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INTRODUCTION

Entire male pigs are used in several countries for meat production. Boar taint is a problem associated with this practice and can cause the reject of meat or meat products by consumers. 5 α -Androst-16-en-3-one (5 α -An) was associated with boar taint by Patterson in 1968 (1). Skatole (3-methylindole) also contributes to boar taint (2-6); skatole formation cannot be directly associated to sex, but entire male pigs have higher skatole levels than castrated pigs or gilts (4). Indole is a skatole related compound usually present in back fat of pigs and castrates generally presents higher concentrations than boars (5).

A correct evaluation of boar taint requires the determination of 5 α -An, skatole and indole. The determination of skatole and indole can be carried out by GC-MS (7) or by spectrophotometric determination measuring the absorbance at 580 nm of the derivative formed by the reaction with 4-dimethylaminobenzaldehyde (8). We recently described a method for the determination of skatole and indole in the back fat of pigs by HPLC (9), and another method for analysis of 5 α -An in the same type of sample by HRGC* (10). The last method allows a more simplified procedure than GC methods with packed column (11-14).

Simultaneous determination of 5 α -An, skatole and indole in back fat of pigs and meat products, specially in cured meat products, was examined. Purification of extracts was carried out by a florisil clean-up to obtain in separated fractions skatole/indole and 5 α -An. The purified extracts were analyzed by HRGC (5 α -An) (10) and by HPLC (skatole/indole) (9). In order to develop a more simplified method for 5 α -An analysis, the determination of 5 α -An by HPLC was explored. Hydrazone derivatives were studied to allow a sensitive and selective detection with spectrophotometric (UV) detector. The principal purpose of the present work was to study a single procedure in order to facilitate the simultaneous analysis of principal compounds associated with boar taint.

MATERIAL AND METHODS

Pretreatment of the samples: 3 g of back fat or 2-3 g of meat product extractable fat were weighted in a 25 ml flask, extraction was carried out as described in a previous work (9) with 3 x 10 ml of methanol. The combined methanol extracts were placed at -20°C during 10 min. in order to precipitate the fat, the solution was then filtered and methanol was evaporated. The residue was redissolved in 2 ml hexane:diethyl ether (80:20).

Clean-up: Extracts clean-up was performed on florisil column chromatography. A column was filled with 0.5 cm sodium sulfate, 6 cm florisil (activated at 120°C, during 24 hours) and 1 cm sodium sulfate (column i. d. was 1 cm). The column was prewashed with 40 ml hexane 100%. Elution of 5 α -An, skatole and indole was checked by elution of a standard solution. The sample was applied to the top of the column dissolved in 2 ml hexane:diethyl ether (80:20) and the fractions collected were:

- 1st) 20 ml hexane:diethyl ether (80:20) (F1)
- 2nd) 20 ml hexane:diethyl ether (60:40) (F2)
- 3rd) 30 ml hexane:diethyl ether (40:60) (F3)
- 4th) 30 ml hexane:ethyl acetate (96:4) (F4)

Skatole and indole were present in F1, 5 α -An was recovered in F3 (90%) and F4 (8%). F1 and F3 were concentrated to dryness and redissolved in 0.5 ml methanol for HPLC analysis or in 0.5 ml ml hexane for HRGC analysis.

The recovery of extraction and clean-up was evaluated by spiking 5 samples of back fat with different concentrations of 5 α -An (range: 0.4-2 μ g/g) and skatole/indole (range: 0.05-1.0 μ g/g).

HPLC analysis of skatole and indole: column Nucleosil RP-18 5 μ m (10 cm x 4 mm) operated at ambient temperature. Mobile phase: methanol:water (40:60) at a flow rate of 1 ml/min. The wavelengths used in detection were: 225 and 280 nm (9). The apparatus was a LKB HPLC.

