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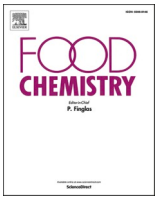

Development and validation of a classification model for boar taint detection in pork fat samples

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ABSTRACT

This study aims to characterize a complete volatile organic compound profile of pork neck fat for boar taint prediction. The objectives are to identify specific compounds related to boar taint and to develop a classification model. In addition to the well-known androstenone, skatole and indole, 10 other features were found to be discriminant according to untargeted volatolomic analyses were conducted on 129 samples using HS-SPME-GC×GC-TOFMS. To select the odor-positive samples among the 129 analyzed, the selection was made by combining human nose evaluations with the skatole and androstenone concentrations determined using UHPLC-MS/MS. A comparison of the data of the two populations was performed and a statistical model analysis was built on 70 samples out of the total of 129 samples fully positive or fully negative through these two orthogonal methods for tainted prediction. Then, the model was applied to the 59 remaining samples. Finally, 7 samples were classified as tainted.

1. Introduction

Historically, the castration of piglets was an integral part of the technical itinerary in pork production across Europe, including the region of Wallonia in Belgium. The reasons behind this practice were to ensure calmer animals and to obtain a better valorization, as castrated pigs were known to exhibit superior meat quality (Andersson et al., 1997). Nowadays, animal welfare concerns are challenging the practice of castration, and solutions are being developed (Lin-Schilstra, Ingenbleek, Font-I-Furnols, Tomasevic, & Bonneau, 2021). Alternatives such as effective pain management during castration (Horn, Marx, & von Borell, 1999), immunocastration (Kress, Weiler, Schmucker, Čandek-Potokar, Vrecl, Fazarinc, Škrlep, Batorek-Lukač, & Stefanski, 2019), or the production of whole (i.e., uncastrated) males (Bonneau & Weiler, 2019) exist. Nevertheless, hurdles must be overcome in terms of meat quality, especially for some males developing an unpleasant odor during meat preparation, which can be unpleasant for many consumers. This odor, called boar taint, results from a mixture of molecules released while heating the meat (Duarte, Schroyen, Mota, Vanderick, & Gengler, 2021) and is mainly caused by the human perceivable presence of androstenone (5α-androst-16-ene-3-one, CAS 18339-16-7) and skatole (3-methylindole, CAS 83-34-1) in the adipose tissue (Buttinger & Wenzl, 2020; Font-i-furnols, M., Martín-bernal, R., Aluwé, M., Bonneau, M., Haugen, J. E., Mörlein, D., Mörlein, J., Panella-riera, N., Škrlep, M. (2020), 2020; Patterson, 1968). Therefore, much research is being undertaken to find reliable and rapid methods to identify smelly carcasses in slaughterhouses (Burgeon et al., 2021; Font-i-furnols, M., Martín-bernal, R., Aluwé, M., Bonneau, M., Haugen, J. E., Mörlein, D., Mörlein, J., Panella-riera, N., Škrlep, M., 2020; Støier, 2019) as a need for harmonized methods is emerging (Haugen, Brunius, & Zamaratskaia,

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2012).

Currently, the discrimination of odor-positive carcasses relies on the expertise of trained human noses, who sniff carcasses at the end of the slaughter line and discard boar tainted ones (Mathur et al., 2012; Trautmann, Gertheiss, Wicke, & Mörlein, 2014). This represents the cheapest and quickest way for odor discrimination (Burgeon et al., 2021) but presents limitations such as variations in detection efficiency among operators and sensory fatigue (Haugen et al., 2012). Five to ten percent of uncastrated pig carcasses are downgraded due to boar taint, resulting in direct financial losses for producers (Bonneau et al., 2000). Consequently, this is why more and more methods for the determination of this boar odor keep emerging (Lund, Borggaard, Birkler, Jensen, & Støier, 2021). Another way to enhance the situation is to investigate the link between the volatile composition (volatolome) of neck fat from boar tainted pigs and the genome. This could allow a better selection of the genetic lines (Bhatt et al., 2022; Burgeon et al., 2023; Burgeon et al., 2021).

