ANALYSIS OF VOLATILE COMPONENTS FROM THE BACKFAT OF PIG AND RELATIONS WITH ANDROSTENONE CONTENT

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SUMMARY

The aim of this study was to detect possible volatile indicators of the 5α-androst-16-en-3-one (androstenone), a steroid implicated in boar taint. For this purpose, two kinds of measurements were made :

- the volatile components from the backfat of 39 entire male pigs were extracted by the dynamic headspace method and identified by Gas-Liquid Chromatography-Mass Spectrometry,

- fat androstenone levels were measured by a specific immunological method.

The different chemical families identified were : aromatic compounds, aliphatic alkanes, aldehydes, alcohols, ketones and chloride compounds.

An analysis of correlations between androstenone and some volatile compounds showed highly significant relations obtained by stepwise linear regression. These relations indicate that the volatile content analysis may be an interesting way for indirect evaluation of boar taint.

INTRODUCTION

Despite well-known economic advantages, pork production from young entire males is still discouraged in most countries because of the possibility of boar taint. Androstenone has been proved to be the most important compound contributing to this problem at least in french conditions (BONNEAU et al. 1992). Thus, several direct or indirect dosage methods have been developed for this steroid. The direct methods use different techniques - chromatographic (GLC) (CLAUS and HOFFMAN 1971 ; GARCIA-REGUEIRO and DIAZ 1985), radio-immunological (CLAUS 1974) and ELISA (CLAUS et al. 1988), but none of them is suitable for a systematic control on the slaughter line. Among the indirect methods (BONNEAU and RUSSEIL 1985 ; SQUIRES 1990 ; SQUIRES et al. 1991), that proposed by BONNEAU and RUSSEIL, which involves measuring the size of the Cowper glands, is indeed simple and quick, but because of the heterogeneity of the genotypes and the sexual precocity of animals slaughtered in France, this method cannot be used.

The object of this study was to determine whether the dosage of the volatile compounds of the backfat of entire male pigs could lead to an indirect evaluation of the androstenone content in these animals.

MATERIALS AND METHODS

Nature and origin of the samples : 39 backfat samples were taken from entire male pigs slaughtered at 105 kg. The animals were of the Large-White (n=11), French Landrace (n=15) and Pietrain (n=13) breeds, and were selected from a wider population in order to obtain a more normal distribution of their androstenone content. Androstenone dosage was carried out according to the radio-immunological method as described by CLAUS (1974).

Storage conditions : Before analysis, samples were wrapped in an aluminium sheet, vacuum stored in a polyethylene film and frozen at -20°C.

Analysis of volatile components : For each sample, volatile compounds were extracted by the dynamic headspace method from three pieces of adipose tissue freed of rind and meat (6 x 1,5 x 0,5 cm). These three fragments were placed in a glass cylindrical extractor (diameter = 35 mm ; height = 160 mm) through which a helium current with a flow of 120 ml min^{-1} was blown for 45 minutes. The extracted volatile compounds were adsorbed on the TENAX trap of an automatised dynamic headspace apparatus (DCI DELSI). Injection of volatile molecules into a Gas-Chromatograph (DELSI DI-700) coupled to a Flame Ionisation Detector was achieved by thermal desorption of the trap at 250°C. Separation was performed with a DB5 fused silica capillary column (length = 60 m ; internal diameter = 0,32 mm ; film thickness = 1µm). Carrier gas was helium (head column pressure : 1 Bar) and the oven was programmed from 40 to 240°C (slope : 3°C min^{-1}). Identification was made by Gas Chromatography-Mass Spectrometry (GC-MS) according to BERDAGUÉ et al. (1992). Kovats Indices (KI) were calculated (TRANCHANT 1982) and compared with reliable literature data.

Statistical analysis : Relations between androstenone content (expressed in µg per g of adipose tissue) and volatile compounds (expressed in arbitrary units of area) were studied by stepwise linear regression according to the model described by TOMASSONE (1989). The probability level for the introduction of the variables into the model was $p<0,05$. Calculations were made from 33 observations. In order to validate the calculated model, androstenone contents from 6 backfat test samples were predicted by analyzing their volatile compounds.

