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

A consumer study was performed in four EU countries to further clarify the acceptability of meat with boar taint. In Denmark, France, Italy and Poland, a total of 476 female consumers evaluated 8 meat patties from boars with varying levels of skatole (0.10-0.40 $\mu$ g/g fat tissue) and androstenone (0.47-2.00  $\mu$ g/g fat tissue), in a pair-wise comparison with patties from castrates. Boar meat patties were always less preferred than the castrate meat patties, regardless 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 reduced acceptability among androstenone sensitive consumers. No clear threshold levels for 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 the meat product were developed, taking into account androstenone sensitivity. Further work is needed, covering the whole range of androstenone and skatole levels found in entire male pigs and for a wider set of meat products.

## Highlights

- Consumer and expert panel evaluations were closely correlated with skatole
- At low skatole level, preference for boar meat patties decreased with increasing androstenone level
  - Effect of androstenone level was only significant for androstenone sensitive consumers
- A map presenting the reduction in preference depending on the level of androstenone
   and skatole was developed
  - No clear threshold/rejection level for skatole or androstenone could be determined

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# Keywords

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

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### 46 1 Introduction

Surgical castration of piglets is still performed in many countries to prevent boar taint. However, societal pressure to ban this practice is increasing and several representatives of the pork production chain in EU countries have signed a declaration of intention to ban surgical castration by 2018 (European Commission, 2010). One prerequisite, however, is that consumer acceptance of meat from boars is ensured. Boar taint has been studied for several decades, but reliable cutoff levels for boar taint as well as an operational definition of boar taint have yet to be agreed. 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 µg/g for skatole, and between 0.5 and 3.0 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 causes: the imperfect link between sensory analysis and chemical analysis of boar taint, the restricted comparability of the chemical analysis of androstenone and skatole between laboratories, individual differences in androstenone sensitivity, the type of product served (percentage of fat, serving temperature, masking ingredients), the attribute that is assessed (cooking odour or flavour), the type of consumer panel used (standardised laboratory conditions versus home-used tests; sensitivity of consumers to androstenone and skatole) and the experimental set-up of the consumer panel (e.g. pairwise, type of reference sample, scale, parameters) (Ampuero et al., 2011; Haugen, Brunius, & Zamaratskaia, 2012; Lundström et al., 2009).

This study aims to further clarify the acceptability of meat from boars presented as meat patties (a standardized meat product with high fat content) for different levels of skatole and androstenone. By combining carcasses with known levels of skatole and androstenone in backfat, meat patties of both boar taint compounds were produced presenting a variation in the back fat level of skatole from 0.10 to 0.40  $\mu$ g/g and of androstenone from 0.47 to 2.00  $\mu$ g/g. Consumer panel studies were conducted in four EU countries (Denmark, France, Italy and Poland). The samples were offered in a pair-wise design, which aimed at deriving consumer rejection thresholds for androstenone and skatole following the methodology of Prescott et al. (2005). The experimental set-up accounted for the level of skatole and androstenone and its interaction, the 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.

### 2 Material and methods

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## 2.1 Preparation of the minced meat patties

Different batches of minced boar meat were prepared at DMRI (Danish Meat Research Institute, 80 Roskilde, Denmark) as a mixture of meat from various animals to achieve 8 tailored levels of 81 androstenone and skatole in order to produce different types of boar meat patties (B1 to B8). 82 The levels were selected based on the results obtained in a previous pilot trial performed in 83 Germany. Moreover, a batch of minced meat from castrated male pigs was included as reference 84 sample for the paired comparison. 85 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 92 assessors using the boiling water method (Aaslyng, Broge, Brockhoff, & Christensen, 2015; Meinert, 2011). Finally, skatole and androstenone level in back fat was analysed using an HPLC 93 method (Aaslyng et al., 2015) and expressed as µg/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  $2.0 \mu g/g$  fat tissue for androstenone and from 0.10 to  $0.40 \mu g/g$  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-Bodzinski-Ratzlaff) according to ISO1443 (1973). The method is modified to be run on HydrotecTM 8000 hydrolysis system and SoxtecTM 8000 extraction system as described in the application note 3981 (2013) (FOSS, Denmark). The samples were treated with 8 M hydrogen chloride, dried and the liberated fat was extracted with petroleum ether. The solvent was then evaporated and the fat weighed. Final fat content of the patties was  $17.2 \pm 1.4\%$  for the boar samples versus 20.1% for the castrate sample. Mean water content was  $64.8 \pm 1.0\%$  for the boar samples versus 62.3% for the castrate sample.

