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Assessing boar taint in Portuguese pork: A small-scale study of prevalence and classification via established detection thresholds

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Abstract

Aim of study: To assess the boar taint prevalence in the Portuguese pork industry through an observational study model by measuring skatole and androstenone levels in fat and to compare it with the estimated thresholds for these compounds among Portuguese consumers.

Area of study: Portugal.

Material and methods: Adipose tissue samples from 140 animals (102 entire males and 38 females) were collected in three Portuguese abattoirs for boar taint quantification. Cut-off limits were determined using best-estimate thresholds (BET) for skatole and androstenone.

Main results: Boar taint quantification for the 140 animals revealed levels of 36.0±4.7 ng/g of skatole and 64.5±21.3 ng/g of androstenone, and values were significantly higher in males. Group BET values were 35.4 ng/g and 566.3 ng/g for skatole and androstenone, respectively. BET values were exceeded in 28.8% of the samples for skatole and 0.7% for androstenone.

Research highlights: The investigation revealed a generally low level of boar taint in the samples of this small-scale study on skatole and androstenone prevalence in Portugal's pork supply chain. However, occasionally a significantly elevated boar taint levels suggest that relying solely on slaughtering sexually immature males might not fully resolve this issue.

Additional keywords: androstenone; skatole; slaughterhouses; sensory limits, pork.

Abbreviations used: 3-AFC (Three-Alternative Forced-Choice test), BET (Best-Estimated Thresholds), HPLC (High-Performance Liquid Chromatography), LoD (Limit of Detection).

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Introduction

The castration of male piglets, a longstanding and widespread practice in most countries, aims to prevent the occurrence of boar taint in the meat of certain male pigs (Bonneau & Weiler, 2019). However, the practice has attracted criticism and has been identified as an animal welfare issue (Sodring et al., 2020). Boar taint is an unpleasant odour found in the carcasses of entire males (EFSA, 2004; Fredriksen et al., 2011) caused mainly by the accumulation of androstenone and skatole (Claus et al., 1994), and commonly found in the meat of approximately 5 to 10% of entire male pigs (Baek et al., 1997; Aluwe et al., 2020). The accumulation of these compounds in the adipose tissue during pubertal development of growing animals leads to off-odours and off-flavours in pork, creating a negative perception of pork quality among consumers (Bonneau & Weiler, 2019).

In prominent pig-producing nations like Germany, France, and Denmark, approximately 75% to 93% of commercial male piglets undergo surgical castration (Van Ferneij, 2022). In other countries, most of the male pigs are raised without undergoing castration (Spain, 80%; Portugal, 85%; Ireland, 100%; United Kingdom, 98%), and are slaughtered before reaching puberty, with around five months of age and 100 kg of body weight. (De Briyne et al., 2016). This practice is seen as the most sustainable in reducing androstenone levels, by feeding rapid-growth diets allowing entire males to reach market weight before becoming sexually mature (Squires et al., 2020). However, there is the risk of some animals reaching puberty earlier and tainting pork in consequence.

The concentrations of skatole and androstenone vary between individuals, with a dispersion caused by several factors such as sex, age, feed, and genotype, among others (Bonneau & Weiler, 2019; Squires et al., 2020). If the concentrations of these compounds are small, the average consumer will not detect the off-odour. But this aspect is still under debate in the literature, with authors proposing different limit values for consumers not being able to detect boar taint (Lundstrom et al., 2009; Prusa et al., 2011; Bonneau & Chevillon, 2012; Borrisser-Pairo et al., 2016; Verplanken et al., 2017; Aluwe et al., 2018; Christensen et al., 2019). These limits can be defined by the sensory detection thresholds. The detection threshold is the lowest concentration of a substance in a medium relating to the lowest perception at which a stimulus is detected (ASTM:E679-04, 2011); however, sensory thresholds may not be generalised to cover different groups of people over extended periods of time (Annor-Frempong et al., 1997).

The objectives of this study were: a) to gain insights into the occurrence of boar taint within the Portuguese pork production industry by analysing levels of skatole and androstenone in the fat; b) to estimate thresholds for skatole and androstenone among Portuguese consumers. With these results, it was also intended to determine if a significant portion of samples exhibit off-odours by cross linking collected boar taint data with determined perception thresholds.

Material and methods

Sample collection

The sample collection study was conducted along the lines of an observational study, wherein genetic crosses, average weight at slaughter, and feeding regimes of the animals were not considered. Consequently, the sampling approach adhered to a cluster sample methodology, wherein adipose tissue samples were randomly gathered from various animals across different origins (organised by lots) from three major abattoirs in the northern region of Portugal. The selection of carcasses for the collection was conducted randomly, with a deliberate effort to include more males in the sample, maintaining a ratio of two males for every female. A total of 140 samples of subcutaneous fat were collected between August 2021 and April 2022. The adipose tissue was collected from the dorsal neck region (commonly referred to as backfat) after slaughtering in the refrigeration chambers where carcasses underwent temperature stabilisation. A sample identification code was assigned to each piece of adipose tissue, allowing the animals' sex and abattoir tracking. Samples were individually labelled, transported at 5 °C to the laboratory, where they were vacuum packed and stored at −20 °C.