RESULTS AND DISCUSSION

Nature and origin of identified compounds : Among the 55 molecules studied by GC-MS, 52 were identified and 24 quantified (Table 1). These compounds belong to different chemical families, i.e. aldehydes (9), ketones (10), alkanes and alkenes (10), alcohols (8), aromatic compounds (8), chloride compounds (4), 1 terpene, 1 lactone, 1 furane and 1 pyrazine.

Chemical names	Kovats Indices (DB5)	Reliability of identification	Relative Area	Chemical names	Kovats Indices (DB5)	Reliability of identification	Relative Area
unknown 1	-	-	1.56	2-hexenal	852	b	n.q.
ethanol	-	a	5.69	3-methyl-2-hexanone	854	d	n.q.
2-propanone	-	a	5.14	m-xylene	866	b	2.92
pentane	500	a	n.q.	p-xylene	874	c	4.23
dichloro-methane	528	b	n.q.	2-heptanone	889	b	n.q.
2,3-butanedione	585	b	15.84	ethenyl-benzene	896	c	n.q.
2-butanone	595	c	n.q.	o-xylene	898	b	1.83
hexane	600	a	1.48	heptanal	900	b	3.57
2-methyl-3-buten-2-ol	609	b	n.q.	nonane	900	a	n.q.
trichloro-methane	617	a	n.q.	2-butoxy-ethanol	906	b	n.q.
1,1,1-trichloro-ethane	645	d	n.q.	γ-butyro-lactone + 2,6-dimethyl-pyrazine	912	b	n.q.
3-methyl-butanal	651	b	1.73	unknown 2 (MP=83,55,41)	957	d	1.88
2-methyl-butanal	661	b	3.61	propyl-benzene	962	d	n.q.
1-penten-3-ol	680	b	1.24	1-ethyl-2-methyl-benzene	970	b	n.q.
2-pentanone	685	b	1.29	aromatic compound (MP=105,120)	977	d	n.q.
2,3-pentanedione	692	c	n.q.	phenol	977	c	n.q.
pentanal	696	c	1.53	1-octen-3-ol	979	a	n.q.
heptane	700	a	1.72	2-methyl-3-octanone	981	c	n.q.
3-hydroxy-2-butanone	709	b	n.q.	6-methyl-5-hepten-2-one	986	b	6.80
3-methyl-1-butanol	732	c	n.q.	2-pentyl-furan	994	b	3.15
1-pentanol	765	b	n.q.	octanal	1003	b	2.07
toluene	770	a	4.54	limonene	1039	c	n.q.
1-octene	791	b	1.75	undecane	1100	a	10.21
hexanal	798	a	14.69	nonanal	1105	b	n.q.
octane	800	a	n.q.	dodecane	1200	a	1.53
4-octene	806	b	n.q.	decanal	1208	b	n.q.
tetrachloro-ethene	815	b	n.q.	tridecane	1300	a	

Table 1 : volatile components identified by GC-MS analysis.
The reliability of the identification is indicated by the following symbols : a = mass spectrum and retention time identical to those of authenthic sample ; b = mass spectrum and Kovats indices in agreement with the corresponding literature data ; c = mass spectrum consistant with spectra found in literature ; d = tentative identification by mass spectrum. MP = Major Peaks.

The likely biochemical origins of these compounds are :

- lipid oxidation which accounts for the production of non branched aliphatic components such as alkanes, alkenes, 2-methyl-ketones, aldehydes, alcohols and furanes,

- catabolism of carbohydrates produces ethanol, 2,3-butanedione, 3-hydroxy-2-butanone in raw meat and fermented products but the origin of these compounds in backfat has never been studied,

- catabolism of branched amino-acids such as valine, leucine or isoleucine generate 2 or 3-methyl-butanal (MAC LEOD and MORGAN 1958) and 3-methyl-butanol.

The origin of chloride compounds can be attributed to pesticide residues which have accumulated in the tissue of the animals, but the origin of the aromatic compounds still remains unknown. These two compound families are consistently identified in raw pork.

Relationships between volatile compounds and androstenone content : The androstenone contents of the 39 analyzed samples were between 0,2 and 1,66 µg per g of adipose tissue.