This work is part of a global project of the Walloon region in Belgium, which aims to produce whole males with a low risk of boar taint. The main approach focuses on genetic selection for low odor risk of entire males. It requires the development of workflows to select low-risk Walloon Pietrain boars in a holistic and sustainable context. Indeed, various recent studies (Duarte et al., 2021) describe the genetic links between boar taint, fertility (Brinke et al., 2021), and meat quality (Dugué et al., 2020). To achieve this aim, it was first needed to establish a robust classification standard for the different animals, which would ultimately provide a robust boar taint phenotype. In this research, a total of 129 neck fat samples from both tainted and untainted pigs were analyzed by untargeted headspace solid-phase microextraction in combination with comprehensive two-dimensional gas chromatography–time–of–flight mass spectrometry (HS-SPME-GC×GC-TOFMS). Recognizing that only 50 % of the variation in boar taint is due to the combination of skatole and androstenone (Hansson, Lundstrom, & Fjelkner-Modig, 1980), the initial goal of this work was to determine the global VOC profile of boar taint. The second objective was to build a robust statistical model for boar taint discrimination to be used as a classification standard for genomic profiling. The fat samples were also analyzed by two human noses and targeted ultra-high-performance liquid-chromatography tandem mass spectrometry (UHPLC-MS/MS) to cross-compare the different methods currently in use.

2. Materials and methods

2.1. Pig neck fat samples

The animal study protocol was approved by the Ethics Committee of the University of Liège (Protocol code #2307; approved on 21 December 2020).

Pig neck fat samples (n = 129) (sex: male, age: 6 months ± 15 days) were randomly selected from a population and collected on-site at the slaughterhouse. Each sample was individual sealed plastic bags, transported, and subsequently stored at −20 °C until analysis. Samples were collected directly on the slaughtering line of a breeding of whole males on which no selection was applied to ensure the population's heterogeneity and similarity and to obtain a percentage of positive and negative animals which closely mirrors reality. The 129 samples were selected at random from a larger panel to obtain a sampling with a higher distribution of fat samples from boar taint pigs. Neck fat samples have been classified by a human nose directly at the slaughterhouse and another one at the laboratory. Results presented in this paper are part of a larger study that aims to develop genetic models to select low-risk Pietrains for breeding.

2.2. Chemicals

UHPLC-MS grade methanol, formic acid and ethanol were purchased from Biosolve (Valkenswaard, the Netherlands). Dimethylsulfoxide was purchased from Sigma Aldrich (Bornem, Belgium). Water was purified with a Sartorius Arium ultra-pure laboratory water purification system (Sartorius, Göttingen, Germany) with conductivity 0.055 μS cm^{-1} at 18 °C.

Skatole (99.6 %), and Androstenone (98 %) were purchased from Sigma-Aldrich (Bornem, Belgium). Androstenone-d_4 (99 %, IS), and Skatol-d_3 (98.8 %, IS) were the internal standards from CDN isotopes (Pointe-Claire, Quebec). Individual standard stock solutions of compounds were accurately prepared at concentrations of 1 mg mL^{-1} in ethanol (with correction depending on the purity of the purchased standard solution). All stock and working solutions were stored for one year at –18 °C.

Mixed working standard solutions (an IS solution and a solution containing all the remaining compounds) were prepared by diluting stock solutions with methanol. For calibration, 9 solutions of different concentrations were prepared for each compound. Concentrations ranged from 0 to 6000 parts-*per*-billion (ppb) for androstenone with androstenone-d_4 as the internal standard at a concentration of 10 ppb. For skatole, concentrations ranged from 50 to 600 ppb, with skatol-d_3 being the norm at a concentration of 200 ppb.

2.3. Sampling and extraction of volatile compounds

For HS-SPME-GC×GC-TOFMS analysis, 1 g of frozen neck fat sample was weighted and added into SPME septum-sealed headspace vials of 20 mL. Samples were first incubated at 100 °C for 10 min with an agitator speed of 250 rpm. Then, the headspace of each sample was extracted for 30 min by a 50/30 μm DVB/CAR/PDMS SPME fiber (Supelco, Bellefonte, PA, USA). Each new SPME fiber was first conditioned according to supplier's instructions prior to use. During measurement, the fiber was pre-conditioned at 270 °C for 10 min and post-conditioned at 270 °C for 2 min.

For UHPLC-MS/MS analysis, 0.5 g of rendered fat was extracted in 900 μL of methanol for 30 min at 60 °C with regular stirring. After freezing for one hour at −70 °C, the extract was centrifuged. The supernatant was then placed in injection vials for analysis by UHPLC-MS/MS. Each run contained a blank, a calibration curve and a control sample (QC). The internal standards (IS) pool was added to all tubes. The calibration curve ranged from 500 to 6000 ppb for androstenone and from 50 to 600 ppb for skatole.

