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Can sensory boar taint levels be explained by fatty acid composition and emitted volatile organic compounds in addition to androstenone and skatole content?

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Abstract:

This study aimed at understanding which molecules were responsible for the differences existing in boar taint sensory evaluation. The latter was therefore linked to the results of skatole and androstenone chemical analyses, fatty acid composition and VOC profiles of heated backfat. This study confirmed that some discrepancy exists between chemical analysis and sensory evaluation of tainted backfats. Significant correlations between human nose scores and fatty acid composition were not revealed. Strong correlations between emissions and contents in skatole and androstenone were found. Oxidation products of polyunsaturated fatty acids, with fatty odor descriptors, were found to be more present in the VOC profiles of boar fat considered untainted through the human nose methodology. Weak coefficient of determination for partial least square regression indicates that other factors, yet unknown, are responsible for sensory evaluation outcomes. These findings hence support the idea that high human nose score is mainly due to boar taint compounds rather than general differences in VOC profiles.

Keywords: boar taint, androstenone, skatole, human nose, fatty acid, VOC

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1. Introduction

Surgical castration of piglets without anesthesia and analgesia is the most common practice to ensure the absence of boar taint (Fredriksen et al., 2009), a strong smell created by a variety of molecules released upon cooking of pork meat (Bonneau et al., 2000) and perceived as unpleasant by 14.3-41.0% of European pork consumers (Blanch et al., 2012). However, due to growing animal welfare concerns in the European Union, there have been some actions, such as the Noordwijk Declaration (2007), the Düsseldolf Declaration (2008) and the Brussels Declaration (2010), to reduce or eliminate this practice. As a result, Norway banned surgical castration without pain relief in 2002, Switzerland banned it in 2016, Sweden in 2016, Germany at the end of 2020 and lastly, France banned surgical castration without anesthesia at the end of 2021 (De Briyne et al., 2016; Lin-Schilstra & Ingenbleek, 2021). Currently, three viable alternatives exist: surgical castration with pain reliefs, immunocastration and finally raising entire males (Bonneau & Veiler, 2019). A main disadvantage of this last option remains that high levels of boar taint are found in 5-10% of the cases (Borrisser-Pairó et al., 2016) and its occurrence could be even higher depending on the pig's characteristics and environmental factors (Aluwé et al., 2020). On the other hand, although immunocastration has been found to be overall very efficient, some non-responders might still occur, with the potential accumulation of boar taint (Čandek-Potokar et al., 2017; Font-i-Furnols et al., 2012; Kress et al., 2020).

Being able to correctly discriminate tainted from untainted pigs is therefore a topic of current interest. Throughout the years, many methods have been developed with the aim of offering rapid, cheap and reliable boar taint detection for slaughterhouses and cutting plants. Amongst these techniques, laser diode thermal desorption-tandem mass spectrometry (LDTD-MS/MS) is a recently developed method that focuses on the specific detection and quantification of skatole and androstenone (Lund et al., 2021) and is currently being tested in Danish slaughterhouse (Burgeon, Debliquy, et al., 2021; Font-i-Furnols et al., 2020). These two molecules are well known to be the two major contributors to boar taint which give respectively a strong fecal and urine smell to pork meat (Patterson, 1968; Vold, 1970). Yet, only 50% of the variation in boar taint is due to the combination of skatole and androstenone (Hansson et al., 1980) and the importance of other compounds to boar taint has been pointed out in several studies on sensory evaluation (Font-i-Furnols et al., 2008; Mathur et al., 2012; Trautmann et al., 2016; Whittington et al., 2011).

A variety of molecules have therefore been suggested to potentially contribute to boar taint. The origin of each molecule and their contribution to the overall boar taint is variable. In fact, some are derived from skatole such as indole and 2-aminoacetophenone, some are related to androstenone, such as 3α-androstenol and 3β-androstenol and others have synthesis pathways which are less clear or

nonetheless not directly linked to skatole and androstenone. The latter include *p*-cresol, 4-ethylphenol found in boar preputial fluid and 1,4-dichlorobenzene found in boar fat, but which occurrence in boar fat could be due to external contamination (Brooks & Pearson, 1989; Fischer et al., 2014; Garcia-Regueiro & Diaz, 1989; Patterson, 1967; Solé & Regueiro, 2001; Watson & Patterson, 1982).

Many of the above-mentioned molecules are found in trace amounts in boar fat samples. Therefore, although they might have an unpleasant odor descriptor and a low odor threshold, which allows them to contribute to the overall boar taint, other compounds are found in much greater concentration in the headspace of heated fat, as pointed it out in a previous study by Burgeon, Markey, et al. (2021). Consequently, even without being directly related to the urine and fecal smell of boar taint, the latter still contribute to the overall smell perceived during sensory analysis. Many of these molecules, such as free fatty acids and aldehydes, originated from the oxidation of lipids starting at 70°C. The presence of such molecules in the headspace of heated fat is explained by the fact that skatole and androstenone are lipophilic molecules with low vapor pressure ($7.3 \times 10{-}4$ kPa and $1.3 \times 10{-}6$ kPa at 25 °C, respectively) and need to be heated at high temperatures to be released and detected by the human nose (Burgeon, Markey, et al., 2021).

Human nose detection (*i.e.* sensory analysis) remains a method of choice given its ease of implementation, low cost per analysis and satisfactory performances results. Additionally, it is to date the only method currently in use, which takes into account all of the volatile organic compounds (VOCs) constituting the complex smell of boar taint. This implies that this method could be the only one able to detect samples which are considered tainted, although chemical analysis would classify them as untainted given skatole and androstenone concentrations below rejection thresholds.

Research has already been performed on fatty acid composition of backfat from pigs of different sex, weight and breed (Font-i-Furnols et al., 2019; Raj et al., 2010). Research on fatty acid composition of backfat with various boar taint levels, and its relationship with skatole and androstenone content as well as sensory analysis has also been reported (Liu et al., 2017; Mörlein & Tholen, 2015). Lastly, the relationship between fatty acid composition and VOC emission of lard was studied, without bringing attention to skatole and androstenone content of the analyzed meat cut, as this was not the aim (Serra et al., 2014).

In this study, the authors consider all four aspects mentioned above: (i) fatty acid composition of boar fats with varying boar taint levels, (ii) sensory evaluation of boar taint, (iii) chemical levels of boar taint compounds, androstenone and skatole, and (iv) the VOCs emitted when heating backfat. Through the comprehension of the correlation between these variables and the human nose score attributed during sensory evaluation, this work aims to understand which factor, other than skatole and androstenone content could explain the human nose scores. In other words, this should clarify the

source of existing discrepancies between backfat classification based on skatole and androstenone content analysis and sensory evaluation.

2. Material and methods

2.1. Samples

Backfat samples from 30 boars were randomly selected from a sample of 106 boars at the slaughterhouse. These were sampled during different days and from animals reared under different production systems with different managing and feeding strategies. To ensure the presence of boar tainted carcasses, one trained panelist smelt the subcutaneous fat of the carcass online, close to the neck, after heating the fat with a gas-powered torch heated plate (human nose method). Carcasses classified as boar tainted were selected together with some untainted samples, to ensure enough variability. Approximately 30g of fat from the neck of the selected carcasses was collected and immediately frozen at -20ºC until further analysis (maximum storage time of 1 year). From all the samples, fatty acid composition and skatole and androstenone were chemically analyzed as described in section 2.3 and 2.4, respectively. The backfat samples were selected in order to fit into four categories based on their skatole and androstenone content and used in further analysis. Samples with low and high skatole content were distinguished using the threshold value of 0.2 µg/g liquid fat. This value has been used in other works (Bonneau et al., 2000; Burgeon et al., 2021) although skatole was measured in fat tissue, which provides lower values than those obtained in liquid fat (Haugen et al., 2012). Nevertheless, it was difficult to find enough samples with high levels of skatole (and low androstenone) within the pre-selected fat, confirming what was suggested by Zamaratskaia & Squires (2009). Low and medium androstenone content were separated by a threshold value of approximately 1.0 µg/g liquid fat, while medium and high androstenone concentrations were hence distinguished by a value of approximately 1.5 µg/g liquid fat. These thresholds have been chosen to ensure variability of androstenone levels, since the global samples were highly variable for this compound. According to this, the four groups created were: low skatole, low androstenone (LS/LA, n=8); low skatole, high androstenone (LS/HA, n=7); high skatole, medium androstenone (HS/MA, n=7); high skatole, high androstenone (HS/HA, n=8).

2.2. Sensory evaluation

Sensory evaluation of boar taint, also known as human nose, was performed, at line, by three trained panelists on the 30 selected carcasses. Each sample was evaluated in duplicate. Subcutaneous fat samples were heated with a soldering iron (Soldering iron station Analogue 58W, 150-450ºC, Basetech, Austria) at approximately 250ºC until the surface of the fat melted. Immediately, fat samples were smelt by the three trained panelists (three androstenone and skatole sensitive women between 40 and 55 years old) and classified according to a 4 points scale: 0: no boar taint; 1: weak boar taint; 2: moderate boar taint; 3: strong boar taint. Each sample was evaluated twice. The average

human nose score (HNS) was obtained and used for further analysis. The global panel performance, evaluated with fat samples with known levels of androstenone and skatole, was sensitivity= 0.74; specificity= 0.70; accuracy= 0.74. Individual panelist performance was characterized and the Positive Predictive Value (PPV) as well as the Negative Predictive Value (NPV) were calculated (Appendix A**)**. Detection thresholds evaluated using smell strips (Meier-Dinkel, Trautmann, et al., 2013) were 0.2 µg/g for androstenone and 0.05 µg/g for skatole.

