Role of 4-Phenyl-3-buten-2-one in Boar Taint: Identification of New Compounds Related to Sensorial Descriptors in Pig Fat

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The possible contribution of other compounds to the development of boar taint in fat samples with low concentrations of skatole and androstenone and classified as tainted was evaluated by GC-MS in the SCAN mode. Skatole and androstenone were determined by normal phase HPLC and GC-MS, respectively. For the identification of other compounds fat samples were purified with a gel filtration column and the second fraction was saponified at room temperature with KOH/3 N MeOH. 4-Phenyl-3-buten-2-one was the main compound identified in the fat samples, and its identification was corroborated by comparison with a standard solution obtained from a commercial source and by HPLC. The sensorial analysis of 4-phenyl-3-buten-2-one showed its possible contribution to boar taint. The possible contribution of phenol derivatives and short-chain fatty acids was also evaluated.

Keywords: Boar taint; skatole; androstenone; 4-phenyl-3-buten-2-one

INTRODUCTION

The production of uncastrated male pigs shows some advantages in terms of efficiency because the carcasses are leaner, the production costs are reduced, and the animals grow faster (1-3). However, castration of young male pigs is practiced in some European countries and in North America in order to avoid the occurrence of boar taint, an unpleasant odor present in the meat of 5-10% of the uncastrated male pigs (4, 5). Two main compounds have been related to boar taint: androstenone (5α -androst-16-en-3-one), a testicular pheromone isolated from boar fat samples exhibiting a urine-like odor (6), and skatole, an intestinal degradation product of tryptophan exhibiting a fecal-like odor (7-9). The structures of skatole and androstenone are shown in Figure 1.

An international study involving seven European countries was done recently to determine the respective contributions of androstenone and skatole to boar taint and their possible variations according to production systems and consumer populations (10, 11). The results obtained showed that the contribution of both compounds to boar taint could be influenced by such factors as the concentration of androstenone and skatole, the different methodologies used for the sensory evaluation (12), the different consumption habits (13), and the different human perceptions of androstenone (14).

In previous studies it was concluded that skatole could not explain the total off-odor of boar taint. The correlation coefficient between skatole and boar taint score determined by sensory evaluation is of the order of 0.7, explaining only 50% of the total score (15). If androstenone content was included, ~66% of the variation in odor score could be explained. The magnitude of these coefficients does not exclude the contribution of other unidentified compounds (10, 16) to boar taint. Patterson (17) described the possible contribution of

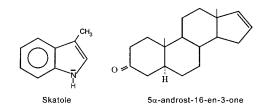


Figure 1. Structures of skatole and 5α -androst-16-en-3-one.

phenolic compounds (mainly *p*-cresol and 4-ethylphenol) to boar odor, and the presence of 1,4-dichlorobenzene was associated with the development of a taint in pig fat samples (*18*). Moreover, the oxidation of lipids leads to the formation of short-chain aldehydes and fatty acids that could be responsible for the presence of certain offflavors in the meat samples mainly related to rancid, pungent, sour, fatty, wax, and almond attributes (*16*).

The aim of this study was to evaluate the possible contribution of other compounds to the development of boar taint in pig backfat samples classified as tainted according to the evaluation of a trained panel test but with low concentrations of skatole and androstenone. Samples with low concentrations of both compounds and classified as control by the panel test were also analyzed in order to compare the results. In addition, the concentrations of indole, 5α -androst-16-en- 3α -ol, and 5α -androst-16-en- 3β -ol were also determined.

MATERIALS AND METHODS

Chemicals. Indole, skatole (3-methylindole), 5α -androst-16-en-3 α -ol, and 5α -androst-16-en- 3β -ol were obtained from Sigma (St. Louis, MO). The internal standards for indolic compounds (7-ethylindole) and for androstenone and androstenols (5α -androstan- 3α -ol) were purchased from Fluka (Buchs, Switzerland) and Research Plus (Bayonne, NJ), respectively. 4-Phenyl-3-buten-2-one was obtained from Aldrich. Hexane, 2-propanol, and methanol were of HPLC grade from Merck (Darmstadt, Germany), and water was of Milli-Q grade. All other chemicals were of analytical reagent grade from Merck.

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Octadecyl columns for the solid phase extraction were from Baker Bond (Phillipsburg, NJ) (500 mg, 6 mL, 40 μm APD, 60 Å), and the gel filtration column (680 mm \times 25 mm) was obtained from Jordi Associated.

Selection of Fat Samples. A total of 4536 animals were raised in six European countries (England, France, Denmark, Holland, Sweden, and Spain) in order to take into account possible differences among countries according to the production systems and to represent different concentration ratios of skatole and androstenone (*10*). Samples of subcutaneous fat (50 g) were taken from the neck area and kept at -20 °C until chemical analysis.

