Talanta 169 (2017) 30–36

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid evaporative ionization mass spectrometry for high-throughput screening in food analysis: The case of boar taint

Kaat Verplanken[a], Sara Stead[b], Renata Jandova[b], Christof Van Poucke[c], Jan Claereboudt[d], Julie Vanden Bussche[a], Sarah De Saeger[e], Zoltan Takats[f], Jella Wauters[a], Lynn Vanhaecke[a,*]

[a] Laboratory of Chemical Analysis, Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[b] Waters Corporation, Altrincham Road, UK-SK9 4AX Wilmslow, United Kingdom
[c] Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Sciences Unit, Brusselsesteenweg 370, B-9090 Melle, Belgium
[d] Waters Corporation, Brusselsesteenweg 500, B-1731 Zellik, Belgium
[e] Laboratory of Food Analysis, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium
[f] Faculty of Medicine, Department of Surgery and Cancer, Imperial College London, London SW7 2AZ, United Kingdom

ARTICLE INFO

Keywords:
Indole
Skatole
Androstenone
High-throughput
REIMS
IKnife

ABSTRACT

Boar taint is a contemporary off-odor present in meat of uncastrated male pigs. As European Member States intend to abandon surgical castration of pigs by 2018, this off-odor has gained a lot of research interest. In this study, rapid evaporative ionization mass spectrometry (REIMS) was explored for the rapid detection of boar taint in neck fat. Untargeted screening of samples (n=150) enabled discrimination between sow, tainted and untainted boars. The obtained OPLS-DA models showed excellent classification accuracy, i.e. 99% and 100% for sow and boar samples or solely boar samples, respectively. Furthermore, the obtained models demonstrated excellent validation characteristics ($R^2(Y)$=0.872–0.969; $Q^2(Y)$=0.756–0.917), which were confirmed by CV-ANOVA ($p < 0.001$) and permutation testing. In conclusion, in this work for the first time highly accurate and high-throughput (< 10 s) classification of tainted and untainted boar samples was achieved, rendering REIMS a promising technique for predictive modelling in food safety and quality applications.

1. Introduction

During the past decades, the public awareness of food safety and quality has significantly increased [1]. The organoleptic properties of food play a crucial part in this, as they are reflective of the first impressions consumers will develop [2]. To effectively ensure the food quality and safety, an analytical platform for the fast and accurate detection of quality parameters in food imposes itself [3]. In this study, rapid evaporative ionization mass spectrometry (REIMS) was proposed as a new analytical approach for in-situ detection of food anomalies and its applicability was demonstrated for an important contemporary off-flavor in meat industry, i.e. boar taint. Boar taint is an off-odor caused by the accumulation of indole (IND), skatole (SK) and androstenone (AEON) in adipose tissue [4–6]. IND and SK are two indolic compounds derived from the biological degradation of L-tryptophan in the hindgut and their odor is often described as fecal-like [4,5]. AEON on the other hand is a pheromone produced in the Leydig cells of the testis and has a urinary- or sweaty-like odor [7]. Initially, the surgical castration of pigs was implemented to prevent boar taint; however, increasing awareness on animal welfare has led to a European intent to voluntarily abandon the surgical castration of piglets by 2018 [8]. Consequently, the rearing of entire male pigs, one of the alternatives to surgical castration could cause adverse consumer reactions due to the re-occurrence of boar taint and thus lead to economic losses in pig husbandry [9,10]. In order to maximize the marketing potential of meat from entire males, sorting strategies to detect boar taint containing carcasses at the slaughter line are in order [11]. One of the main challenges for the detection of boar taint at the slaughter line is the high rate at which pigs are slaughtered, on average 600 per hour. Over the past years, several candidate methods for at-line detection of boar taint have been proposed, including sensory and analytical methods. However, none of these meet the required performance characteristics needed at the slaughter line [12]. Indeed, sensory methods, e.g. the soldering iron method, whereby neck fat is singed with a soldering iron and the released smell assessed by a trained assessor, are directly applicable at the slaughter line and

* Corresponding author.
E-mail address: Lynn.Vanhaecke@UGent.be (L. Vanhaecke).

http://dx.doi.org/10.1016/j.talanta.2017.03.056
Received 7 February 2017; Received in revised form 14 March 2017; Accepted 17 March 2017
Available online 21 March 2017

provide a fast and holistic detection of boar taint, but they rely on the sensory abilities of one trained assessor [13–15]. Consequently, sensory methods are subject to inter-individual variation and moreover are associated with habituation and fatigue [14]. Furthermore, various analytical methods show potential for the at-line detection of boar taint but often lack sensitivity, specificity or high-throughput [12]. Indeed, the use of sensor technology, e.g. thickness shear mode resonator sensors and parasitoid biosensors, offers sensitive and fast detection of the boar taint compounds but these sensors often show poor specificity or lack thorough testing and validation [16–20]. The use of a mass spectrometric based electronic nose for targeted screening of the boar taint compounds was promising, however, results were preliminary, lacking thorough validation [21]. The detection of the boar taint compounds by means of high-throughput gas chromatography coupled to mass spectrometry on the other hand offers satisfactory precision (RSD% < 20%) [22,23]. However, although these methods are fast (run-to-run of 3.5 to 6 min), they do not meet the speed requirements needed at the slaughter line. Moreover, as insufficient sensitivity is obtained on a portable GC-MS instrument, up until now these methods cannot be implemented directly at the slaughter line [22]. More recently, RAMAN spectroscopy was evaluated for the detection of boar taint [24,25]. Targeted detection of IND, SK and AEON was associated with very large prediction errors: 173 μg kg^{-1} and 1460 μg kg^{-1} for SK and AEON, respectively [24]. More accurate results (88% identification accuracy) were obtained with an untargeted classification approach, enabling identification of aberrant adipose tissue samples. However, data acquisition lasted 20 min per sample, limiting the potential use of RAMAN spectroscopy at the slaughter line [25]. To overcome these bottlenecks, rapid evaporative ionization mass spectrometry (REIMS) is proposed as a new emerging technique that circumvents long analysis times by enabling direct ionization from the sample combined with mass spectrometric analysis. As such, REIMS analysis takes only a few seconds and guarantees point-of-control analysis [26–30]. Additionally, REIMS offers highly accurate histological identification of tissues and demonstrated a correct classification performance of 90–98% [26]. Originally it was intended for in vivo identification of tissues during medical interventions, but recently also found its application niche in food analysis as its feasibility was successfully demonstrated for the identification of the species of origin in meat products [31]. In this study, REIMS was explored to develop a predictive model for accurate high-throughput identification of boar taint in pig adipose tissue, a first of its kind step towards achieving at-line classification of boar carcasses.