2.3. Fatty acid composition

For the FA analysis, a portion of 10 g of frozen fat was thawed at 4ºC during 24 hours. Fat was ground with a commercial grinder, and FA were quantified as FAME (FA methyl ester) using 25 mL of sodium methylate and 30mL paratoluensulfonic acid for transesterification. The sample was methylated by incubation in a sand bath at 80 ºC for 50 min. For FAME solubilization 20 mL of heptane was used. An aliquot of 0.4 µL was introduced by split injection into a capillary column (60 m x ID 0.25 mm, Agilent HP88; 0.25-µm film thickness, Barcelona, Spain). Helium was the carrier gas at 1.5 mL/min. Column temperature was initially at 140ºC for 5 min, was increased by 4ºC/min to 240ºC and maintained for 20 min at this temperature. Individual FAME were identified by retention time with reference to Supelco® 37 Component FAME Mix (47885-U Sigma Chemical Co., St. Louis, MO). Data is expressed in mg/100 g of adipose tissue.

2.4. Skatole and androstenone quantification in backfat

Quantification of skatole and androstenone in backfat were performed by stable isotope dilution analysis – headspace solid-phase microextraction – gas chromatography/mass spectrometry (SIDA-HS-SPME-GC/MS) developed by Fischer et al. (2011). Results are expressed as µg/g of liquid fat.

Briefly, thawed and skinless backfat samples are diced and microwave heated for 2 min at 180W. After separation of connective tissue and liquid fat, 500 mg of fat was transferred into a 2 mL plastic vial and spiked with 250 ng of androstenone-d_3 and 50 ng of skatole-d_3 to achieve final concentrations of 500 ng/g androstenone-d_3 and 100 ng/g skatole-d_3. The sealed plastic vial was then shaken for 30s, stored for 10 min at 55°C and mixed again for 30 s in order to allow for equilibration of the standards. Extraction of the compounds of interest was then performed by adding 1 mL of methanol and then shaking 30s, heating for 10 min at 55°C and shaking again 30s. The samples were then centrifuged (10 min, 6500 rpm, -15°C). The methanolic supernatant was transferred into a 10 mL headspace vial and evaporated at 40°C by a gentle stream of air. Once dryness was achieved, the vial was sealed and placed in an autosampler device (Varian Combi Pal, Darmstadt, Germany), operating with a heated agitator and an SPME assembly. HS-SPME was carried out as follows: equilibration for 5 min at 100°C; extraction for 30 min at 100°C with a fused-silica fiber coated with 65 µm

poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) (Supelco, Bellefonte, PA); desorption for 20 min within the injector.

A GC-MS (GC-450 – MS-240 ion trap, Varian, Darmstadt, Germany) equipped with a Varian VF-5ms capillary column (30 m × 250 µm × 0.25 µm, Varian, Darmstadt, Germany) was used for the analyses. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The oven temperature program was set as follows: start at 50°C, hold for 3 min, then raise to 160°C at a rate of 10°C/min, followed by a raise of 5°C/min up to 240°C, hold for 1 min. Injection was performed at 270°C in splitless mode for 3 min, the split valve was then opened in a split ratio 1:100.

Mass spectrometry data was obtained with a full scan acquisition mode using electron impact ionization (EI). The peak area ratios of analyte and internal standard (IS) were later determined by displaying the specific mass fragments of each analyte and its corresponding IS in selected ion monitoring (SIM) mode. The selected mass traces (m/z) were as follows: skatole m/z 130, skatole-d_3 m/z 133 + 134 and androstenone m/z 257 + 272 and androstenone-d_3 m/z 260 + 275.

2.5. Analysis of VOCs generated through heating of backfat

The analysis of VOC profiles produced through the heating of boar backfat was performed following incubation of fat at 150°C and analysis by SPME-GC-MS according to the method described by Burgeon, Markey, et al. (2021).

Briefly, 2.5g of backfat was cut and cooled with liquid nitrogen. The sample was then ground for 5s with an analytical grinder (A11 basic analytical mill, IKA) and a 1.0g of sample was recovered in a vial and stored at -20°C until analysis. The next step of this method consisted in a 20 min incubation at 150°C in a heated agitator (Gerstel, Mülheim an der Ruhr, Germany). VOCs sampling was then achieved by exposing a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm) SPME fiber (Supelco, Darmstadt, Germany) to the headspace for 5 min. The vials were shaken at 250 rpm (agitator on/off time: 10 s/1 s) during incubation and extraction. Fiber desorption took place for 2 min. Injection was performed in splitless mode at 270°C. The fiber was left for 20 min at injection temperature for reconditioning.

A GC-MS (7890A-5975C, Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5 MS capillary column (30 m × 250 µm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA) was used for the analyses. Helium was used as a carrier gas at a flow rate of 1.2 mL/min. The oven temperature program was set as follows: start at 40°C, hold for 3 min, then raise to 300°C at a rate of 5°C/min, hold for 5 min at 300°C. The mass spectrometer was set to have a temperature of 230°C at the ion source and 150°C at the quadrupole. Mass spectrometry data was obtained with a SIM/SCAN acquisition mode. In SIM mode, the targeted ions were: m/z 130 for skatole and m/z 257, and 272 for androstenone. The peak area of these ions (expressed in atomic mass unit, amu) was integrated to

study the relationship between content and emissions as described later, ie. these peak areas constitute the "emission data".

In SCAN mode, mass spectra were scanned from 35 to 500 amu. Component identification was then performed by comparison of the obtained spectra with reference spectra from the NIST17 database (National Institute of Standards and Technology, Gaithersburg, USA). Additionally, experimental retention indices (RI) were calculated following the injection of a n-alkanes C8-C30 mixture (Sigma Aldrich, Darmstadt, Germany) under the same chromatographic conditions as those previously mentioned. This allowed the comparison of these RI with literature RIs obtained from the NIST Mass Spectrometry Data center.

Lastly, pure standards were injected for skatole (CAS n◦ 83-34-1, Sigma Aldrich) and androstenone (CAS n◦ 18339-16-7, Sigma Aldrich, Darmstadt, Germany) to ensure that the peaks were correctly identified.

The only difference to the method described in Burgeon, Markey, et al. (2021) is that the incubation temperature was fixed to 150°C and no internal standard was added for semi-quantification (of skatole and androstenone) as this was not the aim of the article.

2.6. Data analysis

Pearson correlation coefficients were determined between fatty acids and HNS, between HNS and androstenone and skatole content and between the skatole and androstenone emission and content data. Fatty acid compositions of the different chemical classification groups (Appendix B), were analyzed using one-way ANOVA (one fixed factor: taint group). Volatile organic compounds and fatty acid compositions of the different human nose (HN) classification categories were also analyzed using one-way ANOVA (one fixed factor: HN category). When the means were significantly different ($p<0.05$), a Tukey-Kramer comparison test was performed. Both Pearson correlation coefficients and ANOVA were established with Minitab 19 software (Minitab Inc., State College, PA, USA).

Principal components analyses were performed on fatty acid composition data and VOC data to detect existing trends between the different samples. A partial least square regression (PLSR) was used to develop a mathematical model trying to predict human nose score (HNS) by taking into account skatole and androstenone content, fatty acid content and VOC emission. A cross-validation was performed to choose the optimal number of PLS components. The number of components chosen for the model were those that yielded in the lowest cross-validated root mean square of prediction (RSMEP) and highest coefficient of determination (R^2). The variable importance in projection (VIP) scores were then analyzed. Both PCA and PLSR were conducted in R (R 4.0.2 software, R Development Core Team, Boston United States).

PCA individual plots were performed in R. All other graphs and tables were established on Excel (Microsoft Office 2016).

3. Results and discussion

3.1. Agreement between human nose evaluation and chemical analysis

As a reminder, based on the results obtained through chemical analysis, four groups were created: low skatole, low androstenone (LS/LA, n=8); low skatole, high androstenone (LS/HA, n=7); high skatole, medium androstenone (HS/MA, n=7); high skatole, high androstenone (HS/HA, n=8). The samples' taint was also assessed through sensory evaluation. The results obtained for both tests (chemical analysis and human nose evaluation) have been compared and are presented in Figure 1.

Figure 1. Color

It can be noticed from Figure 1 that almost all fat samples scored as untainted through the human nose method are found in the LS/LA part of the graph (6 out of the 7 with HNS=0). Similarly, most of the fats graded as highly tainted (3 out of the 4 fats with HNS=3) are found in the HS/HA region. In this class there are also 4 samples with HNS ≥2 and one fat with HNS=1. There seems to be positive correlation in this region between the two methods – when skatole and androstenone content increases, the HNS increases accordingly. These observations support the idea that the human nose is an overall good representation of the chemical analyses available.

The rest of the fat samples with intermediate HNS are found in analytical groups combining a high level of skatole or androstenone and a lower level of androstenone or skatole. Interestingly, only fats with HNS greater than 1.5 are found in the HS/MA region when most of the samples in the LS/HA region have HNS lower or equal to 1.5 (5 out of 7 fats found in this region). This result shows a greater influence of skatole level in the HNS which can also be seen when the average HNS by class group is studied (Appendix C). This is in agreement with the results presented by Mörlein et al. (2016).

