of carcasses to be examined at the abattoir. It is not, however, specific for skatole, because similar compounds such as indole are detected concurrently. An HPLC method with UV detection is also described, but this does not always match the sensitivity of the GC methods (GRACIA-REGUEIRO et al., 1986).

It is the aim of this study to detect very low concentrations of skatole and indole with the aid of new method of analysis, and to establish a relationship between the two substances in the proportions of backfat and lean meat of a sample.

MATERIALS and METHODS:

Sample material:

The material for investigation comprised 30 samples of muscular and fatty tissue (M. longissimus dorsi with fatty tissue from the region of the lumbar vertebra) from boars, which were divided according to lean meat and backfat and frozen at -30 °C until the time of analysis.

Sample preparation and calibration:

Skatole and indole were determined by means of an HPLC method with fluorimetric detection (GIBIS et al., 1991).

Weigh 1-2 g finely comminuted fatty tissue and 6-7 g meat tissue in a 50-ml centrifuge tube and add the internal standard 2-methylindole (1 µg). Extract the sample twice with 10 ml methanol. In order to remove the fat, freeze the combined methanol extracts at -30 °C for 30 min. After centrifugation (12 min at +4 °C and 13000 rpm), add 60 ml Tris buffer (0.05 mol/l Tris(hydroxymethyl)-aminomethane in 0.05 mol/l NaCl pH 8.3) to the supernatant liquid, mix thoroughly and pour into the prepared solid phase extraction column. Fill the columns with 2 ml Amberlite XAD-8 (32 mm x 10 mm ID), which must first be conditioned with methanol and washed with 2 x 5 ml water (superpure) and 2 x 5 ml Tris buffer. Wash with 2 x 5 ml water (superpure) and 2 x 5 ml Tris buffer and perform elution with 3 x 2 ml acetone. After evaporation of the eluent under a current stream of air at 40 °C to approximately 0.5 ml, fill to 2 ml with eluent A in a graduated reagent tube. Use 50 µl or 20 µl of the solution, passed through a membrane filter, for HPLC separation.

Conduct quantitative evaluation with the aid of the internal standard 2-methylindole. Prepare all standards for the calibration curve in the same way as the samples. The following standards with 0.02 to 1.5 µg indole and skatole and a constant quantity of 2-methylindole (1 µg) are used for the calibration curves. The injection volume is the same as that of the samples.

HPLC conditions:

Column: Supersher 100 RP-18 HPLC column, 4µm (125 mm x 4 mm ID) and Lichrospher RP-18 precolumn (4 mm x 4 mm ID), 5µm at 40 °C

Mobile phase: eluent A: 0.02 M acetic acid/acetonitrile/2-propanol (60:25:15, v/v); eluent B: acetonitrile/2-propanol (70:30, v/v)

Gradient programme: min 0-10 100 % eluent A, min 10-11 transition to 100 % eluent B, min 11-13 eluent B, min 13-14 transition to 100 % eluent A, min 14-20 eluent A

Flow rate: 1 ml/min

Fluorescence detector: excitation 270 nm, emission 370 nm

HPLC system: made by Gynkothek with Marathon autosampler and integrated column oven.

RESULTS and DISCUSSION:

In accordance with the chromatography conditions described above, indole, the internal standard 2-methylindole and skatole can be clearly separated both in fatty tissue and in lean meat with their respective retention times of 3.8 min, 5.2 min and 6.0 min, and can be identified by comparison with the standard chromatograms. After completion of processing and HPLC separation, the average recovery rate for skatole and indole is 104 % and 106 % respectively. The detection limit for this method is approximately 0.005 µg/g.

The advantages of this method of HPLC determination are the sensitivity of the fluorescence measurement and the relative lack of detectable interfering substances.

The skatole and indole contents in lean meat and fatty tissue of the investigated boar samples are shown in Fig. 1 and Fig. 2. The pairs of values were used for the graphs and calculation only if both values lay above the detection limit of the method. The concentration of skatole in backfat is plotted against that in lean meat in Fig. 1. The calculated coefficient of correlation ($r = 0.96$) is highly significant ($P \leq 0.001$; $n = 22$) and exhibits a clear linear relationship between the skatole content in backfat and lean meat. Correspondingly, the concentration of indole in backfat is plotted against that in lean meat in Fig. 2. The coefficient of correlation obtained from linear regression is lower ($r = 0.76$) and likewise highly significant ($P \leq 0.001$; $n = 20$). The two graphs show that there is a direct connection between the contents of indole and skatole in backfat and lean meat. This means that on account of the relationships shown here it would be possible to express an assertion on the variations in odour and taste of the lean meat caused by skatole and indole on the basis of the established concentrations in the fatty tissue.