2. Materials and methods

2.1. Reagents and chemicals

The reference standards indole (IND) or 2,3-benzopyrrole (CAS 120-72-9) and skatole (SK) or 3-methylindole (CAS 83-34-1) were obtained from Sigma Aldrich (St. Louis, MO, USA). The reference standard 5α-androst-16-ene-3-one (AEON, CAS 18339-16-7) was obtained from Steraloids (Newport, RI, USA). For each compound, standard solutions were prepared in isopropyl alcohol at a concentration of 20 μg ml^{-1}. Also a mixture of IND, SK and AEON was prepared in isopropyl alcohol at a concentration of 20 μg ml^{-1}.

2.2. Samples

Both sow (blank) samples and boar neck fat samples were collected at the slaughter line. In order to select boar samples negative and positive for boar taint, boar carcasses were screened for boar taint at the slaughter line by means of the soldering iron method optimized by Bekaert et al. [14] All samples were cooled during transport to the lab and were immediately stored upon arrival at −80 °C until analysis. The presence or absence of boar taint in the samples was confirmed by an in-house validated UHPLC-HR-Orbitrap-MS analysis method [32]. Samples containing levels of IND, SK and/or AEON above and below the odor thresholds (IND: 100 μg kg^{-1}, SK: 200 μg kg^{-1}, AEON: 500 μg kg^{-1}) were considered as positive and negative for boar taint, respectively. In total, 50 samples for each group were collected.

2.3. Instrumentation

The iKnife hand-held sampling device (Waters, Wilmslow, UK) was used to apply a localized high frequency electric current to the surface of each sample, which instantly vaporizes molecules from the latter. It consisted of a monopolar cutting device with a shortened knife blade of approximately 6 mm and was applied in dry cut mode in combination with a diathermy electrosurgical generator at 45 W. Sampling was carried out for 3–5 s and for each sample, 2 technical replicates were analyzed, thus taking into account repeatability of the analysis. For targeted purposes, isopropyl alcohol was used as a dopant to stimulate ionization of the boar taint compounds. Mass spectrometric analysis was carried out on a Xevo G2-XS Q-TOF instrument equipped with a helical coiled ribbon collision surface supplied with a constant current power supply set to 4.5 A (Kanthal D 1.0×0.1 mm) (Waters, Wilmslow, UK). All analysis occurred in REIMS TOF MS sensitivity mode with continuum data acquisition. Isopropyl alcohol was infused directly into the REIMS source at a constant flow rate of 100 μl min^{-1} to promote the ionization of lipid (fatty acid and phospholipid) species. The mass resolution was typically set at 18750 and 19195 for *m/z* 281.2537 and 773.5432, respectively. The cone voltage was set at 100 V. Mass spectrometric analysis was performed in negative ionization mode with a mass range of 50–1200 *m/z* and scan speed of 1 s/scan. Prior to use, the instrument was calibrated using sodium formate. For quality control purposes, the endogenous matrix ion PE (34:1) $[M\text{-}NH4]^-$ C39H76NO8P with *m/z* 699.497 was used as a lock-mass compound. Furthermore, replicate burns of a QC sample (bovine muscle) were collected between every 10 pig neck fat samples. The intensity of the base peak ion at *m/z* 699.497 was recorded and plotted for quality control monitoring. The iKnife, transfer tubing and venturi device were cleaned with methanol between every 10 samples.

2.4. Untargeted identification approach of neck fat samples

An Untargeted mass spectrometric analysis was evaluated for the discrimination between boar taint positive and negative carcasses. Untargeted analysis was performed by profiling both boar (negative and positive) and sow (blank) samples and thus effectively providing a mass spectral fingerprint for the latter. Samples were analyzed in duplicate on 3 consecutive days in order to take into account reproducibility. This experiment was repeated on 3 additional days, with different cone voltage settings (60 or 100 V) of the ionization source, in order to check the robustness of the measurements. Afterwards, the mass spectrometric fingerprints were used to construct predictive models for the classification of sow and boar samples into blank (sow) and boar taint positive and negative groups.

