1	Consumer acceptance of minced meat patties from boars in four European countries
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19 Abstract

A consumer study was performed in four EU countries to further clarify the acceptability of 20 meat with boar taint. In Denmark, France, Italy and Poland, a total of 476 female consumers 21 evaluated 8 meat patties from boars with varying levels of skatole (0.10-0.40 μ g/g fat tissue) 22 and androstenone (0.47-2.00 µg/g fat tissue), in a pair-wise comparison with patties from 23 castrates. Boar meat patties were always less preferred than the castrate meat patties, regardless 24 25 of the level of androstenone and skatole. Acceptability of the boar meat patties decreased with increasing skatole level. In samples with low skatole levels, higher levels of androstenone also 26 reduced acceptability among androstenone sensitive consumers. No clear threshold levels for 27 28 androstenone and skatole could be identified. Maps presenting the reduction in preference due to increasing levels of skatole and androstenone, and corrected for the general acceptance of 29 the meat product were developed, taking into account androstenone sensitivity. Further work is 30 needed, covering the whole range of androstenone and skatole levels found in entire male pigs 31 and for a wider set of meat products. 32

33	H	lighlights
34	•	Consumer and expert panel evaluations were closely correlated with skatole
35	•	At low skatole level, preference for boar meat patties decreased with increasing
36		androstenone level
37	•	Effect of androstenone level was only significant for androstenone sensitive
38		consumers
39	•	A map presenting the reduction in preference depending on the level of androstenone
40		and skatole was developed
41	•	No clear threshold/rejection level for skatole or androstenone could be determined

43 Keywords

44 Androstenone; Skatole; Boar taint; Thresholds; European consumers; Castration

45

46 **1** Introduction

Surgical castration of piglets is still performed in many countries to prevent boar taint. However, 47 societal pressure to ban this practice is increasing and several representatives of the pork 48 production chain in EU countries have signed a declaration of intention to ban surgical castration 49 by 2018 (European Commission, 2010). One prerequisite, however, is that consumer acceptance 50 of meat from boars is ensured. Boar taint has been studied for several decades, but reliable cut-51 off levels for boar taint as well as an operational definition of boar taint have yet to be agreed. 52 53 Two compounds are mainly responsible for this off-odour: skatole and androstenone. In the literature, cut-off levels vary between 0.15 and 0.25 μ g/g for skatole, and between 0.5 and 3.0 54 55 µg/g for androstenone (Bonneau & Chevillon, 2012; Bonneau et al., 2000; Lunde et al., 2010; Lundström, Matthews, & Haugen, 2009; Meier-Dinkel et al., 2013). This lack of clarity has many 56 causes: the imperfect link between sensory analysis and chemical analysis of boar taint, the 57 restricted comparability of the chemical analysis of androstenone and skatole between 58 laboratories, individual differences in androstenone sensitivity, the type of product served 59 (percentage of fat, serving temperature, masking ingredients), the attribute that is assessed 60 (cooking odour or flavour), the type of consumer panel used (standardised laboratory conditions 61 versus home-used tests; sensitivity of consumers to androstenone and skatole) and the 62 experimental set-up of the consumer panel (e.g. pairwise, type of reference sample, scale, 63 parameters) (Ampuero et al., 2011; Haugen, Brunius, & Zamaratskaia, 2012; Lundström et al., 64 2009). 65

This study aims to further clarify the acceptability of meat from boars presented as meat patties 66 (a standardized meat product with high fat content) for different levels of skatole and 67 androstenone. By combining carcasses with known levels of skatole and androstenone in backfat, 68 meat patties of both boar taint compounds were produced presenting a variation in the back fat 69 level of skatole from 0.10 to 0.40 μ g/g and of androstenone from 0.47 to 2.00 μ g/g. Consumer 70 panel studies were conducted in four EU countries (Denmark, France, Italy and Poland). The 71 samples were offered in a pair-wise design, which aimed at deriving consumer rejection 72 thresholds for androstenone and skatole following the methodology of Prescott et al. (2005). The 73 experimental set-up accounted for the level of skatole and androstenone and its interaction, the 74 75 effect of location (country), consumer sensitivity for skatole and androstenone, sequence of the pair and position in the pair as well as general liking of the product. 76

78 2 Material and methods

79 2.1 Preparation of the minced meat patties

Different batches of minced boar meat were prepared at DMRI (Danish Meat Research Institute, Roskilde, Denmark) as a mixture of meat from various animals to achieve 8 tailored levels of androstenone and skatole in order to produce different types of boar meat patties (B1 to B8). The levels were selected based on the results obtained in a previous pilot trial performed in Germany. Moreover, a batch of minced meat from castrated male pigs was included as reference sample for the paired comparison.

