ANALYTICAL APPROACH TO UNRAVEL BOAR TAINT CONTRIBUTING COMPOUNDS

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Abstract – Boar taint is an off-odour and off-flavour that can occur when meat or fat from entire males is heated. The main compounds responsible for this odour are skatole and androstenone and to a lesser degree indole. However, the concentration levels of these compounds do not completely account for the occurrence of boar taint as determined by sensory analyses. A plausible explanation for this would be the contribution of other 'unknown' compounds to boar taint. In this study, a two-way strategy was followed to explore this hypothesis. Firstly, a targeted post-acquisition re-interrogation of a dataset of boar taint samples was performed using high resolution MS. ToxID software identified two additional compounds that were correlated (R^2 resp. 0.105 and 0.075) to boar taint, namely 4,16-androstadie-3-one and isovaleric acid. Within this dataset 67% of de sensory score is explained by skatole and androstenone. This R^2 does however not increase when the two other compounds are added. Secondly, an untargeted screening of the same dataset, using differential and OPLS-DA analyses enabled the elucidation of eight boar taint contributing compounds. In total seven new compounds were identified as correlated to boar taint, with R^2 ranging form 0.142 to 0.424. Skatole, and two of the newly unidentified ions can explain 58% of the sensory score of this database.

Key Words – Androstenone, Indole, Orbitrap, skatole, UHPLC

- INTRODUCTION

Nowadays, the use of entire male pigs for pork production is limited because of the potential occurrence of boar taint, an off-odour and off-flavour in meat. In this context, two compounds, skatole and androstenone, are considered as the main contributing compounds to boar taint [1,2].
However, their level of contribution to boar taint is a subject of discussion. These two compounds do not completely account for the occurrence of boar taint as determined by a trained sensory panel [3]. Several factors may explain these rather low correlations obtained so far i.e. individual variation observed in trained experts [4], the available chemical detection methods lacking sufficient in-house validation and also the lack of consistency in the concentration levels of boar taint compounds between porcine carcasses [5].

In spite of these possible explanations, the possibility of additional compounds contributing to boar taint must be taken into account. Various studies have already recognized that there are other odours associated with the mature boar and that these may also influence the general odour perception of heated boar flesh. Compounds like p-cresol and 4-ethylphenol [1], substances belonging to Δ-16 steroids: 5β-androst-16-en-3α-one and 4,16-androstadien-3-one, 5α-androst-16-en-3α-ol, 5α-androst-16-en-3β-ol and 5,16-androstadien-3β-ol [5-7], styrene and 1,4-dichlorobenzene [8]. Additionally, 4-phenyl-3-buten-2-one but also some aldehydes, aliphatic short chain fatty acids and some alcohols and ketones have been described as tainted [8].

In the present study a two-way strategy was followed to explore the assumption that other compounds may explain for the relatively low correlations observed so far between sensory boar taint analysis and androstenone and skatole concentrations obtained upon chemical analysis. To this extent, an targeted screening for boar taint related compounds based on literature findings was performed by means of a post-acquisition re-interrogation of data collected from a vast dataset of fat samples on HRMS. Finally a smaller dataset was applied to search for unknown compounds using the HRMS dataset and specialized software to statistically evaluate the potential additional correlation that these predefined unknowns may explain for.

- MATERIALS AND METHODS

Samples

Neck fat of 83 slaughtered entire male pigs was collected at the slaughterhouse of the VION Food Group (Eindhoven, The Netherlands) and scored for the presence of boar taint by the slaughterhouse expert at the slaughterline by a gas burner [9].

Chemical analysis

Sample preparation consisted of a melting step, followed by an extraction with methanol and a clean up through freezing and solid phase extraction. Subsequently, the analytes were chromatographically separated using ultra high performance liquid chromatography (UHPLC) and detected with an Exactive™ high-resolution mass spectrometer in the positive and negative ion mode. The Exactive™ high-resolution mass spectrometer allows post-acquisition re-interrogation of data [10].

Data interpretation

Although the analytical method was developed and validated by focusing on the three known boar taint compounds (indole, skatole and androstenone), a screening for unknown compounds was premised. To this extent, two different strategies were employed, namely a targeted approach using ToxID™ software and an untargeted approach using Sieve™ software. Finally, for both datasets simple and multiple linear regression (SPSS™ statistics 20) was used to identify the best model to explain the sensory score given by the experts.