2.5. Chemometric data analysis

All data files were pre-processed, including peak alignment and peak picking, using the Progenesis bridge conversion tool (Waters, Wilmslow, UK). Next, Progenesis QI software (Waters, Wilmslow, UK) was used for lock-mass correction using the endogenous matrix ion with *m/z* 699.497 and background subtraction applying a TIC replicate threshold setting of 100,000. Prior to model building, the data were log-transformed and pareto scaled to generate normally distributed data and reduce noise, respectively. Next, multivariate regression analysis was performed in SIMCA 14 (Umetrics, Umea, Sweden). Principal component analysis (PCA) was used for unsupervised data analysis to reveal outliers, groups and trends. Afterwards, orthogonal

partial least-square discriminant analysis (OPLS-DA) was used to construct prediction models able to predict the Y-variable (classification of samples in groups) from the X-matrix (mass spectrometric fingerprint). In order to avoid over-fitting of the data, the quality of the OPLS-DA models was evaluated through the goodness of fit ($R^2(Y)$) and the predictive ability of the models ($Q^2(Y)$). Permutation testing (20 permutations) was performed to assess the risk that the model is spurious, i.e. that the model fits the training set but does not predict Y well for new observations. Additionally, CV-ANOVA (cross-validated analysis of variance) and cross-validation, according to a leave 1/7 out classification, were performed to confirm the validity of the models. In parallel, OMB version 1.1.29.0 (Waters Corporation, Wilmslow, UK) was used as a model builder recognition software tool. To this end, a linear discriminant analysis (LDA) model including 80% of randomly selected samples of each group was built. The remaining 20% was used as a test set for external validation of the model and run through the recognition software, whereby the observed classifications (based on two burns) were recorded in post-acquisition mode.

3. Results and discussion

3.1. Discrimination between boars (tainted and untainted) and sows

To demonstrate the classification potential of REIMS for boar taint, 50 blank (sow), 50 boar taint positive and 50 negative samples were analyzed. In a first experiment (data not shown), both negative and positive ionization mode were taken into account, to increase the range of detected metabolites, and were considered separately. In negative ionization mode, better classification accuracy (98%) was observed compared to positive ionization mode (94%). For this reason, it was decided to continue all analysis in negative ionization mode for final model building. The PCA plot revealed 17 potential outliers (Fig S1a); however, only 5 of the latter were identified as true suspected outliers using the Hotelling's T2 plot. Four outliers originated from the blank group and 1 outlier from the boar taint positive group. Since the values of these outliers were located between the 95% and 99% confidence limit, they were omitted from further data analysis. The validity of the supervised OPLS-DA model was evaluated through $R^2(Y)$ and $Q^2(Y)$, CV-ANOVA testing and permutation tests. Generally, $Q^2(Y)$ values > 0.5 are regarded as good for biological models.[33] In this study, values obtained for $R^2(Y)$ and $Q^2(Y)$ were 0.872 and 0.756, respectively, indicating an excellent fit and predictive abilities. Moreover, CV-ANOVA analysis ($p < 0.001$) demonstrated that the obtained OPLS-DA model was highly significant. Finally, permutation testing demonstrated that the predictive abilities of the original model ($R^2(Y)$ and $Q^2(Y)$) were higher in comparison to the permutated models (Fig S1b). The obtained OPLS-DA model showed separation between the sow and boar groups (Fig. 1). The two boar groups on the other hand showed some overlap, nevertheless, cross-validation demonstrated that the obtained model had a total correct classification rate of 99% and consequently could be used as a highly accurate predictive tool for the presence of boar taint. All blank and negative samples were correctly classified, whereas of the boar taint positive samples, 98% was correctly classified. The remaining 2% was classified as negative. The classification results obtained by chemical and sensory analysis, which were used as Y-information for model building, could form the basis of this misclassification. Indeed, based on the sensory scores of the neck fat samples, these samples were severely tainted. However, chemical analysis by means of UHPLC-HRMS revealed boar taint levels of SK and AEON barely exceeding the proposed odor thresholds of 200 and 500 μg kg^{-1}, respectively. Since previous studies also report a discrepancy between the presence of SK and AEON on the one hand and the sensory evaluation of boar samples on the other, this could potentially lead to biased class information in the Y-axis, causing misclassification in the OPLS-DA model [34,35]. In parallel to the OPLS-DA model, an LDA model including 80% randomly selected samples was built and loaded into a model builder recognition tool. The remaining 20% of samples were run through the real-time recognition software, which resulted in a 95% correct classification rate for the tainted boar group and thus false negative rate (β error) of ≤5%. Additionally, for the sow group also a correct classification rate of 95% was observed. For the untainted boar group on the other hand, a correct classification rate of 65% was observed due to the presence of 1 outlier and allocation of 5 and 1 samples as tainted and blank (sow), respectively. Despite the high percentage of false positive results, a false positive rate (α-error) of ≤5% was observed for the sow and untainted boar samples combined. Moreover, in contrast to false negatives, false positive classifications will not result in a loss of consumers' confidence in pork industry. However, since tainted boar meat is often subject to penalty fees, the number of false positives should be minimized [12,36].

3.2. Discrimination between tainted and untainted boars

Despite the fact that the risk of boar taint is limited to carcasses of uncastrated pigs, the indolic compounds are also present, although to a lesser extent in sows, barrows and gilts. Nevertheless, only boar carcasses should be screened at the slaughter line. Therefore, also a more simplified OPLS-DA model was constructed including the boar taint negative and positive group. The obtained model was significant ($p < 0.001$) and demonstrated excellent predictive properties ($R^2(Y)$ =0.969, $Q^2(Y)$ =0.917). Moreover, a permutation test showed that the predictive abilities of the latter model were higher than those obtained for the permutated models (Fig S2b). Finally, cross-validation revealed that all samples were correctly allocated to the boar taint positive of negative group, thus indicating 100% accuracy, specificity and sensitivity of the obtained OPLS-DA model (Fig. 2). The obtained results indicate that the untargeted REIMS analysis technique is promising for implementation at the slaughter line. As this technique involves the use of highly expensive, lab based equipment (Xevo G2-XS Q-TOF instrument), implementation in a harsh environment such as the slaughter line is unconventional and remains a challenge. However, as the iKnife