The batches of minced meat were prepared by combining back fat and meat of 3 to 4 boar 86 87 carcasses with known levels of skatole and androstenone as determined in the back fat of the used carcasses. Carcass selection was performed at Danish Crown (Ringsted, Denmark). First, 88 boar carcasses were sorted based on analyses of the online skatole equivalent detection method 89 (a combination of skatole and indole) (Mortensen & Sorensen 1984), and a sample of the back 90 fat was excised. Carcasses were then further selected based on the sensory score given by 2 91 assessors using the boiling water method (Aaslyng, Broge, Brockhoff, & Christensen, 2015; 92 Meinert, 2011). Finally, skatole and androstenone level in back fat was analysed using an HPLC 93 method (Aaslyng et al., 2015) and expressed as $\mu g/g$ fat tissue. The average levels of skatole 94 and androstenone in the back fat of the carcasses used to prepare the eight patties varied from 95 0.5 to 2.0 μ g/g fat tissue for androstenone and from 0.10 to 0.40 μ g/g fat tissue for skatole 96 (Table 1). Of the selected carcasses, back fat and pork from the fore-ends of both carcass sides 97 were excised, vacuum packed, frozen at -20°C and then kept at -40°C until use. For each batch, 98 back fat was added proportionally to the fore-end meat (12% fat content) to achieve minced 99 meat with an average fat content of 20%. Meat and fat were minced using a hole size of 3 mm, 100 then mixed thoroughly and divided into 500 g packages. The packages were vacuum packed 101 and frozen at -20°C until evaluation. Level of skatole and androstenone of the minced meat 102 patties (µg/g minced meat) was analysed at the European Commission Joint Research Centre, 103

Institute for Reference Materials and Measurements (JRC IRMM, Geel, Belgium) using LC MS/MS (Buttinger, 2014).

Final mean fat content was analysed using gravimetric analysis modified after SBR (Schmid-106 Bodzinski-Ratzlaff) according to ISO1443 (1973). The method is modified to be run on 107 HydrotecTM 8000 hydrolysis system and SoxtecTM 8000 extraction system as described in the 108 application note 3981 (2013) (FOSS, Denmark). The samples were treated with 8 M hydrogen 109 chloride, dried and the liberated fat was extracted with petroleum ether. The solvent was then 110 evaporated and the fat weighed. Final fat content of the patties was $17.2 \pm 1.4\%$ for the boar 111 samples versus 20.1% for the castrate sample. Mean water content was $64.8 \pm 1.0\%$ for the boar 112 samples versus 62.3% for the castrate sample. 113

114

115 [Table 1]

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117 2.2 Sensory profile

A sensory profiling analysis of the minced meat patties was carried out at DMRI using a trained 118 119 panel based on ASTM-MNL13 (Manual on Descriptive Analysis Testing for Sensory Evaluation), ISO 4121 (Sensory analysis - Guidelines for the use of quantitative response 120 scales) and ISO 13299 (Sensory analysis - Methodology - General guidance for establishing a 121 sensory profile). The training was based on ISO 8586-1 (Sensory analysis - Part 1: General 122 guidance for the recruitment, selection, training and monitoring of assessors). Eight assessors 123 were used; all were sensitive to androstenone and skatole. They had received a general training 124 in assessing boar tainted meat using references for the attributes (e.g. sweat, manure) (Aaslyng, 125 Broge, Brockhoff, & Christensen, 2016). The trained panel evaluated the eight boar meat patties 126 with varying boar taint concentrations and the castrate meat patties twice, in two sessions. The 127 attributes scored were pork odour/flavour (fried pork; reference: fried pork chop), piggy 128 odour/flavour (piggy, animal-like odour; reference: melted pig fat), manure odour/flavour, 129

pungent odour/flavour (an odour that "sticks" in the nose), urine odour/flavour, sweat odour,
boar odour/flavour and juiciness (amount of juice after 5 chews). Attributes were scored on an
unstructured 15 cm line scale going from "no intensity" to "strong intensity".

133

134 2.3 Consumer panels

Consumer tests were performed in four EU countries, with one location per country: at the 135 Danish Meat Research Institute (DMRI) in Roskilde, Denmark, at the ACTALIA sensory lab 136 of the Centre of Expertise for the Food Industry in Caen, France, at the Research Centre on 137 Animal Production (CRPA) in Reggio Emilia, Italy and at Warsaw University of Life Sciences 138 (CGGW) in Warsaw, Poland, all following the same standardised protocol. In Poland and 139 140 France, consumer tests were performed in eight sessions with 15 and 16 consumers per session, respectively. In Italy and Denmark, the test was performed in 16 sessions, with 8 and 7 141 consumers per session, respectively. The consumer test consisted of three parts. First, 142 consumers evaluated the meat patties. Second, consumers performed a smell test to determine 143 their sensitivity to skatole and androstenone. Third, consumers filled in a post-hoc questionnaire 144 145 on demographics and cooking and pork liking characteristics. The questionnaire for the consumer panel was provided in English; each country's sensory test leader then translated it 146 into the national language. 147

148

149 Selection of consumers

Previous studies have indicated that women are more sensitive to androstenone than men (Bekaert et al., 2011; Weiler et al., 2000). To ensure a sufficiently high prevalence of androstenone sensitive consumers, only women were recruited. Incentives were given as applicable at the sensory test centre. A total of 476 female consumers performed valid consumer tests in 4 EU countries: Denmark, France, Italy and Poland. Parameters for participation were 1) age between 18 and 65 years old and 2) tendency to eat pork in a hot meal at least twice a month. The demographic characteristics of the consumers and their cooking and pork liking characteristics were evaluated in each of the 4 countries. Average age of the consumers was 43 \pm 14 years (Table 2). Pork was consumed more than once per week, up to 3 times a week in a hot or a cold dish by 81% and 78% of the consumers, respectively.