A study of the linear correlations showed up the existence of significant links between these contents and the surfaces of the peaks corresponding to the *ortho*-xylene ($r = 0,44$; $p<0,05$), to unknown 2 (KI = 957) ($r = -0,44$; $p<0,05$) and to the decanal ($r = -0,31$; $p<0,11$). Figure 1 shows that it is also possible, through stepwise regression, to obtain a close relationship ($r = 0,84$; $p<0,001$) between androstenone content and a linear combination of the peak surfaces of *ortho*-xylene, *meta*-xylene, unknown 2 (KI = 957) and 2-propanone. This relationship seems to be relatively independent of the breed concerned for French Landrace and Pietrain animals, but the same cannot be said of Large-White animals, where androstenone content was low and of small variation.

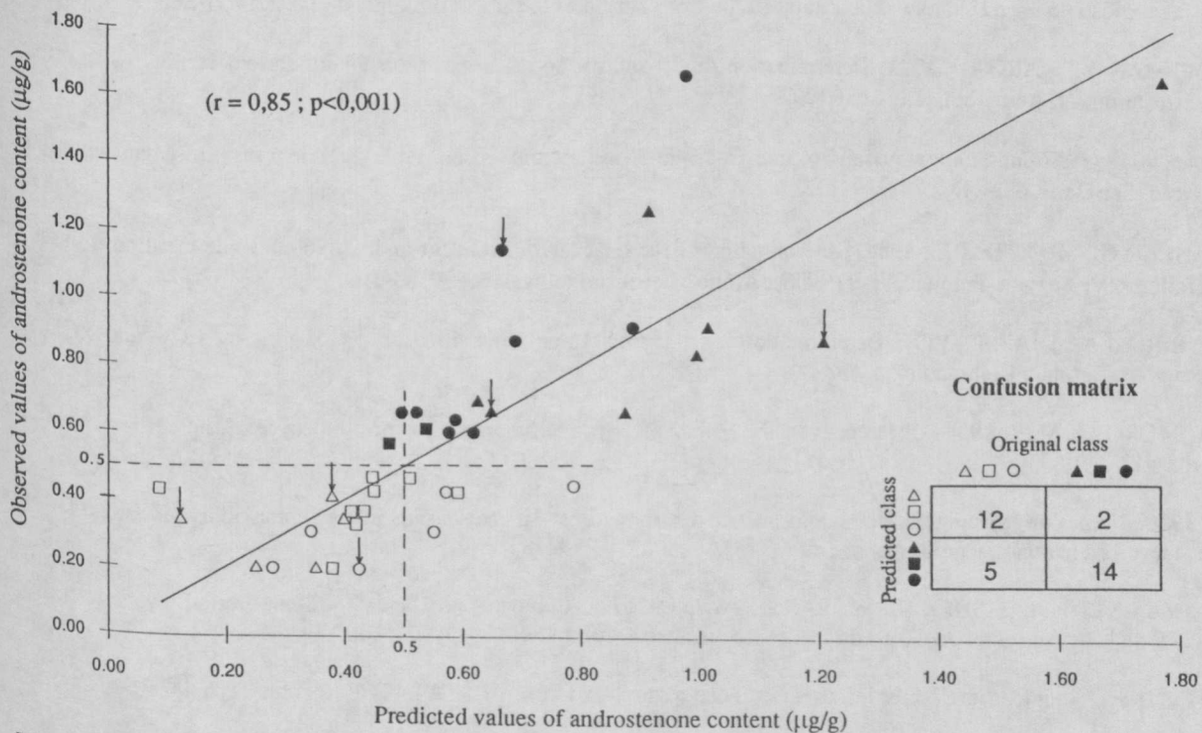

Figure 1 : Relation between observed and predicted values of androstenone content obtained by stepwise regression with 33 samples.

Predicted values = 6.10^{-4} *o*-xylene - $1,7.10^{-4}$ *m*-xylene - $3,5.10^{-4}$ unknown 2 + $0,5.10^{-4}$ 2-propanone + 0,5.
The breed of the pigs and the class of androstenone content (a.c.) are indicated as follow : △ = Pietrain with a.c.≤0,5 µg/g ; ▲ = Pietrain with a.c.>0,5 µg/g ; □ = Large-White with a.c.≤0,5 µg/g ; ■ = Large-white with a.c.>0,5 µg/g ; ○ = French Landrace with a.c.≤0,5 µg/g ; ● = French Landrace with a.c.>0,5 µg/g.
The confusion matrix indicates the number of samples well or wrongly classified by the model according to their androstenone content : △, □, ○ = a.c≤ 0,5 µg/g and ▲, ■, ● = a.c.>0,5 µg/g.
The arrows show the 6 samples used to test the model.

