UNIVERSITEIT GENT

FACULTY OF VETERINARY MEDICINE
approved by EAEVE

RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY FOR HIGH THROUGHPUT SCREENING IN FOOD ANALYSIS: THE CASE OF BOAR TAINT

Kaat Verplanken*[1], Sara Stead[2], Renata Jandova[2], Christof Van Poucke[3], Jan Claereboudt[4], Julie Vanden Bussche[1], Sarah De Saeger[5], Zoltan Takats[6], Jella Wauters[1] and Lynn Vanhaecke[1]

[1] Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Laboratory of Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium
[2] Waters Corporation, Altrincham Road, UK-SK9 4AX Wilmslow, United Kingdkom
[3] Institute of Agricultural and Fischeries Research (ILVO), Technology and Food Sciences Unit, Brusselsesteenweg 370, B-9090 Melle, Belgium
[4] Waters Corporation, Brusselsesteenweg 500, B-1731 Zellik, Belgium
[5] Laboratory of Food Analysis, Department of Bioanalysis, Ghent University, Ottergemsesteenweg 460, Ghent, B-9000, Belgium
[6] Faculty of Medicine, Department of Surgery and Cancer, Imperial College London, London, SW7 2AZ, UK

*E-mail: Kaat.Verplanken@UGent.be Website: http://www.vvv.ugent.be

INTRODUCTION

Increasing awareness on **animal welfare** led to a voluntary intent among European Member States to abandon the surgical castration of pigs by **2018**. However, rearing entire males, one of the alternatives, implies the possible occurrence of **boar taint** in carcasses. Boar taint is an off-odour, provoking negative consumer reactions, and consequently leads to severe economic losses in pig husbandry. For this reason, it is crucial to timely detect boar taint containing carcasses at the slaughter line. Since currently, accurate and fast analysis methods at the slaughter line are lacking, **rapid evaporative ionization mass spectrometry (REIMS)** was evaluated for the latter purpose.

MATERIALS & METHODS

Samples

Sow and boar neck fat samples were collected at the slaughter line (n = 150). The presence or absence of boar taint in the samples was confirmed by **sensory evaluation** and **UHPLC-HR-Orbitrap-MS** analysis(1). Samples containing levels of indole (IND), skatole (SK) and androstenone (AEON) above and below the odour 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.

(1) Bekaert, K.M.; Vanden Bussche, J.; Francois, S.; Tuyttens, F.A.; De Brabander, H.F.; Vandendriessche, F.; Vanhaecke, L. *J Chromatogr A* **2012**, 1239, 49-55.

Instrumentation

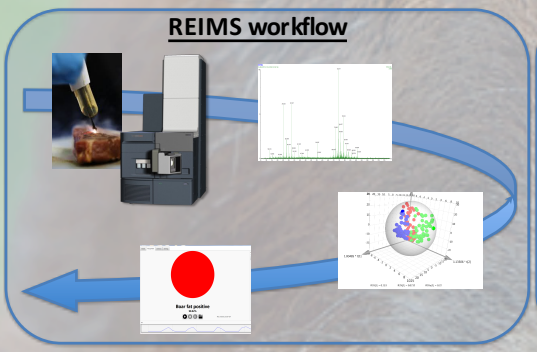
Sampling was carried out for 3 to 5 seconds with an **iKnife** hand-held sampling device (Waters, Wilmslow, UK), which was directly coupled to a **Xevo G2-XS Q-TOF** instrument equipped with a coiled ribbon collision surface (Waters, Wilmslow, UK).

Xevo G2-XS Q-TOF	Setting
Mode	REIMS TOF MS
Data acquisition	Continuum
Cone Voltage	100 V
Ionization mode	Negative
Mass range	50 – 1200 *m/z*
Scan speed	1 s/scan

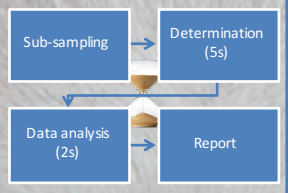
Untargeted profiling & Chemometric data processing

Boar and sow neck fat samples were profiled, providing a **mass spectral fingerprint**. Afterwards, the mass spectral fingerprints were used to a construct predictive **OPLS-DA** model for classification. To this end, **Progenesis QI** and **SIMCA 2.2** software were used for data pre-processing purposes and model building, respectively.

