

The androstenone-skatole dilemma as applied in a consumer test.

WALSTRA, P., ENGEL, B.* and MATEMAN, G.

Research Institute for Animal Production "Schoonoord", Zeist, The Netherlands

*TNO Institute of Applied Computer Science, Delft, The Netherlands.

Introduction

Without any doubt the fattening of young entire male pigs (boars) instead of castrates should be promoted from an economical point of view (Walstra, 1974) as well as with regard to animal welfare. Experiments in many countries have shown that boars have a more efficient feed conversion and have leaner carcasses. The production of meat from boars, however, is limited in most countries, because of the presence in the fatty tissue of the annoying so-called boar taint in a (small) part of the boars.

The compound 5 α -androst-16-ene-3-one (=androstenone) was found to be responsible for the taint (Patterson, 1968). Two years later Walstra and Maarse (1970) and Vold (1970) found another compound in the fatty tissue of boars which also has an annoying odour: 3-methylindole (=skatole).

Much research effort was put on research on androstenone, because the functioning of this compound as a pheromone and appearance later on in the fatty tissue was readily found and this made it understandable that it could be held responsible for the occurrence of boar taint. Little was known about skatole. In a Swedish experiment (Hansson et al., 1980) was found that androstenone was the most important compound and that skatole contributed an about extra 7% to the variance in boar taint. In a combined Swedish/Danish experiment, however, just the opposite was found (Lundström et al., 1984). In two further Dutch/Danish experiments (to be published) skatole was found to be the most important compound; in one case androstenone contributed less than 1% extra and in another androstenone gave an extra contribution of about 7% to the variance in boar taint. So from these four studies it must be concluded that skatole seems to be more important than androstenone.

A consumer test was planned with three different androstenone levels in meat from boars. Because of the more recent knowledge mentioned above, indicating that skatole cannot be neglected, we abandoned the original plan and changed it into two different levels for each of the compounds androstenone and skatole. The lower levels were chosen below given threshold values, where no complaints were expected. Furthermore a possible synergistic effect was examined.

Material and methods

About 380 boars were screened for their concentrations of androstenone and skatole in backfat samples. Androstenone was measured by means of the ELISA-method in The Netherlands as described by Storm (1984) and skatole in Denmark as described by Mortensen and Sørensen (1984).

From these boars four groups of 15 boars each were selected with combinations of low and high concentrations: low androstenone and low skatole (LL), low androstenone and high skatole (LH), high androstenone and low skatole (HL) and high androstenone and high skatole (HH). The low and high concentrations were either below or above the threshold levels which were set earlier at 1.0 $\mu\text{g/g}$ fat for androstenone in The Netherlands (Punter and Van Gemert, 1984) and at 0.2 $\mu\text{g/g}$ fat for skatole in Denmark (Lundström et al., 1985). Belly cuts of each boar group

were compared with those of a gilt group as a control. The average concentrations are listed in Table 1.

Table 1. Means (and range) of concentrations of androstenone and skatole of the various groups (in $\mu\text{g/g}$ fat).

	Gilts	LL	LH	HL	HH
N	15	15	15	15	15
Androstenone	-	0.76 (0.69 - 0.86)	0.74 (0.48 - 0.96)	1.28 (1.02 - 1.57)	1.31 (1.06 - 1.58)
Skatole	0.08 (0.05 - 0.16)	0.15 (0.13 - 0.18)	0.33 (0.29 - 0.39)	0.15 (0.10 - 0.20)	0.34 (0.28 - 0.45)

The consumer panel comprised 395 families consisting of 1055 individuals. The panel was accustomed to test other agricultural products in their home situation, but no meat or meat products were tested before. Beside the more general questions concerning storage time, the way of frying etc., the most interesting questions were those asking for judgement of the quality of the belly cuts.

Those family members responsible for cooking were asked to judge the general appearance before cooking as good, no remark or bad and to rate the odour during preparation as pleasant, no remark or unpleasant. All family members (so including the cooks) were asked to judge tenderness, odour and taste as good, reasonable or bad. Statistical analyses were carried out on the observations obtained during the preparation of the slices (the cook's response) and on the observations obtained after the meat was served (the family response).