160

161 [Table 2]

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163 *Sample preparation, serving and evaluation*

Meat was thawed for 48 hours at 5°C. On the day of the consumer test, meat patties of 110 g 164 were prepared with a patty press of 120 mm outer diameter (Gräwe, Germany) to ensure that 165 166 all patties had the same thickness. Patties were prepared in a separate pan for each sample. Patties were fried for 10 to 12 minutes, until core temperature reached 80°C, while turning the 167 samples every two minutes. For each serving (for eight consumers) four boar patties were fried. 168 Samples were served on a preheated plate without covering them with a lid. Time between 169 frying and serving was kept as short as possible, so that serving (core) temperature was 170 approximately 70°C. 171

Each consumer received five paired meat samples. The first pair only consisted of two castrate 172 meat patties. This pair was considered as warm-up pair to avoid first sample effects and to get 173 used to the served meat patties. The following four paired samples each consisted of one castrate 174 and one type of boar meat patty (B1-B8), with varying in concentrations of androstenone and 175 skatole. Paired samples were served at the same time and according to a balanced design 176 considering in each session the type of boar meat patty (following the sub-block structure of 177 the boar taint levels: B1/B3; B2/B4; B5/B7 and B6/B8), the position of the boar within each 178 pair (left=first or right=second) and the sequence of each pair (2nd to 5th pair). Before the first 179

and after each serving, consumers were advised to eat a small amount of bread and drink somewater to cleanse the palate.

For each pair, consumers were first asked to indicate which patty they preferred for odour and flavour. Subsequently, they were asked to give a liking score for each of the patties on a 9-point scale from 'dislike extremely' (1) to 'like extremely' (9) without the level 'neither like nor dislike' (5) to force consumers to give a more specific (i.e. either positive or negative) answer. Difference in liking score (boar-castrate) instead of liking score was used for statistical analysis to correct for overall liking of the meat product.

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189 Androstenone and skatole sensitivity

Sensitivity to androstenone and skatole was tested by using paper smell strips spiked with either 20 µl odour solution or the pure solvent (propylene glycol) (Mörlein, Meier-Dinkel, Moritz, Sharifi, & Knorr, 2013). Odour solutions (high androstenone: $5.0 \mu g/g$; low androstenone: 0.5 $\mu g/g$; and skatole: $1.0 \mu g/g$) were provided by the University of Göttingen. All sensory test leaders in the four countries followed the same protocol for the preparation and application of the sniffing strips.

To assess consumers' olfactory acuity, ten triangles were presented and consumers were asked to discriminate the odd sample. The first triangle was presented to learn the principle of a triangle test: one tube with mint-like odour (d-Carvone), two tubes with odourless solvent. The following triangles included low androstenone, high androstenone and finally, skatole – three triangles each. Consumers were instructed to sniff each strip only once and to give their best guess in case they did not smell a difference between each of the three samples in a triangle.

202 Consumers were classified as 'very sensitive' to androstenone if all three low and all three high 203 odd samples in the androstenone triangles were discriminated correctly. They were classified 204 as 'sensitive' if only all three high androstenone triangles were discriminated correctly. 205 Consumers were classified as sensitive to skatole if all three triangles with skatole were 206 discriminated correctly.

207

208 2.4 Statistical analysis

Sensory profiling of the expert panel was analysed by principal component analysis for all the
attributes by means of the FACTOR procedure of SAS ver. 9.2 (SAS institute Inc, Cary, NC,
USA). The CORR procedure of SAS was used to determine Pearson's correlation between
androstenone and skatole levels, overall liking scores and by country and average scores of first
and second principal components (PC1 and PC2).

Consumers' preferences for odour and flavour of the boar meat patty in the pair were analysed 214 using the GLIMMIX procedure of SAS and differences in overall liking (boar-castrate) were 215 216 analysed with the MIXED procedure of SAS. In all the models, type of boar meat patty (B1 to B8), sequence (pair 2 to 5), position (left/first - right/second), country (Italy, France, Poland, 217 Denmark), sensitivity to androstenone (not sensitive, sensitive, very sensitive) and sensitivity 218 to skatole (not sensitive, sensitive) were included as fixed factors. Consumer within a country 219 was considered to be a random effect. Two-way interactions between sensitivity, country and 220 type of boar were removed because they were not significant. For flavour preference, skatole 221 sensitivity was removed (P>0.05) and for differences in overall liking (P>0.05), sequence was 222 removed from the model. 223

Regression for odour preference and flavour preference of the boar sample in the pair, liking and percentage dissatisfaction on androstenone and skatole levels were calculated separately for androstenone-sensitive and non-sensitive consumers. For sensitive consumers, the model included the level of androstenone, the level of skatole (log-transformed values to ensure normal distribution) and the interaction between both compounds. For non-sensitive consumers, the model only included the level of skatole, as the level of androstenone is notrelevant for these consumers.

The equations for odour and flavour preference for the overall population were computed by weighting the results by the proportion of androstenone sensitive consumers (34.2%) in this study, resulting in:

234	Odour preference $_{\text{Sensitive}}$ (%) = -1.60 + 0.45*LnA -0.49*LnS +0.41*LnS*LnA
235	Flavour preference $_{\text{Sensitive}}$ (%) =-1.87 + 0.76*LnA -0.55*LnS +0.52*LnS*LnA
236	Odour preference _{All} (%) = -1.32 + 0.15*LnA -0.48*LnS +0.14*LnS*LnA
237	Flavour preference All (%) = $-1.45 + 0.26*LnA - 0.51*LnS + 0.18*LnS*LnA$
238	with LnA and LnS as the natural logarithm of androstenone and skatole content, respectively.
239	

The final maps representing the reduction in preference with increasing boar taint levels were built based on these equations for odour and flavour preference of the overall and the androstenone sensitive consumers, and reduced with 0.5, representing the chance of preferring the boar meat patty in the case that a consumer is not able to differentiate between the boar meat patty and the castrate meat patty.