2.4. Human nose evaluations

Boar odor at the slaughterhouse of whole males was determined according to the slaughterhouse procedure by a slaughterhouse operator trained in the detection of odorous carcasses with a binary determination: odor / no odor. This agent performed the boar odor evaluation using the soldering iron technique applied at room temperature to the fat sample, iron was cleaned between 2 samples, waiting time arranged, worked in a fume hood, rating on a 2-gradation scale: 0, when there is no odor and 1 for a strong odor. Before each series of sensory evaluations, the agent must correctly identify a random sequence of 6 tubes: 2 with androstenone, 2 with scatole and 2 with water.

2.5. Instrumentations

2.5.1. GC×GC–TOFMS analysis

GC×GC–TOFMS analysis were performed using a Pegasus BT 4D GC×GC-TOFMS system with cryogenic LN2 modulator (LECO Corp., St. Joseph, MI, USA). To achieve an adequate separation, the column set consisted of a normal column set (non-polar × polar) composed of a first dimension (^{1}D) column Rxi-5Sil MS (30 m × 0.25 mm i.d. × 0.25 μm df, Restek Corp., Bellefonte, PA, USA) and a second dimension (^{2}D) column Rxi-17Sil MS (1.5 m × 0.25 mm i.d. × 0.25 μm df, Restek Corp.). Neck fat headspace samples were desorbed from SPME fiber in the GC injector

at 250 °C for 10 min. The main GC oven was set at 40 °C for 1 min, then increased to 200 °C with a ramp of 5 °C min^{-1}, and finally increased to 270 °C (held for 5 min) at a ramp of 15 °C min^{-1} for a total run of 40 min with a Helium flow of 1.4 mL min^{-1}. The secondary oven temperature offset was 20 °C. The transfer line temperature was 250 °C. The modulation period (P_M) was 2.5 s with a hot jet duration of 0.08 s. The temperature of the 70 eV electron ionization (EI) source was 250 °C. Mass range was 40–550 amu. MS acquisition rate was 200 Hz. All 129 samples were injected randomly on 12 different days according to the laboratory protocol mainly to avoid batch effect or systemic errors (Bhatt et al., 2023).

2.5.2. Experimental set up for volatile compound identification

Analytical conditions chosen for this study were selected according to the laboratory prior experiences on biological samples (Dubois, Stefanuto, Heudt, Focant, & Perrault, 2018; Stadler, Stefanuto, Brokl, Forbes, & Focant, 2013; Stefanuto et al., 2017) to obtain the best information from the neck fat samples.

In order to validate these conditions, 24 neck fat samples from whole pigs matching purebreds were injected in triplicate randomly on 7 different days. No variation nor bias were observed and comparison of pick intensity for different compounds of interest – including, skatole, androstenone and indole – was done giving relative standard deviation calculated on areas ranging from 22 % for androstenone to 35 % for skatole and indole (Table SM2 in Supplementary Material).

An example of a 2D chromatogram of neck fat sample of the animal n°17 is presented in Figure SM1 in Supplementary Material.

2.5.3. UHPLC-MS/MS analysis

Liquid chromatographic analyses were performed with an Acquity UPLC system (Waters, Milford, MA) and separations were done on an Acquity UPLC HSS T3 column (150 × 2.1 mm, 1.8 μm particle size; Waters). The column was equilibrated at 60 °C and the injection volume was 10 μL. Solutions used for the mobile phase were 0.1 % formic acid in water (A) and 0.1 % formic acid in methanol (B). The gradient used was as follows: 0–1 min: 55 % B, 1–3 min: increase to 84 % B, 3–5 min: 84 % B; 5–6.5 min, increase to 90 % B; 6.5–7 min, increase to 92 % B; 7–8 min, decrease to 55 %B, 8–9 min, increase to 100 % B, and finally, 55 % B up to 11 min. The gradient was run at a flow rate of 0.3 mL min^{-1}. The column and autosampler were maintained, respectively, at 60 °C and 15 °C.