[Table 1]

# 2.2 Sensory profile

A sensory profiling analysis of the minced meat patties was carried out at DMRI using a trained panel based on ASTM-MNL 13 (Manual on Descriptive Analysis Testing for Sensory Evaluation), ISO 4121 (Sensory analysis - Guidelines for the use of quantitative response scales) and ISO 13299 (Sensory analysis - Methodology - General guidance for establishing a sensory profile). The training was based on ISO 8586-1 (Sensory analysis - Part 1: General guidance for the recruitment, selection, training and monitoring of assessors). Eight assessors were used; all were sensitive to androstenone and skatole. They had received a general training in assessing boar tainted meat using references for the attributes (e.g. sweat, manure) (Aaslyng, Broge, Brockhoff, & Christensen, 2016). The trained panel evaluated the eight boar meat patties with varying boar taint concentrations and the castrate meat patties twice, in two sessions. The attributes scored were pork odour/flavour (fried pork; reference: fried pork chop), piggy odour/flavour (piggy, animal-like odour; reference: melted pig fat), manure odour/flavour,

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".

## 2.3 Consumer panels

Consumer tests were performed in four EU countries, with one location per country: at the Danish Meat Research Institute (DMRI) in Roskilde, Denmark, at the ACTALIA sensory lab of the Centre of Expertise for the Food Industry in Caen, France, at the Research Centre on Animal Production (CRPA) in Reggio Emilia, Italy and at Warsaw University of Life Sciences (CGGW) in Warsaw, Poland, all following the same standardised protocol. In Poland and 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 consumers per session, respectively. The consumer test consisted of three parts. First, consumers evaluated the meat patties. Second, consumers performed a smell test to determine their sensitivity to skatole and androstenone. Third, consumers filled in a post-hoc questionnaire 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 into the national language.

## 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.

Meat was thawed for 48 hours at 5°C. On the day of the consumer test, meat patties of 110 g

[Table 2]

Sample preparation, serving and evaluation

were prepared with a patty press of 120 mm outer diameter (Gräwe, Germany) to ensure that 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 samples every two minutes. For each serving (for eight consumers) four boar patties were fried. Samples were served on a preheated plate without covering them with a lid. Time between frying and serving was kept as short as possible, so that serving (core) temperature was approximately 70°C.

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

and after each serving, consumers were advised to eat a small amount of bread and drink some water 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.

# Androstenone and skatole sensitivity

Sensitivity to androstenone and skatole was tested by using paper smell strips spiked with either 20  $\mu$ l odour solution or the pure solvent (propylene glycol) (Mörlein, Meier-Dinkel, Moritz, Sharifi, & Knorr, 2013). Odour solutions (high androstenone:  $5.0\,\mu\text{g/g}$ ; low androstenone:  $0.5\,\mu\text{g/g}$ ; and skatole:  $1.0\,\mu\text{g/g}$ ) 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. Consumers were classified as 'very sensitive' to androstenone if all three low and all three high odd samples in the androstenone triangles were discriminated correctly. They were classified as 'sensitive' if only all three high androstenone triangles were discriminated correctly.

Consumers were classified as sensitive to skatole if all three triangles with skatole were discriminated correctly.