HPLC analysis of 5 α -An: A light excess (50-100 μ g) of 2,4-dinitrophenylhydrazine (2,4-DNPH) was added to the F3 concentrated and redissolved in 1 ml of methanol. The reaction was carried out at pH=5.5 and at 60°C during 30 min. The solution is microfiltered for HPLC analysis and then concentrated to 0.2-0.5 ml of methanol. HPLC conditions were: column Nucleosil RP-18 5 μ m (10 cm x 4 mm) operated at ambient temperature. Mobile phase: acetonitrile:water (90:10) at 1.5 ml/min. Wavelengths used: 254 and 360 nm. 50 μ l of standard and sample solutions were injected via a Rheodyne injector 7215.

HRGC analysis of 5 α -An: Columns: FSOT BP-1 (30 m x 0.3 mm) (SGE, Australia) and FSOT SE-52 (15 m x 0.3 mm) (Alltech, Belgium). Detectors: FID and ECD (Ni, 10 mCi). A PTV (Programmed Temperature Vaporizer) injector was employed, cold and hot splitless modes were used. Carrier gas was helium at 30 cm/seg (BP-1) and 26 cm/seg (SE-52). Make up gas for ECD detection was nitrogen at 50 ml/min. For a selective detection with ECD were obtained oxime derivatives of 5 α -An by the reaction with PFBHA (O-pentafluorobenzylhydroxylamine) (10). The apparatus used was a DANI gas chromatograph 3800 HR-PTV.

RESULTS AND DISCUSSION

Extraction recoveries of 5 α -An, skatole and indole were 90, 98 and 90 % respectively, these values allow the use of the same extraction procedure for the three compounds. The elimination of fat was accomplished by precipitation at -20°C (9), 5 α -An recovery in this step was 85%.

Florisil clean-up allows the elimination of the interference compounds presents in the extract and provides a exhaustive purification without a excessive long time-consumption procedure. In F1 skatole and indole were present and in F2 were collected several compounds that can interfere with skatole and indole.

Fig. 1 show chromatograms obtained in the GC analysis of F1 and F3, it is possible to analyze F1 and F3 together because they have not present interference peaks in the retention time zones of 5 α -An, skatole and indole in the corresponding chromatograms.

Also Fl can be analyzed by HPLC (9); this technique allows to assure the identification/quantification by comparison of the responses of skatole and indole at the two wavelenghts used: 220 nm and 280 nm (9). HRGC analysis of 5 α -An was carried out with two detection methods: FID and ECD. ECD provides a more selective analysis, and the results obtained with FID and ECD were very closed, showing that HRGC-FID is a reliable method to quantify 5 α -An.

In order to evaluate 5 α -An by HPLC, hydrazone derivatives of the hormone were prepared. 5 α -An showed a low absorbance in the UV region; to avoid this problem hydrazone derivatives were very useful. Recovery of hydrazone derivatives by reaction of 5 α -An with 2,4-DNPH was 75 %, 4-NPH provides better results (85%), but 2,4-DNPH derivatives showed better absorbance characteristics. Fig. 2 shows a HPLC chromatogram of 2,4-DNPH hydrazone derivative of 5 α -An. The detection was carried out at 254 nm and 360 nm, the last wavelenght allows a more selective analysis. 5 α -An quantification in back fat and meat products was more difficult with this technique than by HRGC. Accurate results were obtained only in samples with strong boar taint.

Fig. 3 shows a HPLC chromatogram of Fl of a sample of cured ham. Skatole identification is possible without problems, but indole determination needed the comparison of the responses at 220 and 280 nm (9) to assure the identification/quantification. These problems are due to the presence of several degradation products. 5 α -An is affected by the same problem but in this case derivatization with PFBHA avoids these difficulties. Fig. 4 shows a chromatogram of Fl+F3 of a cured ham sample obtained by HRGC-FID.

5 α -An, skatole and indole levels found in boar meat products were similar to those obtained from back fat of non castrated pigs (table I). 5 α -An levels in fresh and cured ham were similar, but skatole showed a decrease in several samples of cured ham (15).