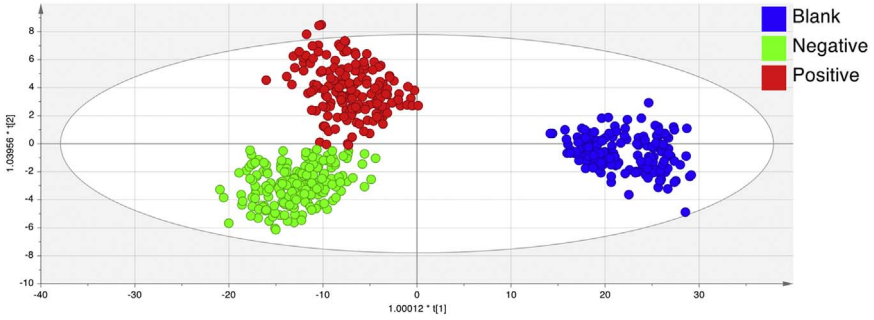

Fig. 1. Score plot of a partial least-squares discriminant analysis model for a dataset containing blank (sow) (n=50), negative (untainted) (n=50) and positive (tainted) (n=50) boar neck fat samples in negative ionization mode.

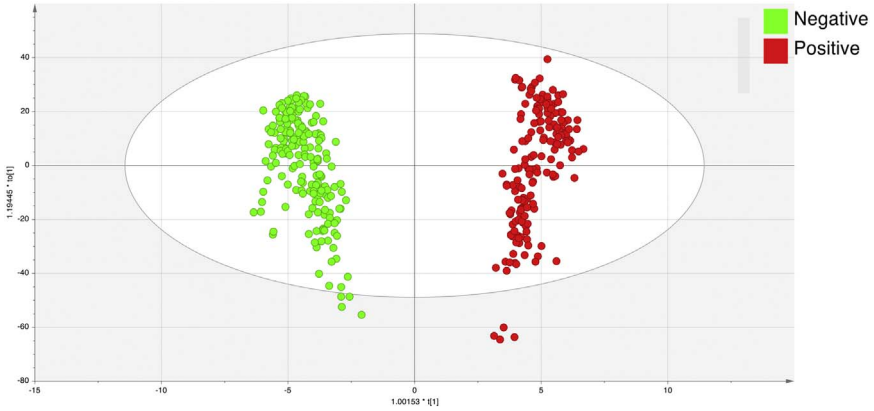

Fig. 2. Score plot of a partial least-squares discriminant analysis model for a dataset containing negative (untainted) (n=50) and positive (tainted) (n=50) boar neck fat samples in negative ionization mode.

is connected through a 4 m long tubing to the Xevo G2-XS Q-TOF instrument, the instrument itself could be placed in a separate room next to the slaughter line where humidity and temperature are controlled. As such, the at-line application of boar taint screening remains ensured and the need for sampling is excluded. Apart from the practical challenges, implementation also involves a high investment cost for abattoirs. However, because of the high number of pigs slaughtered in an average abattoir, i.e. 600/h in Belgium and in light of the increasing number of entire male pigs that will need to be slaughtered, costs per analysis per carcass are estimated to remain below 1 euro. This estimate was based on an annual slaughter of 20–50% entire male pigs, whereby 10–25% of all slaughtered carcasses should be screened for boar taint, starting from a 50/50 distribution between male and female carcasses. This indicates that it is practically and economically feasible to implement the REIMS technique at the slaughter line for routine boar taint screening.

Compared to previously reported studies, much higher classification accuracy for tainted and untainted boar carcasses was obtained by REIMS. Sensitivity and specificity of sensory methods ranged between 36–88% and 11–85%, respectively, and fluctuated greatly, depending on the trained assessor [37,38]. Recently, a classification accuracy between tainted and untainted boar samples of 81% was obtained using a portable RAMAN device. However, it should be noted that only true positive and negative samples were taken into account, whereby a cut-off of 1500 μg kg^{-1} was chosen for AEON, while in this study, a cut-off value of 500 μg kg^{-1} was considered. Moreover, an uncertainty range of ± 20% of the threshold level was considered for chemical analysis for sample inclusion in the RAMAN experiment [25]. Furthermore, compared to the targeted detection of IND, SK and AEON, applying an untargeted approach could benefit the true identification of aberrant carcasses. Indeed, up until now, the presence of SK and AEON in neck fat samples of boars only accounts for 76% of the explained variance between the presence of the latter compounds and the intensity of boar taint assessed by trained experts, indicating that also other unknown compounds attribute to the presence of boar taint [34,39]. This was confirmed by a second OPLS-DA model ($R^2(Y)$ =0.582; $Q^2(Y)$ =0.529) using quantitative UHPLC-HR-Orbitrap-MS data of IND, SK and AEON as predictive information to classify the samples under investigation as tainted or untainted, as a decrease in accuracy (89%), specificity (82%) and sensitivity (97%) was observed in comparison to the applied untargeted approach (100%) (Fig S3).