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# 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 using the GLIMMIX procedure of SAS and differences in overall liking (boar-castrate) were 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, Denmark), sensitivity to androstenone (not sensitive, sensitive, very sensitive) and sensitivity to skatole (not sensitive, sensitive) were included as fixed factors. Consumer within a country was considered to be a random effect. Two-way interactions between sensitivity, country and type of boar were removed because they were not significant. For flavour preference, skatole sensitivity was removed (P>0.05) and for differences in overall liking (P>0.05), sequence was removed from the model. 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 not relevant 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:

Odour preference Sensitive (%) = -1.60 + 0.45\*LnA -0.49\*LnS +0.41\*LnS\*LnA

Flavour preference Sensitive (%) =-1.87 + 0.76\*LnA -0.55\*LnS +0.52\*LnS\*LnA

Odour preference All (%) = -1.32 + 0.15\*LnA -0.48\*LnS +0.14\*LnS\*LnA

Flavour preference All (%) = -1.45 + 0.26\*LnA -0.51\*LnS +0.18\*LnS\*LnA

with LnA and LnS as the natural logarithm of androstenone and skatole content, respectively.

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.

### 3 Results

### 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

- 253 correlated well (P<0.05) with this sensory profile, with r=0.95 for PC1 and r=0.60 for PC2.
- 254 Correlations with androstenone were low and non-significant.
- 255 [Fig 1]

# 3.2 Factors influencing consumers' acceptance

## 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 on the scale from 1 to 9. For the boar meat patties served in the second to fifth pair, average liking scores varied from 4.9 to 6.2 (Table 1). The average liking score of the castrate meat patty served in these pairs varied between 6.6 and 6.9, with a higher score for the castrate meat patty in those pairs where the boar meat patty was liked less. Consumer liking score decreased with increasing skatole level (r=-0.62, P<0.05), whereas the correlation with androstenone was 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 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]

## 3.2.2 Effect of skatole and androstenone sensitivity

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

287 [Table 3]

288 [Fig 3]

## 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.

## 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. For androstenone sensitive consumers, preference also decreased with increasing androstenone level if skatole levels were low (Table 4). As the effect of skatole and androstenone was gradual, no clear thresholds for androstenone and skatole could be defined in this study. The effect of skatole and androstenone level, as well as the effect of androstenone sensitivity, is further evaluated and presented in a map illustrating the reduction in preference with increasing skatole 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-sensitive consumers minus 50%.

318 [Table 4]

319 [Table 5]

### 4 Discussion

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# 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 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 in which consumers indicate their preference of different test samples compared to a fixed reference sample. Indeed, if threshold values for boar taint are studied, it is relevant to know at 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 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 affected by the previously served samples. 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).

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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 with low skatole levels. The same association was found for the sensory profile. These results reflect the importance of skatole in the evaluation of the boar taint attributes as already observed in previous studies (AnnorFrempong, Nute, Whittington, & Wood, 1997; Dijksterhuis et al., 2000; Hansson, Lundström, Fjelknermodig, & Persson, 1980; Meier-Dinkel, Gertheiss, Muller, Wesoly, & Mörlein, 2015), and especially for products served warm (de Kock, Heinze, Potgieter, Dijksterhuis, & Minnaar, 2001; Diestre, Oliver, Gispert, Arpa, & Arnau, 1990). The 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 µg/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 µg/g) (Bonneau & Chevillon, 2012; Bonneau et al., 2000; Lunde et al.; Meier-Dinkel et al., 2013) whereas the range of skatole levels (0.10 to 0.40 µg/g) in the patties explored concentration ranges far above the skatole thresholds found in the literature (0.15 and 0.25 µg/g) (Bonneau et al., 2000; Lunde et al., 2010; Lundström et al., 2009). Based on recent literature (> 2010) the proportion of pigs with androstenone  $> 2.0 \mu g/g$  and skatole  $> 0.4 \mu g/g$  was 11 and 2% (n=119) (Mörlein, Lungershausen, Steinke, Sharifi, & Knorr, 2012); 5 and 3% (n=1031) (Mörlein et al., 2016); 18 and 3% (n=575), 12 and 3% (n=488) (IFIP, unpublished), and 11 and 2% (n=53) (Aluwé et al., 2013). The range of skatole levels considered in this study sufficiently covered the range around the consumer detection threshold values proposed in the literature and the range present in entire male pigs. The range of androstenone levels considered in this study only covered the lower spectrum of threshold values and did not cover the range of higher androstenone values common in entire male pigs. While skatole was more important compared to androstenone in this study, results can be different if higher androstenone levels were included. Further research using the same methodology and exploring the higher ranges of androstenone concentrations as in the present study will be needed.