Classification of Selected Samples. A total of 378 uncastrated males were classified in a 3×3 table representing three categories of skatole and androstenone concentrations (low, medium, and high) (*10, 19*). Rapid measurements for skatole were done with the colorimetric assay developed by Mortensen and Sørensen (*20*) at The Danish Meat Research Institute.

Androstenone determinations were based on the ELISA method (*21*) and were carried out at the Institute for Animal Science and Health (The Netherlands). The cutoff levels for each compound were established according to previous studies and were $\leq 0.10 \ \mu g/g$ (low), $0.11-0.22 \ \mu g/g$ (medium), and $\geq 0.22 \ \mu g/g$ (high) for skatole and $\leq 0.50 \ \mu g/g$ (low), $0.51-1.0 \ \mu g/g$ (medium), and $\geq 1.0 \ \mu g/g$ (high) for androstenone (*19*).

Determination of Skatole, Indole, Androstenone, and Androstenols Content by Laboratory Methods. A total of 20 fat samples presenting boar taint and classified previously with low concentrations of skatole and androstenone were analyzed by laboratory methods. Skatole and indole were determined by normal phase HPLC (22). Indolic compounds were extracted at room temperature with 10 mL of hexane/ 2-propanol (92:8) and separated on a Hypersil aminopropylsilica column (Teknokroma) (5 μ m) (250 mm \times 4.6 mm i.d.). The mobile phase was hexane/2-propanol (92:8), and detection was performed by fluorescence (excitation at 280 nm and emission at 360 nm). Androstenone and androstenols were extracted with 2 \times 20 mL of dichloromethane at room temperature. Five milliliters was taken, evaporated to dryness, and dissolved in 2 mL of methanol to allow fat precipitation. The sample was applied to an octadecyl column prewashed with methanol (23). The fraction eluted was recovered, and the column was washed with 2 mL of methanol. The fractions collected were analyzed by GC-MS. The compounds were separated on an HP-5MS column (30 m \times 0.25 mm i.d., 0.25 μ m film), and the temperature program was as follows: 70 °C held for 1 min, raised at 10 °C/min to 190 °C, raised at 5 °C/ min to 270 °C, and then held for 5 min. Detection was performed in the SIM mode, and the ions selected were m/z272 and 257 for androstenone and *m*/*z* 241 and 274 for both androstenols.

Identification of Other Compounds by GC-MS. Extraction and Cleanup Procedure. A total of 20 fat samples with low concentrations of skatole and androstenone and classified as tainted were analyzed by gel filtration chromatography to evaluate the contribution of other compounds to boar taint (16). Twenty fat samples classified as untainted were analyzed according to the same procedure in order to compare the results obtained. Five grams of back fat was extracted with 200 mL of chloroform/methanol (2:1) at room temperature. An aliquot of 1 g dissolved in dichloromethane was applied to a gel filtration column (680 mm imes 25 mm i.d., Jordi Associated) coated with polyvinylbenzene and with an exclusion limit of 1000 Da. Dichloromethane was used as mobile phase at a flow rate of 5 mL/min, and three fractions were collected corresponding to 0-20, 20-45, and 45-60 min. The second fraction was evaporated to dryness, and an aliquot was saponified at room temperature with 3 N KOH/MeOH. The nonsaponifiable fraction was extracted with 3×2 mL of dichloromethane, redissolved in 20 μ L of iso-octane, and analyzed by GC-MS. The same fraction was analyzed by reversed-phase HPLC without any saponification procedure applied.

Chromatographic Conditions. The chromatographic analysis was done with an HP-5890 GC coupled to an HP-5970 selective

Table 1. Concentrations (Micrograms per Gram) ofAndrostenone, Skatole, and 4-Phenyl-3-buten-2-oneAdded to Fat Samples

androstenone	skatole	4-phenyl-3-buten-2-one
		0.25
		1.00
0.50		0.25
0.50		1.00
1.00		0.25
1.00		1.00
	0.10	0.25
	0.10	1.00
	0.25	0.25
	0.25	1.00

mass detector. The column used was an HP-5MS ($25 \text{ m} \times 0.25 \text{ mm}$ i.d., $0.25 \mu \text{m}$ film), and the temperature program was as follows: 70 °C held for 1 min, raised at 10 °C/min to 200 °C, raised at 7 °C/min to 270 °C, and then held for 10 min. The temperatures of the injector and detector were set at 270 and 280 °C, respectively. The injection volume was 2 μ L, and detection was performed in the SCAN mode (40–400 Da/e). The mass spectra were obtained by electronic impact at 70 eV.

HPLC analysis was done with a Waters Multisolvent Delivery System (Waters 600) coupled to a diode array detector (Waters 990) (190–600 nm). The column used was an RP-18 (Teknokroma) (5 μ m) (150 \times 4 mm i.d.) operated at room temperature, and the mobile phase consisted of methanol/ water (80:20, v/v) at a flow rate of 1.5 mL/min.