245 **3 Results**

246 **3.1** Characterisation of the meat patties

Based on the sensory profile of the meat patties by the trained panel, two principal components (PC) were determined explaining 54% and 16% of the variation (Fig. 1). The negative side of PC1 is related to the castrate meat patty and patties with low skatole levels (B1 to B4) and associated with pork flavour and the absence of boar taint attributes. The positive part of PC2 is mainly associated with juiciness and pig related attributes, i.e. piggy (related to pork fat) and pork (related to fried pork chops). The back fat skatole level defining the boar meat patties correlated well (P<0.05) with this sensory profile, with r=0.95 for PC1 and r=0.60 for PC2. Correlations with androstenone were low and non-significant.

255 [Fig 1]

256

3.2 Factors influencing consumers' acceptance

258 **3.2.1** Effect of type of boar meat patty (B1 to B8)

The average consumer liking score of the castrate meat patty, served in the first pair, was 6.6 259 on the scale from 1 to 9. For the boar meat patties served in the second to fifth pair, average 260 liking scores varied from 4.9 to 6.2 (Table 1). The average liking score of the castrate meat 261 patty served in these pairs varied between 6.6 and 6.9, with a higher score for the castrate meat 262 patty in those pairs where the boar meat patty was liked less. Consumer liking score decreased 263 with increasing skatole level (r=-0.62, P<0.05), whereas the correlation with androstenone was 264 265 not significant (r=-0.07, p>0.05). Overall, consumer liking scores correlated well with the expert panel evaluation of boar taint, mainly with PC1 (r=-0.69, P<0.05)). Correlation between 266 267 liking score and PC2 was lower (r=-0.45, P<0.05).

Difference in liking score as well as odour and flavour preferences of the boar meat patty was significantly affected by the type of boar meat patty that was served in the pair (P<0.001; Fig. 2). The smallest difference in liking score between the boar meat patty and the castrate meat patty was observed for B3 and B4, followed by B1. The largest difference was observed for B6 and B7 followed by B8 (these 3 samples had the highest SKA levels). In line with these results, the preference for the boar sample in the pair with B7 was lower than in the pairs with B1, B2, B3 and B4.

275 [Fig 2]

277 **3.2.2** Effect of skatole and androstenone sensitivity

Of all consumers, 25% were classified as sensitive for androstenone and 9% were classified as 278 very sensitive (Table 3). For skatole, average percentage of sensitivity was 60%. For consumers 279 very sensitive to androstenone, odour (P=0.002) and flavour (P<0.001) reduction in preference 280 for boar meat patties was greater as compared to sensitive and non-sensitive consumers (Fig. 281 3b,c). Also differences in liking score were larger for very sensitive compared to non-sensitive 282 consumers (P<0.001). For consumers sensitive to skatole, preference for boar meat patties was 283 also reduced (P=0.007) and difference in liking score (P<0.001) was larger compared to the 284 non-sensitive consumers. The effect of skatole sensitivity was not significant for flavour 285 preference (P>0.05) (Fig. 3a). 286

287 [Table 3]

288 [Fig 3]

289

290 **3.2.3** Effect of country, serving order and sequence

The percentage of consumers that preferred the boar meat patties regarding odour and flavour differed between countries (Fig. 3b). For the odour of the boar meat patties (P<0.001), the French consumers showed a higher preference compared to the Danish and Polish consumers. For flavour (P=0.006), results were slightly different: the French and Italian consumers made less differentiation between the boar and the castrate patty in a pair compared to the Danish consumers.

The effect of position of the boar meat patty within the pair (first versus second) was significant for the difference in liking score and for odour preference (P<0.001), but not for flavour. Boar meat patties that were evaluated in the first position within a pair were more often preferred for odour than those evaluated in the second position (38 versus 25%) and difference in liking score was also smaller (-1.31 versus -17.71). The effect of sequence of the pair was significant for odour preference (P<0.001) and showed a tendency for flavour preference (P=0.093). The boar meat patties in the 2^{nd} and 3^{rd} pair were more often preferred for odour than those evaluated in the 4^{th} and 5^{th} pair (Fig. 3c). The effect of sequence was not significant for difference in liking score.