MS analysis was carried out with a Waters Acquity TQ mass spectrometer (Waters, Manchester, UK). ESI-MS/MS conditions were optimized by individual direct injection of each compound at a concentration of 10 μg mL^{-1} and a flow rate of 5 μL min^{-1}. The instrument was operated with an electrospray ionization source in positive mode (ESI+). The ESI parameters were set as follows: capillary voltage 2.5 kV, source temperature 150 °C, desolvation temperature 450 °C, cone gas (nitrogen) flow 250 L/h, desolvation gas (also nitrogen) flow 1200 L/h. Collision-induced dissociation was done with argon as the collision gas 3×10^{-3} mbar pressure in the collision cell. Data acquisition was done with MassLynx 4.1 and TargetLynx 4.1 software (Waters). Table SM1 in supplementary material presents the different MS/MS parameters used for each compound.

2.6. Data processing and statistical analysis

Raw data acquisition and pre-processing were performed on ChromaTOF® (ver. 4.72, LECO Corp., St. Joseph, MI, USA) with a S/N threshold of 100 and a minimum similarity score of 800 (masses from 35 to 550 amu). The putative identification of analytes was conducted against the NIST17 mass spectral library. Chromatograms of all the neck fat samples were compared using ChromaTOF® Tile (ver. 1.01, LECO Corp.) with a tile size of 8 modulations (^{1}D) for 40 spectra (^{2}D), and a S/N threshold of 100.

Chemometric tests (i.e., unsupervised screening (PCA), random forest (RF), hierarchical clustering, and prediction score) were obtained using MetaboAnalyst 5.0 (Xia Lab, McGill University, Montréal, QC, Canada) (Xia, Psychogios, Young, & Wishart, 2009) and a lab-based script running on R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) (Stefanuto et al., 2022). In the data processing workflow, the classification model was built on a fraction of the entire data set (training set) and tested on the validation set. Since the validation data were not used to create the model, it ensures a robust evaluation of the model performances. Information about the data splitting is included in a data processing workflow chart in Supplementary Materials (Figure SM2).

3. Results and discussion

3.1. Odor discrimination according to human nose evaluation

Due to the remarkable sensitivity of human nose, capable of perceiving boar taint at parts-*per*-trillion (ppt) concentrations (Breer, 2003) and its cost-effectiveness (Burgeon et al., 2021), this method remains the predominant choice for on-line detection in Europe. Briefly, the human nose operator heats the neck fat of a carcass with a soldering iron and then smells it to detect the presence of boar taint (Mathur et al., 2012; Trautmann et al., 2014). Carcasses are then categorized as either tainted or untainted. For this study, two human noses were part of our panel to classify samples, one operating directly at the slaughterhouse and another one in an external laboratory, the Walloon Agricultural Research Centre (CRA-W). Samples were considered as tainted according to the noses' assessment when at least one of the noses classified the sample as such. The results given by the two noses indicate 51 neck fat samples over the 129 were distinguished as tainted and 78 as untainted.

3.2. UHPLC-MS/MS results

As human nose has an important variability caused by inter- and intra-individual sensory performance (Burgeon et al., 2023), the 129 samples were also analyzed using UHPLC-MS/MS as described in the section Materials and Methods for skatole and androstenone quantification. A total of 106 neck fat samples had both skatole and androstenone measured concentration under the 200 and 2000 parts-*per*-billion (ppb) cut off levels, respectively, and 23 samples above (Brooks & Pearson, 1989; Ferrer & Arboix, 1986). Details of the determined concentrations for both compounds are presented in Table SM3 in Supplementary Material.

3.3. Data processing and statistical analysis

3.3.1. Model building

Due to the discrepancies between noses evaluations and UHPLC-MS/MS analysis, a robust classification tool was needed to mitigate biased decision-making. Finally, to provide a classification standard and therefore an innovative phenotype for genomic evaluation and based on the results of the human nose odor evaluations and UHPLC-MS/MS analysis, 70 samples were selected as a training set for model construction. Indeed, 16 samples were both classified as tainted (T) by both human noses and the chemical quantification using UHPLC-MS/MS and 54 samples as untainted (UT). The 59 remaining samples were treated as unknown samples (UN).

Firstly, Tile-based Fisher ratio analysis (Marney et al., 2013; Stefanuto, Smolinska, & Focant, 2021) was performed on the training set to find discriminant features (here defined as distinctive compounds) on both population (T and UT). Subsequently, based on higher F-ratios (the ratio of the between group variance to the within group variance), the top 13 discriminant features were selected for model building. Table 1 presents the 13 features selected for model building with retention times for both dimensions and associated F-ratios. Among the 13 selected features, androstenone, skatole and indole were identified within the

Table 1
Top 13 features selected for model building with compound identification, similarity and probability scores, quantification mass, retention times (^{1}D and ^{2}D) and F-ratios associated.