Sample preparation

Fat extraction from adipose tissue involved microwave heating of thinly sliced backfat in glass test tubes for 1 minute at 800 W. About 1 mL of water-free fat was accurately measured and mixed with 1.0 mL of 95% methanol. This mixture underwent 30 s of vigorous vortexing and a 10 min ultrasonic bath at 40 ºC. Samples were centrifuged at 1100 g for 15 min and then cooled in an ice-water bath to solidify the fat. After solidification, the resulting supernatant was collected in 1.5 mL Eppendorf tubes. These tubes were stored at either 4 ºC or -20 ºC if not analysed on the same day.

Skatole and androstenone analysis

Skatole and androstenone content were determined by high performance liquid chromatography (HPLC), according to the method described by Pereira-Pinto et al. (2024). The reference standards androstenone (5α-androst-16-ene-3-one, CAS number 18339-16-7) and skatole (3-methylindole, CAS number 83-34-1) were obtained from Sigma Aldrich (St. Louis, MO, USA). Reagents were obtained from VWR International (Merck, Darmstadt, Germany) and were of analytical grade or HPLC grade. Dansylhydrazine and boron trifluoride were obtained from

Sigma Aldrich (St. Louis, MO, USA). Demineralised water was treated in a Milli-Q Plus water purification system from Millipore (Bedford, MA, USA). Prior to injection, the derivatization process was carried out manually at room temperature. This involved the sequential addition of 75 µL of dansylhydrazine (dissolved in methanol at 0.1% v/v), 50 µL of deionized water, and 40 µL of BF3 to a 500 µL sample within an Eppendorf tube. After shaking the mixture for 10 s, it was allowed to stand for 5 min before injection. An HPLC system (Thermo Scientific Dionex UltiMate 3000, Waltham, MA, USA) was employed, featuring a quaternary pump, a column oven, a fluorescence detector, and a Kromasil 100-5-C18 250 × 4.6 mm 5 µm column (AkzoNobel, Bohus, Sweden), operating at 40 ºC. The mobile phase composition included the following eluents: (A) 0.1% acetic acid (v/v), (B) acetonitrile, (C) tetrahydrofuran, and (D) 95% methanol (v/v). The gradient profile used was as follows: (A) acetic acid 0,1% (v/v), (B) acetonitrile; (C) tetrahydrofuran, (D) 95% methanol (v/v), with the following gradient profile: 0.0-5.0 min: 45% A, 55% B; 5.0-6.0 min: 40% A, 55% B, 5% C; 6.0-6.1 min: 20% A, 30% B, 30% C, 20% D; 6.1-12.0 min: 40% B, 40% C, 20% D; 12.0-12.1 min: 45% A, 55% B; 12.1-13.0 min: 45% A, 55% B. Fluorescence detection involved excitation at 285 nm and emission at 340 nm for the initial 6 min, followed by excitation at 346 nm and emission at 521 nm from 6.1 to 13 min. A 20 µL sample was injected in all experiments, and chromatograms and data integration were managed using Thermo Scientific Dionex Chromeleon version 7.2 SR5 from Thermo Scientific, Waltham, MA, USA. Limits of detection (LoD) were 16.0 and 1.5 ng/g for androstenone and skatole, respectively. Recovery values were 102.8% for androstenone and 99.7% for skatole.

Skatole and androstenone odour thresholds determination

The skatole and androstenone odour thresholds were determined using the Three-Alternative Forced-Choice test (3-AFC), following the guidelines ASTM E679-04. A total of 12 panellists (10 women and 2 men, aged between 23 and 61 years old) with prior experience in sensory analysis of meat and meat products were recruited within the technical and teaching staff receiving basic training on the discrimination and recognition of skatole and androstenone. Samples were prepared in a liquid vaseline medium with varying concentrations of skatole and androstenone (Sigma Aldrich, St. Louis, MO, USA), and 20 mL of each solution were placed in 50 mL amber glass screw-top flasks and maintained in a water bath at 60±5 ºC. Six ascending concentrations of skatole (25, 50, 100, 200, 400 and 800 ng/g) and six ascending concentrations of androstenone (150, 300, 600, 1200, 2400, 4800 ng/g) were used. The concentrations were selected following the study by Annor-Frempong et al. (1997) and after previous sensitivity tests with the panellists. Each assessor was seated separately in well-ventilated booths and received a single set of blind-coded samples using the 3-AFC method on each occasion. These sets comprised the test sample being examined and two samples without the stimulus (skatole or androstenone). The sample presentation among panellists and within each set was randomised. Assessors had the freedom to smell the samples repeatedly until they reached a conclusion regarding which sample stood out as distinct within the set. The computations for the best-estimated threshold (BET) followed the guidelines outlined in ASTM E679-04: the BET concentration for each panellist is determined as the geometric average of the concentration at which the most recent misjudgement occurred and the succeeding higher concentration. The panel threshold is established as the geometric mean of the individual panellists' BET values. In instances where the panellist's response was accurate at the lowest given concentration, the computation involves the geometric mean between that minimum concentration and half of its value.