An exhaustive screening of the full scan data for preselected compounds relevant to boar taint as derived from literature (Table 1), was executed using ToxID™ 2.1.2 software (Thermo Fisher Scientific, San Jose, USA). For this purpose, a ToxID™ database was constructed by implementing the molecular formula of 13 relevant compounds (Table 1). The isotopic ion (^{13}C, ^{37}Cl) was found suitable as additional diagnostic ion to the corresponding $[M+H]^+$ ion. The main ToxID™ settings concerned a minimum peak intensity of 1000 and a maximum mass deviation set at 5 ppm.

For the untargeted screening of potential boar taint compounds differential analysis with Sieve™ 2.0 (Thermo Fisher Scientific, San Jose, USA) was applied. To build up a prediction model using this kind of dataset, the use of multivariate techniques is essential. In this study the Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) for determination of potential boar taint compounds with SIMCA13 (Umetrics, Sweden) was selected. To determine the number of significant components, the model used cross-validation rules. For all samples, data were $\log^2$ transformed and scaled according to the Pareto method.

- RESULTS AND DISCUSSION

The samples used for targeted post-acquisition re-interrogation of data using ToxID™ were selected based on sensory evaluation (cat. 0-4).

The samples used for untargeted post-acquisition re-interrogation of data using Sieve™, were selected from the 83 samples of the ToxID™ database. In total 30 samples were retained of which 15 without boar taint characterized with concentrations of skatole and androstenone below the cut-off values and the sensory scores equal to 0. For the 15 positive boar taint samples, a minimum concentration of skatole or androstenone was set above the cut-off value (respectively 0.2 mg kg^{-1} and 0.5 mg kg^{-1}) and a sensory score of at least 3, was presumed.

Table 1 Preselected compounds relevant to boar taint as derived from literature (*: P-value < 0.05).

Compound	Chemical formula	Mol. weight	R^2
5α-androst-16-en-3α-ol	$C_{19}H_{30}O$	274.22911	
4,16-androstadien-3-one	$C_{19}H_{26}O$	270.19781	0.105*
4-ethylphenol	$C_8H_{10}O$	122.07261	0.011
p-cresol	C_7H_8O	108.05696	
Styrene	C_8H_8	104.06205	0.008
4-phenyl-3-buten-2-one	$C_{10}H_{10}O$	146.07261	
5β-androst-16-en-3α-one	$C_{19}H_{28}O$	272.21346	
5,16-androstadien-3β-ol	$C_{19}H_{28}O$	272.21346	
1,4-dichlorobenzene	$C_6H_4Cl_2$	145.96845	
5-methyl-3-heptene-2-one	$C_8H_{14}O$	126.10391	0.002
2,4-heptadienal	$C_7H_{10}O$	110.07261	0.013
isovaleric acid	$C_5H_{10}O_2$	102.06753	0.075*
cis-2-nonenal	$C_9H_{16}O$	140.11956	0.012

ToxID™

For the application of ToxID™, 13 compounds (Table 1) selected in terms of relevance to boar taint according to literature were implemented in a database and evaluated on their presence in the samples (n = 83) for which data were available from previous UHPLC-HRMS analyses. The following compounds could not be detected in the samples under investigation: 5α-androst-16-en-3α-ol, 1,4-dichlorobenzene, 4-phenyl-3-buten-2-one and p-cresol. For the other compounds, only two compounds (characterized by a specific elemental composition and with high likelihood corresponding to 4,16-androstadiene-3-one and isovaleric acid) could be significantly correlated with boar taint (Table 1). Regression coefficients of 0.105 and 0.075 were obtained for 4,16-androstadien-3-one and isovaleric acid, respectively. Using multiple linear regression, it was demonstrated that 67% of the sensory score may be explained by skatole and androstenone. This percentage remained the same upon inclusion of indole, 4,16-androstadiene-3-one and isovaleric acid to the model. However, we believe that the three compounds may impact boar taint in general, as every compound is positively correlated with the odour separately.