For the cook's response per animal the fraction f of good or no remark (or pleasant or no remark) was calculated. An overall test was carried out on the rank numbers of those fractions with the Kruskal-Wallis test, comparing the various groups simultaneously. For the family response first the average scores per family was obtained (1 = good, 2 = reasonable, 3 = bad). For each animal the average of the values s was determined. On the basis of these averages per animal the groups were compared with the Kruskal-Wallis test as well.

Pairwise comparisons for the cook's response based on the fraction f were made using a normal approximation (Van Ryzin, 1975). On the assumption that the extra variation due to the fact that repeated observations are made on the same animal is negligible, the fraction f may be analysed on the basis of the binomial distribution. This approach leads to similar qualitative conclusions. Pairwise comparisons for the family response between groups were made with Wilcoxon's rank-sum test.

Results

In Tables 2 and 3 the percentages for the judgements of the various quality characteristics are given. As expected the most severe objections are those made by the cooks for odour during preparation of the belly slices.

Table 2. The judgement for odour given by the cook and all family members (in percentages).

	cooks			all family members		
	pleasant	no remark	un-pleasant	good	reason-able	bad
Gilts	49.4	48.1	2.5	66.2	32.4	1.5
LL	30.8	55.1	14.1	63.9	27.4	8.7
LH	40.0	41.3	18.7	54.8	34.7	10.6
HL	43.8	42.5	13.8	65.2	25.6	9.3
HH	29.9	42.9	27.3	47.9	35.0	17.1

Table 3. The judgement for tenderness and taste given by all family members (in percentages).

	tenderness			taste		
	good	reason-able	bad	good	reason-able	bad
Gilts	66.5	29.1	4.4	70.6	27.5	2.0
LL	67.8	30.3	1.9	67.8	29.3	2.9
LH	62.3	33.7	4.0	66.2	27.3	6.6
HL	67.5	29.8	2.6	74.1	22.4	3.5
HH	63.6	32.3	4.1	57.1	35.0	7.8

During eating the percentages for a bad odour were more than one-third lower than during cooking. The overall test results are shown in Table 4.

Table 4. The P-values from the overall tests for the different quality criteria.

	cooks		all family members		
	general appearance	odour	tenderness	odour	taste
Incl. gilts (n = 75)	0.89	<0.001	0.98	0.001	0.37
Excl. gilts (n = 60)	0.90	0.06	0.95	0.01	0.55

Table 4 shows that there is no significant effect for general appearance and tenderness. But also for taste there is no significant effect, whereas for odour the effect is very clear. The table also shows that an important difference exists between the gilts and the other groups. In Table 5 the results for the pairwise comparisons between the groups are listed for odour.

Table 5. Pairwise comparisons between groups for odour score during preparation and eating.

	cooks				all family members			
	♀	LL	LH	HL	♀	LL	LH	HL
LL	*				NS			
LH	*	NS			*			
HL	*	NS	NS		NS	NS	NS	
HH	*	*	NS	*	*	*	NS	*

NS = not significant; * = significant at the 5 % level ($P < 0.05$).

During preparation the percentage unpleasant odour in gilts is significantly below the four boar groups, while further significant differences were found between the HH and the LL and HL groups. The latter means that a demonstrable skatole effect was found at high androstenone concentrations, but not at low androstenone concentrations, and that no androstenone effect could be demonstrated. During eating a significant skatole effect was found at both androstenone levels. Again no significant effect of androstenone was found. Here the gilts did not differ significantly from the LL and HL groups, i.e. the groups with the low skatole concentrations.

Discussion and conclusions

The results given in Tables 2 and 3 show that dependent on the concentration of androstenone and/or skatole consumers will react on boar taint. In a small number of cases an off-flavour will also be found in gilts. The percentages found here are in the same order as in an earlier consumer test (Walstra and Maarse, 1970) as well as in other countries (Malmfors and Lundström, 1983). But also the percentages unpleasant for the HH-group are in the same order as in our earlier consumer test.