Identification of Compounds. Identification of compounds was performed by gas chromatography-mass spectrometry (GC-MS). Compounds were tentatively identified by comparing their mass spectra with those contained in the National Bureau of Standards (NBS) library, and only the compounds that showed a quality match >80% were selected. Identification of some of these compounds was confirmed by matching their spectral data with those of authentic reference compounds analyzed under the same chromatographic conditions. Kovats indices (KI) were calculated (24) and compared with those reported in the literature (25). Quantification of the identified compounds was performed using 5α -androstan-3-one as internal standard. The results are expressed as nanograms per gram of fat sample.

Confirmatory Analysis (Analysis of 4-Phenyl-3-buten-2-one in Fat Samples). The fraction eluted from the gel filtration column between 20 and 45 min was also analyzed by reversed phase HPLC without the saponification procedure described previously. The aim was to corroborate the presence of the compound identified as 4-phenyl-3-buten-2-one by GC-MS in the fat samples analyzed.

Sensory Evaluation. The sensorial attributes of 4-phenyl-3-buten-2-one were evaluated by a total of seven panelists selected according to their ability to detect skatole and androstenone. Fat samples that did not exhibit boar taint were used as blank samples and were fortified with different concentrations of androstenone, skatole, and 4-phenyl-3-buten-2-one as shown in Table 1. The upper and lower concentrations for skatole and androstenone were chosen according to the cutoff levels for these compounds, whereas the upper and lower concentrations for 4-phenyl-3-buten-2-one were chosen arbitrarily (16). About 5 g of fortified fat sample was placed in a 250 mL Pyrex bottle. Bottles were closed hermetically and placed in a water bath set at 60 °C for 1 h. Fat samples were cooled and then were presented to the members of the test panel. The selected individuals were asked for a number of attributes associated with the presence of boar taint in fat samples: pig, urine, sweat, manure, naphthalene, rancid, and abnormal odor (10, 12). The attributes were rated on a hedonic scale with five categories from "like very much" to "dislike very much".

Statistical Analysis. The effect of the presence of boar taint in the fat samples analyzed on the concentration of the identified compounds was evaluated by analysis of variance

using the GLM procedure (SAS) (*26*). Principal component analysis (PCA) was performed using the results from the identified compounds as variables.

RESULTS AND DISCUSSION

Concentration of Boar Taint Compounds in the Fat Samples Evaluated. The mean concentrations of skatole and indole in the fat samples determined by normal phase HPLC were 0.05 and 0.04 μ g/g, respectively (16). The highest concentrations for skatole and indole were 0.07 and 0.08 μ g/g, respectively, and the lowest concentration for both compounds was 0.01 μ g/ g. The mean concentration of androstenone determined by GC-MS was 0.18 μ g/g, and the highest and lowest concentrations of this compound were 0.34 and 0.07 μ g/ g, respectively (16). The mean concentration of both androstenols was $<0.05 \,\mu$ g/g. The results obtained confirm that androstenone and skatole could not be responsible for the development of boar taint in the samples selected because their concentrations were lower than the cutoff levels established for these compounds (19).

Compounds Identified in the Analysis by GC-MS. Gel filtration chromatography was applied to separate triglycerides from compounds of lower molecular weight, such as skatole and androstenone. Nevertheless, GC-MS analysis of the fraction collected from the gel filtration column was not possible due to the high amount of free fatty acids that made the interpretation of the chromatogram too difficult. It was therefore necessary to apply a cold saponification to perform GC-MS analysis (*16*).

Although >75 peaks were detected, only a part of them were studied because in some cases the ion pattern was too difficult to resolve with confidence and the profiles were very similar between boar taint and control samples. The compounds identified and their reliability of identification are shown in Table 2. Aldehydes were the main group of compounds identified, representing the major proportion of the total chromatographic area, although the presence of hydrocarbons and long-chain fatty acids as their methyl and ethyl esters derivatives was also important. These compounds mainly come from the oxidative degradation of unsaturated fatty acids (oleic, linoleic, and linolenic) and contribute to the development of the aroma of a food product due to their low olfactory thresholds and their high percentage of formation during the oxidative process (27). Phthalates were also identified, and these were derived from plastics, possibly from the material used for packaging the fat samples (28).