The higher difficulty to evaluate androstenone than skatole in the boar taint perception carried out with trained panelist is in agreement with previous works (Aluwé et al., 2022; Dijksterhuis et al., 2000; Font et al., 2009; Lunde et al., 2010). In this regard, HNS is higher and similar in HS/HA and HS/MA group (2.38$\pm$0.69 and 2.36$\pm$0.48, respectively, both with high levels of skatole), is intermediate in the LS/HA class (1.29$\pm$0.95, with low levels of skatole) and is low in the LS/LA class (0.31$\pm$0.70; with low levels of skatole). Having high levels of androstenone classified differently with the HNS points out the difficulty of this sensory classification, especially when skatole is absent. This can increase the false negative if androstenone levels are high and skatole are low, which agrees with the higher false negative with high androstenone and low skatole levels reported by Meier-Dinkel et

al. (2015). This agrees with the higher correlation between HNS and skatole (0.68, P<0.001) compared to those between HNS and androstenone (0.37, P=0.042), that are in line with those reported by Mathur et al. (2012), 0.69 between HNS and skatole and 0.42 between HNS and androstenone. In general, these intermediate levels are more difficult to be detected with the sensory evaluation.

Several hypotheses could explain such difference between HNS of boar taint and chemical evaluation of androstenone and skatole. In fact, the first important difference is that HNS measures boar taint while the present chemical evaluation only quantified androstenone and skatole. Although these two compounds are the main responsible for boar taint, they are not the only ones (Rius & García-Regueiro, 2001). A general hypothesis which is valid for the observations made in the LS/HA group, is that regardless of whether androstenone is considered pleasant or unpleasant, its perception is related to the individual ability and detection threshold of the substance (Font-i-Furnols et al., 2003, 2016; Meier-Dinkel, Sharifi, et al., 2013). Secondly, some VOCs associated with the HS/MA group, which are absent in the VOC profiles of LS/HA, might possess unpleasant odor descriptors leading to higher HNS. Thirdly, interaction between androstenone and skatole (Aluwé et al., 2018; Font-i-Furnols et al., 2003; Garrido et al., 2016; Mörlein et al., 2016) can also influence the perception of boar taint. Lastly, high levels of androstenone can produce saturation of the panelist's nose, as experienced by the trained sensory panel. This temporal lack of perception of androstenone could be confused by inability to perceive androstenone or non-sensitivity towards this substance. It is further necessary to study the parameters that influence this saturation that are probably related to the individual conditions of the panelist, the resting time between samples, the concentration of androstenone of the sample (and maybe its interaction with other compounds such as skatole) and the matrix used for the evaluation (i.e. fat, smell strips). Moreover, this could explain why samples in the LS/HA group with androstenone content greater than 3 µg/g scored lower HNS compared to other samples in this category. However, when levels of skatole were also high (HS/HA group), it was possible to identify tainted samples by the HNS, probably due to the less saturation characteristic of skatole, as experienced by the trained sensory panelists. This might also indicate that probably HS/HA scores are mainly due to high skatole levels.

Similarly to what is observed here, Meier-Dinkel et al. (2015) pointed out in their study, that sensory panelists who were able to clearly identify samples with high skatole content as tainted, had trouble discriminating some samples with high androstenone from untainted backfat samples.

The confrontation of the results obtained for the chemical analysis and that of the sensory evaluation also shows and confirms a certain level of discordance between both methods. For instance, one sample with HNS = 1 (*i.e.* weak boar taint) is found in the HS/HA region. Similarly, some fats

considered tainted through sensory evaluation are found in the LS/LA region, although close to the threshold established.

A difference in the type of sample used for the two analyses could in part explain the discordance. In fact, the chemical analyses were performed on methanolic extracts of liquefied and tissue-free fat, while sensory analyses were done on native fat (Trautmann et al., 2014). The sensory analyses therefore allow a good representation of all interactions occurring within the fat, including between fatty acids constituting the majority of boar fat, and gives a complete vision of the VOCs emitted along with skatole and androstenone (Mathur et al., 2012; Meier-Dinkel et al., 2015; Trautmann et al., 2016; Whittington et al., 2011). Lastly, the presence of samples considered tainted through sensory evaluation in the LS/LA region, could be explained by the fact that human olfaction is largely variable with sensitivity ranging several orders of magnitude between individuals, with individuals having specific anosmia, some specific hyposmia (reduced olfactory acuity) and others specific hyperosmia (increased olfactory acuity) (Genva et al., 2019) which affects the obtained results at the detection threshold. Given that some people present anosmia to androstenone (Font-i-Furnols, 2012), the assessors that carried out the human nose evaluation were selected to be sensitive to androstenone and skatole, some might even present specific hyperosmia (detection threshold= 0.2 µg/g for androstenone and 0.05 µg/g for skatole) and hence grade LS/LA fats as tainted samples.

Moreover, only androstenone and skatole have been considered as chemical compounds. Androstenols and indoles, which are also related to boar taint, were not analyzed and they could help to better explain HNS scores across the whole set of samples.

To better understand part of the disagreement between the human nose evaluation and the chemical analysis, a deeper look into the fatty acid composition of the samples as well as the emission of VOCs by the heated fat were performed. In fact, boar fat is a highly complex matrix, firstly due to matrix effects occurring with the fatty acids impacting the release of boar taint compounds and secondly given the numerous other VOCs present, which interact and result in a complex smell which cannot be associated to a unique and constant odor descriptor (Haugen et al., 2012; Trautmann et al., 2014).

3.2. Understanding the attributed scores in the human nose evaluation

3.2.1. Fatty acid composition

Fatty acid composition was analyzed for each fat samples and is summarized in Table 1. For easier interpretation of the results, four categories of HN appreciation were created to represent the backfat samples: no (HNS = 0), weak (HNS= 0.5 and 1), moderate (HNS= 1.5 and 2), strong (HNS= 2.5 and 3) boar taint.

Table 1. Black and white

From this table it can be observed that the fatty acid profiles are made up of a majority of monounsaturated fatty acids (MUFA) followed by the saturated fatty acids (SFA) and lastly polyunsaturated fatty acids (PUFA) considerably lower than the two other categories.

MUFA and PUFA are predominated by two molecules. In fact, the MUFA make up approximately 46% of the total fatty acid profile, with C18:1n-9 cis constituting 41.90% to 42.83% of the profile by itself. Similarly, the PUFA category makes up approximately 16% of the profiles, and is itself made up of 12.43% to 15.83% of C18:2 n-6 cis. For SFA, two molecules are present in much greater quantities compared to the rest of the profile, *i.e.* C16:0 and C18:0 making up approximately 23% and 12.5% of each group.

The overall trends between the three categories and the major constituents of the profiles observed above have also been found in previous studies on pig carcasses *i.e.* higher SFA and lower PUFA are related to higher boar taint levels (Liu et al., 2017; Mörlein & Wicholen, 2015; Raj et al., 2010).

A principal component analysis was then performed exclusively with the fatty acid data of the backfats to highlight the diversity of fatty acid profiles between samples and link it to the evaluated HNS. From Figure 2, biplot of principal components (PC) 1 and 2, it appears going from right to left along PC1, that "weak" and "moderate" boar taint samples appear first, followed by the majority of the "strong" boar taint samples on the left side of principal component 1. When it comes to the "no" boar taint samples it can be said that these are randomly spread across PC1.

Figure 2. Color

The correlation between PC 1 and the variables that compose it was analyzed and the top 10 greatest contributors to PC 1 were pointed out (represented by arrows in Figure 2). PUFAs were the most correlated with positive values for PC 1 and SFA the most negatively correlated with PC1. When considering tainted samples only (all samples to the exception of "no" boar taint samples), it seems that the amount of SFA increases along with HNS. On the other hand, PUFA is inversely correlated to SFA and it can be said that these tend to decrease with increasing HNS (also visible in Table 1) . What remains unclear however is the reason behind such spreading along PC1 for the "no" boar taint backfats.

To supplement this PCA, the correlation between SFA, MUFA and PUFA and HNS were looked at and correlations of 0.23, -0.03 and -0.23 were obtained, but were in all cases non-significant. This is in accordance with the observations made above, however being non-significant they further encourage the finding of Liu et al. (2017) who states that fatty acids cannot predict the score given during human nose evaluation.

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The relationship between fatty acid composition and skatole and androstenone content was also analyzed (Appendix B). As opposed to previous research, it appears from the data gathered here that there is no relationship between fatty acids constituting the fat and skatole and androstenone contents. ANOVAs were performed for each fatty acid in the hope of determining differences based on varying skatole and androstenone concentrations and to the exception of C8:0, no other fatty acids presented a significant difference between the groups. When looking at the case of C8:0, it appears that this molecule is present in very small concentrations (from 0.01 to 0.02% of the profiles), although a significant difference is observed from a statistical point of view, this does not necessarily mean that there are biological implications. The authors do not believe in a cause-to-effect relationship between skatole and androstenone concentrations and the variation in C8:0 in the profiles given the low concentrations and the absence of significance for this molecule in previous studies. Such statement is supported by the findings of Mörlein & Tholen (2015) who describe C8:0 as a molecule that does not contribute to discriminating fat samples with varying boar taint levels. Additionally, the correlation between the fatty acids' classes were determined - the correlations between SFA, MUFA and PUFA with skatole were 0.12, -0.06 and -0.09, respectively, and with androstenone were -0.08, 0.06 and 0.04, respectively, all of them non-significant. This weak correlation is explained by the low relation between the compounds in terms of biosynthesis pathways but also by the low number of samples. In fact, if the correlation was determined considering all 106 samples initially selected, it would be slightly higher. In this case, correlations of 0.12, -0.09 and -0.30 are obtained between skatole and SFA, MUFA and PUFA, respectively and are significant in the case of SFA and PUFA. Correlations of 0.10, 0.20 and -0.26 are obtained between androstenone and SFA, MUFA and PUFA, respectively and are significant in the case of MUFA and PUFA.