Data analysis

Skatole and androstenone levels in fat means, median and interquartile ranges were determined. Furthermore, a confidence interval was computed at a 95% confidence level, leading to a significance threshold set at $p < 0.05$. The statistical analysis was performed using TIBCO® Statistica®, v.14.0.0, TIBCO Software Inc, Palo Alto, CA, USA.

Results

The skatole and androstenone levels in fat are shown in Table 1. A total of 140 samples were assessed and categorised based on the sampling location (abattoirs A, B, and C) and gender. In this survey, 140 pigs (100%) had measurements of skatole above the limit of detection (LoD), and 72 pigs (51.4%) had androstenone below the LoD. The peak concentrations detected were 169 ng/g for skatole and 1215 ng/g for androstenone, respectively.

Mean and confidence interval (95%) for skatole concentration for all samples was 36.0 ± 4.7 ng per gram of liquid fat. The average concentration of androstenone in all the 140 samples was 64.5 ± 21.3 ng per gram of liquid fat.

Skatole levels were significantly different ($p<0.05$) between males and females (40.3 ± 5.9 ng/g and 24.7 ± 5.4 ng/g respectively). As expected, there was a substantial discrepancy in the androstenone values obtained between males and females (84.1 ± 28.4 ng/g and 11.7 ± 0.7 ng/g respectively). In fact, the median value for females aligns with the calculated value for cases falling below the detection limit (Fig. 1).

Discrepancies were observed in individual odour thresholds, with detection limits ranging from 17.7 to 141 ng/g for skatole (Table 2) and 106.1 to 3394.1 ng/g for androstenone (Table 3). The group's BET for skatole was

Table 1. Skatole and androstenone mean, confidence interval, median and interquartile range.

Sex	n	Skatole (ng/g)			Androstenone (ng/g)		
		Mean ± CI	Median	IQR	Mean ± CI	Median	IQR
M	102	40.3 ± 5.9	31.4	[22.5; 47.6]	84.1 ± 28.4	42.6	[11.3; 89.8]
F	38	24.7 ± 5.4	20.6	[14.8; 31.5]	11.7 ± 0.7	11.3	[11.3; 11.3]
Total	140	36.0 ± 4.7	27.5	[19.9; 42.0]	64.5 ± 21.3	11.3	[11.3; 70.0]

M, males. F, females. CI, confidence interval (95%). IQR, interquartile range.

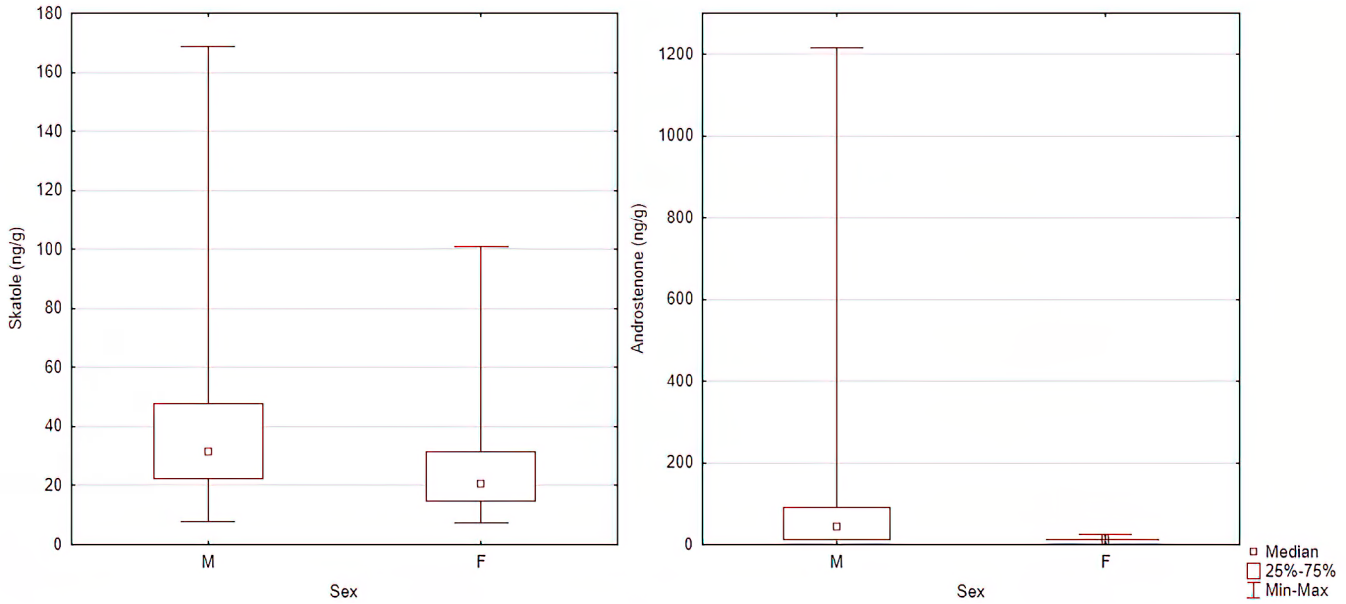

Figure 1. Boxplot representing skatole and androstenone content distribution according to gender (M, male; F, female).