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307 3.3 Effect of boar taint compound level and androstenone sensitivity

For all consumers, preference of the boar meat patty decreased with increasing skatole content. 308 For androstenone sensitive consumers, preference also decreased with increasing androstenone 309 level if skatole levels were low (Table 4). As the effect of skatole and androstenone was gradual, 310 no clear thresholds for androstenone and skatole could be defined in this study. The effect of 311 skatole and androstenone level, as well as the effect of androstenone sensitivity, is further 312 evaluated and presented in a map illustrating the reduction in preference with increasing skatole 313 314 and androstenone level. These maps (Table 5) illustrate the reduction in preference based on the prediction equations for odour and flavour preference for androstenone sensitive and non-315 sensitive consumers minus 50%. 316

317

318 [Table 4]

319 [Table 5]

320

322 **4 Discussion**

323 4.1 Effect of type of boar meat patty

Consumers gave higher liking scores to the meat patties from the castrated male pigs when the 324 325 meat patty from the boar was liked less. This variation in liking score of the castrate meat patty indicates the relevance of our approach in experimental set-up, i.e. a pair-wise serving design 326 in which consumers indicate their preference of different test samples compared to a fixed 327 reference sample. Indeed, if threshold values for boar taint are studied, it is relevant to know at 328 329 which boar taint level consumers start to reject boar samples compared to the reference sample and not e.g. in comparison with a sample with more or less boar taint. Indeed, the consumer 330 331 liking of a patty with boar meat may not only depend on the presence of boar taint, but may also be affected by how the consumers generally like the presented meat product and may be 332 affected by the previously served samples. 333

This experimental set-up was based on the study of Prescott, Norris, Kunst and Kim et al. (2005), in which the same set-up was used to determine the consumer rejection threshold for cork taint in white wine. Studies also show that it is more easy for consumers to rank samples than to score samples on a hedonic scale (Wichchukit and Mahony, 2014). In line with our experimental approach and to account for the expected bias in liking score, the preference and the within pair difference in liking score between the boar and castrate meat patties instead of the liking score of the boar meat patty itself was used for further evaluation.

Regardless of the level of androstenone and skatole, consumers preferred boar meat patties clearly less than the reference patty from castrated male pigs (<50%). This could be due to differences in texture or juiciness of boars compared to castrated male pigs as shown in the sensory profile as a result of the lower fat content of these boar meat patties compared to the castrate meat patty. Based on these results, it would be relevant to further optimise sample preparation to yield comparable fat concentrations in all meat patties. Difference may also be due to the low concentrations of skatole and androstenone which were present in the low-level
boar taint patty. It can be of interest to include lower ranges of both skatole and androstenone
levels in further research, down to concentrations that can be found in castrated male pigs and
gilts, even though this is mainly academic interest and not relevant for setting sorting limits.
Indeed, such low concentrations are also very common in entire male pig populations and its
influence is not clear since some studies showed that they have an effect on acceptability (Font
i Furnols et al., 2008) while others did not confirm these findings (Bonneau & Chevillon, 2012).

354

355 The consumer study indicates that preference for a boar meat patty served in a pair with a castrate meat patty was more reduced for boar meat patties with higher skatole levels than those 356 with low skatole levels. The same association was found for the sensory profile. These results 357 reflect the importance of skatole in the evaluation of the boar taint attributes as already observed 358 in previous studies (AnnorFrempong, Nute, Whittington, & Wood, 1997; Dijksterhuis et al., 359 2000; Hansson, Lundström, Fjelknermodig, & Persson, 1980; Meier-Dinkel, Gertheiss, Muller, 360 Wesoly, & Mörlein, 2015), and especially for products served warm (de Kock, Heinze, 361 Potgieter, Dijksterhuis, & Minnaar, 2001; Diestre, Oliver, Gispert, Arpa, & Arnau, 1990). The 362 363 lack of a clear, distinguishable effect of androstenone, even when tested by androstenone sensitive experts, may be due to the fact that the range of androstenone levels (0.47 to 2.00 364 365 $\mu g/g$) in the patties included in this study did not exceed the higher detection thresholds suggested for androstenone in the literature $(1.5-3.0 \ \mu g/g)$ (Bonneau & Chevillon, 2012; 366 Bonneau et al., 2000; Lunde et al.; Meier-Dinkel et al., 2013) whereas the range of skatole 367 levels (0.10 to 0.40 μ g/g) in the patties explored concentration ranges far above the skatole 368 369 thresholds found in the literature (0.15 and 0.25 μ g/g) (Bonneau et al., 2000; Lunde et al., 2010; Lundström et al., 2009). Based on recent literature (> 2010) the proportion of pigs with 370 androstenone > 2.0 μ g/g and skatole > 0.4 μ g/g was 11 and 2% (n=119) (Mörlein, 371

Lungershausen, Steinke, Sharifi, & Knorr, 2012); 5 and 3% (n=1031) (Mörlein et al., 2016); 18 372 and 3% (n= 575), 12 and 3% (n=488) (IFIP, unpublished), and 11 and 2% (n=53) (Aluwé et al., 373 2013). The range of skatole levels considered in this study sufficiently covered the range around 374 the consumer detection threshold values proposed in the literature and the range present in entire 375 male pigs. The range of androstenone levels considered in this study only covered the lower 376 spectrum of threshold values and did not cover the range of higher androstenone values 377 common in entire male pigs. While skatole was more important compared to androstenone in 378 this study, results can be different if higher androstenone levels were included. Further research 379 using the same methodology and exploring the higher ranges of androstenone concentrations 380 as in the present study will be needed. 381