The relationship between fatty acid composition and skatole and androstenone level is a complicated topic to elucidate. In fact, although several studies have been performed and general trends are similar between them, it appears that specific relationships observed between fatty acids and boar taint compounds are not the same from one study to another. Mörlein & Tholen (2015) for example reported that LS/LA fat samples had higher levels of PUFA compared to HS/HA which hence presented higher levels of SFA, such as C16:0, C18:0 and high levels of MUFA, such as C18:1. Verplanken et al. (2017), in their study on the use of rapid evaporative ionization mass spectrometry to discriminate tainted from untainted carcasses observed similar trends between tainted and untainted boar samples with higher abundance of some MUFA such as C18:1 and C22:1 in tainted boar fats. On the other hand, Liu et al. (2017) reported PUFA levels to be positively correlated with androstenone content, while MUFA were negatively correlated with both androstenone and skatole.

From this section it seems that although some trends appear between fatty acids and HNS, these are not significant. What could explain in part the absence of trends in the above-presented data is that the backfat samples composing the different taint groups were taken from boars reared under different

production systems with various feeding strategies and different genetics. In fact, as a monogastric species, the fatty acid composition of pork is a direct reflection of the fatty acid composition in the feed. Similarly, pigs originated from different breeds, with varying genetics, will have varying fatty acid composition (De Smet et al., 2004; Johansson et al., 2002; Wood et al., 2008) as well as different likelihood of presenting boar taint (Xue et al., 1996).

3.3. VOC analysis

3.3.1. Analysis of skatole and androstenone emissions

The emissions of skatole and androstenone in the headspace of heated fat were related to its associated content (Figure 3). It appears that the emissions in skatole and androstenone in the headspace of heated fat are a good representation of their corresponding content. Two other main observations can be made. Firstly, the correlation was stronger for skatole than androstenone probably because of the higher vapor pressure of skatole compared to androstenone (7.3×10^{-4} kPa and 1.3×10^{-6} kPa at 25 °C, respectively). Another explanation could be that some of the androstenone present in the fat is found as conjugates, less volatile than free androstenone and subsequently less easily released from the fat matrix (i.e. leading to a poorer correlation). Such conjugates include androstenone sulfates such as androst-3-enol-3-sulfate and androstenone-4-sulfate (Bone & Squires, 2021; Squires et al., 2020). Secondly, correlation is lower at higher levels of androstenone than at lower ones. This could in part explain the difficulty to classify tainted samples with high androstenone (and low skatole) with the human nose methodology as presented in section 3.1.

Figure 3. Color

The correlation between skatole and androstenone content and emissions has already been described in a previous paper (Bruggeo Markey, et al., 2021) and will not be further developed here.

These correlation plots, simply confirm that the skatole and androstenone concentrations perceived by the human nose during sensory evaluation of boar taint, were overall representative of their actual content. Part of the explanation between the existing discrepancy between sensory evaluation and chemical analyses of boar fat must therefore reside elsewhere.

3.3.2. Analysis of general VOC profiles

The best possible representation of the VOC profile of heated fat was performed in this study by sampling the headspace compounds with a DVB/CAR/PDMS SPME fiber. This fiber is frequently used to perform untargeted analyses of VOCs given its ability to capture a great variety of VOCs (both in terms of volatility and polarity). One should however remember that the analyzed VOCs are dependent of the sampling conditions (amongst which are found the SPME fiber coating) and could

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therefore differ from one study to another. In this study, the VOC profiles obtained are composed of 61 molecules (Table 2). Amongst these are found more than 6 different chemical families. The profiles are, however, constituted mainly from fatty acids, making up approximately 73.07% of the VOC profiles, followed by aldehydes, which make up approximately 20.69% of the VOC profiles. Alcohols, alkenes, ketones and furans are the other chemical families observed.

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Table 2. Black and white

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Many of these molecules are known to be typical products of fatty acids oxidation and have already been previously observed in studies on the VOC profiles of heated fat (Burgeon, Markey, et al., 2021; Rius et al., 2005; Serra et al., 2014; Sørensen & Engelsen, 2014). In fact, PUFAs such as C18:2n-6 cis, play an important role in the odor of pork due to the multitude of VOCs that are produced through its oxidation (Aaslyng & Schäfer, 2008). Typical VOCs produced include alcohols (e.g. pentan-1-ol), fatty acids (e.g. octanoic acid) and aldehydes (e.g. hexanal) (Domínguez et al., 2019). SFAs on the other hand, contribute less to the generation of VOCs and hence to the general smell of heated pork. In fact, these are 100 times less reactive than PUFAs (Parker, 2015). As observed in Table 2, hexadecanoic acid (i.e. C16:0) is found in great abundance in the headspace of heated fat, which depicts well the smaller reactivity of SFAs. Such free fatty acids, which are not degraded and found intact in the headspace, have high sensory thresholds which further emphasizes their low contribution to the smell of heated fat (*e.g.* C16:0 has detection threshold of 10 000 mg/kg in oil (van Gemert, 2011)).

Although the SPME-GC-MS method used in this study is identical to that used in a Burgeon, Markey, et al., (2021)'s paper and the results being qualitatively similar, the relative abundance of the compounds are different. In fact, in this study the VOC profiles are majorly constituted of fatty acids when aldehydes were the major constituents of the profiles in the above-mentioned study for the analysis at 150°C.

Another difference between the two studies is that skatole is part the VOC profiles obtained here by analyzing the data in SCAN mode, while this compound was only observed in SIM mode in Burgeon, Markey, et al., (2021)'s study. This can be explained by the fact that tainted samples with much higher skatole content were analyzed in this case and is consequently found in higher headspace concentrations and thus observed in SCAN mode for some samples here. Although the skatole emissions were significantly correlated to the skatole content as shown in Figure 3, it is not surprising that no significant differences exist between the groups presented in Table 2. In fact, the data was gathered here in SCAN mode and is presented in percentage of the total VOCs profiles where skatole only constitutes minor percentages.

In this article, a focus is brought on the comprehension of which molecules could be responsible of the varying perception between the different HN appreciation groups. A principal component analysis (Figure 4) was performed here to understand whether analyzing exclusively general VOC profiles obtained through an untargeted approach can point out differences between HNS scores.

Figure 4. Color

As opposed to the PCA performed with fatty acid content (Figure 2), the "no" boar taint backfat samples are less randomly distributed and are found on the right side of PC 1 (to the exception of one sample), whereas the majority of strong boar taint samples are found on the left side of PC 1.

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Separation of the other taint groups is less evident. However, analyzing the top 10 molecules that are the most correlated to PC1 should help to point out trends between "no" boar taint and "strong boar taint" which have not been perceivable with fatty acid composition.

As it can be noticed by the arrows represented in Figure 4, 9 out of 10 top contributors to PC1 are aldehydes, all positively correlated to this principal component. Most of these molecules have in common that they are direct or indirect products of the oxidation of fatty acids (Burgeon, Markey, et al., 2021; Serra et al., 2014). For example, characteristic MUFA oxidation products are found, undec-2-enal and dec-2-enal are C18:1 oxidation products (Domínguez et al., 2019). Similarly, deca-2,4-dienal is a characteristic VOC of the oxidation of PUFA C18:2.

Having the total emitted fatty acids strongly negatively correlated to PC 1, i.e. going towards the "strong" boar taint samples, joins the idea of having a positive trend between increasing HNS and SFA content as developed in section 3.2.1 . In fact, as described earlier, SFA being less prone to oxidation will lead to more intact fatty acids in the headspace of heated backfat samples, as seen here for strong boar taint samples. Similarly, observing more aldehydes and in particular (*E,Z*)-deca-2,4-dienal (oxidation product of C18:2) towards the "no" boar taint group joins the idea of increased PUFA content for lower HNS mentioned in section 3.2.1. These observations which distinguish "no" boar taint samples from "high" boar taint samples was also confirmed in Table 2. In fact, one can notice that the results obtained for the above mentioned molecules are significantly different between these two HN appreciation groups.

To the exception of (*E*)-non-2-enal, which has been attributed various odor descriptors depending on its concentration, some more pleasant than others – cucumber, green, fat and cardboard odors (Han, Zhang, & Fauconnier, 2021; Han, Zhang, Fauconnier, et al., 2021; Ross & Smith, 2006; Ullrich & Grosch, 1987; Zhao et al., 2017) the majority of other aldehydes which appear to have a positive trend between their emissions and "no" boar taint backfats possess unpleasant odor descriptors. (*E,Z*)-deca-2,4-dienal, for example, which makes up 7.75% of the total VOC profile has an odor quality described as "fatty".

One might wonder how are untainted fat samples attributed low HNS (no taint group) given the fatty odor of most of the molecules described above. The explanation resides in the fact that recognized boar taint compounds, such as skatole and androstenone, have much greater odor activity values (OAVs) compared to other molecules cited earlier. In fact, Gerlach et al. (2018) who determined the OAVs of key molecules in different types of fats, including boar fat found that androstenone and skatole had OAVs of 25 and 40 respectively as opposed to hepta-2,4-dienal and deca-2,4-dienal which had OAVs of 2 and 1. This implies that boar taint compounds have a much greater impact on the perception by an assessor evaluating boar taint, compared to oxidation products cited earlier. The results obtained concerning the correlation between skatole content and HNS and androstenone

content and HNS presented in section 3.1 further supports this idea. Lastly, it should be stated that the appreciation of fatty odor descriptors is dependent on the food matrix considered. Having fatty and fried odors in cooked meat is normal and often desired. Hence, these fatty odors most probably do not negatively contribute to the sensory analysis of backfat.

