AT-LINE RAPID INSTRUMENTAL METHOD FOR MEASURING THE BOAR TAINT COMPONENTS ANDROSTENONE AND SKATOLE IN PORK FAT

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Abstract – An instrumental method for simultaneous measurement of androstenone and skatole in backfat samples from entire male pigs was developed at the Danish Meat Research Institute (DMRI). The method is based on Laser Diode Thermal Desorption (LDTD) and Atmospheric Pressure Chemical Ionization (APCI) coupled with MS-MS detection. With this instrumentation, the assay meets requirements set by large Danish pork slaughterhouses for maximum cost per sample, short time from a sample is extracted from the carcass on the slaughter line to the result of the analysis is in the slaughterhouse database and requirements for the speed of operation. With an automated sample pre-treatment, it will be possible with a single LDTD-MS-MS system to keep up with a line speed of 360 male pig carcasses per hour and to run 16 hours per workday.

Key Words – Male pigs, Laser diode thermal desorption, LDTD, MS-MS.

I. INTRODUCTION

A declaration has been put forward by the EU to voluntarily end the use of surgical castration without anaesthesia, of pigs in Europe, by 2018. As meat from some uncastrated male pigs develop a boar taint flavour perceived as unpleasant by many consumers, the pig industry is concerned about maintaining market shares after such a ban on castration. Therefore, the European pig industry needs a screening method for identifying male pig carcasses containing high levels of the boar taint components androstenone and skatole at the slaughterhouse, before they leave the slaughter line. The task of identifying and developing a suitable screening method was given to the DMRI by the Danish pork industry.

Haugen et al. [1] present a comprehensive list of analytical methods of which some may have potential as at-line or even on-line assays capable of working under slaughterhouse conditions. However, the methods all lack either sensitivity, robustness or speed to satisfy specifications set by the industry.

System specifications.

The method must be instrumental and objective. The method must be able to measure both androstenone and skatole simultaneously, using the same sample pre-treatment and equipment. The time from sample extraction from the carcass to the result is available for the slaughterhouse must be less than 30 minutes. The cost of consumables for analysing a carcass must be less than 1 €, and finally the instrumentation must be robust, requiring little maintenance with system components being purchasable as “off-the-shelf” equipment. The method should be selective for the identified boar taint components, with good reproducibility between samples from the same carcass. Limits of quantification for both androstenone and skatole should lie well below the anticipated sorting thresholds for the two compounds in backfat samples.

The aim of the work.

Identify instrumentation that will be robust under industrial conditions and with an appropriate sample pre-treatment, which can meet the specifications set up by the slaughter industry.

II. MATERIALS AND METHODS

Instrumentation

The LDTD system with APCI ionization (Phytronix, Quebec, Canada) front ending a MS-MS Sciex 6500 QTrap detector (AB Sciex, 500 Old Connecticut Path, Framingham, MA 01701, USA) were selected as the basic instrumentations that could meet the requirements for robustness of operation, accuracy, selectivity and with a throughput capable of keeping up with line speeds at a large Danish slaughterhouse.

In the LDTD, the dried sample is desorbed from the bottom of a well in a special microtiter plate that has a metal foil bottom plate. Desorption is done by a focused laser beam that heats the metal foil resulting in the deposited analytes being brought into the gas phase. Desorbed analytes are carried by a flow of air past a corona discharge needle where they are ionized. The ionized sample is then directly measured in the attached mass spectrometer. The laser heating typically lasts only about 5 seconds and there is no need for time-consuming chromatographic separation [2, 3].

Sample handling and pre-treatment

Approximately 0.4 g of fat is extracted from the carcass for analysis. On the slaughter line, the fat sample is placed in a tared plastic centrifugue tube and its weight is determined. Samples are subsequently transported to an in-house
laboratory for analysis. A salt assisted liquid-liquid extraction (SALLE) is now applied. The extraction buffer contains internal standards for the two measured compounds to compensate for any variation in the sample preparation steps, laser desorption, ionization and MS analysis. The sample with extraction buffer is then homogenized using a shaft blender. After equilibration and centrifugation, an aliquot from the supernatant is transferred to the LazWell™ plate. The organic phase evaporates after approximately 1 minute, and the LazWell™ plate is successively inserted in the LDTD for laser desorption, ionization and detection by the MS-MS system.

III. RESULTS AND DISCUSSION
Blank backfat from a young castrated male pig was used to create the calibration curves shown in figures 1 and 2. For this the acetonitrile was additionally spiked with 6 levels of androstenone and 6 levels of skatole. The mass spectrometer is run in positive ionization and selected reaction monitoring modes.

![Androstenone calibration curve](Image1)

![Skatole calibration curve](Image2)

With these calibration curves the concentration of analytes in new samples can be calculated by measuring the peak areas of analytes and internal standards.

When implemented at a slaughterhouse, as a fully automated process, samples will be treated in batches of 24 or 48 as the homogenization and centrifugation steps are bottle necks for the work flow in the analysis.

IV. CONCLUSION
A potentially robust and specific method, based on LDTD-MS-MS, for measuring androstenone and skatole in backfat from entire male pigs was developed. Sample pre-treatment is very simple and no chromatographic steps are involved. In the future work, either an automated or semi-automated fat sample extraction from carcasses will be developed. When automated at a slaughterhouse laboratory, the cost for consumables per carcass is approx. 0.7 €. Analysis time is 10 seconds and the method thus fulfills industries needs for throughput.

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