## 4.2 Effect of androstenone and skatole sensitivity

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

androstenone sensitivity has been shown for women than for men (Mörlein et al., 2015; Weiler et al., 2000), these relatively low numbers for sensitivity were not expected, but can probably be attributed to differences in methodology (e.g. criteria of all three triangles being correctly discriminated). It can be assumed that the concentrations spiked on the paper strips used to evaluate sensitivity were sufficiently high (20  $\mu$ l of 5  $\mu$ g/g androstenone and 1  $\mu$ g/g skatole) to be detected. In a study with trained panellists using the same methodology with spiked paper strips, the odour thresholds found were 0.24  $\mu$ g/g for androstenone and 0.18  $\mu$ g/g for skatole (Heyrman et al. 2016). In another study using non-trained assessors and odorants diluted in oil, the odour threshold was comparable (0.21  $\mu$ g/g for androstenone and 0.10  $\mu$ g/g for skatole) (Font i Furnols, Guerrero, Serra, Rius, & Oliver, 2000). Despite the great pains taken to supply 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 well enough at the end of the strips where the solution was spiked, or consumer comprehension of the triangular test task. Either or both could result in a lower number of sensitive consumers than expected. In future studies, further effort should be done to standardise, optimise and simplify this methodology to enable a better comparison between studies.

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# 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 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 of sequence: boar meat patties served in the 2<sup>nd</sup> and 3<sup>rd</sup> pair showed a higher odour preference than those served in the 4<sup>th</sup> and 5<sup>th</sup> pair. This may indicate that the consumers become more aware of boar taint after evaluating several patties, which makes it easier for them to differentiate boar meat patties from castrate meat patties. The study of Mörlein et al. (2015) showed no effect of repeated exposure when evaluating boar meat samples. Their experimental set-up was not comparable, however, as their test was performed with some delay between the two sensory tests and at home versus in a sensory lab. In accordance with our results, Heyrman et al. (2015) showed that assessors who were familiar with boar taint (by previous exposure with smell strips or tainted samples, but otherwise untrained) were better able to detect fat samples with boar taint than assessors without prior experience. The effect of position and sequence highlights the importance of a well-designed serving order within and across the pairs to overcome these potential effects.

# 4.4 Effect of boar taint compound level and androstenone sensitivity

Consumer preference and liking of the boar meat patty gradually declined with increasing 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 to the maps reflecting the reduction in consumer preference per level of boar taint (for those levels included in this test). For example, if a stakeholder is willing to take the risk of a reduction in flavour preference of 10% compared to the reference sample population, androstenone levels up to 2.07  $\mu$ g/g in back fat are acceptable if skatole levels are low ( $\leq$ 0.10  $\mu$ g/g), while skatole levels in back fat higher than 0.10  $\mu$ g/g are not acceptable at any androstenone level. However, these levels will imply a reduction in preference of up to 22% for the androstenone sensitive consumers, or in other words, 72% of the sensitive consumers will prefer the reference sample. In line with previous studies, results indicated that the interaction of androstenone and skatole needs to be taken into account (Mörlein et al., 2016). At low skatole levels, an increase of 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 0.18  $\mu$ g/g.

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.