Table 2. Results of the panellist's judgements and calculated best-estimate threshold (BET) for skatole.

Panellists	Judgements [1]						BET
	Concentrations of skatole (ng/g)						
	25	50	100	200	400	800	
1	+	+	+	+	+	+	17.7
2	0	+	+	+	+	+	35.4
3	+	+	+	+	+	+	17.7
4	+	0	+	+	+	+	70.7
5	0	0	+	+	+	+	70.7
6	0	+	+	+	+	+	35.4
7	+	0	0	+	+	+	141.4
8	+	+	+	+	+	+	17.7
9	0	0	+	+	+	+	70.7
10	+	+	+	+	+	+	17.7
11	+	+	+	+	+	+	17.7
12	0	+	+	+	+	+	35.4
Group BET = 35.4 ng/g							

[1] "0" indicates that the panellist selected the wrong sample of the set of three. "+" indicates that the panellist selected the correct sample.

determined to be 35.4 ng/g, while for androstenone, it was 566.3 ng/g.

Considering the 140 samples under study and using the determined BET group values, it was found that the determined skatole content exceeded the BET values in 46 samples (28.8%), while only one sample (0.7%) exceeded the BET value for androstenone (Fig. 2).

Discussion

This study is grounded in an observational study approach, utilising various carcasses sourced from the slaughterhouse. Its primary objective is to establish the prevalence of boar taint, although with a limited number of samples, without controlling for prior feeding programs,

Table 3. Results of the panellist's judgements and calculated best-estimate threshold (BET) for androstenone.

Panellists	Judgements [1]						BET
	Concentrations of androstenone (ng/g)						
	150	300	600	1200	2400	4800	
1	+	+	+	+	+	+	106.1
2	0	0	0	0	+	+	1697.1
3	+	+	+	+	+	+	106.1
4	0	+	0	0	+	+	1697.1
5	0	0	+	+	+	+	424.3
6	0	+	+	+	+	+	212.1
7	+	0	0	0	+	+	1697.1
8	0	+	+	0	+	+	1697.1
9	0	0	+	+	+	+	424.3
10	+	+	+	+	+	+	106.1
11	0	+	0	+	+	+	848.5
12	0	0	0	0	0	+	3394.1
Group BET = 566.3 ng/g							

[1] "0" indicates that the panellist selected the wrong sample of the set of three. "+" indicates that the panellist selected the correct sample.