3.4. Linking content and emissions analysis

Given the observed trends found for both content (fatty acid composition and content in skatole and androstenone) as well as those observed with VOCs, a PLS-R analysis was used to develop a model taking into account all analyses performed on the fat matrix to determine whether taken altogether, the measurements were good predictors of the HNS obtained during sensory evaluation.

The PLS regression chosen was one containing only the intercepts and 1 component given that this yielded the lowest cross-validated root mean square of prediction (RSMEP= 0.8957) and highest coefficient of determination (R^2=0.35). This coefficient of determination indicates that the model has a certain power of prediction but however is not very strong (an R^2 of 1 signifies that the observed values can be predicted with 100% accuracy by the model).

The VIP scores (VIP= variable importance in the projection) have then been analyzed. The VIP score is a measure of the contribution of a variable in the model considering the variance explained by each component (the given variable having a certain impact on each PLS component). It is generally accepted that a variable should be selected when VIP > 1 (Mehmood et al., 2012). When generating the VIPs, it was observed that the highest VIP was no greater than 0.15, this indicates that no specific molecule stands out in the explanation of the model but that the predictive ability of the model is a consequence of several molecules having a small impact individually. As mentioned in section 3.1, boar taint perception can be influenced by interactions between skatole and androstenone. This is also true for interactions with other molecules. However, PLS regression is an additive linear model, interactions between molecules are therefore not looked at.

Yet, the predictive ability of the model could be increased by looking at other factors that are not considered in this study. Molecules with higher OAVs and recognized as contributing to the smell most probably play an important role and could explain part of the incoherence between chemical analyses and sensory evaluation.

One must remember that the goal of boar taint detection methods in slaughterhouses is to ensure that no tainted meat reaches the consumer. To meet this objective, the exact knowledge of the concentration in boar taint compounds is not required. In fact, what prevails is the overall appreciation of the smell of pork meat during cooking and consumption. The method that mimics the most this practice is sensory analysis. Additionally, this method is the only one able to perceive all the generated molecules (provided they are above detection threshold), and the interactions that occur

between them (as presented in this section). Sensory analysis will therefore remain one of the preferred methods for slaughterhouse detection of boar taint. However, it is necessary that the panelist who perform the analysis are well trained and sensitive to androstenone and skatole to reduce the false positive and false negative scores. Furthermore, HNS determine boar taint which, as reported here, is not exactly the same as chemical analysis of specific and impactful boar taint compounds and this could be a limitation. Other methods, such as Raman spectroscopy and sensor-based methods, should however still be exploited to offer a larger and complete variety of options for slaughterhouses in the coming years.

4. Conclusions

Although the human nose method was an overall good representation of the results obtained with the chemical analysis, some existing incoherencies can be found. These are mainly due to the greater influence of skatole on sensory evaluation, especially in fat with intermediate HNS. Additionally, this could also be due to the fact that human olfaction is variable.

Even though a relationship can be perceived between skatole and androstenone content and fatty acid content, this relationship was not evident when HNS and fatty acid contest were considered, thus, indicating that the fatty acid composition is not a good predictor of the score attributed during human nose evaluation.

When looking at VOC emissions when heating fat, significant positive correlations between content and emissions in skatole and androstenone were obtained. Additionally, backfat samples considered untainted had greater amounts of aldehydes in the VOC profiles as opposed to highly tainted backfat samples which were correlated to higher amounts of emitted fatty acids in the VOC profiles. This observation joined the idea that a positive trend exists between HNS and SFA content. SFA are less prone to oxidation and therefore lead to more intact fatty acids in the headspace. Lastly, the generated PLS regression pointed out a positive correlation between the actual and the predicted HNS scores however the predictive ability of the model remained weak, suggesting that other factors play a role in sensory evaluation. Investigating VOCs with higher OAVs and recognized as contributing to boar taint was suggested to increase the coefficient of determination of the PLS regression.

Altogether, this support the idea that elevated HNS attributed to tainted meat is mainly due to recognized boar taint compounds rather than general modifications of VOC profiles composed of pleasant and unpleasant odors.

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Appendix

Appendix A. Black and white

	Panelist 1	Panelist 2	Panelist 3	**General**
Sensitivity	0.70	0.86	0.86	0.74
Specificity	0.58	1.00	0.87	0.70
Accuracy	0.68	0.90	0.86	0.74
PPV	0.98	0.70	0.96	0.99
NPV	0.54	0.46	0.50	0.51

Appendix B. Fatty acid composition (%, mean±standard error) according to the taint groups based on the chemical concentrations of skatole and androstenone: high skatole/high androstenone (HS/HA),

low skatole/high androstenone (LS/HA), low skatole/low androstenone (LS/LA), high skatole/medium androstenone (HS/MA). Means with a different superscript are significantly different ($p<0.05$) according to Tukey-Kramer's test.

	HS/HA	LS/HA	LS/LA	HS/MA
C8:0	0.02±0.00[a,b]	0.02±0.00[a]	0.01±0.00[b]	0.01±0.00[a,b]
C10:0	0.07±0.01	0.07±0.01	0.07±0.01	0.06±0.00
C12:0	0.09±0.01	0.08±0.01	0.08±0.01	0.09±0.01
C14:0	1.24±0.06	1.18±0.05	1.17±0.03	1.25±0.04
C15:0	0.06±0.01	0.08±0.01	0.08±0.0	0.08±0.01
C16:0	23±0.66	22.44±0.35	22.96±0.49	23.96±0.35
C17:0	0.34±0.03	0.41±0.05	0.43±0.06	0.38±0.05
C18:0	13.06±0.47	11.67±0.63	12.37±0.73	13.17±0.67
C20:0	0.23±0.01	0.17±0.02	0.18±0.01	0.20±0.02
C21:0	0.01±0.00	0.02±0.00	0.02±0.00	0.01±0.00
C22:0	0.12±0.01	0.13±0.01	0.12±0.01	0.12±0.02
Σ SFA	38.23±1.04	36.26±0.84	37.48±1.17	39.35±0.97
C14:1	0.02±0.00	0.03±0.00	0.03±0.01	0.04±0.02
C16:1	2.32±0.10	2.53±0.11	2.47±0.18	2.53±0.13
C17:1	0.28±0.02	0.35±0.03	0.33±0.03	0.31±0.04
C18:1 n – 9 cis	42.63±0.78	42.84±0.53	42.65±0.93	42.19±0.59
C18:1 n – 9 trans	0.24±0.01	0.24±0.01	0.26±0.02	0.25±0.02
C20:1 n – 9	0.85±0.04	0.78±0.04	0.77±0.06	0.74±0.03
Σ MUFA	46.34±0.84	46.78±0.57	46.51±1.06	46.06±0.65
C18:2 n – 6 cis	13.51±0.94	14.91±1.01	14.06±0.83	12.85±0.64
C18:2 n – 6 trans	0.11±0.01	0.12±0.01	0.13±0.02	0.14±0.01
C18:3 trans	0.03±0.00	0.03±0.00	0.03±0.01	0.02±0.00
C18:3 n3	0.70±0.06	0.74±0.06	0.69±0.05	0.64±0.04
C20:2	0.62±0.05	0.62±0.01	0.59±0.03	0.51±0.03
C20:3 n – 6	0.10±0.01	0.10±0.00	0.09±0.01	0.09±0.01
C20:4 n – 6	0.26±0.05	0.32±0.04	0.29±0.03	0.26±0.03
C20:5 n – 3	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00
C22:5:DPA	0.04±0.01	0.03±0.01	0.05±0.00	0.04±0.00
C22:6DHA:n3	0.06±0.01	0.05±0.00	0.08±0.02	0.03±0.00
Σ PUFA	15.44±1.07	16.96±1.11	16.02±0.94	14.59±0.72
Total	100	100	100	100

Appendix C. Mean and standard deviation of the androstenone and skatole contents and human nose scores by class group.

Class group[1]	HS/HA	HS/MA	LS/HA	LS/LA
n	8	7	7	8
Androstenone (µg/g liquid fat)	4.72±1.68	1.35±0.19	4.11±1.45	0.39±0.35
Skatole (µg/g liquid fat)	0.52±0.21	0.27±0.08	0.06±0.02	0.06±0.06
Human nose score[2]	2.38±0.69	2.36±0.48	1.29±0.95	0.31±0.70

[1]high skatole/high androstenone (HS/HA), low skatole/high androstenone (LS/HA), low skatole/low androstenone (LS/LA), high skatole/medium androstenone (HS/MA)

[2] Scores: 0: no boar taint, 1: weak boar taint, 2: moderate boar taint; 3: strong boar taint

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Tables:

Table 1. Fatty acid composition (%, mean±standard error) classified according to the HN appreciation category (no, weak, moderate and strong boar taint). Means with a different superscript are significantly different (p<0.05) according to Tukey-Kramer's test.