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TABLE I. 5 α -An, SKATOLE AND INDOLE CONCENTRATIONS IN BOAR CURED HAM

Sample	5 α -An	Skatole	Indole
1	2,78	0.02	0.02
2	1,45	0.25	0.10
3	1.06	0.15	0.04
4	0.52	0.03	0.02
5	1.10	0.02	0.02
6	1.00	0.02	ND
7	2.00	ND	ND
8	1.00	0.10	ND
9	0.78	0.03	0.02
10	0.27	0.02	ND

All results in μ g/g extractable fat

ND, not determined

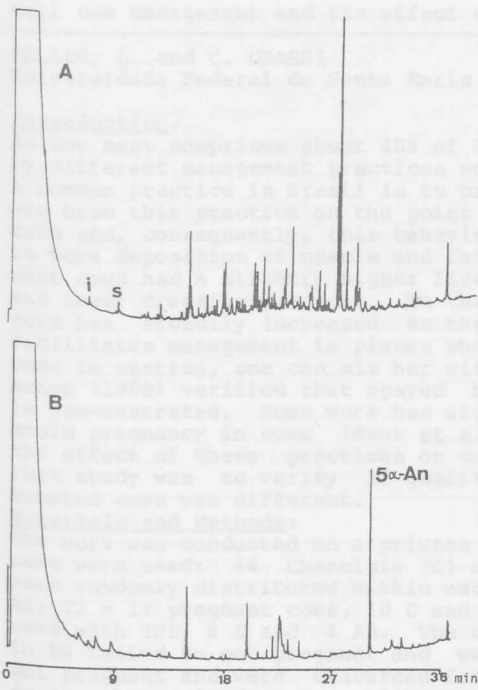


Fig. 1. FID chromatograms of back fat sample. A: F1, i: indole and s:skatole. B: F3. Conditions: column FSOT SE-52, 15 m x 0.3 mm. Temperature program: 80°C-4°C/min-250°C. Carrier gas: He at 26 cm/seg. Splitless injection

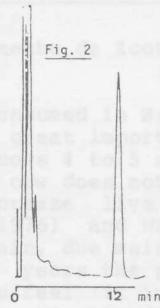


Fig. 2. HPLC chromatogram of hydrazone derivative of 5α-An (2,4-DNPH) Conditions: see text.

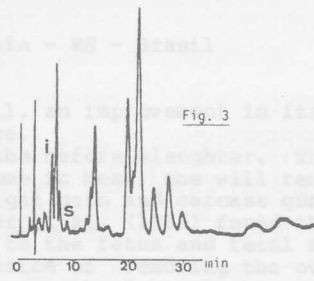


Fig. 3. HPLC chromatogram of cured ham sample. F1. Conditions: see text.

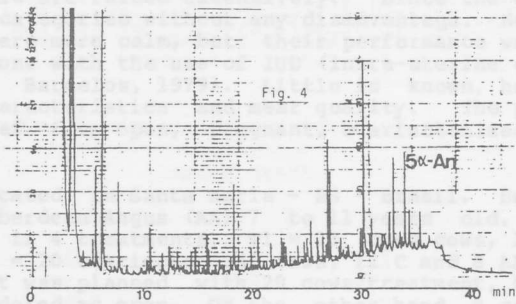


Fig. 4. FID chromatogram of cured ham sample. Conditions: column - BP-1 25 m x 0.3 mm FSOT. Temperature program: 80°C-4°C/min-260°C, carrier gas: He, 30 cm/seg. Splitless injection.

Results and Discussion:

The effect of treatments on warm and cold carcass weight, dressing percent and carcass shrinkage is presented in table 1.

Table 1. EFFECT OF DIFFERENT TREATMENTS ON COW YIELD AND SHRINKAGE

Treatment	n	Carcass weight - kg		Dressing %		% Shrinkage
		Warm	Cold	Warm	Cold	
Open	13	184.93	183.63	49.24 ^a	49.51 ^b	.76
Pregnant	17	187.02	183.91	49.13 ^a	47.59 ^b	1.12
Overfed	20	192.49	181.07	49.92 ^a	48.54 ^b	.78
Mid top	12	188.68	186.92	50.10 ^a	49.04 ^b	.93

Means in a column with different superscripts differ (P<.05).