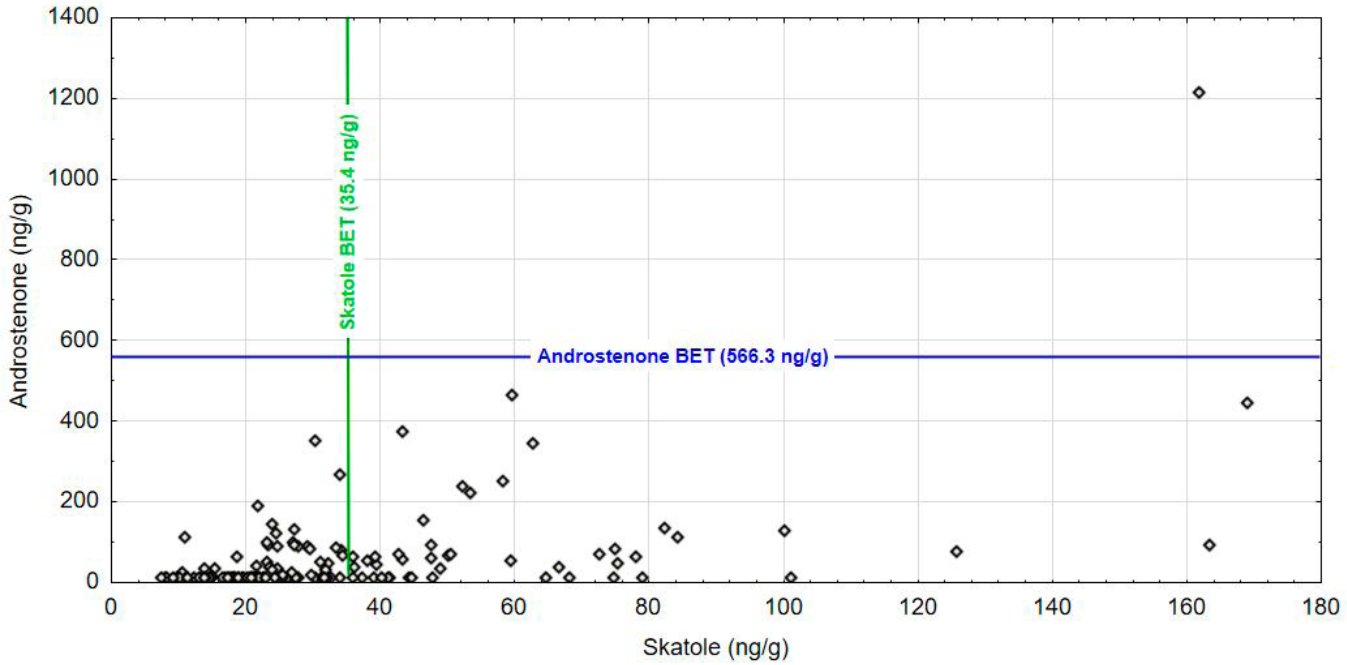

Figure 2. Distribution of skatole and androstenone levels (nanogram per gram of liquid fat) in 140 pigs from Portuguese abattoirs and odour thresholds determined in this study.

production systems during the growing-finishing period, or the specific crossbreeds included in the sample. Very low concentrations were not possible to measure due to limitations in chemical analysis techniques, and are typically referred to as being below the LoD. These data points are frequently censored in statistical assessments and replaced with a fixed value. According to Croghan & Egeghy (2003), opting to replace zero or LoD values with LoD divided by the square root of 2 (LoD/) stands out as the most appropriate choice when addressing measurements that fall below the LoD. This approach is favoured due to its tendency to yield a diminished bias. Given that the values acquired for skatole and androstenone do not conform to a normal distribution supplementary to the mean and standard deviation, this study also presented median values and interquartile ranges. The low sample size (140 adipose tissue samples collected from 140 animals) can also be addressed, representing a limitation of this study and indicating its small-scale nature. A study conducted by Prusa et al. (2011), aimed at defining the prevalence of skatole and androstenone in the fat of pigs within the United States. The findings revealed an average of 197.3 ng/g of skatole and 2363 ng/g in entire males, while female pigs exhibited levels of 34.1 ng/g for skatole and 120 ng/g for androstenone. When comparing these results to the ones obtained in the current study, significantly higher levels of boar taint were observed in the male pigs. The pigs processed in the Iberian market are usually slaughtered between five and six months old, which is insufficient time to reach sexual maturity. In contrast, the pigs examined in the United States were sourced from farms and had attained full maturity, hence the expected presence of elevated quantities of skatole and androstenone. Concerning the skatole values observed in female pigs, they seem to be closely aligned with the results from our study. However, it is noteworthy that the androstenone levels in the females appear unusually elevated. In a study by Walstra et al. (1999), skatole and androstenone levels were assessed in male and female pigs across six European countries (Spain, France, Netherlands, United Kingdom, Denmark and Sweden). The results from this study indicated higher values when compared to the results obtained in our study. In average skatole and androstenone values were reported to be 70 ng/g and 30 ng/g, respectively. For entire males, the skatole averages ranged from 100 ng/g to 170 ng/g, and androstenone levels varied between 800 ng/g and 1270 ng/g. Borrisser-Pairo et al. (2016) published a study examining the occurrence of boar taint in commercial pigs from Spain. They specifically included entire males with comparable ages and weights to those in our study sample. In the study, involving 903 animals, the average skatole level in the fat was 40 ng/g, identical to the values found in the males analysed in our study. However, concerning androstenone, the overall values reported by this author were 200 ng/g, higher than those detected in 102 males examined in this study. Assessing boar taint in adipose tissue, melted fat, or pure fat may yield disparate values (with lower values typically in adipose tissue, attributed to the presence of additional constituents). This disparity complicates direct comparison of values found in literature and underscores the importance of harmonising boar taint detection methods, since additional validation of current methods and standardisation of methodologies are required to accurately quantify boar taint compounds (Haugen et al., 2012). The presence of androstenone doesn't seem to be limited exclusively to entire males. It is expected that, androstenone should only be present in males, but there are reports suggesting its presence in females as well, as noted by van Oeckel et al. (1996) and Prusa et al. (2011). This phenomenon may be attributed to the conversion of progesterone into androstadienone and subsequently into androstenone (Robic et al., 2014). Also, while skatole levels are typically higher in intact male pigs, it's worth noting that skatole can also be found in barrows and females, albeit in smaller quantities (Aldal et al., 2005). The primary reason for the elevated skatole levels in intact male pigs compared to barrows or gilts is the reduced hepatic degradation of skatole. This reduction is attributed to the inhibition of hepatic metabolism by androstenone, testosterone, or 17β-estradiol (Zamaratskaia & Squires, 2009; Bonneau & Weiler, 2019). Additionally, environmental conditions and housing facilities can play a role in skatole levels, since it can be absorbed through the skin via contact with faeces or through the lungs by inhalation (Thomsen et al., 2015). Moreover, the dietary composition plays a crucial role in regulating intestinal skatole synthesis. Adding fermentable carbohydrates can reduce skatole levels by changing gut microflora activity and composition, leading to less L-tryptophan availability in the colon and decreased intestinal lumen apoptosis (Bee et al., 2020; Squires et al., 2020). In this study, it was also possible to observe a positive correlation between androstenone and skatole concentrations ($r=0.5382$, $p<0.01$). This correlation has also been reported by Walstra et al. (1999) and Borrisser-Pairo et al. (2016). Certain studies have reached the conclusion that the existence of androstenone hampers the metabolism of skatole (Doran et al., 2004; Panella-Riera et al., 2008), potentially elucidating the observed positive correlation between these two compounds responsible for boar taint.