	% of total fatty acid profile			
HN appreciation (n)	No BT (n=7)	Weak BT (n=5)	Moderate BT (n=6)	Strong BT (n=12)
C8:0	0.01±0.00^{b}	0.02±0.00^{a}	0.02±0.00^{a,b}	0.01±0.00^{b}
C10:0	0.07±0.00	0.08±0.00	0.07±0.00	0.05±0.00
C12:0	0.07±0.00^{b}	0.09±0.00^{a,b}	0.11±0.00^{a}	0.08±0.00^{b}
C14:0	1.13±0.02	1.21±0.02	1.23±0.02	1.26±0.01
C15:0	0.07±0.00^{a,b}	0.09±0.00^{a,b}	0.10±0.01^{a}	0.06±0.00^{b}
C16:0	23.03±0.21	22.30±0.11	22.48±0.14	23.77±0.12
C17:0	0.38±0.02	0.44±0.02	0.45±0.03	0.33±0.01
C18:0	12.79±0.32	11.42±0.35	11.50±0.19	13.28±0.11
C20:0	0.19±0.01	0.17±0.01	0.17±0.01	0.23±0.00
C21:0	0.02±0.00	0.02±0.00	0.02±0.00	0.01±0.00
C22:0	0.11±0.00^{b}	0.14±0.00^{a,b}	0.15±0.01^{a}	0.11±0.00^{b}
ΣSFA[1]	37.90±0.49	35.95±0.41	36.29±0.29	39.20±0.21
C14:1	0.03±0.00	0.04±0.04	0.05±0.01	0.02±0.00
C16:1	2.43±0.08	2.58±0.09	2.48±0.06	2.48±0.02
C17:1	0.31±0.01	0.36±0.02	0.33±0.01	0.29±0.01
C18:1 n – 9 cis	42.83±0.22	42.62±0.54	41.90±0.37	42.81±0.14
C18:1 n – 9 trans	0.25±0.01	0.24±0.00	0.25±0.01	0.24±0.00
C20:1 n – 9	0.81±0.02	0.75±0.02	0.73±0.03	0.81±0.01
Σ MUFA[2]	46.66±0.28	46.59±0.60	45.74±0.40	46.65±0.15

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C18:2 n – 6 cis	13.56±0.27[a,b]	15.30±0.29[a]	15.83±0.41[a]	12.43±0.15[b]
C18:2 n – 6 trans	0.13±0.01	0.12±0.00	0.14±0.01	0.11±0.00
C18:3 trans	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
C18:3 n3	0.67±0.02[a,b]	0.79±0.02[a]	0.82±0.02[a]	0.62±0.01[b]
C20:2	0.60±0.01	0.64±0.02	0.59±0.02	0.55±0.01
C20:3 n – 6	0.09±0.00	0.10±0.02	0.10±0.00	0.09±0.00
C20:4 n – 6	0.25±0.01[b,c]	0.34±0.01[a,b]	0.35±0.02[a]	0.24±0.00[c]
C20:5 n – 3	0.01±0.00	0.02±0.00	0.01±0.00	0.01±0.00
C22:5:DPA	0.09±0.01	0.11±0.01	0.08±0.01	0.06±0.00
C22:6DHA:n3	0.02±0.00	0.02±0.00	0.02±0.02	0.01±0.00
Σ PUFA[3]	15.44±0.31[a,b]	17.46±0.32[a]	17.97±0.[illegible][a]	14.15±0.16[b]
TOTAL	100	100	100	100

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Table 2. GC-MS[1] results of VOCs[2] (%, mean± standard error) found in the headspace of heated fat, sampled with the DVB/CAR/PDMS fiber and detected in SCAN mode. The VOCs are classified according to the HN appreciation category (no, weak, moderate and strong boar taint). All reference RIs are issued from the NIST Mass Spectrometry Data Center, to the exception of skatole's which is its injected standard's RI. Means with a different superscript are significantly different ($p<0.05$) according to Tukey-Kramer's test.

				% of total VOC profile			
	CAS[3] number	Reference RI[4] (VF-5ms[5])	Literature RI	No BT[6] (n=7)	Weak BT (n=5)	Moderate BT (n=6)	Strong BT (n=12)
Alcohols							
pentan-1-ol	71-41-0	771	771	0.47±0.16	0.49±0.11	0.34±0.09	0.25±0.08
heptan-1-ol	111-70-6	971	970	0.18±0.04	0.18±0.04	0.12±0.06	0.10±0.03
oct-1-en-3-ol	3391-86-4	980	979	0.45±0.12	0.41±0.15	0.30±0.11	0.18±0.06
octan-1-ol	111-87-5	1072	107	0.44±0.09	0.44±0.08	0.30±0.09	0.22±0.07
Total alcohols				1.53±0.29	1.52±0.32	1.06±0.34	0.75±0.23
Aldehydes							
Unknown aldehyde	NA	702	NA	0.02±0.01	0.03±0.01	0.03±0.01	0.01±0.01
pentanal	110-62-3	720	717	0.35±0.10	0.38±0.08	0.16±0.04	0.26±0.10
hexanal	66-25-1	797	799	1.32±0.34	1.18±0.22	0.83±0.22	0.99±0.26
(*E*)-hex-2-enal	505-57-7	850	850	0.17±0.04	0.08±0.04	0.06±0.02	0.06±0.02
heptanal	111-71-7	900	900	0.47±0.11	0.30±0.16	0.38±0.12	0.26±0.07
(*E*)-hept-2-enal	18829-55-5	955	955	2.52±0.49	2.03±0.44	1.78±0.40	1.27±0.21
benzaldehyde	100-52-7	959	960	0.53±0.05[a]	0.15±0.07[b]	0.04±0.04[b]	0.16±0.04[b]
octanal	124-13-0	1002	1002	0.64±0.13	0.53±0.10	0.46±0.10	0.40±0.09
(*2E,4E*)-hepta-2,4-dienal	5910-85-0	1010	1012	2.65±0.28[a]	1.56±0.22[a,b]	0.87±0.29[b]	1.24±0.22[b]
5-ethylcyclopentene-1-carbaldehyde	36431-51-3	1030	1035	0.17±0.05	0.12±0.06	0.09±0.04	0.06±0.03

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2-phenylacetaldehyde	122-78-1	1043	1042	0.14±0.04	0.07±0.02	0.08±0.02	0.08±0.01
(*E*)-oct-2-enal	2548-87-0	1058	1058	1.07±0.19	0.83±0.17	0.65±0.15	0.56±0.11
nonanal	124-19-6	1104	1104	2.15±0.38	1.74±0.30	1.48±0.30	1.37±0.22
(*E*)-non-2-enal	18829-56-6	1160	1160	0.56±0.09[a]	0.36±0.06[a,b]	0.29±0.07[a,b]	0.28±0.07[b]
3-ethylbenzaldehyde	34246-54-3	1163	1168	0.01±0.01	0.00±0.01	0.00±0.00	0.00±0.00
decanal	112-31-2	1205	1205	0.03±0.01	0.01±0.01	0.01±0.01	0.01±0.01
(*2E,4E*)-nona-2,4-dienal	5910-87-2	1214	1215	0.28±0.04[a]	0.14±0.04[a,b]	0.06±0.04[b]	0.11±0.03[b]
(*E*)-dec-2-enal	3913-81-3	1262	1262	2.47±0.36	1.60±0.22	1.29±0.30	1.59±0.27
(*2E,4Z*)-deca-2,4-dienal	25152-83-4	1294	1295	12.04±1.27[a]	7.33±0.84[a,b]	5.87±1.21[b]	6.37±1.08[b]
(*E*)-undec-2-enal	2463-77-6	1364	1365	3.17±0.53[a]	1.99±0.32[a,b]	1.50±0.39[b]	1.87±0.35[b]
dodecanal	112-54-9	1408	1409	0.11±0.04[a]	0.02±0.02[a,b]	0.00±0.00[b]	0.00±0.00[b]
tetradecanal	124-25-4	1612	1611	0.08±0.05	0.01±0.01	0.01±0.01	0.00±0.00
hexadecanal	629-80-1	1816	1815	0.00±0.00	0.00±0.00	0.03±0.03	0.05±0.02
Total aldehydes				31.28±4.04[a]	20.46±2.97[a,b]	15.96±3.34[b]	16.99±2.96[b]
Alkenes							
heptadec-1-ene	6765-39-5	1678	1673	0.08±0.03[a]	0.00±0.00[b]	0.00±0.00[b]	0.01±0.01[b]
Total alkenes				0.08	0	0.01	0.02
(Emitted) Fatty acids							
2-methylpentanoic acid	97-61-0	984	983	0.00±0.01	0.00±0.00	0.00±0.01	0.00±0.00
hexanoic acid	142-61-1	1021	1020	0.00±0.00	0.09±0.09	0.35±0.27	0.24±0.14
octanoic acid	124-07-2	1184	1180	0.22±0.08	0.23±0.10	0.19±0.09	0.26±0.11
nonanoic acid	112-05-0	1275	1273	0.45±0.10	0.26±0.07	0.13±0.06	0.24±0.06
decanoic acid	334-48-5	1371	1373	0.60±0.17	0.22±0.14	0.30±0.14	0.30±0.13