3.3. Candidate biomarkers

After model building, S-plots were constructed in order to reveal significant ions responsible for sample allocation (Fig. 3). In the S-plot, the x-axis corresponds to the contribution (covariance (p)) of the ion to the variance of the observations, e.g. absence or presence of boar taint. The y-axis on the other hand represents the correlation (p(corr)) between samples and the reliability of the results. In order for an ion or a combination of ions to be relevant, cut-off values of |p| ≥0.03 and |p(corr)| ≥0.5 are advised in metabolomics studies [40,41]. In total, 60 ions demonstrated a high contribution to the presence of boar taint in neck fat. However, none of the latter or a combination of the 4 most relevant compounds were reliable (|p(corr)| < 0.5) to allocate samples in the boar taint negative or positive group. Consequently, in order to

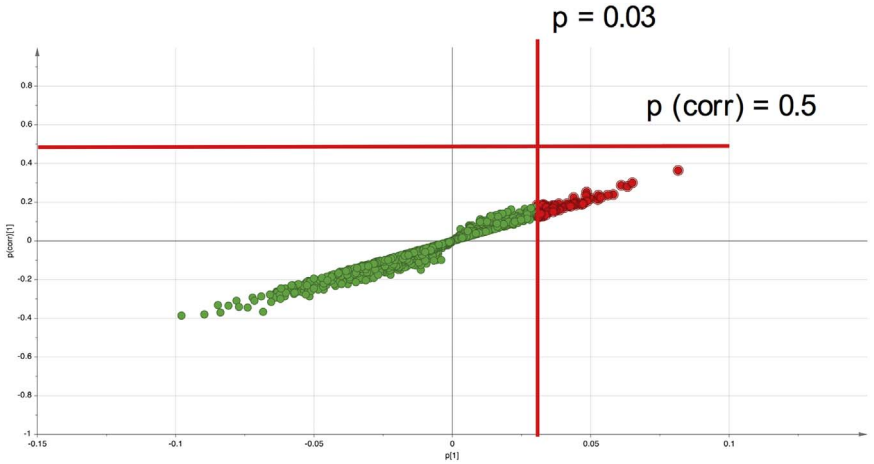

Fig. 3. Loading S-plot representing the contribution ions obtained in negative ionization mode towards the presence of boar taint. Cut-off values of |p(corr)|≥0.5 and |p|≥0.03 were applied.

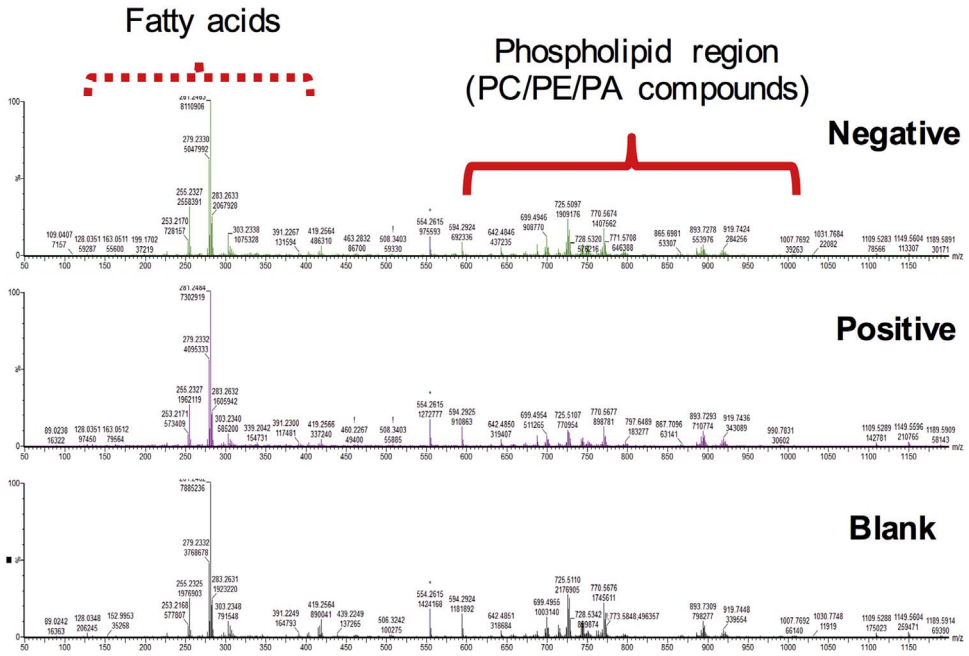

Fig. 4. Mass spectral fingerprint for negative (untainted (n=50), positive (tainted) (n=50) boar and blank (sow) (n=50) neck fat samples obtained in negative ionization mode.

correctly classify between tainted and untainted boar carcasses, the complete mass spectrum should be taken into account. A possible explanation for the lack of reliable candidate biomarkers could be the observed matrix effect. Indeed, analysis of the boar taint compounds in adipose tissue resulted in ion suppression effects whereby IND, SK and AEON could not be detected above the background matrix ions. Although a different ionization technique was applied, ion suppression of the boar taint compounds was also observed using gas chromatography coupled to electron impact mass spectrometric analysis in full scan mode [22]. Consequently, the ion suppression effects led to insufficient sensitivity to apply a targeted approach to identify aberrant boar carcasses.

3.4. Validation

To monitor and guarantee the repeatability of the measurements, a QC sample was analyzed after every 10 pig neck fat samples. Repeatability was then plotted as the intensity of the base peak ion of the endogenous lock-mass compound. In total, 5% and only 0.57% of the measurements exceeded the 2 SD and 3 SD warning limits, respectively. As over 94% of the measurements lay within these limits, good repeatability may be concluded. In a final experiment, also the robustness of REIMS for sample classification was evaluated. To this end, all data were re-acquired on different days but with a change in heater power settings of the collision surface. When taking into account the three sample groups (blank, boar taint negative and positive), a decreased classification accuracy (89%) was observed when working with a lower heater power, whereby an even percentage of false positive and negative results was obtained (16%). Since ionization of compounds is enhanced by higher energy and thus a higher heater power, the decrease in accuracy was most likely due to a loss of sensitivity in ion intensity. When omitting the blank group from the model and considering only the two boar groups, excellent classification accuracy (100%) was achieved. This indicates that despite the change in heater power settings, the REIMS spectra are very reproducible. However, it should be noted that when applying a lower heater power, the obtained OPLS-DA model showed less reliable predictive abilities as $Q^2(Y)$ and $R^2(Y)$ were 0.291 and 0.939, respectively, most likely originating from the decrease in sensitivity. This was confirmed in a permutation test, which indicated that the model fits the data well but cannot be used to accurately predict new observations (Fig S4). Consequently, careful consideration should be given to the MS settings in order to ensure the validity of each model.