With the purpose of comparing and classifying the values obtained in the study of the prevalence of boar taint, the olfactory thresholds of skatole and androstenone were determined using Portuguese consumers. Sensory thresholds cannot be universally applied to diverse groups of people over extended periods, as their success in detecting specific substances in threshold tests depends on momentary individual sensitivity, which has been shown to change over time (Annor-Frempong et al., 1997). It is important to highlight that the optimal approach for establishing boar taint sensitivity thresholds would involve utilising pork fat free of androstenone and skatole initially instead of liquid vaseline, in order to imitate the meat odour as closely as possible, taking into consideration any potential interference it may introduce. Comparing this study group BET with a publication by Annor Frempong et

al. (1997), the calculated BET values were slightly lower, being 26 ng/g for skatole and 426 ng/g for androstenone. The lowest individual odour thresholds of this study were obtained by women. Worth notice is that females seem to exhibit a higher sensitivity to androstenone: a study carried out in Spain and Germany determined that 31% of the Spanish and 18% of German respondents to a survey revealed higher sensitivity to androstenone, and among these sensitive individuals, a notably larger proportion were women (Weiler et al., 2000). Roughly 50% of the adult human population cannot detect the characteristic scent when they inhale androstenone (Wysocki et al., 1989). This finding is corroborated by a recent investigation by Klyuchnikova et al. (2022), which evaluated the ability of 807 individuals residing in central Russia to perceive the odour of androstenone; it was found that 49% of these participants could be classified as potentially having a specific inability to detect this substance through smell. On the other hand, skatole is perceived by 99% of consumers (Weiler et al., 2000), thus explaining why panellists exhibit a higher level of proficiency in detecting odour.

Industrial procedures for quality control concerning meat classification according to the level of boar taint are usually based on consumer's perception threshold values. Thus, it is essential to set threshold values for androstenone, skatole, or boar taint levels, determining whether these carcasses should be categorised as having boar taint or not. This threshold should be determined considering consumer sensitivity, acceptability, and the prevalence of boar taint in the swine population (Aluwe et al., 2018; Font-i-Furnols et al., 2020). From the results, given the lowest BET, it can be inferred that panellists with higher olfactory sensitivity would be able to detect the odour of skatole in 114 of the tested samples (constituting 81.4%), while they would detect the odour of androstenone in 19 samples (equivalent to 13.6%). Nonetheless, it's important to note that factors such as panel training can significantly influence the odour detection thresholds by a substantial margin. Additionally, test conditions, such as the size of sniff bottles employed, can be relevant to the results and some how justifies the variability found on the published data (Annor-Frempong et al., 1997). The thresholds for evaluating boar taint, frequently discussed in the literature, exhibit variability. This divergence might arise from the fact that the current acceptability standards are mostly based on the concentrations of these odour compounds in uncooked meat (Annor-Frempong et al., 1997). Researchers have suggested a range between 500 and 1000 ng/g for androstenone as a potential limit (Prusa et al., 2011; Borrisser-Pairo et al., 2016). In cases where skatole is absent, some studies propose even higher limits, falling within the range of 2000 to 3000 ng/g (Bonneau & Chevillon, 2012). As for skatole, the acceptable limits can differ, with some sources suggesting a range of 100 to 200 ng/g (Prusa et al., 2011; Borrisser-Pairo et al., 2016). However, Aluwe et al. (2018) has reported that skatole values exceeding 100 ng/g are not considered acceptable, regardless of the androstenone level, probably caused by the synergistic relationship between androstenone and skatole when it comes to defining the intensity of odours (Annor-Frempong et al., 1997). Furthermore, Bonneau & Chevillon (2012) deems skatole values in the 200 to 250 ng/g range to be excessively high. The determination of any sorting threshold is contingent upon the tolerance for adverse consumer responses that stakeholders are prepared to accept. Hence, Aluwe et al. (2018) undertook a study to illustrate the decline in preference for boar meat patties as the levels of androstenone and skatole increased. The preference for boar meat patties diminished with higher skatole content. Additionally, for consumers sensitive to androstenone, preference decreased as androstenone levels increased, particularly when skatole levels were low. Moreover, Christensen et al. (2019) stated that the anticipated risks of aversion among an average consumer, a discerning consumer, and the general consumer population aligned with sorting thresholds averaging 250 ng/g of skatole and 4000 ng/g of androstenone.