382 4.2 Effect of androstenone and skatole sensitivity

The results on androstenone and skatole sensitivity show that sensitive consumers were better 383 able to differentiate boar meat patties from castrate meat patties. As found in previous studies 384 (Aluwé et al., 2011; Font i Furnols et al., 2016), androstenone sensitivity increases the chance 385 that boar meat products are disliked at higher boar taint levels, especially if these consumers 386 dislike androstenone (Bonneau & Chevillon, 2012; Font i Furnols, Gispert, Diestre, & Oliver, 387 2003). However, other studies evaluating pork chops and schnitzels (Aaslyng et al., 2016) or 388 pork patties (Lunde et al., 2010) did not find any significant effect of sensitivity. When 389 discussing the effect of androstenone and skatole sensitivity, it is important to note that 390 comparison of sensitivity numbers between studies is difficult. Part of the differences can be 391 attributed to differences in methodology, e.g. concentrations, solutions or strips, number of 392 replicates (e.g. triangles), and the definition of (in)sensitivity (Bekaert et al. 2011; Lunde et al. 393 2010; Weiler et al. 2000). The sensitivity figures found in our study are similar to those found 394 in the study performed in parallel in Russia and China (Font i Furnols et al., 2016) using the 395 same methodology, but other studies reported 80 to 100% skatole sensitivity (Font i Furnols, 396 2012; Meier-Dinkel et al., 2013). As only women were included in our test and higher 397

androstenone sensitivity has been shown for women than for men (Mörlein et al., 2015; Weiler 398 et al., 2000), these relatively low numbers for sensitivity were not expected, but can probably 399 be attributed to differences in methodology (e.g. criteria of all three triangles being correctly 400 discriminated). It can be assumed that the concentrations spiked on the paper strips used to 401 evaluate sensitivity were sufficiently high (20 μ l of 5 μ g/g androstenone and 1 μ g/g skatole) to 402 be detected. In a study with trained panellists using the same methodology with spiked paper 403 strips, the odour thresholds found were 0.24 μ g/g for androstenone and 0.18 μ g/g for skatole 404 (Heyrman et al. 2016). In another study using non-trained assessors and odorants diluted in oil, 405 the odour threshold was comparable (0.21 μ g/g for androstenone and 0.10 μ g/g for skatole) 406 407 (Font i Furnols, Guerrero, Serra, Rius, & Oliver, 2000). Despite the great pains taken to supply 408 all sensory test facilities with a detailed protocol as well as odour solutions to prepare the strips, we cannot rule out problems with the consumers' performance of the task, such as not sniffing 409 well enough at the end of the strips where the solution was spiked, or consumer comprehension 410 of the triangular test task. Either or both could result in a lower number of sensitive consumers 411 than expected. In future studies, further effort should be done to standardise, optimise and 412 simplify this methodology to enable a better comparison between studies. 413

414

415 4.3 Effect of country, serving order and sequence

The interaction between country and type of boar meat patty was not significant in the current test in the 4 EU countries, indicating that the acceptance of boar meat in a certain country or test location was independent of the level of boar taint. In the parallel study in China and Russia, however, an interaction of sensitivity with type of boar meat patty (related to the androstenone and skatole level) was found with a decrease in preference and liking score with increasing concentration of boar taint in Russia, but not in China (Font i Furnols et al., 2016). In general, French consumers made the least differentiation between castrate and boar meat patties. Possibly cultural habits and habituation to typical French meat products such as andouillette, which is made from pig intestines and has a very specific odour, might explain the differences. In order to better understand the possible differences between countries, further research should be done including consumers representing the different regions (with cultural variation) per country. It could then be interesting to investigate if these consumers indeed associate tainted samples with specific food products, in order to better understand the cultural link.

Serving was balanced for position within the pair and for sequence. Indeed, boar meat patties 429 430 that were served first (left) in a pair showed least reduction in preference for odour and least difference in liking score compare to the castrate meat patty. The same was seen for the effect 431 of sequence: boar meat patties served in the 2nd and 3rd pair showed a higher odour preference 432 than those served in the 4th and 5th pair. This may indicate that the consumers become more 433 aware of boar taint after evaluating several patties, which makes it easier for them to 434 differentiate boar meat patties from castrate meat patties. The study of Mörlein et al. (2015) 435 showed no effect of repeated exposure when evaluating boar meat samples. Their experimental 436 set-up was not comparable, however, as their test was performed with some delay between the 437 two sensory tests and at home versus in a sensory lab. In accordance with our results, Heyrman 438 et al. (2015) showed that assessors who were familiar with boar taint (by previous exposure 439 with smell strips or tainted samples, but otherwise untrained) were better able to detect fat 440 samples with boar taint than assessors without prior experience. The effect of position and 441 sequence highlights the importance of a well-designed serving order within and across the pairs 442 to overcome these potential effects. 443

444

445 **4.4** Effect of boar taint compound level and androstenone sensitivity

446 Consumer preference and liking of the boar meat patty gradually declined with increasing447 skatole and androstenone level. In other words, the present results made it impossible to define

clear cut-off values for skatole and androstenone. For practical purposes, stakeholders can refer 448 to the maps reflecting the reduction in consumer preference per level of boar taint (for those 449 levels included in this test). For example, if a stakeholder is willing to take the risk of a reduction 450 in flavour preference of 10% compared to the reference sample population, androstenone levels 451 up to 2.07 μ g/g in back fat are acceptable if skatole levels are low ($\leq 0.10 \mu$ g/g), while skatole 452 levels in back fat higher than $0.10 \,\mu g/g$ are not acceptable at any androstenone level. However, 453 these levels will imply a reduction in preference of up to 22% for the androstenone sensitive 454 consumers, or in other words, 72% of the sensitive consumers will prefer the reference sample. 455 In line with previous studies, results indicated that the interaction of androstenone and skatole 456 needs to be taken into account (Mörlein et al., 2016). At low skatole levels, an increase of 457 458 androstenone reduced the preference for boar meat patties, while this affected preference less negatively if the level of skatole in the back fat of the boar meat patties was already higher than 459 $0.18 \,\mu g/g$. 460

Further research should be done for a wide range of boar meat products, including very low levels of androstenone and skatole as well as higher levels of androstenone. This will create a better understanding of the impact of boar taint compounds on the perception of consumers. The resulting preference maps will allow stakeholders to choose the threshold for both compounds according to the stakeholders' constraints and risk management policy. Furthermore, it will also help to clarify the possibilities of valorising tainted boar carcasses.

