ANALYSIS OF ANDROSTENONE IN PIG BACK FAT BY SOLID-PHASE EXTRACTION AND GC-MS.

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BACKGROUND

Compared with castrates, the production of entire male pigs shows some advantages: the carcasses are leaner, the production costs are reduced and the animal welfare is improved (Walstra, 1974; Bonneau, 1982). However castration of young male pigs has been practiced in some countries in order to avoid the occurrence of boar taint, an unpleasant odour present in the meat from 5-10% of the animals (Diestre et al.,1982).
Patterson (1968) identified the hormone 5α-Androst-16-en-3-one (5α-An) as the compound responsible for the urine like-odour associated with boar taint. The contribution of other steroids like androstenols is not clear (Bonneau, 1982; García-Regueiro and Díaz, 1989). Skatole and indole, which are produced in the intestine as a result of the breakdown of tryptophan, were also associated with boar taint (Vold, 1970; Hanson et al.,1980).
Radioimmunological analysis (RIA) (Claus et al.,1971), ELISA and chromatographic methods using GC-MS (Magard and Berg, 1995; García-Regueiro and Díaz, 1989), GC-FID (Kaufmann et al.,1976) or GC-ECD (Brabander and Verbeke, 1986; García-Regueiro and Díaz, 1985) were developed for the determination of androstenone in back fat samples. Further, chromatographic methods for determining skatole and indole in back fat using normal-phase (García-Regueiro and Díaz, 1989; García-Regueiro and Rius, 1998), and reversed phase HPLC (Hansen-Moller, 1992,1994) or GC (Peleran and Bories, 1985; Porter et al.,1988) were likewise described. Mortensen and Sϕrensen (1984) developed a routine UV method that allows on line determination of skatole.

OBJECTIVES

A simplified clean-up procedure was evaluated in order to develop a rapid and selective procedure for determining the 5α-An concentration in back fat samples by GC-MS.

MATERIAL AND METHODS

Pretreatment of the samples: 2μg of 5α-Androstan-3-one as internal standard (IS) was added to 1g of fat. Extraction was achieved with 2x20ml of dichloromethane. Dichloromethane was decanted into 50ml flash. 5ml were taken, evaporated to dryness and the residue was dissolved in 2ml of methanol to produce the precipitation of the fat at room temperature.
Clean-up: Clean-up was performed over octadecyl columns (C18) prewashed with 10ml of methanol. The sample was applied to the top of the column and the fraction eluted was recovered. The column was washed with 2ml of methanol. The two fractions collected were evaporated and redissolved in 20μl of iso-octane. The solution was analyzed by GC-MS.
Chromatographic conditions:. The column used was a HP-5MS (30m x 250μm, 0.25μm) and the temperature program was 70°C(1min)-10°C/min-190°C-5°C/min-270°C(5min). Temperatures of the injector and detector were set at 270°C and 280°C, respectively. Detection was performed in the SIM mode and four ions were selected: m/z (272, 257) for 5α-An and m/z (274, 202) for 5α-Androstan-3-one (IS). The equipment used was a GC HP-5890 and a selective mass detector HP-5970.
The validation of the method was performed to spiking fat samples with known concentrations of 5α-An. The linearity was determined in the range of 0.5 to 2μg/g of 5α-An. The efficiency of recovery was evaluated in these samples. The intra- and inter-assay variation was determined in 8 back fat samples spiked with 2μg/g of 5α-An. The samples were analyzed on seven different days. The factor response was evaluated varying the concentrations of 5α-An (0.5, 1, 1.5, 2μg/g) versus a fixed concentration of the internal standard (2μg/g).

RESULTS AND DISCUSSION

Dichloromethane allowed the extraction of the compounds studied and the solution could be evaporated easily. The elimination of the fat by precipitation in methanol at -20°C has been described in different works (García-Regueiro and Díaz, 1989; Tuomola et al., 1996) but the coprecipitation of compounds can occur. In this study the precipitation of fat was done at room temperature to minimize this effect. However it was not possible to analyze directly the extract obtained by GC-MS. Clean-up of the extract using a octadecylsilica column allowed the elimination of the remaining lipids without a long time consumption procedure. The samples were analyzed by GC-MS in the selective ion monitoring (SIM) mode because it provides more sensitivity than SCAN mode.
The linearity of the method was evaluated with spiked fat samples at the concentrations range of 0.5, 1, 1.5 and 2μg/g. The regression equation obtained was $y=3x10^{+06}$ x + 387662 with a correlation of $R^2=0.9998$. Since, the determination of 5α-An was possible in the range of concentrations in which it can be found in fat samples. The relative response of 5α-An with reference to internal standard was also evaluated. The linear equation obtained($Area_{5\alpha\text{-An}}/Area_{IS}$ *versus* $mass_{5\alpha\text{-An}}/mass_{IS}$) was y=0.6748 x + 0.0205 ($R^2=0.9982$), and the relative response of 5α-An/internal standard was 0.6748.
The relative recoveries obtained at different concentrations of androstenone and the C.V. values obtained are shown in Table1. In general, the relative recovery of androstenone was superior at 90%. The C.V. values of recovery was superior at low concentrations of 5α-An (0.5μg/g), although the values obtained were inferior at 6% in all the samples.