Feature	Compound	CAS number	Molecular formula	Similarity score	Probability score (%)	Quant mass	F-ratio	$^{1}t_R$ (s)	$^{2}t_R$ (s)
F1	Androstenone	18339-16-7	$C_{19}H_{28}O$	831	37.46	94	154	2184.9	2.03
F2	Skatole	83-34-1	C_9H_9N	909	19.86	90	108	1199.9	0.43
F3	2-aminoacetophenone	613-89-8	C_8H_9NO	755	9.26	92	91	1069.9	0.41
F4	Indole	120-72-9	C_8H_7N	925	46.24	92	84	1054.9	0.48
F5	Benzonitrile	100-47-0	C_7H_5N	873	35.72	76	61	525	2.29
F6	2,5-dimethyl-Pyrazine	123-32-0	$C_6H_8N_2$.	884	63.62	108	32	417.5	1.75
F7	1-methoxy-4-methyl-benzene	104-93-8	$C_8H_{10}O$	912	36.15	122	31	587.5	1.87
F8	2-propenyl-benzene	487-11-6	C_9H_{10}	853	28.52	117	26	600.0	1.78
F9	2,3-dimethylphenyl isocyanate	1591-99-7	C_9H_9NO	808	32.87	104	76	1359.9	0.65
F10	Benzenemethanol	100-51-6	C_7H_8O	791	52.06	107	64	660.0	2.11
F11	Biphenyl	92-52-4	$C_{12}H_{10}$	852	43.56	152	20	1192.4	2.46
F12	Pyrazine	290-37-9	$C_4H_4N_2$	912	77.72	80	18	190.0	1.38
F13	2-dodecanone	6175-49-1	$C_{12}H_{24}O$	926	31.32	211	9	1644.9	1.59

odorous samples. The detection of other molecules, like 2-aminoacetophenone (F3) describes as a hepatic skatole metabolite and a potential contributor to boar taint (Fischer et al., 2014) support those results.

Prior to statistical evaluations, probabilistic quotient normalization (PQN) of the data and an autoscaling (mean-centered and divided by the standard deviation of each variable) were applied. The hierarchical clustering result presented as a heat map on Fig. 1 (a) for the top 13 features shows that F13 is overexpressed in untainted neck fat sample. In contrast, F1 to F12 are overexpressed in boar-tainted neck fat samples.

Principal component analysis (PCA) was also performed to visualize a potential clustering trend between the boar-tainted and untainted pig neck fat samples. As represented on the PCA Fig. 1 (b), PC 1 and PC 2 contributed to 68.7 % of the variance. A clustering trend was observed for the untainted group (in green) but a great dispersion was observed