Sieve™

Prior to the untargeted screening it was decided to broaden the range of the detection method (i.e. changing gradient elution, enlarging *m/z* range, including positive and negative ionization mode etc.). To this purpose, the commercial standards of the ToxID™ database were purchased and and injected onto the analytical system. 1,4-dichlorobenzene and p-cresol could not be detected under the provided circumstances, which explains their absence in the ToxID™ screened samples. After the optimization, 30 samples were selected from the larger dataset as described above, to be processed with Sieve™. Executing the differential analysis allowed the detection of 6334 ions, each characterized by a specific mass to charge ratio and retention time. Next, these detected ions were statistically evaluated, resulting in 83 ions considered as potentially relevant boar taint compounds (Fig. 1).

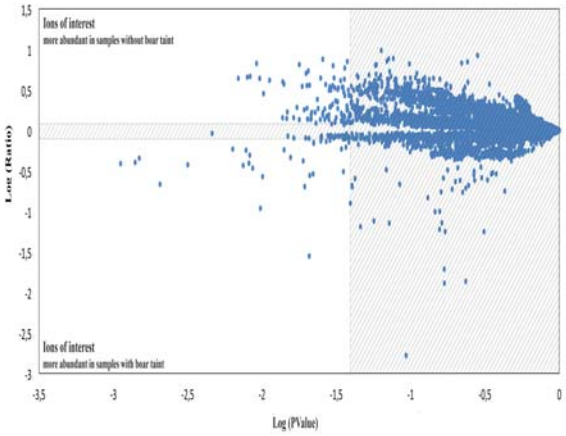
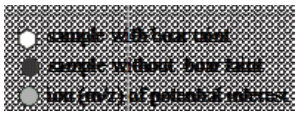
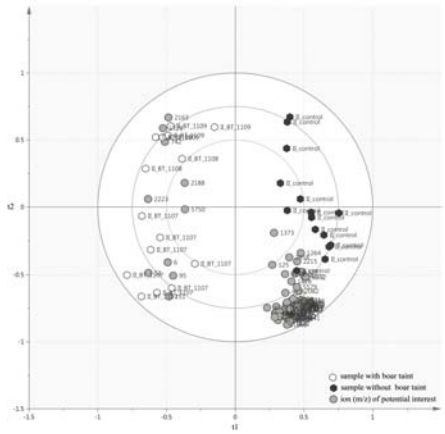


Figure 1: Volcano plot of the 6334 ions obtained after Sieve analysis of samples (n = 83) with and without boar taint, resulting in 83 significant ions (white zone).

Subsequently, OPLS-DA analyses were conducted to single out the boar relevant compounds. As output, the biplot clearly indicated a distinct discrimination between the group with and without boar taint along the x-axis (left samples with boar taint, right samples without boar taint) as well as the differences within the same sample group along the y-axis (Fig. 2).





In this experiment, the focus laid on those ions that were significantly more available in samples with boar taint. In total, ten ions were situated nearby the samples with boar taint, which implies that these ions are potential candidates to contribute to boar taint (Fig. 2).

The quality of the OPLS-DA model was verified with cross-validated predictive residuals (CV-ANOVA, P=0.01).

Afterwards, individual regression coefficients were calculated for the ten ions marked as potentially boar taint contributing compounds. Eight of the ten ions displayed a significant ($P < 0.05$) R^2, which implies that eight of the ten compounds contribute to boar taint when interpreting every compound individually. When taking skatole and androstenone together,

53% of the sensory score could be explained. However, skatole and ions 1328 and 51 together explained for 58% of the sensory score. This was the best model found for this dataset. Ultimately, eight ions were retained as potential candidate contributors to the boar taint odour.

- CONCLUSION

Due to the low correlation between sensory analyses and chemical analyses, it has been difficult to unambiguously define sensory threshold levels for boar taint without negative perception of the consumer. One of the hypotheses is that compounds other than the three acknowledged ones contribute to boar taint and may therefore increase the correlation. The targeted re-interogation of UHPLC-HRMS data showed the positive correlation of 4,16-androstadien-3-one and isovaleric acid with the boar taint odour determined through sensory analysis. Application of the untargeted screening approach by using differential and OPLS-DA analyses on a smaller database, allowed to retain another eight compounds out of 6334 ions. In total (targeted and untargeted approach) ten ions were postulated as possible contributing compounds to boar taint of which three were identified as the acknowledged boar taint compounds indole, skatole and androstenone. As for the identification of the other suggested compounds, isovaleric acid, hexanoic acid, 6-methoxy indole and 4,16-androstadien-3-one have been put forward. The latter will be the subject of future experiments.

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