467 **5** Conclusion

468 Consumer preference of the boar meat patty served pair-wise with a castrate meat patty 469 decreased with increasing skatole content for the boar meat patties made from carcasses with 470 back fat concentrations of skatole varying from 0.10 to 0.40 μ g/g fat tissue and of androstenone 471 from 0.47 to 2.00 μ g/g fat tissue. At low skatole content, the preference for the boar meat patties 472 also decreased with increasing androstenone level, even more so in the subpopulation of

consumers that were sensitive to androstenone. It was not possible to determine a clear 473 preference/rejection threshold for androstenone and skatole, as liking or preference decreased 474 gradually. Any sorting limit therefore depends on the risk for negative consumer reactions that 475 stakeholders are willing to take. For this reason, a map presenting the reduction in preference 476 for the boar meat patties with increasing level of androstenone and skatole compared to the 477 reference sample was built based a general population weighted by the prevalence of sensitivity 478 and only androstenone sensitive consumers. Further research is needed to finalise these maps 479 for lower skatole and androstenone levels as well as higher androstenone levels and other meat 480 products. 481

482

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- 597 598

- **Table 1** *Skatole (S) and androstenone (A) content of the boar meat patties based on chemical analysis*
- *of the back fat and of the meat patty, and the consumer liking score of the boar meat patties and the*

castrate meat patty served in the same pair

	Boar								
	Ba	ck fat			Meat patty ³				
Sample code	S	А		S	А	Liking	Liking		
Boar meat patty	(µg/g)	(µg/g)	(μ <u></u>	g/g)	(µg/g)	score ^{1,2}	score ^{1,2}		
B1	0.12	0.48	<0	.05	0.25	5.9	6.6		
B2	0.10	2.00	<0	.05	0.51	5.8	6.6		
B3	0.15	0.90	<0	.05	0.34	6.0	6.6		
B4	0.15	1.48	<0	.05	0.85	6.2	6.6		
B5	0.28	0.75	0.	07	0.38	5.4	6.7		
B6	0.33	1.54	0.	07	0.39	5.1	6.8		
B7	0.40	0.47	0.	06	0.13	4.9	6.9		
B8	0.39	2.00	0.	08	0.85	5.2	6.6		

¹Scored on a scale from 1 (dislike extremely) to 9 (like extremely)

⁶⁰⁴ ² The number of observations for each pairwise comparison varied from n=224 to n=247

³ Served pairwise with boar meat patty B1 to B8

609	Table 2 Demographic and	d pork consumption	characteristics of the consu	mers participating in the
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610	sensory study in Denmark, France	. Italv. Poland and overall	l (as % of the number of	^c consumers)
010	sensory siddy in Dennard, I rance	, naiy, i olana ana overali	(as 700) inc number of	consumersy

	Denmark	France	Italy	Poland	Total
Number of consumers	109	128	121	118	476
Age					
<25 yr	5.7	21.8	13.2	22.9	16.4
25-44 yr	29.8	49.2	43.0	22.9	36.7
45-59 yr	43.3	18.8	24.0	33.9	29.3
>60 yr	21.2	10.2	19.8	20.3	17.6
Education level					
Primary	19.8	3.9	6.6	0.8	7.4
Secondary	13.2	20.3	24.0	57.6	29.0
Higher	56.6	27.4	44.7	7.6	33.4
University	10.4	48.4	24.8	33.9	30.2
Cooking main dishes at home					
Mainly me	69.5	76.6	66.1	72.0	71.2
Mainly others	10.5	5.4	6.6	6.0	7.0
Sometimes	20.0	18.0	27.3	22.0	21.8
Pork liking					
Dislike	0.0	0.0	4.1	0.8	1.2
Neither like nor dislike	12.5	25.7	10.7	10.1	15
Like	87.5	74.3	85.2	89.1	83.8
Consume pork in a hot dish (times/week)					
> 4	8.6	3.9	4.1	9.3	6.4
3-4	20.0	14.8	13.2	36.4	21.0
2-3	54.3	54.7	59.5	46.6	53.8
< 1	17.1	26.6	23.1	7.6	18.9
Consume pork in a cold dish					
>4	16.0	2.3	5.8	22.0	11.2
3-4	27.4	17.2	34.7	29.7	27.1
2-3	38.7	43.0	50.4	28.0	40.3
< 1	17.9	37.5	9.1	20.3	21.6
Do you currently suffer a cold or allergy	17.1	8.6	11.6	19.5	14.0