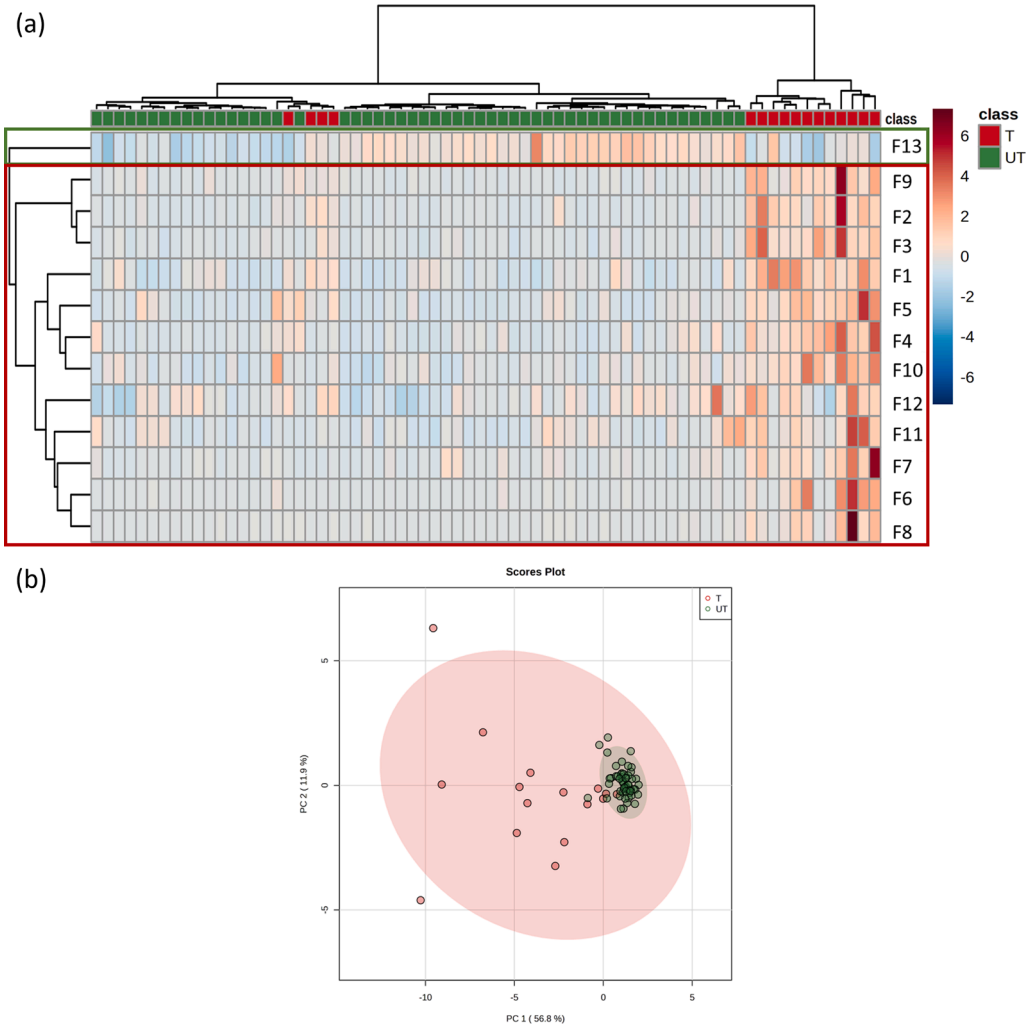

Fig. 1. (a) Heat map displaying the overexpression of the top 13 significant features selected [tainted (red) and untainted (green)]. (b) PCA score plot using the top 13 selected features for the two different groups: tainted (red), untainted (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for the tainted group (in red). This indicated that the features selected were able to describe the untainted pigs, but no significant clustering trend was observed between the two groups using PCA.

In order to build a random forest (RF) based statistical model for boar-taint differentiation, the 70 selected samples were normalized and scaled before model development using 35 samples as a training set and 35 other samples as a test set (randomly split 50/50). The model robustness and efficiency were assessed through area under the curve