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dodecanoic acid	143-07-7	1565	1565	0.96±0.11	0.61±0.11	0.59±0.09	0.55±0.12
tetradecanoic acid	544-63-8	1768	1768	6.36±0.60	4.65±0.62	5.14±0.68	6.69±1.46
pentadecanoic acid	1002-84-2	1861	1859	0.15±0.05	0.03±0.03	0.04±0.04	0.05±0.02
(*Z*)-hexadec-9-enoic acid	373-49-9	1953	1953	10.9±1.42	8.44±2.16	12.23±2.00	10.01±1.57
hexadecanoic acid	57-10-3	1974	1972	17.34±1.73	16.63±0.84	19.50±3.07	22.84±4.09
(*Z*)-heptadec-10-enoic acid	29743-97-3	2075	2073	0.42±0.09	0.43±0.07	0.57±0.12	0.79±0.26
(*E*)-octadec-13-enoic acid	693-71-0	2161	2163	22.13±6.13	35.15±1.94	37.38±7.99	33.91±6.28
octadecanoic acid	57-11-4	2174	2175	0.76±0.33	2.11±0.88	0.99±0.67	2.05±0.66
Unknown fatty acid	NA	2286	NA	0.15±0.07	0.21±0.07	0.31±0.09	0.19±0.05
Total emitted fatty acids				60.43±4.76^{b}	73.08±3.61^{a,b}	77.71±4.37^{a,b}	78.12±3.69^{a}
Furans							
2-pentylfuran	3777-69-3	991	990	0.95±0.24	0.97±0.26	0.75±0.17	0.57±0.11
2-[(*E*)-pent-1-enyl]furan	20992-69-2	1000	1000	0.02±0.01	0.01±0.01	0.01±0.01	0.00±0.01
2-heptylfuran	3777-71-7	1192	1193	0.18±0.06^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.01±0.01^{b}
Total furans				1.15±0.27	0.97±0.26	0.76±0.18	0.59±0.11
Ketones							
heptan-2-one	110-43-0	889	889	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00
(*E*)-oct-3-en-2-one	1669-44-9	1039	1038	0.03±0.02	0.03±0.02	0.00±0.00	0.00±0.00
(*E*)-non-3-en-2-one	18402-83-0	1139	1137	0.03±0.02	0.04±0.02	0.00±0.00	0.00±0.00
1-phenylhexan-1-one	942-92-7	1462	1459	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00
pentadecan-2-one	2345-28-0	1699	1700	1.72±0.16^{a}	0.80±0.15^{b}	0.80±0.11^{b}	0.81±0.14^{b}
heptadecan-2-one	2922-51-2	1902	1900	0.93±0.16	0.56±0.10	0.67±0.13	0.59±0.11
Total ketones				2.72±0.32^{a}	1.44±0.23^{b}	1.48±0.24^{b}	1.40±0.25^{b}

Others							
Unknown other A	NA	685	NA	1.71±0.24	1.88±0.33	2.36±0.68	1.56±0.27
Unknown other B	NA	910	NA	0.03±0.02	0.01±0.01	0.03±0.02	0.00±0.00
Unknown other C	NA	945	NA	0.03±0.02	0.01±0.01	0.01±0.01	0.00±0.00
2-pentylpyridine	2294-76-0	1197	1201	0.38±0.05[a]	0.08±0.06[b]	0.08±0.04[b]	0.08±0.03[b]
Skatole	83-34-1	1389	1389	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01
[(*9Z,12E*)-tetradeca-9,12-dienyl] acetate	30507-70-1	1795	1795	0.23±0.05	0.15±0.06	0.13±0.03	0.10±0.03
delta-Tetradecalactone	2721-22-4	1924	1912	0.12±0.05	0.03±0.03	0.02±0.02	0.09±0.04
gamma-Palmitolactone	730-46-1	2104	2104	0.27±0.09	0.34±0.05	0.38±0.10	0.29±0.06
bis(2-ethylhexyl) hexanedioate	103-23-1	2398	2398	0.05±0.02[a]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
Squalene	111-02-4	2833	2833	0.00±0.00	0.02±0.02	0.02±0.02	0.01±0.01
Total others				2.81±0.30	2.52±0.29	3.04±0.68	2.14±0.29
Total				100	100	100	100

[1]Gas chromatography – mass spectrometry (GC-MS), [2]Volatile organic compounds (VOCs), [3]Chemical abstracts service (CAS), [4]Retention index (RI), [5]VF-5ms is the type of column used, [6]Boar taint (BT)

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Figure captions:

Figure 1. Graphical representation combining chemical analysis for skatole and androstenone content (ng/g liquid fat) on the x- and y-axis respectively, and the score obtained when performing sensory evaluation through the human nose method. The appreciation corresponding to the human nose score (HNS) are the following:0: no boar taint, 1: weak boar taint, 2: moderate boar taint and 3: strong boar taint perceived for HNS. The black lines allow the separation of the different taint groups – high skatole/high androstenone (HS/HA), low skatole/high androstenone (LS/HA), low skatole/low androstenone (LS/LA), high skatole/medium androstenone (HS/MA).

Figure 2. Principal component analysis biplot representing backfats (i.e. the individuals) based on their fatty acid composition and the top 10 contributors (i.e. the variables) for principal component 1. The backfat samples are represented in four categories based on HN appreciation: no (HNS = 0), weak (HNS= 0.5 and 1), moderate (HNS= 1.5 and 2), high (HNS= 2.5 and 3) boar taint. The top contributors are represented with arrows (all are very highly significantly correlated to PC1).

Figure 3. Correlation plots between the emission (peak area) and content (ng/g) of skatole (a) and androstenone (b) (***: P<0.001).

Figure 4. Principal component analysis biplot representing backfats (i.e. the individuals) based on their VOC profiles and the top 10 contributors (i.e. the variables) for principal component 1. For easier graphical visualization, four categories of HN appreciation were created to represent the backfat samples: no (HNS = 0), weak (HNS = 0.5 and 1), moderate (HNS= 1.5 and 2), high (HNS= 2.5 and 3) boar taint. The top contributors are represented with arrows (all are very highly significantly correlated to PC1).

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Author statement :

Clément Burgeon: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization, writing - original draft, writing - review and editing.
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Gerard Sabeña: Investigation, methodology, writing-review & editing
Marie-Laure Fauconnier: conceptualization, formal analysis, funding aquisition, supervision, validation, methodology, writing - review & editing
Núria Panella-Riera: conceptualization, investigation, data curation, writing-review & editing, funding acquisition

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Declarations of interest: none

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1. Sensory evaluation is a method of choice for abattoir detection of boar taint.
2. Sensory evaluation was linked to VOC, boar taint compounds and fatty acid content.
3. Discrepancies exists between sensory and chemical analysis of boar taint.
4. Oxidation products of PUFAs were abundant for untainted fat samples.
5. Differences in VOC profiles do not impact significantly sensory evaluation.

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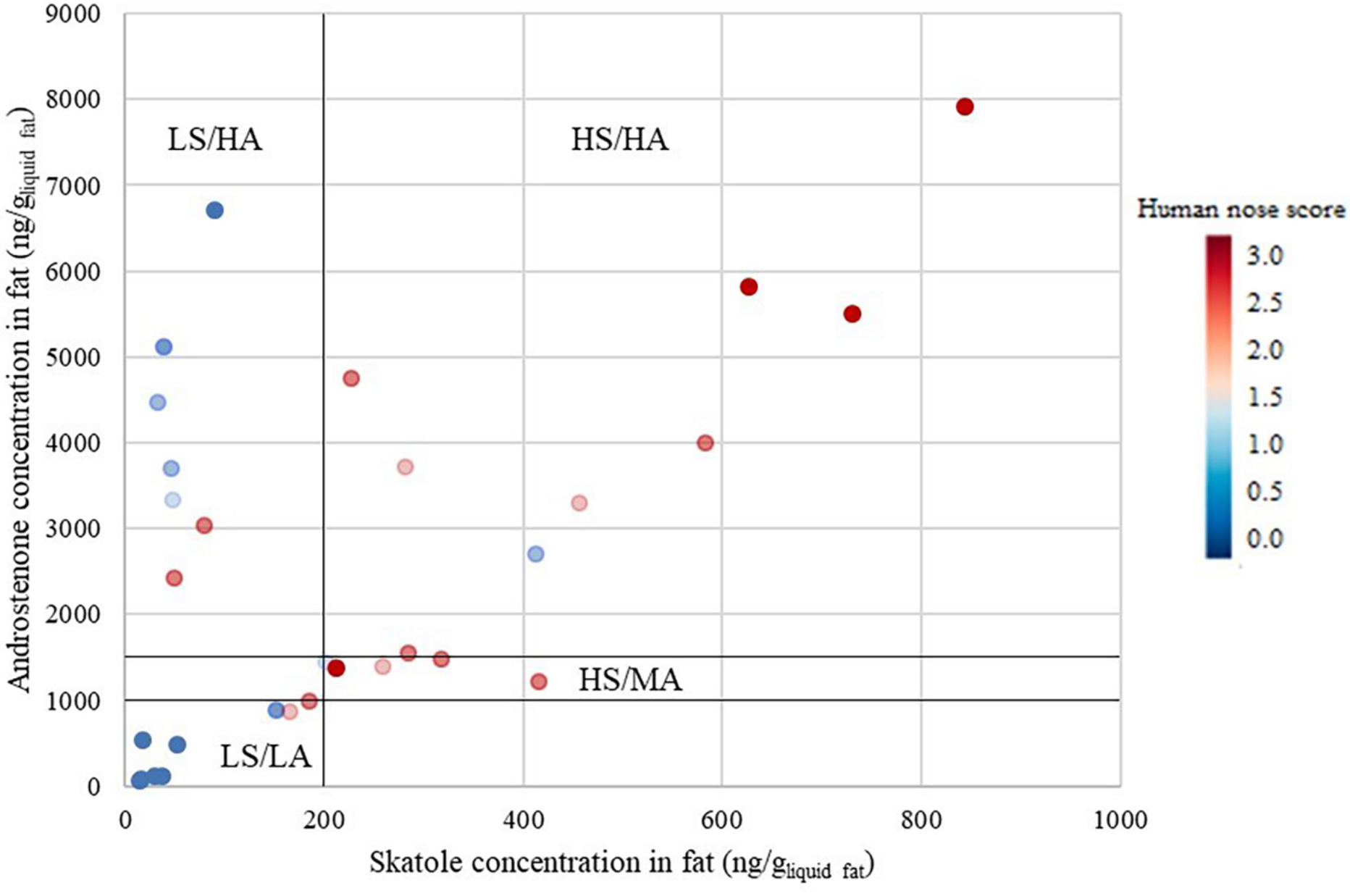