Some of the peaks detected in the chromatographic profile showed a greater abundance in the fat samples classified as tainted but with low concentrations of skatole and androstenone. One of these peaks had a retention time near 9 min and was identified as 4-phenyl-3-buten-2-one by comparing its mass spectrum with those in the NBS library (Figure 2A). The EI mass spectrum obtained on this compound showed a molecular ion of 146, corresponding to the molecular weight of 4-phenyl-3-buten-2-one. The chromatographic analysis of a standard solution of 4-phenyl-3-buten-2-one allowed comparison of the retention time of this compound and the spectral data to confirm the identification. The minor abundance of 4-phenyl-3-buten-2-one in the control samples analyzed is shown in Figure 2B. The source of 4-phenyl-3-buten-2-one is not clear, but its chemical structure seems to be related with cinnamic

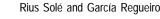
Table 2. Compounds Identified in Pig Back Fat SamplesClassified with Low Concentrations of Androstenone andSkatole (Analysis by GC-MS)

family	compound	reliability (%)
aldehydes	nonanal	86
	decanal	81
	2-decenal	86
	2,4-decadienal	91
	hexadecanal	93
	octadecanal	84
acids (methyl esters)	aminocaproic	88
	decanoic	94
	dodecanoic	96
	tetradecanoic	98
	hexadecanoic	99
	9-hexadecenoic	99
	octadecanoic	99
	8,11-octadecadienoic	99
	11-octadecenoic	99
acids (ethyl esters)	tetradecanoic	95
	hexadecanoic	95
	octadecanoic	90
	linoleic	97
alcohols	1-pentadecanetiol	81
	1-nonadecanol	91
	1-eicosanol	95
hydrocarbons	docosane	99
	tetradecane	95
	1-tetradecene	98
	pentadecane	97
	1-hexadecene	98
	heptadecane	97
	octadecane	98
	3-octadecene	98
	2,6-dimethyloctadecane	90
	eicosane	97
	3-eicosene	99
	cyclododecane	95
	cyclotetradecane	96
	cyclohexadecane	98
others	4-phenyl-3-buten-2-one ^a	95
	ethanone derived ^b	84
	ethanol derived ^c	80
	phenol derived d	97
	indole	93
	skatole	81
	androstenone	88
	squalene	87
	cholesterol	99
	phthalate	90
	diethyl phthalate	97

^{*a*} Compound identified by comparison of its spectrum with the spectrum of a standard solution. ^{*b*} Compound identified tentatively as 2-(2-methylpropoxy)-1,2-diphenylethanone by comparison of its spectrum with the spectrum in the NBS library. ^{*c*} Compound identified tentatively as 2-(2-butoxy)ethanol by comparison of its spectrum with the spectrum in the NBS library. ^{*d*} Compound identified tentatively as 2,4-bis(1,1-dimethylethyl)phenol by comparison of its spectrum with the spectrum in the NBS library.

acid metabolism and with phenylalanine degradation. However, specific experiments should be carried out to establish the origin of this compound.

Another peak eluted at a retention time of 17.60 min showed an EI mass spectrum with a base peak of 202 and a major abundance of the fragments at m/z 147, 163, and 105. This compound was first identified as 2-(2methylpropoxy)-1,2-diphenylethanone by comparing the mass spectrum with those in the NBS library. However, the mass spectrum of this compound differed from the mass spectrum obtained from a standard solution of 2-(2-methylpropoxy)-1,2-diphenylethanone, mainly due



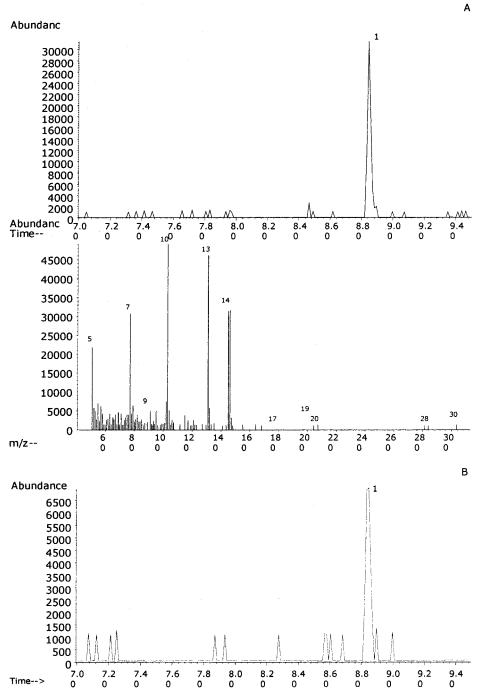


Figure 2. (A) GC-MS chromatogram and EI mass spectrum of the compound identified as 4-phenyl-3-buten-2-one in the fat samples classified as tainted and with low concentrations of skatole and androstenone. (B) GC-MS chromatogram of the compound identified as 4-phenyl-3-buten-2-one in the fat samples classified as control and with low concentrations of skatole and androstenone

to the presence of fragment ions at m/z 201 and 202 and the relative abundance of the ions at m/z 105 and 79. The differences observed did not permit identification. The structures of 4-phenyl-3-buten-2-one and 2-(2methylpropoxy)-1,2-diphenylethanone are shown in Figure 3.

