A PRACTICAL ENZYME-LINKED IMMUNOASSAY FOR QUANTITATION OF SKATOLE

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SUMMARY

Skatole (3-methyl indole) has been identified as a major chemical component responsible for off flavors in many animal products. For example, its concentration above 0.2 ppm in pork fat has been considered an indicator for boar taint. The currently available analytical methods to quantitate skatole are either non-specific or involve lengthy protocols. We have developed a practical enzyme linked immunoassay (ELISA) for this purpose. The test utilizes skatole antibodies immobilized on microtitration wells and skatole-enzyme conjugate as a tracer. Using a direct competitive ELISA, the test quantitates this analyte in boar sera up to 0.02 ppm in less than an hour. Recoveries of skatole in hog serum are between 80-105%. The test may be adapted for quantitation of skatole in dairy products in which it has also been identified as one of the flavour contributors.

INTRODUCTION

Boar taint has been a major problem for the pork industry. About 5-10% of the male swine population are known to produce the boar taint, a cause for offensive odour and poor meat tenderness in slaughtered animals. Hog farmers castrate young male pigs to relieve this problem, which often results in retarded weight gain and decreased lean meat content.

While Patterson (1968) reported androstenone (5- a -androst-16-en-3-one) as the major component of the boar taint, others have indicated skatole to be mostly responsible for this odour (Vold 1970; Walstra et al. 1970). Carefully planned studies on the effect of skatole and androstenone on the boar taint, tenderness and taste of the meat showed skatole to be a better determinant than androstenone (Lundstroem et al. 1984; Mortensen et al. 1986). The skatole above 0.2 ppm in the backfat of male pigs is considered an indicator for the offensive odour in pork.

A number of gas chromatographic and high pressure liquid chromatographic methods have been reported for quantitation of skatole (Hansson et al. 1980; Garcia-Regueiro et al. 1986). The methods suffer from lengthy sample processing and involve extraction of skatole from animals' backfat by organic solvents thereby, making it impractical for routine analysis.

A semi-automated method developed at Danish Pork Research Institute also relies on extraction of skatole from the backfat and measurement of the colour intensity formed by reaction between the sample filtrate and Ehrlich's reagent (Mortensen et al. 1984). The procedure, however, is not specific for skatole since the reagent reacts with many primary and secondary amines as well as other indole derivatives and alkaloids. The method is thus likely to produce a large number of false positives.

There is a need for a test which is specific, requires little or no sample preparation and can be automated to screen a large number of animals. We report an enzyme-linked immunoassay (ELISA) for quantitation of skatole in hog sera which meets these criteria. Since one would expect a state of equilibrium to exist for most chemicals between the body fluids and tissues of animals, our hypothesis was that serum skatole level may provide an indicator of the levels in animal fat.

MATERIALS AND METHODS

Preparation of Anti-Skatole Antibodies.

The anti-skatole antibodies were prepared by injecting BSA conjugate of indole-3-acetic acid into rabbits (Weiler 1981).

Skatole Alkaline Phosphatase Conjugate.

Indole-3-butyric acid (0.25 mg) was activated with EDAC and conjugated with 1 mg of alkaline phosphatase. The conjugate was purified by extensive dialysis with TBS containing 0.1% sodium azide and used in the assay.

Titration Curve of Skatole.

Anti-IAA antibodies in 50 mM carbonate-bicarbonate buffer, pH 9.5, were immobilized on wells of microtitration plates by incubation at 4°C. After 20 hrs the plates were washed with saline-water. Each well was filled with 100 μL of enzyme conjugate (1:10000 dilution) and skatole standards in TBS containing porcine serum (1:8 ratio). The plates were incubated for 1 hr at 37°C and washed. A 200 μL of substrate solution (0.1% p-nitrophenylphosphate in TBS) was added into each well and the plates incubated at 37°C. After 30 min the reaction was stopped with 0.1% sodium hydroxide and the absorbance of the colour measured in an EIA reader. Percent binding was calculated from the absorbance

Table 1. Recovery Studies

Skatole Added, Picomoles	Skatole Found, Picomoles	% Recovery
20,000	17,738	89
30,000	28,506	95
50,000	51,700	103

Table 2. Skatole Concentration in Serum and Backfat of Boars

Skatole Concentration, ppm Backfat[1]	Serum[2]
0.19	2.53
0.12	12.31
0.14	5.29
0.50	0.19
0.12	0.19
0.04	4.16
0.23	5.77
0.12	0.22
0.14	0.39
0.51	3.93
0.17	3.14
0.10	20.76
0.32	0.18
0.04	3.13