Fig. 1:
Regression between the content of skatole in backfat and lean meat

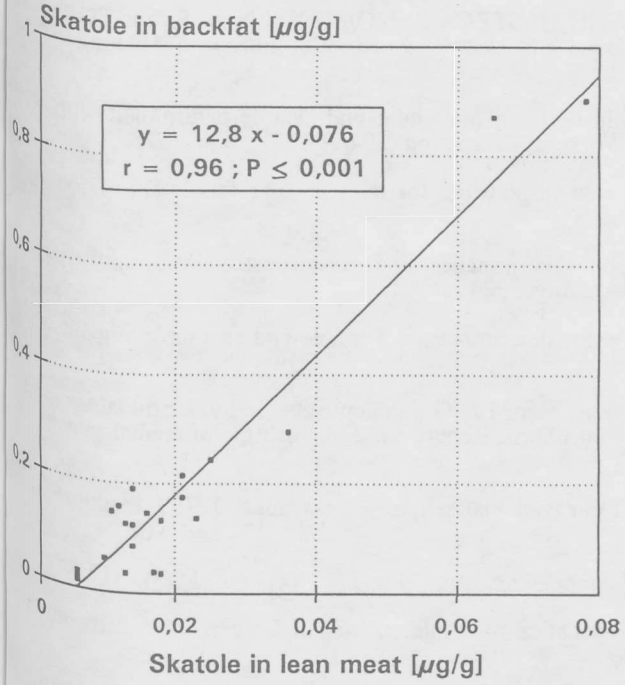
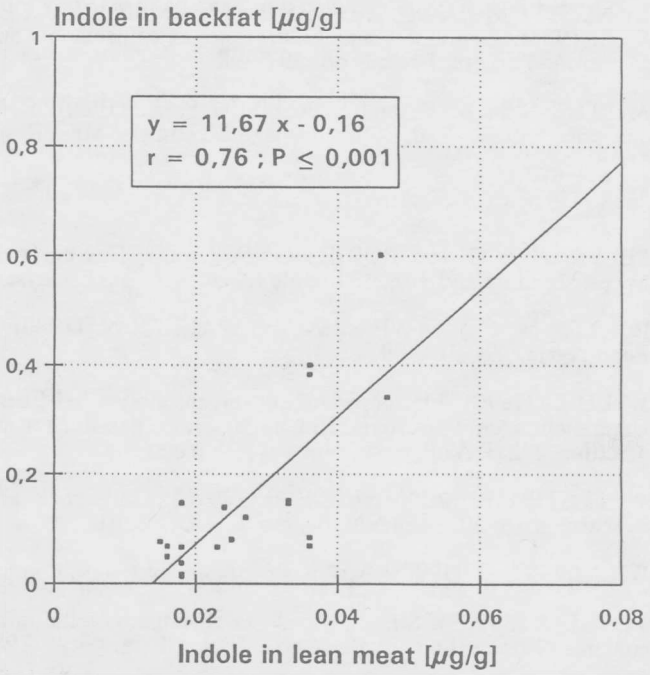


Fig. 2:
Regression between the content of indole in backfat and lean meat



Furthermore, the correlation for the variables skatole and indole in backfat ($r = 0.63$, $P \leq 0.001$, $n = 30$) was calculated, as was the corresponding correlation for lean meat ($r = 0.50$, $P \leq 0.05$, $n = 20$). In comparison with the skatole/indole correlation in backfat established by GRACIA-REGUEIRO and DIAZ (1989) ($r = 0.29$; $n = 15$; not significant), the correlation calculated in this study is considerably higher and significant, even though the indole and skatole contents are highly scattered and in some of the samples the indole content is actually clearly increased in comparison with the skatole content.

CONCLUSIONS:

The HPLC method described here allows the highly sensitive and specific determination of skatole and indole both in fatty tissue and in lean meat. The investigation shows that there is a direct relationship between the examined backfat and lean meat of a boar sample.

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