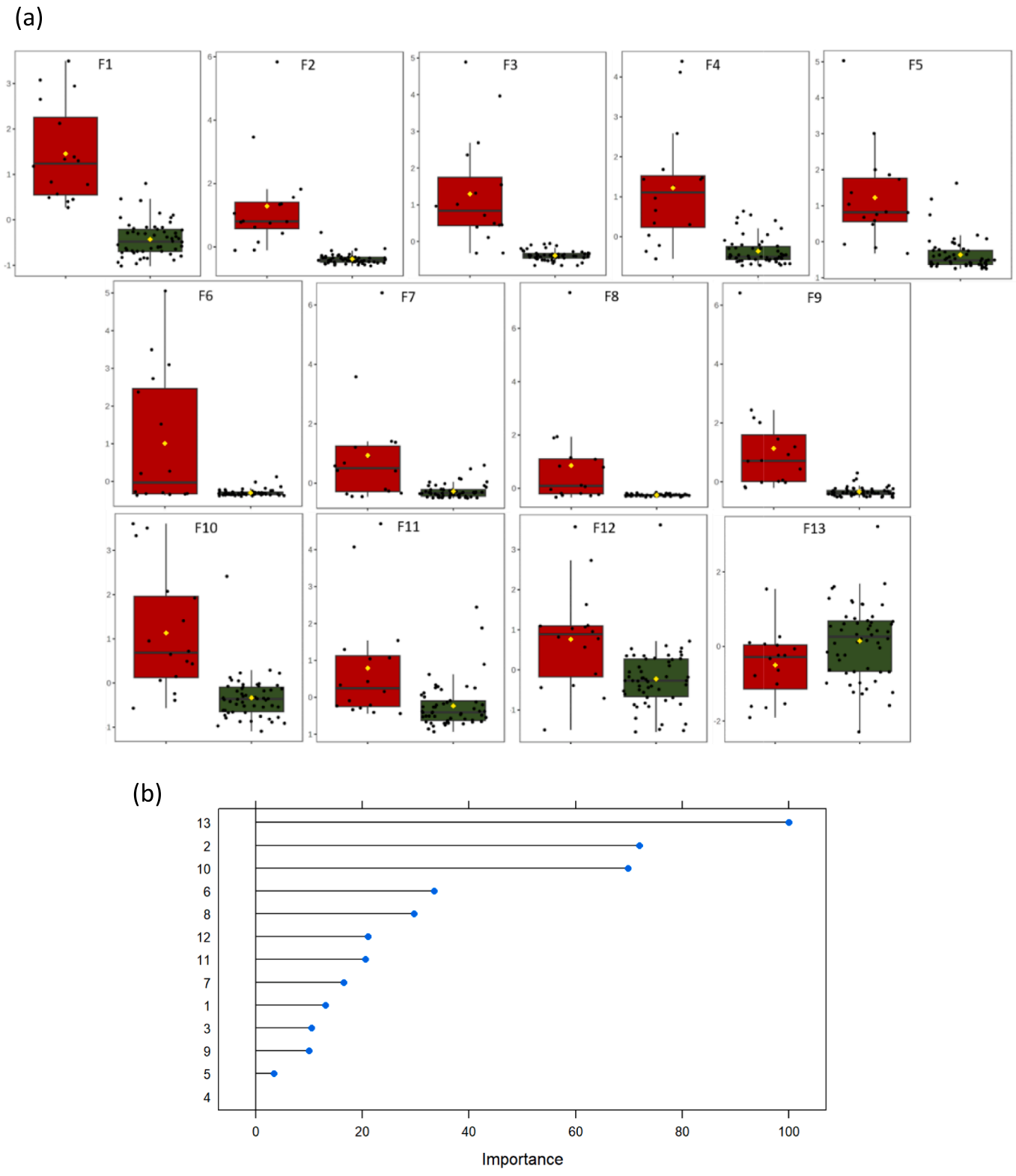

Fig. 2. (a) Comparison of the normalized concentration range for tainted (in red) and untainted (in green) samples of the top 13 significant features (F1–F13). (b) Relative VIP score plot determined by the random forest model predicting the importance of the model classified from 0 to 100% on the x-axis for features F1–F13 (number 1–13 on the y-axis). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(AUC), sensitivity and specificity determination.

The resulting model exhibited remarkable sensitivity and specificity, both at 100 % along with an AUC of 1. This high level of performance is inherently linked to the sample selection. It is based on 70 samples for which the two human noses and the UHPLC-MS/MS were providing the same classification. This model also enabled the prediction of variable importance of the top 13 features considering their normalized concentrations range (Fig. 2). This subset of 13 selected features achieved high classification accuracy in discriminating between tainted and untainted samples. Fig. 2 (b) highlights that F13, corresponding to 2-dodecanone, has the highest variable importance score (VIP). This result can be explained by the fact that F13 is the only feature downregulated in tainted samples in this model. Androstenone (F2) exhibits a VIP close to 75 % while skatole and indole have VIPs less than 20 % compared to F13. Indeed, even if skatole, androstenone and, in a minor way indole, are well known to be responsible for boar taint, the results indicate the potential of monitoring other molecule for boar taint detection, such as 2-dodecanone (F13) and benzenemethanol (F10), both recognized as odorous compounds (Vera, Canellas, & Nerín, 2020; Zhang, Mi, Liu, Sang, & Wang, 2020). The latter has been described by Vera et al. as responsible for a floral odor, while 2-dodecanone (F13) has been perceived by Zhang et al. as an "orange, grass, fresh" odor.