If the threshold for the perception of sexual odours is fixed at 0,5 µg of androstenone per g of adipose tissue (BONNEAU 1992, personnal communication) to define two groups of animals according to their androstenone content (figure 1), it would appear that 78% (i.e. : (12+14)x100/33) of the samples are correctly classified into their original class after analysis of volatile compounds.

Moreover, an indirect estimate of the androstenone content of the 6 test samples of adipose tissue from the linear combination of the 4 volatile compounds shows that it is possible to distinguish contents lower or higher than 0,5 µg/g of adipose tissue, which apparently confirms the ability of the calculated relation to make predictions.

CONCLUSION

Analyses carried out show that there is a wide variety of volatile compounds in the backfat of entire male pigs. A study of the correlations between these components and androstenone content showed there were highly significant relations. These relations - as yet unexplained from a biochemical point of view - indicate that some volatile compounds make an indirect evaluation of androstenone content possible. However, the analytical techniques used are lengthy and delicate and cannot yet be applied for quality control operations on the slaughter line.

REFERENCES

BERDAGUÉ J.L., MONTEIL P., TALON R., MONTEL C., 1992. Effect of starter cultures on the formation of flavour compounds in dry sausage. Meat Sci., submitted for publication.

BONNEAU M., RUSSEIL P., 1985. The size of Cowper's (bulbo-urethral) glands as an estimate of boar taint on the slaughter line. Livest. Prod. Sci., 13, 169-178.

BONNEAU M., LE DENMAT M., VAUDELET J.C., VELOSO-NUNES J.R., MORTENSEN A.B., MORTENSEN H.P., 1992. Contributions of fat androstenone and skatole to boar taint : I. Sensory attributes of fat and pork meat. Livest. Prod. Sci. (in press).

CLAUS R., HOFFMAN B., KARG H., 1971. Determination of 5α-androst-16-en-3-one, a boar taint steroid in pigs, with reference to relationships to testosterone. J. Anim. Sci., 33, 1293-1297.

CLAUS R., 1974. Dosage radio-immunologique du 5α-androst-16-en-3-one, stéroïde responsable de l'odeur de verrat, dans le tissu adipeux des porcs. C.R. Acad. Sci. Paris, Série D, 278, 299-302.

CLAUS R., MAHLER G., MÜNSTER E., 1988. Determination of the boar taint steroid 5α-androst-16-en-3-one in adipose tissue of pigs with a rapid microtitre plate enzyme-immunoassay (MTE). Archiv. Lebensmittelhygiene, 39, 85-108.

GARCIA-REGUEIRO J.A., DIAZ I., 1985. Determination of 5-androst-16-en-3-one in back fat of pigs by CGC and ECD. J. High Resolution Chromat. & Chromat. Communic., 8, 698-699.

MAC LEOD P., MORGAN M.E., 1958. Differences in the ability of *Lactic streptococci* to form aldehydes from certain amino acids. J. Dairy Sci., 41, 908-913.

SQUIRES E.J., 1990. Studies on the suitability of a colorimetric test for androst-16-ene steroids in the submaxillary gland and fat of pigs as a simple chemical test for boar taint. Can. J. Anim. Sci., 70, 1029.

SQUIRES E.J., GULLETT E.A., FISHER K.R.S., PARTLOW G.D., 1991. Comparison of androst-16-ene steroid levels determined by a colorimetric assay with boar taint estimated by a trained sensory panel. J. Anim. Sci., 69, 1092-1100.

TOMASSONE R., 1989. Comment interpréter les résultats d'une régression linéaire ? STAT-ITCF, Institut Technique des Céréales et des Fourrages.

TRANCHANT J., 1982. Manuel pratique de chromatographie en phase gazeuse. MASSON ed., 504p.