The identification of other compounds was done by comparing their mass spectra with the NBS library data. The phenol derived [2,4-bis(1,1-dimethylethyl)phenol] showed a retention time near 11 min and an EI mass spectrum with a base peak of 206 and a major abundance of the fragments at m/z 191 and 57. Shortchain aliphatic acids play a significant role in the development of undesirable aromas in many foodstuffs

(buttery, rancid, musty, and soapy). However, because of the higher flavor thresholds of fatty acids, they may not always be present in sufficient quantity to contribute to off-flavors arising from oxidative deterioration of unsaturated lipids (27, 29-31). The development of the characteristic sheepmeat odor has been related to the presence of 4-methyloctanoic acid in subcutaneous fat (32), and a panel test associated the presence of 4methylnonanoic acid, together with skatole, to the animal odor detected in pig meat (33). In the same way, 3-methylbutanoic acid derived from intestinal fermentation could be responsible for the development of offflavors in pig meat due to its attributes described as sweaty, manure-like, and putrid (34).

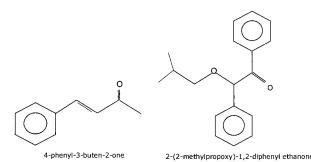


Figure 3. Structures of 4-phenyl-3-buten-2-one and 2-(2-methylpropoxy)-1,2-diphenylethanone.

Table 3. Influence of the Presence of Boar Taint on theConcentration of the Compounds Identified in FatSamples by GC-MS Expressed as Nanograms per Gram(Mean and Standard Deviation of Mean)

	BT ^a	C ^{<i>b</i>}	SD	significance ^c
phenol derived ^d	1043.66 ^a	226.45 ^b	156.99	**
ethanol derived ^e	159.76	374.18	78.10	NS
ethanone derived f	1352.45 ^a	125.54 ^b	282.65	*
4-phenyl-3-buten-2-one ^g	194.24 ^a	41.40 ^b	25.56	*
aminocaproic ^h	328.85 ^a	42.63 ^b	80.96	*

^{*a*} BT, fat samples classified with boar taint. ^{*b*} C, fat samples classified without boar taint. ^{*c*} Level of significance: ** (P < 0.01); * (P < 0.05); NS ($P \ge 0.05$). ^{*d*} Compound identified tentatively as 2,4-bis(1,1-dimethylethyl)phenol by comparison of its spectrum with the spectrum in the NBS library. ^{*c*} Compound identified tentatively as 2-(2-butoxy)ethanol by comparison of its spectrum with the spectrum in the NBS library. ^{*f*} Compound identified tentatively as 2-(2-methylpropoxy)-1,2-diphenylethanone by comparison of its spectrum with the spectrum with the spectrum in the NBS library. ^{*f*} Compound identified tentatively as 2-(2-methylpropoxy)-1,2-diphenylethanone by comparison of its spectrum with the spectrum of a standard solution. ^{*h*} Compound identified by comparison of its spectrum with the spectrum of a standard solution. ^{*h*} Compound identified by comparison of its spectrum with the spectrum with the spectrum with the spectrum of a standard solution. ^{*h*} Compound identified by comparison of its spectrum with the spectrum in the NBS library.

Phenols have been identified as the source of carbolictype taints in foods due to their volatility, high concentration, and capacity to remain absorbed into the food (*35*). 4-Methylphenol (*p*-cresol) was one of the main compounds that have been studied extensively and was previously related to boar taint (*17*). It has a taste threshold of 2 μ g/L and an odor threshold of 200 μ g/L, both measured in water (*35*). The attributes associated with *p*-cresol were similar to the attributes used to describe the presence of boar taint such as mothball, shoe polish, indole, and animal-like (*34*, *35*). Moreover, the presence of *p*-cresol and *p*-ethylphenol together with high concentrations of 3-methylbutanoic acid has been related with the development of off-flavor in pig meat (*30*, *34*).

Concentrations of the Compounds Identified in the Fat Samples Analyzed. The chromatographic profile obtained from fat samples that had been classified previously with boar taint differed from the chromatographic profile obtained from fat samples that did not exhibit this off-flavor. The mean concentrations of some of the compounds identified tentatively by comparing their mass spectra with those contained in the library or identified with standard solutions were significantly higher in the samples classified as tainted (P < 0.01 and P < 0.05) (Table 3).