3.3.2. *Unknown samples evaluation (validation set)*

Based on the classification model built as described above, the 59 remaining unknown samples (UN) were classified into UT and T. For the pre-processing, UN samples followed the same normalization and scaling procedure as the training set. Then, random forest algorithm was applied to classify UN samples without a priori knowledge of the samples. All samples with a prediction score $\geq$ 0.5 were classified as UT whereas others were categorized as T, as illustrated on the box plot on Fig. 3. From this classification, 52 neck fat samples over the 59 UN were classified as UT. 10 % of tainted samples (n = 7) correspond to the normal proportion of tainted pig in our study population. Of these seven UN samples classified as tainted, four presented androstenone value under the cut-off limit of 2000 ppb, with concentrations ranging from 544 to 1645 ppb. However, none of the samples had a skatole concentration value under the cut-off limit of 200 ppb. Concerning the human nose evaluations, 6 out of 7 had at least one positive evaluation. Of the 52 neck fat samples classified as untainted, 24 were classified as non-odorous by both human noses but classified as odorous by UHPLC-MS/MS analysis, still with concentrations very close to the 200 and 2000 ppb limits for skatole and androstenone respectively. For instance, one of the sample close to 50 %, classified as untainted in this model, had a skatole value determined by UHPLC-MS/MS analysis upside the threshold of 200 ppb (obtained concentration $\geq$ 600 ppb with an estimation of 905.7 ppb) but an androstenone one under the 2000 ppb threshold (1357.7 ppb). This shows again that classification of samples focusing only on threshold values of one or two compounds is not accurate enough for boar taint evaluation. Thus, the classification obtained here could be a robust combination of orthogonal classification methods, i.e, human nose, UHPLC-MS/MS, and GC×GC–TOFMS. The model obtained provides an unbiased classification for samples close to the threshold of UHPLC-MS/MS. Unbiased method is required to establish gold standard classification to perform further genomic studies.

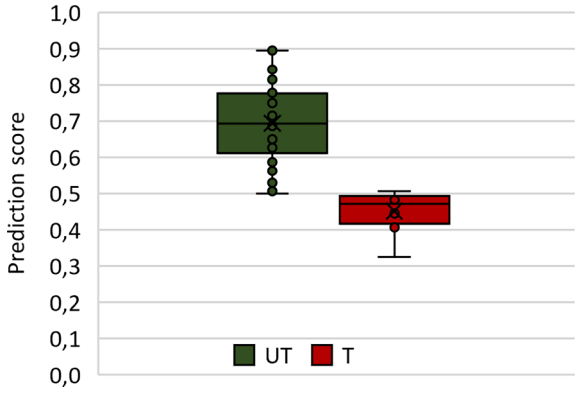

Fig. 3. Classification of the 59 unknown samples as tainted (T in red) and untainted (UT in green) according to the prediction score of the statistical model.

4. Conclusions

HS-SPME-GC×GC-TOFMS provided high separation resolution for the detection and identification of complex VOC profile from the neck fat samples (see 2D chromatogram in SM1 for demonstration of the separation achieved through multidimensional analysis showing potential 1D coelution). A validation set was used to assess the figure of merits for the analytical workflow. The characterization of pig neck fat volatolome allowed to distinguish discriminant molecules for BT detection. In addition to the well-known androstenone, skatole and indole, 10 other features were found to be discriminant including 2-aminoacetophenone, benzonitrile, 2,5-dimethyl-pyrazine, 1-methoxy-4-methylbenzene, 2-propenyl-benzene, 2,3-dimethylphenyl isocyanate, benzenemethanol, biphenyl, pyrazine and 2-dodecanone. These 13 features were used to establish a robust and accurate classification model. In the future, this model will be used to provide objective sample classification for improved phenotyping of boar-taint on whole male pigs, and ultimately to improve opportunities to develop genomic selection for this complex trait.

This new approach relied on the combination of human noses and UHPLC-MS/MS information to establish preliminary classification. The model classes were built on 70 samples out of the total of 129 samples fully positive or fully negative through these two orthogonal methods. Then, the classification model developed was applied to the 59 remaining samples for which the UHPLC-MS/MS and human noses results were not in agreement. Finally, following the developed model, 7 samples out of 59 unknown samples were classified as tainted and 52 as untainted. Combining targeted with untargeted analyses revealed an immensely powerful potential for boar taint evaluation and discrimination as a classification tool for genomic profiling. This study is a significant step for the construction of a statistical evaluation model that could further be used as classification standard (i.e., innovative phenotype) for the genomic evaluation of breeding boars for low odor risk of their entire male descendants.

5. Deontological

The animal study protocol was approved by the Ethics Committee of the University of Liège (Protocol code #2307; approved on 21 December 2020).

CRediT authorship contribution statement

Anaïs Rodrigues: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thibault Massenet:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lena M. Dubois:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Anne-Catherine Huet:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Alice Markey:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **José Wavreille:** Writing – review & editing, Resources, Project administration. **Nicolas Gengler:** Writing – review & editing, Resources, Project administration,

Funding acquisition, Conceptualization. **Pierre-Hugues Stefanuto:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Conceptualization. **Jean-François Focant:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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The co-author Lena M. Dubois is currently working at LECO Instrument GmbH, Deutschland.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138572.

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