[1] Colorimetric Analysis [2] ELISA quantitation

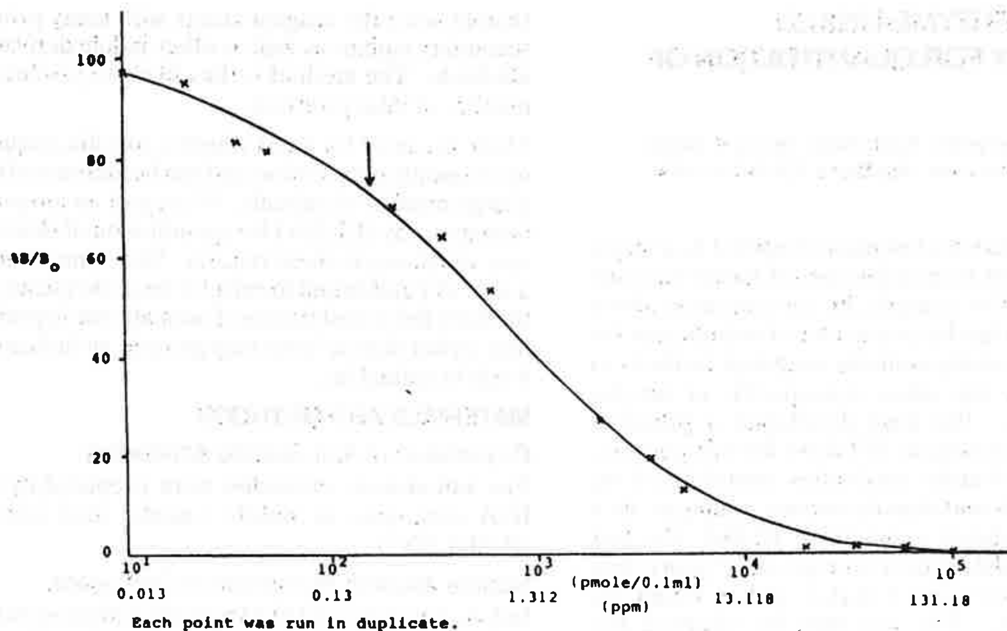

FIG. 1 Skatole Standard Curve

obtained in the absence (Bo) and presence (B) of the analyte and plotted against skatole concentration to get the titration/standard curve (Fig.1). Skatole in unknown samples was determined from the standard curve.

RESULTS

Recovery of Skatole.

A set of known skatole standards prepared in 10% porcine serum were analyzed by the method described above. Recovery of skatole ranged from 89-103% with a mean of 96% (Table 1).

Skatole Analysis in Boars.

Serum samples from 14 Danish boars were analyzed by ELISA and by a colorimetric method using backfat (Mortensen and Sorensen 1984). The results are compared in Table 2.

DISCUSSION

A competitive ELISA for quantitation of skatole (3-methylindole) in porcine serum has been developed. The test is rapid, highly specific and shows insignificant cross-reactivity (%) with tryptophan, a commonly present amino acid in animal serum. The ELISA method, being highly specific, is expected to provide true skatole levels in boars. In addition, the test should also be practical for screening thousands of animals in high speed slaughter lines.

Chemicals generally exist in equilibrium between blood and tissues. We anticipated, therefore, that the serum level of skatole in boars would be an indicator of its concentration in the animal backfat, a likely indicator of the boar taint. However, evaluation of skatole levels in serum and backfat in 14 Danish boars (Table 2) did not show any correlation. Experimental data indicates that skatole in blood may not be an indicator of the boar taint. Serum levels are found to vary from animal to animal and are independent of animal sex as determined by ELISA on five hundred randomly selected male and female pigs' sera. We now believe that a skatole derivative in backfat of male pigs could be a better indicator of boar taint. Skatole may be transported from blood to the fat of animals by a hydrophobic molecule such as androstenone, the hormone present in males only. A reversible, chemical equilibrium between skatole A, androstenone B, to form the enamine D (via the imminium ion C) can be envisioned to take place.

In fact, rapid reaction of skatole with androstenone has been shown *in vitro* by TLC analysis. The enamine compound D can readily form the colored compound under conditions used by Mortensen and Sorensen (1984) for their test.

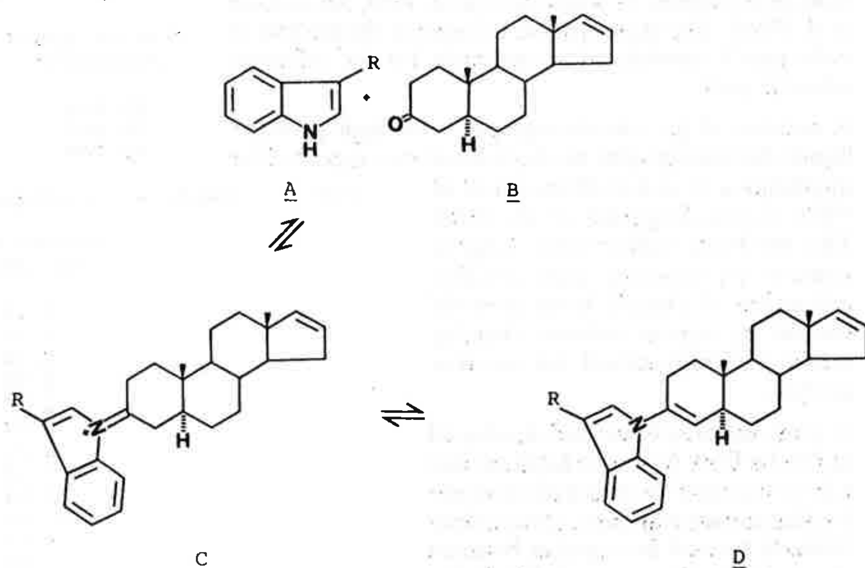

FIG. 2 Proposed Reaction Between Skatole and 5-α-andros-16-ene-3-one

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