3.5. Mass spectral content

Untargeted profiling of neck fat samples revealed differences in spectra between sow, tainted and untainted boars (Fig. 4). In order to situate the spectral differences between the latter groups, putative identification was performed of the most abundant ions in the fatty acid and phospholipid region, providing class information of the compounds. To this end, the selected ions were cross-referenced to the LipidMaps (www.lipidmaps.org) and Lipidblast database (http://fiehnlab.ucdavis.edu/projects/LipidBlast). This search was based on the obtained accurate masses and a mass tolerance window of ± 0.01 Da was applied. In most cases, the *m/z* value could not be assigned to one single compound. Nevertheless, the lipid classes could be revealed. The spectral differences in negative ionization mode between the sow, boar taint positive and negative group were mainly situated in the fatty acid and phospholipid region of the obtained mass spectra. Moreover, primary clustering between the spectra of the boar taint positive and negative group was observed (Fig. 5). In general, monounsaturated fatty acids (MUFAs) (16:1, 18:1, 22:1), tentatively identified as palmitoleic acid, oleic acid and erucic acid, respectively, were predominantly present in the boar taint positive group. Intermediate levels were observed in the boar taint negative group

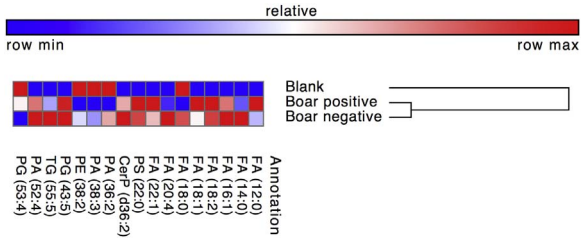

Fig. 5. Heat map (GEN-E software, http://www.broadinstitute.org/cancer/software/GEN-E/index.html) visualizing a selected number of putatively identified compounds in blank (sow) (n=50), positive (tainted) (n=50) and negative (untainted) (n=50) neck fat samples, with hierarchical clustering of the different samples.

and the lowest levels in the blank or sow group (Fig. 5). Similarly, also polyunsaturated fatty acids (PUFAs) (18:2 & 20:4), tentatively identified as linoleic acid and arachidonic acid, were mostly abundant in the boar taint positive and negative groups. The saturated fatty acids (SFAs) lauric acid (12:0) and myristic acid (14:0) on the other hand were predominantly present in the boar taint positive and negative groups, respectively. Furthermore, stearic acid (18:0) was mainly present in the blank or sow group in comparison to the two boar groups. These differences are most likely associated with the differences found in the phospholipid region of the mass spectra as the majority of the signal intensity in the fatty acid region originates from fragmentation of these phospholipids (Fig. 5). Recent studies demonstrated similar trends in fatty acid composition in boars, sows and surgically castrated pigs [25,39,42]. Pauly et al. [39] found a significantly lower amount of SFAs and higher amount of PUFAs in entire males in comparison to surgically castrated pigs and immunocastrates. Similar trends were reported by Mackay et al. [43], who observed a decrease of 21% in n-6-PUFAs in entire males in comparison to boars. Furthermore, in a recent study, significantly higher amounts of total PUFAs were observed in boar carcasses with low levels of AEON (23.4%) in comparison to boar carcasses with high levels of AEON (19.7%). This was due to increased levels of linoleic acid and alpha-linolenic acid [42]. However, these results were not conclusive as higher PUFA and MUFA levels were observed in highly tainted fat samples by Liu et al. [25]. The mechanism behind these differences in fatty acid composition lies in the regulation of fat deposition and differences in lipid synthesis and metabolism. Indeed, recently an increased expression of stearoyl-CoA desaturase and delta-6-desaturase, two enzymes involved in lipid synthesis, was demonstrated in boar adipose tissue in comparison to castrates [43]. The latter enzymes are responsible for the formation of unsaturated fatty acids, explaining the higher amount of PUFA found in boars. Not only differences in lipid composition between boars and sows were observed but also significant reciprocal differences between boars with high and low boar taint levels [25,42,44]. Although the mechanisms behind the influence of high SK and AEON levels on lipid synthesis and metabolism are not completely unraveled, it has been reported that high SK levels induce CYP2E1 activity, an enzyme involved in lipid peroxidation, consequently lowering PUFA levels in adipose tissue. High levels of AEON on the other hand inhibit gene expression of CYP2E1 and block induction of the latter by SK [45]. Since phospholipids are partly composed out of fatty acids, alterations in fatty acid composition can also manifest itself in the phospholipid region [42]. Based on the differences of the latter between sows and boars but more importantly, boars with high and low boar taint levels, lipid profiles could explain the observed discrimination between these three groups and classify carcasses as tainted or untainted.

4. Conclusions

The results obtained in this study demonstrated tainted carcasses could be correctly classified by an untargeted approach. This makes REIMS suitable not only for discrimination between gender samples (sow versus boar) but also for discrimination within gender (tainted versus untainted boars). This discrimination originated from alterations in lipid profiles, mainly situated in the fatty acid and phospholipid region. However, to this end, a fingerprinting approach was necessary as no reliable candidate biomarkers could be identified. Moreover, as REIMS eliminates extensive sample pre-treatment procedures, analysis takes under 10 s, which makes it the first technique that enables in-situ detection of boar taint combined with highly accurate classification. Finally, in view of implementing this untargeted approach in an at-line environment, the MVA software further empowers the applied technology as it enables real time recognition of unknown samples through screening against a known database. For this reason, this new analytical in-situ monitoring platform is very promising for other applications in food safety or quality, whereby rapid characterization of food products is requisite.