# 5 Conclusion

Consumer preference of the boar meat patty served pair-wise with a castrate meat patty decreased with increasing skatole content for the boar meat patties made from carcasses with back fat concentrations of skatole varying from 0.10 to  $0.40 \,\mu\text{g/g}$  fat tissue and of androstenone from 0.47 to  $2.00 \,\mu\text{g/g}$  fat tissue. At low skatole content, the preference for the boar meat patties 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 preference/rejection threshold for androstenone and skatole, as liking or preference decreased gradually. Any sorting limit therefore depends on the risk for negative consumer reactions that stakeholders are willing to take. For this reason, a map presenting the reduction in preference for the boar meat patties with increasing level of androstenone and skatole compared to the reference sample was built based a general population weighted by the prevalence of sensitivity and only androstenone sensitive consumers. Further research is needed to finalise these maps for lower skatole and androstenone levels as well as higher androstenone levels and other meat products.

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**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* 

		Castrate							
	Ba	ck fat		Meat patty					
Sample code	S	A	S	A	Liking	Liking			
Boar meat patty	$(\mu g/g)$	(µg/g)	$(\mu g/g)$	$(\mu g/g)$	score <sup>1,2</sup>	score <sup>1,2</sup>			
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			
В3	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			
В7	0.40	0.47	0.06	0.13	4.9	6.9			
В8	0.39	2.00	0.08	0.85	5.2	6.6			

<sup>&</sup>lt;sup>1</sup> Scored on a scale from 1 (dislike extremely) to 9 (like extremely)

<sup>&</sup>lt;sup>2</sup> The number of observations for each pairwise comparison varied from n=224 to n=247

<sup>&</sup>lt;sup>3</sup> Served pairwise with boar meat patty B1 to B8

**Table 2** Demographic and pork consumption characteristics of the consumers participating in the sensory study in Denmark, France, Italy, Poland and overall (as % of the number of consumers)

	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	17 1	0.7	11.6	10.5	140
allergy	17.1	8.6	11.6	19.5	14.0

 Table 3 Percentage of consumers sensitive to androstenone and/or skatole by country and overall

		Andros			
Country	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

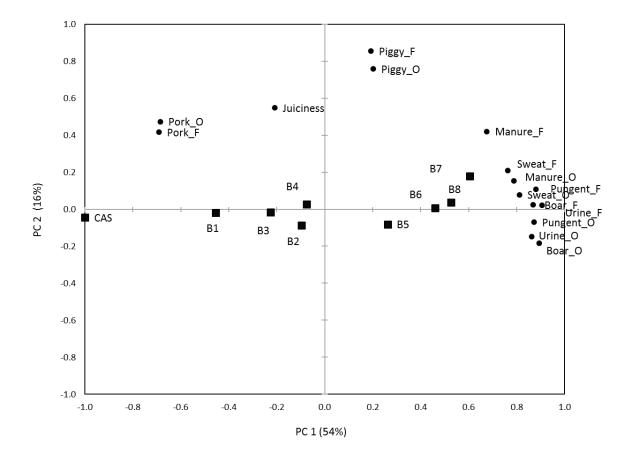
**Table 4** Logistic regression results for odour and flavour preferences of the boar meat patties served pairwise with a castrate meat patty based on the levels and the interaction of androstenone (A) and skatole (S) of the boar meat patty and according to the consumers' sensitivity to androstenone

Odour										
Effect	Estimate	s.e.	Pr >  t	Confidence limit (95%)		Estimate	s.e.	Pr >  t	Conf	idence
						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

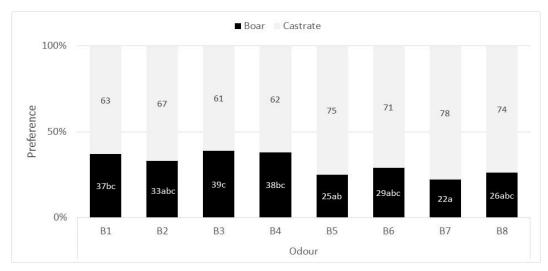
**Table 5.** Reduction in consumers' preferences (%) compared to a 50% chance of preference for odour and flavour of the boar meat patties over the castrate meat patties depending on their level of androstenone (AND) and skatole (SKA) for all consumers based on 34% androstenone sensitivity and considering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