PCA permitted a better overall idea of the behavior of data and variables. The percentages of total variance explained by the first and second components were 37.44 and 22.90%, respectively (Figure 4). Component 1 was determined mainly by skatole, 4-phenyl-3-buten-2-one, 2,4-bis(1,1-dimethylethyl)phenol, and 2-(2-methylpropoxy)-1,2-diphenylethanone. The contribution to component 2 of all these compounds was very low. The distribution in the plane PC1-PC2 showed that these compounds were quite clustered, which indicated that they might be correlated. The distribution of fat samples in the PC1–PC2 plane agrees with the results obtained in the analysis of variance. Fat samples with low concentrations of skatole and androstenone and classified as tainted by the panel test were associated with the presence of the substances identified in this study. These results showed that these compounds could promote the perception of skatole and androstenone or could be responsible for the development of off-flavors with attributes similar to those used to describe the

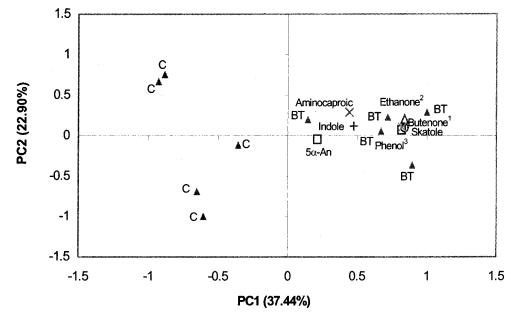


Figure 4. Variable loading and average score of boar taint and control samples plot from PC analysis on content of identified compounds by GC-MS (PC1–PC2 plane). BT, boar tainted samples; C, control samples. Compounds identified: 5α -An, 5α -androst-16-en-3-one; butenone¹, 4-phenyl-3-buten-2-one; ethanone², 2-(2-methylpropoxy)-1,2-diphenylethanone; phenol³, 2,4-bis(1,1-dimethylethyl)phenol.

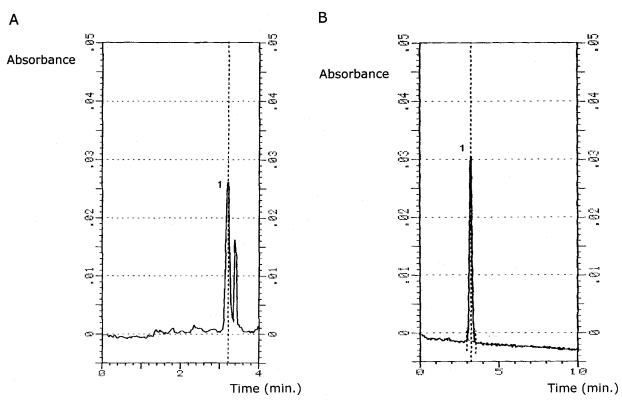


Figure 5. RP-HPLC chromatogram obtained from a fat sample with low concentration of skatole and androstenone and classified as tainted (A) and from a standard solution of 4-phenyl-3-buten-2-one (B). Peak 1: 4-phenyl-3-buten-2-one.

 Table 4. Descriptors Used for the Selected Individuals in

 the Sensorial Evaluation of Fat Samples Spiked with

 4-Phenyl-3-buten-2-one, Androstenone, and Skatole

compound	attributes	
4-phenyl-3-buten-2-one	naphthalene, mothballs	
androstenone skatole	urine, pig, sweat manure, fecal	
androstenone ^{a} + 4-phenyl-3-buten-2-one androstenone ^{b} + 4-phenyl-3-buten-2-one skatole + 4-phenyl-3-buten-2-one ^{c}	urine, pig, sweat urine, pig, sweat manure, fecal	
skatole + 4-phenyl-3-buten-2-one ^{d}	manure, fecal, naphthalene, mothballs	

^{*a*} Androstenone at low concentration (<0.5 μ g/g). ^{*b*} Androstenone at high concentration (>0.5 μ g/g). ^{*c*} 4-Phenyl-3-buten-2-one at low concentration (0.25 μ g/g). ^{*d*} 4-Phenyl-3-buten-2-one at high concentration (1 μ g/g).

presence of boar taint in the samples evaluated by the panelists (rancid, naphthalene, manure, and sweat).

Identification of 4-Phenyl-3-buten-2-one in Fat Samples by Reversed-Phase HPLC. The second fraction eluted from the gel filtration column was also analyzed by HPLC-UV, without any saponification procedure applied, to corroborate the identification of 4-phenyl-3-buten-2-one. The detection of 4-phenyl-3buten-2-one in the analysis by HPLC indicated that this compound did not originate from the saponification procedure applied. The RP-HPLC chromatogram obtained from a fat sample purified by gel filtration chromatography and the RP-HPLC chromatogram obtained from a standard solution of 4-phenyl-3-buten-2one are shown in Figure 5. In both chromatograms it is possible to observe the elution of a peak at a retention time of 3.22 min that showed a maximum absorbance at a wavelength of 280-290 nm. A comparison of the spectra showed the same profile for the standard and for the compound identified by GC-MS.