Acknowledgements

Kaat Verplanken is supported by Flanders Innovation and Entrepreneurship (VLAIO, IWT/SB131420).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2017.03.056.

References

[1] E. Commission, Laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, DOI (2002).
[2] K. Ridgway, S.P.D. Lalljie, R.M. Smith, Analysis of food taints and off-flavours: a review, Food Addit. Contam. Part a-Chem. Anal. Control Expo. Risk Assess. 27 (2010) 146–168.
[3] J.N. Seiber, New analytical advances for addressing healthful constituents in foods, J. Food Drug Anal. 20 (2012) 408–410.
[4] P. Walstra, H. Maarse, Onderzoek geslachtgeur van mannelijke mestvarkens, Researchgroup Vlees en Vleeswaren, T.N.O., Zeist. The Netherlands, 1970, pp. 1–30.
[5] E. Vold, Fleishproduktionseigenschaften bei ebern und kastraten. IV. Organoleptische und gaschromatografische untersuchungen wassedampfflüchtiger stooffe des rückenspeckes von ebern, Meld. Nord. 49 (1970) 1–25.
[6] R.L.S. Patterson, 5alpha-androst-16-ene-3-one: compound responsible for taint in boar fat, J. Sci. Food Agric. 25 (1968) 692–703.
[7] K. Lundstrom, K.R. Matthews, J.E. Haugen, Pig meat quality from entire males, Animal 3 (2009) 1497–1507.
[8] B. Fredriksen, M. Font-i-Furnols, K. Lundstrom, W. Migdal, A. Prunier, F.A. Tuyttens, M. Bonneau, Practice on castration of piglets in Europe, Animal 3 (2009) 1480–1487.
[9] M. Font-i-Furnols, Consumer studies on sensory acceptability of boar taint: a review, Meat Sci. 92 (2012) 319–329.
[10] M. Aluwé, S. Meirlaen, J. Van Meensel, S. Millet, F.A. Tuyttens, CASPRAK: vergelijkende studie op praktijkbedrijven van alternatieven voor onverdoofde castratie van beerbiggen, ILVO Meded. 112 (2012).
[11] European Food Safety Authority, Welfare aspects of the castration of piglets: scientific report of the scientific panel for animal health and welfare on a request from the commission related to welfare aspects of the castration of piglets, EFSA J. 91 (2004) 1–18.
[12] J.E. Haugen, C. Brunius, G. Zamaratskaia, Review of analytical methods to measure boar taint compounds in porcine adipose tissue: the need for harmonised methods, Meat Sci. 90 (2012) 9–19.
[13] M. Aluwé, F.A.M. Tuyttens, K.M. Bekaert, S. De Smet, D.L. De Brabander, S. Millet, Evaluation of various boar taint detection methods, Animal 6 (2012) 1868–1877.
[14] K.M. Bekaert, M. Aluwé, L. Vanhaecke, L. Heres, L. Duchateau, F. Vandendriessche, F.A. Tuyttens, Evaluation of different heating methods for the detection of boar taint by means of the human nose, Meat Sci. 94 (2013) 125–132.
[15] L. Jarmoluk, A.H. Martin, H.T. Fredeen, Detection of Taint (Sex Odor) in Pork, Can. J. Anim. Sci. 50 (1970) (750- &).
[16] C. Di Natale, G. Pennazza, A. Macagnano, E. Martinelli, R. Paolesse, A. D'Amico, Thickness shear mode resonator sensors for the detection of androstenone in pork fat, Sens. Actuators B-Chem. 91 (2003) 169–174.
[17] J.E. Haugen, The use of chemical sensor array technology, the electronic nose, for detection of boar taint, Acta Vet. Scand. 48 (2006).
[18] J.S. Vestergaard, J.E. Haugen, D.V. Byrne, Application of an electronic nose for measurements of boar taint in entire male pigs, Meat Sci. 74 (2006) 564–577.
[19] B. Bourrounet, T. Talou, A. Gaset, Application of a multi-gas-sensor device in the meat industry for boar-taint detection, Sens. Actuators B-Chem. 27 (1995) 250–254.
[20] F. Wackers, D. Olson, G. Rains, F. Lundby, J.E. Haugen, Boar taint detection using parasitoid biosensors, J. Food Sci. 76 (2011) S41–S47.
[21] S. Ampuero, G. Bee, The potential to detect boar tainted carcasses by using an electronic nose based on mass spectrometry, Acta Vet. Scand. 48 (2006).
[22] K. Verplanken, J. Wauters, J. Van Durme, D. Claus, J. Vercammen, S. De Saeger, L. Vanhaecke, Rapid method for the simultaneous detection of boar taint compounds by means of solid phase microextraction coupled to gas chromatography/mass spectrometry, J. Chromatogr. A 1462 (2016) 124–133.
[23] K.M. Sorensen, S.B. Engelsen, Measurement of boar taint in porcine fat using a high-throughput gas chromatography-mass spectrometry protocol, J. Agr. Food Chem. 62 (2014) 9420–9427.
[24] K.M. Sorensen, C. Westley, R. Goodacre, S.B. Engelsen, Simultaneous quantification of the boar-taint compounds skatole and androstenone by surface-enhanced Raman scattering (SERS) and multivariate data analysis, Anal. Bioanal. Chem. 407 (2015) 7787–7795.
[25] X.Y. Liu, H. Schmidt, D. Morlein, Feasibility of boar taint classification using a