In conclusion, a low level of boar taint was found in this study aiming at investigating the prevalence of skatole and androstenone in pork entering the food chain in Portugal. There were little worth notice number of samples classified as having high levels of boar taint (4.2% exceeded the threshold value of 100 ng/g for skatole, and 0.7% surpassed the value of 500 ng/g of androstenone), however 28.8% of the samples exceeded the estimated BET values for skatole, which underscores that the practice of slaughtering immature males alone may not be sufficient to resolve this issue. Additionally, high skatole levels in females may indicate the possibility of skatole resorption, possibly stemming from inadequate hygiene management. An inherent limitation of this study lies in its observational study design, lacking control over preceding factors such as diet, production system, or genetic crossbreeding. Nonetheless, and despite the limitation of a small sample size in this study, the knowledge about the percentage of carcasses with elevated levels of androstenone and skatole offers valuable and objective insights to the sector regarding boar taint, which directly impacts the sensory quality of pork. Nevertheless, it's essential to tailor the acceptable limits of these compounds to the particular sensitivity of a given population or culture. This adaptation can be achieved through sensory tests like the best-estimated threshold.

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Investigation. **Diana Barros:** Investigation. **Manuela Vaz-Velho:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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References

Aldal I, Andresen O, Egeli AK, Haugen JE, Grodum A, Fjetland O, et al., 2005 Levels of androstenone and skatole and the occurrence of boar taint in fat from young boars. Livest Prod Sci 95(1-2): 121-129. https://doi.org/10.1016/j.livprodsci.2004.12.010

Aluwe M, Aaslyng M, Backus G, Bonneau M, Chevillon P, Haugen JE, et al., 2018. Consumer acceptance of minced meat patties from boars in four European countries. Meat Sci 137: 235-243. https://doi.org/10.1016/j.meatsci.2017.11.034

Aluwe M, Heyrman, E, Almeida JM, Babol J, Battacone G, Citek J, et al., 2020. Exploratory survey on European consumer and stakeholder attitudes towards alternatives for surgical castration of piglets. Animals 10(10): 1758. https://doi.org/10.3390/ani10101758

Annor-Frempong IE, Nute GR, Whittington FW, Wood JD, 1997. The problem of taint in pork 1. Detection thresholds and odour profiles of androstenone and skatole in a model system Meat Sci 46(1): 45-55. https://doi.org/10.1016/S0309-1740(97)00003-X

ASTM:E679-04, 2011. Standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits. ASTM International.

Baek C, Hansen-Moller J, Friis C, Cornett C, Hansen SH, 1997. Identification of selected metabolites of skatole in plasma and urine from pigs. J Agr Food Chem 45(6): 2332-2340. https://doi.org/10.1021/jf9605862

Bee G, Quiniou N, Maribo H, Zamaratskaia G, Lawlor PG, 2020. Strategies to meet nutritional requirements and reduce boar taint in meat from entire male pigs and immunocastrates. Animals 10(11): 1950. https://doi.org/10.3390/ani10111950

Bonneau M, Chevillon P, 2012. Acceptability of entire male pork with various levels of androstenone and skatole by consumers according to their sensitivity to androstenone. Meat Sci 90(2): 330-337. https://doi.org/10.1016/j.meatsci.2011.07.019

Bonneau M, Weiler U, 2019. Pros and cons of alternatives to piglet castration: welfare, boar taint, and other meat quality traits. Animals 9(11): 884. https://doi.org/10.3390/ani9110884

Borrisser-Pairo F, Panella-Riera N, Zammerini D, Olivares A, Garrido MD, Martinez B, et al., 2016. Prevalence of boar taint in commercial pigs from Spanish farms. Meat Sci 111: 177-182. https://doi.org/10.1016/j.meatsci.2015.10.001

Christensen RH, Nielsen DB, Aaslyng MD, 2019. Estimating the risk of dislike: An industry tool for setting sorting limits for boar taint compounds. Food Qual Prefer 71: 209-216. https://doi.org/10.1016/j.foodqual.2018.07.008

Claus R, Weiler U, Herzog A, 1994. Physiological-aspects of androstenone and skatole formation in the boar - a review with experimental-data. Meat Sci 38(2): 289-305. https://doi.org/10.1016/0309-1740(94)90118-X

Croghan W, Egeghy PP, 2003. Methods of dealing with values below the limit of detection using SAS Carry. Proc 2003 SouthEast SAS Users Group Conf; Sept 22-24; St. Petersburg, FL. Cary, NC: SAS Users Group, 1-4.

De Briyne N, Berg C, Blaha T, Temple D, 2016. Pig castration: will the EU manage to ban pig castration by 2018? Porc Health Manag 2: 29. https://doi.org/10.1186/s40813-016-0046-x

Doran E, Whittington FA, Wood JD, McGivan JD, 2004. Characterisation of androstenone metabolism in pig liver microsomes. Chem Biol Interact 147(2): 141-149. https://doi.org/10.1016/j.cbi.2003.12.002

EFSA, 2004. Welfare aspects of the castration of piglets. The EFSA Journal 91.