Sensorial Analysis. The results obtained in the sensorial analysis showed that the contribution of 4-phenyl-3-buten-2-one to the responses of the panelists depends on the concentration of androstenone and skatole (Table 4) (16). When 4-phenyl-3-buten-2-one was added to fat samples at a concentration of 1.0 μ g/g, the members of the panel were able to detect androstenone at a very low concentration (<0.5 μ g/g), but when the concentration of androstenone was higher (>0.50 μ g/ g), the panelists recognized the sensory attributes used in this study to describe the presence of the pheromone (urine, pig, and sweat), independently of the concentration of 4-phenyl-3-buten-2-one. The presence of skatole was recognized by the members of the panel in all of the fat samples analyzed, but when the samples were fortified with high concentrations of 4-phenyl-3-buten-2-one, the sensory attributes of naphthalene or mothballs were also described. Those results showed that the presence of 4-phenyl-3-buten-2-one in fat samples classified as tainted but with low concentrations of boar taint compounds could promote the perception of androstenone or the perception of certain attributes used to describe the presence of skatole in some studies (12).

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LITERATURE CITED

- Walstra, P. Fattening young boars: quantification of negative and positive aspects. *Livest. Prod. Sci.* 1974, *1*, 187–196.
- (2) Desmoulin, B. Sex effects on the deposition and quality of fat in lean pigs. *J. Rech. Porcine Fr.* **1983**, *25*, 130– 144.

- (3) Fortin, A.; Friend, D. W.; Sarkar, N. K. A note on the carcass composition of Yorkshire boars and barrows. *Can. J. Anim. Sci.* **1983**, *63*, 711–714.
- (4) Malmfors, B.; Lundström, K. Consumer reaction to boar meat—a review. *Livest. Prod. Sci.* 1983, 10, 187–196.
- (5) Diestre, A.; Oliver, M. A.; Gispert, M.; Arpa, I.; Arnau, J. Consumer responses to fresh meat and meat products from barrows and boars with different levels of boar taint. *Anim. Prod.* **1990**, *50*, 519–530.
- (6) Patterson, R. L. S. 5α-androst-16-ene-3-one: compound responsible for taint in boar fat. *J. Sci. Food Agric*. **1968**, *19*, 31–37.
- (7) Vold, E. Meat Production from Boars and Castrates. IV. Organoleptic and Gas Chromatographic Studies on the Steam Distillate of Back Fat from Boars, Report 238; Institute of Animal Genetics and Breeding, N.L.H.: Vollabekk, Norway, 1970.
- (8) Walstra, P.; Maarse, H. *IVO Report 2*; Researchgroep Vlees en Vleeswaren TNO: Zeist, The Netherlands, 1970.
- (9) Hanson, K. E.; Lundström, K.; Fjelkner-Modig, S.; Pearson, J. The importance of androstenone and skatole for boar taint. *Swed. J. Agric. Res.* **1980**, *10*, 167–173.
- (10) Bonneau, M.; Kempster, A. J.; Claus, R.; Claudi-Magnussen, C.; Diestre, A.; Tornberg, E.; Walstra, P.; Chevillon, P.; Weiler, U.; Cook, G. L. An international study on the importance of androstenone and skatole for boar taint: I. Presentation of the programme and measurement of boar taint compounds with different analytical procedures. *Meat Sci.* **2000**, *54*, 251–259.
- (11) Bonneau, M.; Walstra, P.; Claudi-Magnussen, C.; Kempster, A. J.; Tornberg, E.; Fisher, K.; Diestre, A.; Siret, F.; Chevillon, P.; Claus, R.; Dijksterhuis, G.; Punter, P.; Matthews, K. R.; Agerhem, H.; Béague, M. P.; Oliver, M. A.; Gispert, M.; Weiler, U.; von Seth, G.; Leask, H.; Font, M.; Homer, D. B.; Cook, G. L. An international study on the importance of androstenone and skatole for boar taint: IV. Simulation studies on consumer dissatisfaction with entire male pork and the effect of sorting carcasses on the slaughter line, main conclusions and recommendations. *Meat Sci.* 2000, *54*, 285–295.
- (12) Dijksterhuis, G.; Engel, B.; Walstra, P.; Font, M.; Agerhem, H.; Fisher, K.; Oliver, M. A.; Claudi-Magnussen, C.; Siret, F.; Béague, M. P.; Homer, D. B.; Bonneau, M. An international study on the importance of androstenone and skatole for boar taint: II. Sensory evaluation by trained panels in seven European countries. *Meat Sci.* **2000**, *54*, 261–269.
- (13) Matthews, K. R.; Homer, D. B.; Punter, P.; Béague, M. P.; Gispert, M.; Kempster, A. J.; Agerhem, H.; Claudi-Magnussen, C.; Fisher, K.; Siret, F.; Leask, H.; Font, M.; Bonneau, M. An international study on the importance of androstenone and skatole for boar taint: III. Consumer survey in seven European countries. *Meat Sci.* 2000, *54*, 271–283.
- (14) Weiler, U.; Font, M.; Fisher, K.; Kemmer, H.; Oliver, M. A.; Gispert, M.; Dobrowolski, A.; Claus, R. Influence of differences in sensitivity of Spanish and German consumers to perceive androstenone on the acceptance of boar meat differing in skatole and androstenone concentrations. *Meat Sci.* **2000**, *54*, 297–304.
- (15) Bejerholm, C.; Barton-Gade, P. The relationship between skatole/androstenone and odour/flavour of meat from entire male pigs. In *Proceedings of a Meeting of the EAAP Working Group: Production and Utilisation of Meat from Entire Male Pigs*; Bonneau, M., Ed.; INRA: Paris, France, 1993; pp 75–79.
- (16) Rius, M. A. Evaluation of other compounds related with the development of boar taint in pig back fat samples. Ph.D. Thesis, Universidad de Gerona, 1999.
- (17) Patterson, R. L. S. A possible contribution of phenolic components to boar odour. J. Sci. Food Agric. 1967, 18, 8–10.