There was no significant difference between treatments in warm or cold carcass weight. Pregnant cows, however, displayed a lower (P<.05) warm and cold dressing percent. Any advantage, therefore, that the 2 cows had presented in live weight gain was due to the weight of the pregnant uterus (Matua, membranes and fetal liquids) that in the present work presented an average weight of 19.35 kg, with a range of 7.5 to 27.2 kg. The present results agree with the findings of Bert et al. (1940), Solt (1976) and Walker et al. (1979). In this last work the total uterus weight averaged 18.35 kg with an age of about 6.49 months. In the present work the average age of the fetuses was around 3 months. Pregnant cows also presented a nonsignificant higher cold shrinkage. Kohn (1974) reported that pregnant cows had a higher percentage of water in their muscles, which could explain the higher losses in the cold room. The different treatments did not affect carcass characteristics (table 1). None of the characteristics measured were significantly affected by the 4 treatments. Cows with HD displayed a nonsignificant larger ribeye area and lower deposition of subcutaneous fat. In this treatment, however, the proportion of cholesterol in relation to lipids was a little higher, (60%), whereas in the other 3 treatments it was around 50%. Miller and Sorensen (1977) and Walker et al. (1973) also failed to detect any difference in carcasses from pregnant and open beef females. Fat thickness, as measured between the 12th and 13th rib, was higher for pregnant cows, but the difference was nonsignificant. The organoleptic characteristics of the meat can be visualized in table 3.

Also FI can be analyzed by HPLC (21). This technique allows to assure the identification/quantification by comparison of the responses of skatole and indole at the two wavelengths used: 250 nm and 290 nm (2). HPLC analysis of 5α-AI was carried out with two detection methods: FID and ECD. ECD provides a more selective analysis and the results obtained with FID and ECD were very similar, showing that HPLC-FID is a reliable method to quantify 5α-AI.

In order to evaluate 5α-AI by HPLC, hydrazide derivatives of the hormone were prepared. 5α-AI showed a low absorbance in the UV region. To avoid this problem hydrazide derivatives were very useful. Recovery of hydrazide derivatives by reaction of the hormone with 2,4-DNP was 75%. 2,4-DNP provides better results (25%), but 2,4-DNP derivatives showed better absorption characteristics. Fig. 2 shows a HPLC chromatogram of 2,4-DNP hydrazide derivative of 5α-AI. The detection was carried out at 250 nm and 290 nm, the last wave length allows a more select analysis. Skatole quantification in fresh and cured ham was more difficult with this technique than by HPLC. Accurate results were obtained only in samples with strong bear is 5α-AI.

Fig. 3 shows a HPLC chromatogram of FI of a sample of cured ham. Skatole identification is possible without problems, but indole determination needed the comparison of the responses of skatole and indole. In order to assure the identification/quantification, these problems are due to the presence of several degradation products. 5α-AI is affected by the same problem but in this case derivatization with 2,4-DNP avoids these difficulties. Fig. 4 shows a chromatogram of FI-FI of a cured ham sample obtained by HPLC-FID.

5α-AI, skatole and indole levels in ham meat products were similar to those obtained from back fat of non salted pigs (table 2). 5α-AI levels in fresh and cured ham were similar, but skatole showed a decrease in several samples of cured ham (table 2).



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TABLE 2. 5α-AI, SKATOLE AND INDOLE CONCENTRATIONS IN HAM CURED HAM

Sample	5α-AI	Skatole	Indole
1	2.70	0.02	0.02
2	1.95	0.75	0.70
3	1.05	0.15	0.05
4	0.32	0.03	0.02
5	1.10	0.02	0.02
6	1.00	0.02	ND
7	1.00	ND	ND
8	1.00	0.70	ND
9	0.70	0.03	0.02
10	0.27	0.02	ND

ND: results in partly extractable fat
 ND: not determined