portable Raman device, Meat Sci. 116 (2016) 133–139.
[26] J. Balog, S. Kumar, J. Alexander, O. Golf, J.Z. Huang, T. Wiggins, N. Abbassi-Ghadi, A. Enyedi, S. Kacska, J. Kinross, G.B. Hanna, J.K. Nicholson, Z. Takats, In vivo endoscopic tissue identification by Rapid Evaporative Ionization Mass Spectrometry (REIMS), Angew. Chem.-Int. Ed. 54 (2015) 11059–11062.
[27] O. Golf, N. Strittmatter, T. Karancsi, S.D. Pringle, A.V.M. Speller, A. Mroz, J.M. Kinrosss, N. Abbassi-Ghadi, E.A. Jones, Z. Takats, Rapid evaporative ionization mass spectrometry imaging platform for direct mapping from bulk tissue and bacterial growth media, Anal. Chem. 87 (2015) 2527–2534.
[28] L. Muirhead, J.M. Kinross, R. Preece, A. Speller, O. Golf, R. Goldin, A. Darzi, Z. Takats, A. Prospective, Observational study of surgical and endoscopic rapid evaporative ionisation mass spectrometry (Reims) for real time analysis of the colonic mucosal lipidome in colorectal cancer, Gut 64 (2015) A50–A51.
[29] K.C. Schafer, J. Denes, K. Albrecht, T. Szaniszlo, J. Balog, R. Skoumal, M. Katona, M. Toth, L. Balogh, Z. Takats, In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry, Angew. Chem.-Int. Ed. 48 (2009) 8240–8242.
[30] N. Strittmatter, M. Rebec, E.A. Jones, O. Golf, A. Abdolrasouli, J. Balog, V. Behrends, K.A. Veselkov, Z. Takats, Characterization and identification of clinically relevant microorganisms using rapid evaporative ionization mass spectrometry, Anal. Chem. 86 (2014) 6555–6562.
[31] J. Balog, D. Perenyi, C. Guallar-Hoyas, A. Egri, S.D. Pringle, S. Stead, O.P. Chevallier, C.T. Elliott, Z. Takats, Identification of the species of origin for meat products by rapid evaporative ionization mass spectrometry, J. Agr. Food Chem. 64 (2016) 4793–4800.
[32] K.M. Bekaert, J. Vanden Bussche, S. Francois, F.A. Tuyttens, H.F. De Brabander, F. Vandendriessche, L. Vanhaecke, A validated ultra-high performance liquid chromatography coupled to high resolution mass spectrometry analysis for the simultaneous quantification of the three known boar taint compounds, J. Chromatogr. A 1239 (2012) 49–55.
[33] D.I. Broadhurst, D.B. Kell, Statistical strategies for avoiding false discoveries in metabolomics and related experiments, Metabolomics 2 (2006) 171–196.
[34] M. Bonneau, Compounds responsible for boar taint, with special emphasis on androstenone - a review, Livest. Prod. Sci. 9 (1982) 687–705.
[35] C. Pauly, W. Luginbuhl, S. Ampuero, G. Bee, Expected effects on carcass and pork quality when surgical castration is omitted - Results of a meta-analysis study, Meat Sci. 92 (2012) 858–862.
[36] R.E. Klont, E. Kurt, L. Heres, B. Urlings, Production of entire males - challenges and opportunities, Fleischwirtschaft 90 (2010) 107–109.
[37] L. Meier-Dinkel, J. Gertheiss, S. Muller, R. Wesoly, D. Morlein, Evaluating the performance of sensory quality control: the case of boar taint, Meat Sci. 100 (2015) 73–84.
[38] J. Trautmann, L. Meier-Dinkel, J. Gertheiss, D. Morlein, Boar taint detection: a comparison of three sensory protocols, Meat Sci. 111 (2016) 92–100.
[39] C. Pauly, P. Spring, J.V. O'Doherty, S.A. Kragten, G. Bee, Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac (R)) and entire male pigs and individually penned entire male pigs, Animal 3 (2009) 1057–1066.
[40] S. Wiklund, E. Johansson, L. Sjostrom, E.J. Mellerowicz, U. Edlund, J.P. Shockcor, J. Gottfries, T. Moritz, J. Trygg, Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models, Anal. Chem. 80 (2008) 115–122.
[41] S.Y. Xue, Z.Y. Li, H.J. Zhi, H.F. Sun, L.Z. Zhang, X.Q. Guo, X.M. Qin, Metabolic fingerprinting investigation of Tussilago farfara L. by GC-MS and multivariate data analysis, Biochem. Syst. Ecol. 41 (2012) 6–12.
[42] D. Morlein, E. Tholen, Fatty acid composition of subcutaneous adipose tissue from entire male pigs with extremely divergent levels of boar taint compounds - An exploratory study, Meat Sci. 99 (2015) 1–7.
[43] J. Mackay, M.C. Pearce, S. Thevasagayam, O. Doran, Fatty acid composition and lipogenic enzyme protein expression in subcutaneous adipose tissue of male pigs vaccinated against boar taint, barrows, and entire boars, J. Anim. Sci. 91 (2013) 395–404.
[44] A.J. Elsbernd, K.J. Stalder, L.A. Karriker, J.F. Patience, Comparison among gilts, physical castrates, entire males, and immunological castrates in terms of growth performance, nitrogen and phosphorus retention, and carcass fat iodine value, J. Anim. Sci. 93 (2015) 5702–5710.
[45] E. Doran, F.W. Whittington, J.D. Wood, J.D. McGivan, Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes, Chem.-Biol. Interact. 140 (2002) 81–92.