- (18) Watson, A.; Patterson, R. L. S. Tainting of pork meat by 1,4-dichlorobenzene. J. Sci. Food Agric. 1982, 33, 103–105
- (19) Bonneau, M.; Cook, G. Characteristics of the samples selected for sensory evaluations and consumer surveys. In *Proceedings of a Meeting of the EAAP Working Group: Production and Utilisation of Meat from Entire Male Pigs*; Bonneau, M., Lundström, K., Malmfors, B., Eds.; Wageningen: The Netherlands, 1997; pp 16–19.
- (20) Mortensen, A. B.; Sørensen, S. E. Relationship between boar taint and skatole determined with a new analysis method. In *Proceedings of the 30th International Congress of Meat Science and Technology*; Bristol, U.K., 1984; pp 394–396
- (21) Claus, R.; Herbert, E.; Denhard, M. Comparative determination of the boar taint steroid androstenone in pig adipose tissue by a rapid enzyme immunoassay and a HPLC-method. *Arch. Lebensmittelhyg.* **199**7, *48*, 25– 28.
- (22) García-Regueiro, J. A.; Rius, M. A. Rapid determination of skatole and indole in pig back fat by normal-phase liquid chromatography. *J. Chromatogr. B* 1998, *809*, 246–251.
- (23) Rius, M. A.; García-Regueiro, J. A. Analysis of androstenone in pig back fat by solid-phase extraction and GC-MS. In *Proceedings of the 44th International Congress* of *Meat Science and Technology*; Barcelona, Spain, 1998; pp 584–586
- (24) Tranchant, J. Manuel Practique de Chromatographie en Phase Gazeuse; Masson: Paris, France, 1982; pp 301– 337.
- (25) Berdagué, J. L.; Denoyer, C.; Le Quéré, J. L.; Semon, E. Volatile components of dry-cured ham. *J. Agric. Food Chem.* **1991**, *39*, 1257–1261.
- (26) SAS. SAS/STAT User's Guide: Statistics; SAS Institute: Cary, NC, 1997.
- (27) Kochhar, S. P. Oxidative pathways to the formation of off-flavours. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed.; Blackie Academic and Professional: Glasgow, U.K., 1993; pp 150–201.
- (28) Tice, P. Packaging material as a source of taints. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed.; Blackie Academic and Professional: Glasgow, U.K., 1993; pp 202–235.
- (29) Cramer, D. A. Chemical compounds implicated in lamb flavour. *Food Technol.* **1983**, *5*, 249–253.
- (30) Ha, J. K.; Lindsay, R. C. Volatile branched-chain fatty acids and phenolic compounds in aged Italian cheese flavors. *J. Food Sci.* **1991**, *59*, 2312–2320.
- (31) Berdagué, J. L.; Bonnaud, N.; Rousset, S.; Touraille, C. Volatile compounds of dry-cured ham: identification and sensory characterisation by sniffing. In *Proceedings of the 37th International Congress of Meat Science and Technology*; Kulmbach, Germany, Sept 1–6, 1991; pp 1135–1137.
- (32) Rousset-Akrim, S.; Young, O. A.; Berdagué, J. L. Diet and growth effects in panel assessment of sheepmeat odour and flavour. *Meat Sci.* **1997**, *45*, 169–181.
- (33) Young, O. A.; Berdagué, J. L.; Viallon, C.; Rousset-Akrim, S.; Theriez, M. Fat-borne volatiles and sheepmeat odour. *Meat Sci.* **1997**, *45*, 183–200.
- (34) Ha, J. K.; Lindsay, R. C. Volatile alkylphenols and thiophenol in species-related characterising flavours of red meats. *J. Food Sci.* **1991**, *56*, 1197–1210.
- (35) Saxby, M. J. A survey of chemicals causing taints and off-flavours in foods. In *Food Taints and Off-Flavours*, Saxby, M. J., Ed.; Blackie Academic and Professional: Glasgow, U.K., 1993; pp 35–62.

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