Font-i-Furnols M, Martin-Bernal R, Aluwe M, Bonneau M, Haugen JE, Moerlein D, et al., 2020. Feasibility of on/at line methods to determine boar taint and boar taint compounds: an overview. Animals 10(10): 1886. https://doi.org/10.3390/ani10101886

Fredriksen B, Johnsen AMS, Skuterud E, 2011. Consumer attitudes towards castration of piglets and alternatives to surgical castration. Res Vet Sci 90(2): 352-357. https://doi.org/10.1016/j.rvsc.2010.06.018

Haugen JE, Brunius C, Zamaratskaia G, 2012. Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods. Meat Sci 90(1): 9-19. https://doi.org/10.1016/j.meatsci.2011.07.005

Klyuchnikova MA, Kvasha I, Laktionova T, Voznessenskaya VV, 2022. Olfactory perception of 5 alpha-androst-16-en-3-one: Data obtained in the residents of central Russia. Data Br 45: 108704. https://doi.org/10.1016/j.dib.2022.108704

Lundstrom K, Matthews KR, Haugen JE, 2009. Pig meat quality from entire males. Animal 3(11): 1497-1507. https://doi.org/10.1017/S1751731109990693

Panella-Riera N, Moe M, Grindflek E, Oliver MA, Wood JD, Doran O, 2008. Effect of sex steroids on expression of sulfotransferase 2B1 immunoreactive protein in primary cultured porcine hepatocytes. Livest Sci 118(3): 223-230. https://doi.org/10.1016/j.livsci.2008.01.021

Pereira-Pinto R, Barros M, Vaz-Velho M, Mata F, Pires P, 2024. Enhanced HPLC method for boar taint quantification. ChemistryOpen e202300283. https://doi.org/10.1002/open.202300283

Prusa K, Nederveld H, Runnels PL, Li R, King VL, Crane JP, 2011. Prevalence and relationships of sensory taint, 5 alpha-

androstenone and skatole in fat and lean tissue from the loin (Longissimus dorsi) of barrows, gilts, sows, and boars from selected abattoirs in the United States. Meat Sci 88(1): 96-101. https://doi.org/10.1016/j.meatsci.2010.12.008

Robic A, Faraut T, Prunier A, 2014. Pathways and genes involved in steroid hormone metabolism in male pigs: A review and update. J Steroid Biochem Mol Bio 140: 44-55. https://doi.org/10.1016/j.jsbmb.2013.11.001

Sodring M, Nafstad O, Haseth TT, 2020. Change in Norwegian consumer attitudes towards piglet castration: increased emphasis on animal welfare. Acta Vet Scand 62(1): 22-22. https://doi.org/10.1186/s13028-020-00522-6

Squires EJ, Bone C, Cameron J, 2020. Pork production with entire males: directions for control of boar taint. Animals 10(9): 1665. https://doi.org/10.3390/ani10091665

Thomsen R, Edwards SA, Jensen BB, Rousing T, Sorensen JT, 2015. Effect of faecal soiling on skatole and androstenone occurrence in organic entire male pigs. Animal 9(9): 1587-1596. https://doi.org/10.1017/S1751731115000798

Van Ferneij JP, 2022. The pig castration situation in the European Union. pig333com - Professional Pigs Community Webpage. https://wwwpig333com/articles/the-pig-castration-situation-in-the-european-union_18100/ [March 11, 2022].

van Oeckel MJ, Casteels M, Warnants N, De Boever JL, van Renterghem R, Boucque CV, 1996. Production of entire males with Belgian Landrace and hybrid pigs: The incidence of sensory aberrations. J Anim Physiol Anim Nutr 76(2-3): 111-121. https://doi.org/10.1111/j.1439-0396.1996.tb00681.x

Verplanken K, Wauters J, Vercruysse V, Aluwe M, Vanhaecke L, 2017. Sensory evaluation of boar-taint-containing minced meat, dry-cured ham and dry fermented sausage by a trained expert panel and consumers Food Chem 233: 247-255. https://doi.org/10.1016/j.foodchem.2017.04.105

Walstra P, Claudi-Magnussen C, Chevillon P, Von Seth G, Diestre A, Matthews KR, et al., 1999. An international study on the importance of androstenone and skatole for boar taint: levels of androstenone and skatole by country and season. Livest Prod Sci 62(1): 15-28. https://doi.org/10.1016/S0301-6226(99)00054-8

Weiler U, Furnols MFI, Fischer K, Kemmer H, Oliver MA, Gispert M, et al., 2000. 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 54(3): 297-304. https://doi.org/10.1016/S0309-1740(99)00106-0

Wysocki CJ, Dorries KM, Beauchamp GK, 1989. Ability to perceive androstenone can be acquired by ostensibly anosmic people. P Nat Acad Sci USA 86(20): 7976-7978. https://doi.org/10.1073/pnas.86.20.7976

Zamaratskaia G, Squires EJ, 2009. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal 3(11): 1508-1521. https://doi.org/10.1017/S1751731108003674