		Androstenone sensitivity (%)						
(n=106) France (n=128) Italy (n=121) Poland (n=118) Overall	Skatole sensitivity (%)	No	Yes	Very	Total			
Denmark	No	33.9	12.8	1.8	48.6			
(n=106)	Yes	30.3	16.5	4.6	51.4			
	Total	64.2	29.4	6.4	100.0			
France	No	31.3	7.8	0.8	39.8			
(n=128)	Yes	34.4	21.1	4.7	60.2			
	Total	65.6	28.9	5.5	100.0			
Italy	No	24.8	7.4	0.8	33.1			
(n=121)	Yes	37.2	14.0	15.7	66.9			
	Total	62.0	21.5	16.5	100.0			
Poland	No	30.5	9.3	1.7	41.5			
(n=118)	Yes	40.7	11.9	5.9	58.5			
	Total	71.2	21.2	7.6	100.0			
Overall	No	30.0	9.2	1.3	40.5			
(n=476)	Yes	35.7	16.0	7.8	59.5			
	Total	65.8	25.2	9.0	100.0			

- **Table 4** *Logistic regression results for odour and flavour preferences of the boar meat patties served*
- 617 pairwise with a castrate meat patty based on the levels and the interaction of androstenone (A) and
- *skatole (S) of the boar meat patty and according to the consumers' sensitivity to androstenone*

	Odour					Flavour				
Effect	Estimate	s.e.	Pr > t	Confi	dence	Estimate	s.e.	Pr > t	Conf	idence
				limit ((95%)				limit (95%)	
				Lower	Upper				Lower	Upper
Non-sensitive										
Intercept	-1.17	0.21	< 0.001	-1.31	-1.03	-1.24	0.21	< 0.001	-1.38	-1.10
LnS	-0.48	0.12	< 0.001	-0.55	-0.40	-0.49	0.11	< 0.001	-0.57	-0.41
Sensitive										
Intercept	-1.60	0.37	< 0.001	-1.86	-1.35	-1.87	0.35	< 0.001	-2.11	-1.63
LnA	0.45	0.47	0.344	0.13	0.77	0.76	0.49	0.120	0.43	1.08
LnS	-0.49	0.18	0.007	-0.61	-0.37	-0.55	0.18	0.003	-0.67	-0.43
LnA x LnS	0.41	0.28	0.151	0.22	0.60	0.52	0.29	0.072	0.32	0.71

Table 5. Reduction in consumers' preferences (%) compared to a 50% chance of preference for odour and flavour of the boar meat patties over the castrate meat patties depending on their level of androstenone (AND) and skatole (SKA) for all consumers based on 34% androstenone sensitivity and

considering only androstenone sensitive consumers

				Redu	iction i	n consun	ners preference (%)			
		Ove	erall po	pulatio	on	Sensitive consumers					
Odour		SKA (ppm)					SKA (ppm)				
	AND (ppm)	0.10	0.18	0.26	0.34	0.42	0.10	0.18	0.26	0.34	0.42
	0.47	-2	-11	-16	-19	-21	-3	-14	-20	-25	-27
	0.87	-5	-12	-16	-19	-21	-10	-17	-22	-25	-26
	1.27	-6	-13	-16	-19	-21	-14	-20	-23	-25	-26
	1.67	-7	-13	-17	-19	-20	-17	-21	-23	-25	-25
	2.07	-8	-14	-17	-19	-20	-20	-22	-23	-24	-25
Flavour											
	AND (ppm)										
	0.47	-4	-13	-19	-22	-25	-7	-20	-26	-31	-34
	0.87	-6	-14	-18	-21	-24	-13	-21	-26	-29	-31
	1.27	-8	-14	-18	-21	-23	-17	-22	-25	-27	-29
	1.67	-9	-15	-18	-20	-22	-20	-23	-25	-26	-28
	2.07	-10	-15	-18	-20	-22	-22	-24	-25	-26	-26

627 **Figure captions**

- **Fig. 1** Scores of the first (PC1) and second (PC2) principal components for the different sensory
- attributes and the averaged coordinates for the boar meat patties (B1 to B8) and the castrate meat patty
- (CAS) as determined by the trained panel, with skatole levels of 0.12, 0.10, 0.15, 0.15, 0.28, 0.33,
- 631 $0.40, 0.39 \ \mu$ g/g and androstenone levels of 0.48, 2.00, 0.90, 1.48, 0.75, 1.54, 0.47, 2.00 for B1 to B8
- 632 respectively.

633

- **Fig 2.** Consumers' preferences of the boar and the castrate meat patties in the pair for a) odour and b)
- flavour and c) difference in liking score (boar castrate) according to type of boar meat patty (B1-B8)
- 636 with skatole levels of 0.12, 0.10, 0.15, 0.15, 0.28, 0.33, 0.40, 0.39 μ g/g and androstenone levels of
- 637 0.48, 2.00, 0.90, 1.48, 0.75, 1.54, 0.47, 2.00 for B1 to B8 respectively.
- abc Different letter indicates significant differences per reported variable (P<0.05).
- 639

Fig 3. Consumers' preferences of the boar and the castrate meat patty in the pairs and difference in
liking score (boar – castrate) according to a) effect of skatole (no, yes) and androstenone sensitivity (no,
yes, very) b) effect of country (Denmark, France, Italy, Poland), and c) effect of sequence (pair 2, pair 3,
pair 4 and pair 5).

 abc Different letter indicates significant differences per reported variable (P<0.05)

645