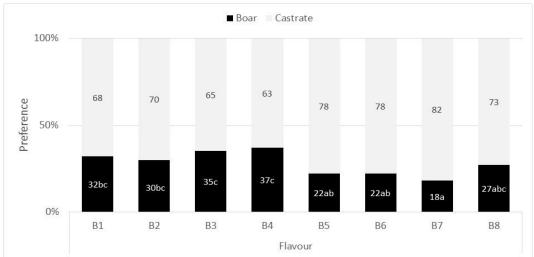
		Reduction in consumers preference (%)									
		Overall population					Sens	itive co	onsum	ers	
Odour		SKA (ppm)					SKA (ppm)				
	AND (ppm)	0.10	0.18	0.26	0.34	0.42	0.10	0.18	0.26	0.34	0.42
	0.47	-2	-11	-16	-19	-21	-3	-14	-20	-25	-27
	0.87	-5	-12	-16	-19	-21	-10	-17	-22	-25	-26
	1.27	-6	-13	-16	-19	-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

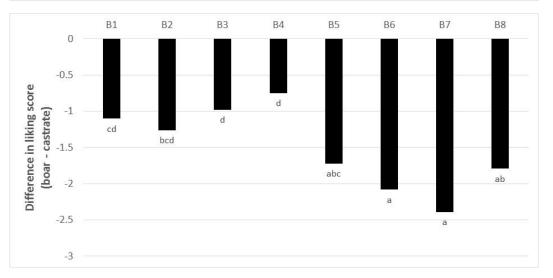
# 626 Figures



**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, 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 respectively.

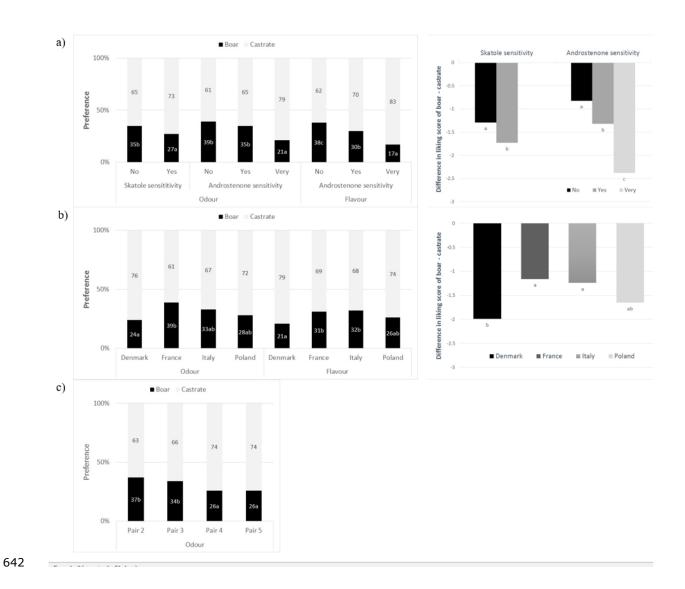






**Fig 2.** Consumers' preferences of the boar and the castrate meat patties in the pair for a) odour and b) flavour and c) difference in liking score (boar – castrate) according to type of boar meat patty (B1-B8) with skatole levels of 0.12, 0.10, 0.15, 0.15, 0.28, 0.33, 0.40, 0.39  $\mu$ g/g and androstenone levels of 0.48, 2.00, 0.90, 1.48, 0.75, 1.54, 0.47, 2.00 for B1 to B8 respectively.

<sup>abc</sup> Different letter indicates significant differences per reported variable (P<0.05).



**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).

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