The within-day (n=5) C.V. for 5α-An and the internal standard 5α-Androstan-3-one in back fat samples are given in Table2. Retention times repeatability showed a C.V. value below 0.03% for both compounds. C.V. for absolute areas were 11.50% for 5α-An and 12.91% for internal standard. Figure 1 shows the chromatograms at selected m/z of 272 and 274 for a back fat sample spiked with 1μg/g of 5α-An.

Table1. Recovery of the method.

5α-Androst-16-en-3-one		
Added (μg/g)	Recovery(%)	C.V.(%)
0.5	92.39	5.36
1	100.48	2.47
1.5	99.44	1.64
2	99.39	1.58

Table2. Intra-assay variability

	5α-Androst-16-en-3-one		5α-Androstan-3-one	
Sample	tr	Area	tr	Area
1	20.93	2471689	21.64	3268223
2	20.93	2174127	21.64	2848076
3	20.93	2316988	21.64	2976970
4	20.94	2821815	21.63	3543385
5	20.93	2152540	21.64	2523052
X	20.932	2387431.8	21.638	3031941.2
S_{n-1}	0.005	274510.23	0.005	391535.54
C.V.	0.021	11.50	0.021	12.91

tr: retention time; X: mean; S_{n-1}: standard desviation; C.V.: coefficient of variation.

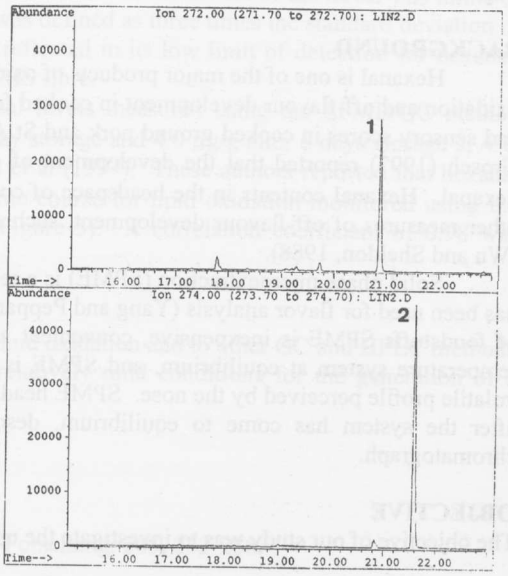

Fig1.CGC-SIM chromatograms. (a) m/z272 and (b) m/z274. Peaks:1, 5α-An; 2, internal standard. Conditions see text.

CONCLUSIONS

A rapid, reproducible and selective method for the determination of androstenone in back fat samples from pigs was developed. Elimination of fat is not achieved completely precipitating with methanol at room temperature. The solid phase extraction allowed the retention of the interfering fat while the compounds of interest were not retained by the column. The presence of interfering peaks was not observed in the GC-MS analysis in the SIM mode. The developed method was useful only as a laboratory method for determining the concentration of androstenone but is not suitable for on-line determination of boar taint compounds in abattoir.

ACKNOWLEDGEMENTS

This work was supported by the project E.U. research programme AIR 3-PL94-2482. M. A. Rius thanks to CeRTA (CIRIT) by a grant FIAP/96-21.704.

REFERENCES

Walstra, P. (1974). *Livest. Prod. Sci.*,**1**:187
Bonneau, M. (1982). *Livest. Prod. Sci.*,**9**:687
Diestre, A.; Calsina, D.; Casademont, G and Monfort, J.M. (1982). *28 th Europ. Meet. Meat Res. Workers*, Madrid:464
Patterson, R. L. S. (1968). *J. Sci. Food Agric.***19**:31
Vold, E. (1970). Report no. 238,.Institute of Animal Genetics and Breeding, NLH, Volebekk, Norway.
Hanson, K.E.; Lundstrom, K.; Fjelkner Modig, S.and Pearson, J. (1980). *Swed. J. Agric. Res.*,**10**:167
Claus, R.; Hoffmann, B. and Karg, H. (1971). *J. Anim. Sci.*, **33**:1293
Magard, M. A. and Hans Berg, E. B. (1995). *J. Agric. Food Chem.*,**43**:114
Garcia-Regueiro, J.A. and Diaz, I. (1989). *Meat Sci.*, **25**:307
Kaufmann, G.; Ritter, F. and Schubert, K. (1976). *J. Steroid Biochem.*, 7:593
Brabander, H.F. and Verbeke, R. (1986). *J. Chromatogr.*, **363**:293
García-Regueiro, J.A. and Diaz, I. (1985). *J. High Resolut. Chromatogr.*, **8**:10580
García-Regueiro, J.A. and Rius, M.A. (1998). *J. Chromatogr.*, (in press)
Hansen-Moller, J. (1992). *J. Chromatogr.*, **624**:479
Hansen-Moller, J. (1994). *J. Chromatogr.*,**661**:219
Peleran, J. C.and Bories, G. F. (1985). *J. Chromatogr.*,**324**:469
Porter, M. G.; Hawe, S. M. and Walker, N. (1988). *J. Sci. Food Agric.*,**49**:203
Mortensen, A. B. and Sφrensen, S. E. (1984). *Proc 30th Europ. Meet. Meat Res. Workers*, Bristol:394
Tuomola, M.; Vativa, M. and Kallio, H. (1996). *J. Agric. Food Chem.*, **44**:1265