LS/HA
HS/HA
HS/MA
LS/LA
Androstenone concentration in fat (ng/g liquid fat)
Skatole concentration in fat (ng/g liquid fat)
Human nose score
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
0
200
400
600
800
1000

Figure 1

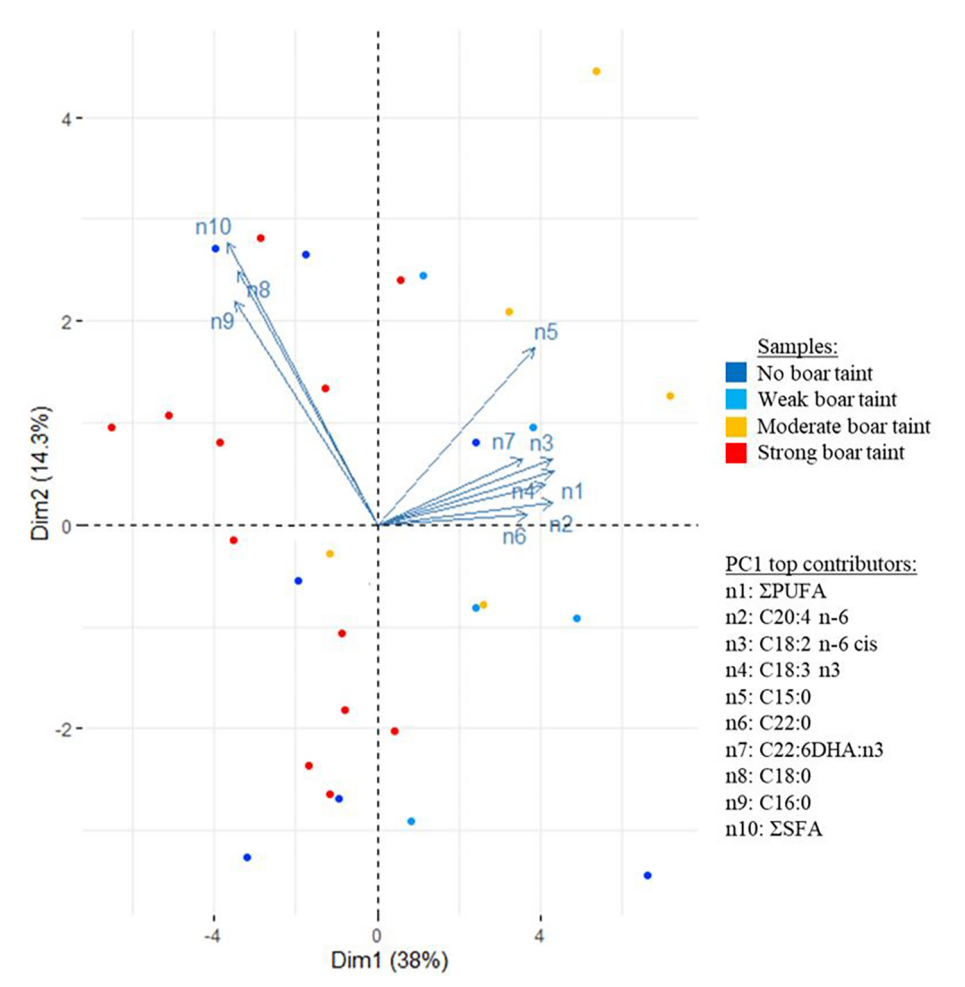
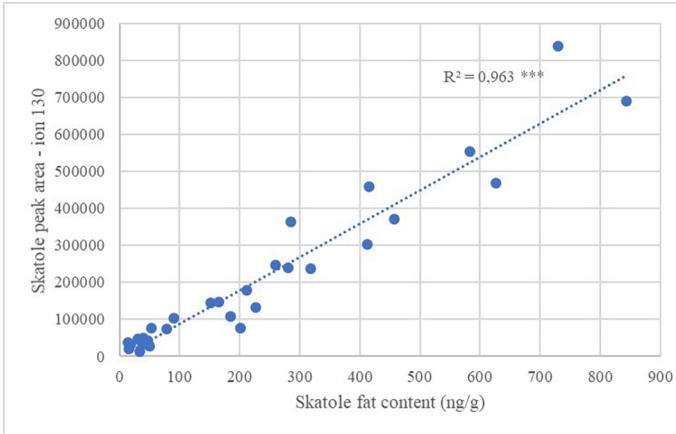

4
2
0
-2
-4
0
4
Dim2 (14.3%)
Dim1 (38%)
n10
n8
n9
n5
n7
n3
n4
n1
n2
n6
Samples:
No boar taint
Weak boar taint
Moderate boar taint
Strong boar taint
PC1 top contributors:
n1: ΣPUFA
n2: C20:4 n-6
n3: C18:2 n-6 cis
n4: C18:3 n3
n5: C15:0
n6: C22:0
n7: C22:6DHA:n3
n8: C18:0
n9: C16:0
n10: ΣSFA

Figure 2


900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Skatole peak area - ion 130
R² = 0,963 ***
0
100
200
300
400
500
600
700
800
900
Skatole fat content (ng/g)

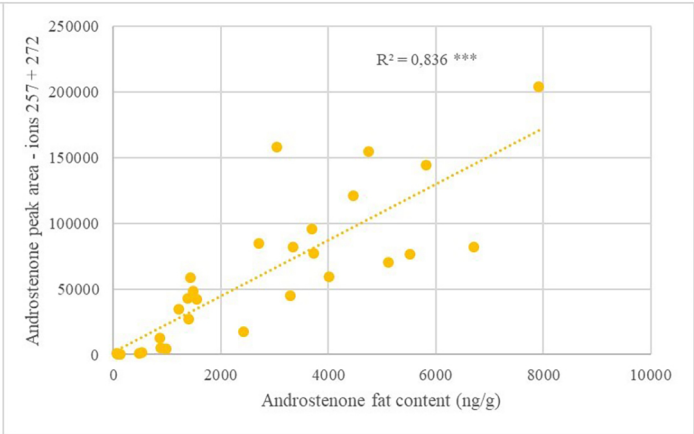

250000
200000
150000
100000
50000
0
Androstenone peak area - ions 257 + 272
R² = 0,836 ***
0
2000
4000
6000
8000
10000
Androstenone fat content (ng/g)

Figure 3

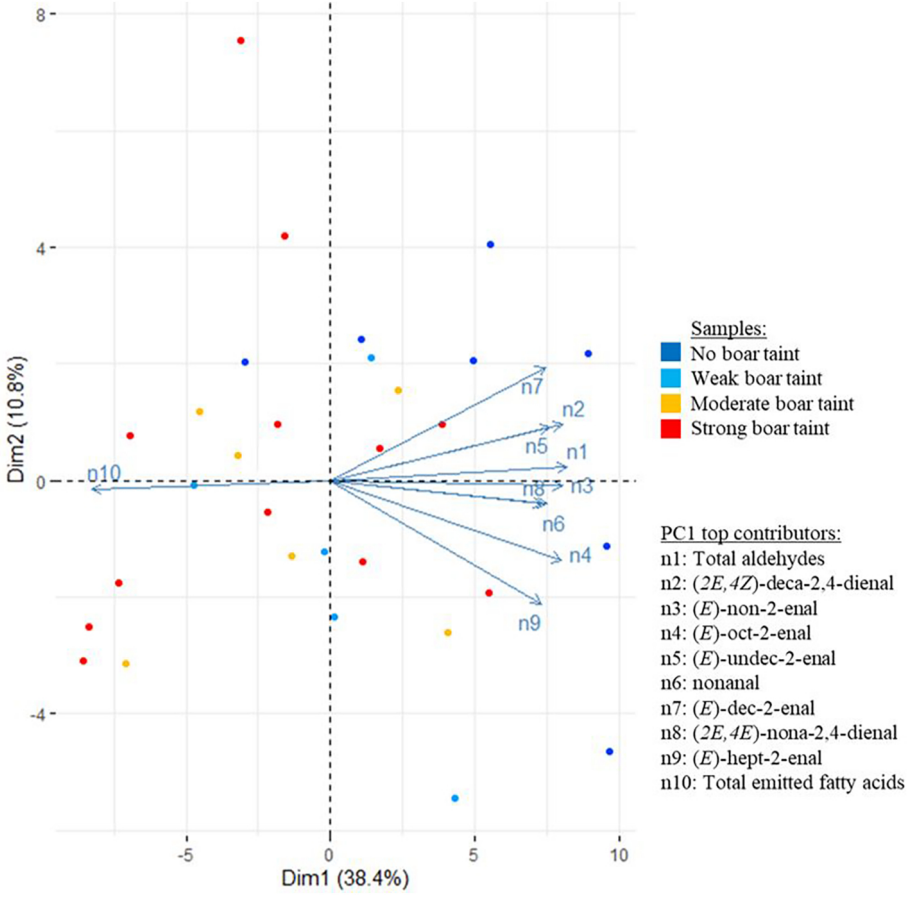

8
4
0
-4
-5
0
5
10
Dim2 (10.8%)
Dim1 (38.4%)
n10
n7
n2
n5
n1
n8
n3
n6
n4
n9
Samples:
No boar taint
Weak boar taint
Moderate boar taint
Strong boar taint
PC1 top contributors:
n1: Total aldehydes
n2: (2E,4Z)-deca-2,4-dienal
n3: (E)-non-2-enal
n4: (E)-oct-2-enal
n5: (E)-undec-2-enal
n6: nonanal
n7: (E)-dec-2-enal
n8: (2E,4E)-nona-2,4-dienal
n9: (E)-hept-2-enal
n10: Total emitted fatty acids

Figure 4