Looking at the percentages unpleasant or bad of the HH-group, one would suggest a synergistic effect of both compounds. It, however, is not supported by the statistics, because the HH-group significantly differs from the HL-group indeed, but not from the LH-group.

A very awkward finding in this consumer test is that the percentage 'unpleasant odour' during preparation significantly differs between gilts and all four boar groups, so also from the LL-group. Based on this consumer test and for the given threshold values one therefore has to conclude that analytical assessment of androstenone or skatol is not yet fully reliable as an objective test method to exclude tainted boar meat from the market. Of course we probably have introduced the maximum differences with the choice of gilts in stead of castrates as controls on the one hand and consumption of belly cuts with high percentages of fat on the other. Nevertheless this product has to be sold as well and about 14 % in the LL-group judged as unpleasant remains a high percentage.

The percentages unpleasant or bad suggest, though the differences between the LH- and HL-groups were not significant, that skatole seems to be more important than androstenone for boar taint as was also referred to in the introduction, there based on multiple correlation coefficients and/or discriminant analyses. The small diffe-

rences in the percentages unpleasant or bad between the LL- and HL-groups might indicate that the average concentration of androstenone (ca. 0.75 µg/g fat) in the LL-group, is already too high and therefore evokes a similar reaction in consumers as an average higher concentration. It would mean that the threshold value found was wrong. Lowering, however, the threshold value for androstenone to e.g. 0.5 µg/g fat implicates a too high proportion of rejected boars for direct consumption as based on the frequency distribution for androstenone in The Netherlands. One could consider to select against androstenone, so that in the whole pig population the incidence decreases and then the threshold value might be lowered. Therefore production of boar meat on a large scale in countries with the higher carcass weights seems to be restricted for the near future.

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Details and indole were present in F1. 5 α -An was recovered in F1 (100%) and F4 (25%). F1 and F3 were concentrated to dryness and redissolved in 0.5 ml methanol for HPL analysis or in 0.5 ml of toluene for MS 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: 2.4-2 µg/g) and skatole/indole (range: 0.25-1.0 µg/g).

HPLC analysis of skatole and indole. Column: Nucleosil RP-18 5 µm (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: 220 and 280 nm. The apparatus was a LKB HPLC.

HPLC analysis of 5 α -An. A 100 µl (except 150-1 µg) 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 60°C and at 60°C during 30 min. The solution is microfiltered for HPLC analysis and then concentrated to 0.1-0.5 ml of methanol. HPLC conditions were: column Nucleosil RP-18 5 µm (10 cm x 4 mm) operated at ambient temperature. Mobile phase: acetonitrile/water (50/50) at 1.5 ml/min. Wavelengths used: 250 and 300 nm. 10 µl of standard and sample solutions were injected via a Rheodyne injector 1115.

MS analysis of 5 α -An. Columns: F30T RP-1 (10 x 0.25 mm, 5 µm, Analytical) and F30T RP-18 (15 x 0.25 mm, 5 µm, Analytical). Detectors: FID and ECD (10 ml/min, 10 ml/min). A PIV (Programmable Temperature Injector) injector was employed. Cold and hot splitless modes were used. Carrier gas was helium at 30 ml/min (F30T) and 25 ml/min (F30T-18). Make-up gas for ECD detection was nitrogen at 50 ml/min. For a selective detection with ECD were obtained with derivatives of 5 α -An by the reaction with DTBA (Diphenylfluorenylmethylsilylating) (15). The apparatus used was a DANI gas chromatograph 1800 RP-PIV.

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 proxi-vitamine at -20°C (9). 5 α -An recovery in this step was 85%.

Final clean-up allows the elimination of the interference compounds present in the extract and provides a definitive purification without a excessive long time-consuming procedure. In F1 skatole and indole were separated and in F3 were collected several compounds that can interfere with skatole and indole.

Fig. 1 show chromatogram obtained in the HPL analysis of F1 and F3. It is possible to analyze F1 and F3 together because they have not present interference peaks in the retained time zone of 5 α -An, skatole and indole in the corresponding chromatograms.