REIMS workflow

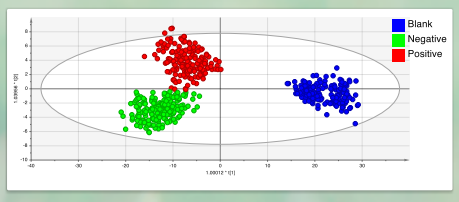


Sample to report within seconds



RESULTS

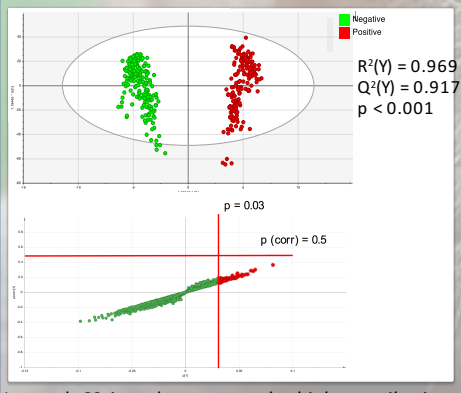
Predictive OPLS-DA model



	Members	Correct	Blank	Negative	Positive
Blank	170	100%	170	0	0
Negative	192	100%	0	192	0
Positive	174	97.7%	0	4	170
No class	0		0	0	0
Total	536	99.25%	170	196	170

Evaluation of the obtained OPLS-DA model showed good validation characteristics **$R^2(Y)$ = 0.872, $Q^2(Y)$ = 0.756**, indicating a good fit and predictive properties of the model. CV-ANOVA analysis ($p < 0.001$) and permutation testing (20 permutations) confirmed the reliability of the obtained OPLS-DA model. A total **classification accuracy of 99%** was achieved.

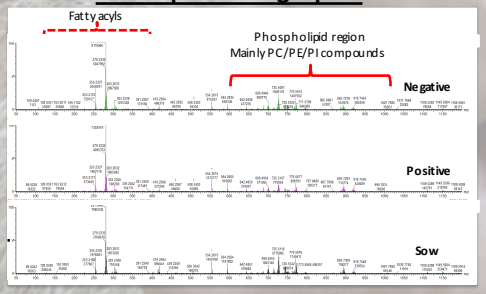
Candidate discriminating compounds



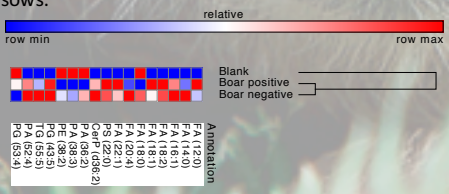
$R^2(Y) = 0.969$
$Q^2(Y) = 0.917$
$p < 0.001$

In total, 60 ions demonstrated a high contribution to the presence of boar taint in neck fat **$|p| \geq 0.03$**. However, none of the latter compounds were reliable **$|p(corr)| \geq 0.5$** for allocation of the samples in the boar taint negative or positive group. Consequently, in order to correctly classify between tainted and untainted boar carcasses, the complete mass spectrum should be taken into account.

Mass spectral fingerprint



Spectral differences between the three groups were mainly situated in the **phospholipid and fatty acid region**. MUFAs were predominantly present in the boar taint positive group. PUFAs and SFAs were most abundant in the 2 boar groups in comparison to sows.



CONCLUSIONS

In this study, **REIMS** was able to correctly (**99% accuracy**) identify tainted boar neck fat samples **within a couple of seconds**, based on an untargeted profiling approach. The discrimination between boars (tainted & untainted) and sows originated from alterations in **lipid profiles**, mainly situated in the fatty acid and phospholipid region. Moreover, as REIMS enables **in-situ analysis**, guaranteeing **point-of-control monitoring**, it is a very promising and powerful tool for a diverse range of applications in **food safety and quality**.

ACKNOWLEDGMENTS

Kaat Verplanken is supported by Flanders Innovation & Entrepreneurship (VLAIO) (IWT: SB 131420).

AGENTSCHAP INNOVEREN & ONDERNEMEN IWT