POTENTIAL OF PTR-TOF-MS FOR MEASURING THE BOAR TAINT COMPONENTS: ANDROSTENONE, SKATOLE AND INDOLE.

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Abstract – The European Union intends to work towards a total cessation of surgical castration of male pigs within the next 6 years. In some countries immuno castration is not an option as it is expected to be unacceptable by large portions of the European consumers of pork. Therefore, the Danish Meat Research Institute has made an effort to find instrumental methods that have the potential for at-line measurement of boar taint components at slaughter line speeds. The most promising candidate is a method known as Proton Transfer Reaction-Time of Flight – Mass Spectroscopy PTR-TOF-MS. Experiments have shown that with this method it is possible to measure Skatole and Indole directely in the headspace over a fat sample and that androstenone can be measured using an SPME-fibre to adsorb boar taint components from the headspace over a heated fat sample. When heated, the fibre can release the androstenone which subsequently can be measured with the PTR-TOF-MS.

Key Words – SPME, solid phase micro extraction, headstace, mass spectroscopy.

I. INTRODUCTION

The European Union intends to work towards a total cessation of surgical castration of male pigs within the next 6 years. In some countries immuno castration is not an option as it is expected to be considered unacceptable by large portions of the European consumers of pork. For these reasons it will be necessary to develop a system for measurement of boar taint on the slaughter line so carcasses having an off- odour can be used for productions where this is of no significance. The human nose method is not an acceptable method at large abattoirs, where many hundreds of entire males are slaughtered per hour. This is due to the fact that a person's sense of smell is dulled after prolonged use.

Therefore, the Danish Meat Research Institute has made an effort to find instrumental methods that have the potential for at-line measuring boar taint components (skatole, indole, androstenone) at slaughter line speeds. The most promising candidate is a method known as Proton Transfer Reaction - Time of Flight – Mass Spectroscopy PTR-TOF-MS. With this instrument, sampling can be made directly from the headspace over a heated sample. Its reacts instantaneously when coming into contact with the headspace and its detector system needs no purging after having its inlet removed from the sample.

II. MATERIALS AND METHODS

On a commercial slaughter line, back fat samples approximately 10x10 cm in size were taken from the shoulder region of 74 entire male pigs and frozen until measuring. In the laboratory sub-samples were used for analyzing for Androstenone, Skatole and Indole using the so called ASI HPLC reference method [1, 2].

The PTR-TOF-MS that we used was the PTR-TOF 8000 High-Resolution from Ionicon Analytik Ges.m.b.H [3] Austria.

From the 74 samples, a smaller piece roughly 0.2 grams each were placed in 15 ml vials that were loosely closed using an aluminum foil cap. The vials were heated to roughly 170°C on a hot plate. Aluminum foil was packed around the vials and in contact with the heating plate.

After 4 minutes of heating the inlet tube from the PTR-TOF-MS was introduced in to the headspace over the melted fat sample in the vial. The instrument was tuned in on the masses 117.2 u, 131.2 u and 272.4 u for indole, skatole and androstenone respectively. The detected ion yields were registered and these values were related to the concentrations as measured by the ASI method.

A less direct approach was also tested on a smaller scale. SPME (solid phase micro extraction) fibres [4,5] were placed in the headspace of the heated vials with the fat samples. After 10 minutes, volatile organic compounds (VOC) in the fat had been adsorbed on the surface of the SPME fibre.

When inserted into the heated inlet of the PTR-TOF-MS the adsorbed molecules are released whereby the masses of the VOC's can be determined.

III. RESULTS AND DISCUSSION

With the headspace measurement, it is in general not necessary to measure the weight of the fat samples in the vials. This is due to the fact that the equilibrium between boar taint component in the fat and the headspace is a heterogenic one. Concentrations in the fat samples are several orders of magnitude greater than in the headspace. In such cases the headspace concentration will only depend on the VOC concentration in the solid fat phase and on temperature. If equilibrium has not been reached before measuring, the headspace concentrations will also depend on the surface area of the sample and the exposure time of headspace to sample. In this set of experiments the measurements were carried out before equilibrium was reached. For the headspace measurements the skatole and Indole concentrations were pooled in a variable known as skatole equivalents (se) [2] which is a simple linear combination of skatole and Indole.

$$Se = 100*(0.72*[skatole]+0.41*[indole]+0.27)$$

In figure 1 are shown the PTR-TOF-MS determined skatole equivalents versus the HPLC reference method (N=74).

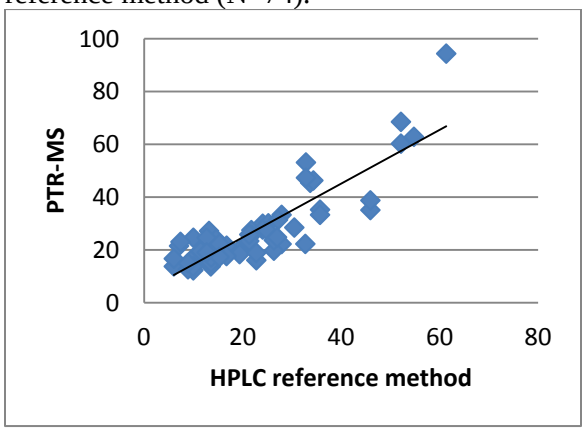

Figure 1. PTR-TOF-MS method versus HPLC reference method for skatole equivalents.

Even though the results are far from perfect is should be seen as a preliminary test where there is ample room for improvements.
In figure 2 is shown the reproducibility of the PTR-TOF-MS method on 22 pigs measured on to different days.

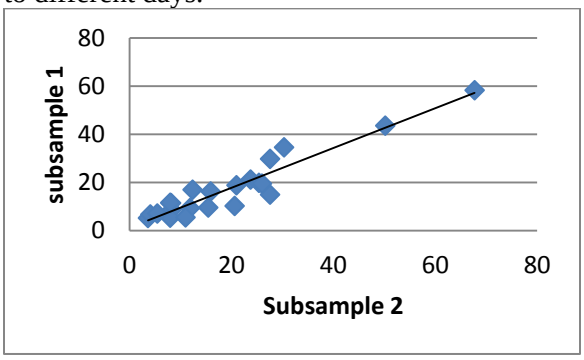

Figure 2. Reproducibility, when measuring Skatole equivalents.

It proved difficult to drive the androstenone out of the fat samples if they were only heated. This is due to the fact that androstenone is very lipophilic and that it has as a much greater molecular weight than skatole.
In figure 3 is shown a PTR-TOF-MS measurement on headspace from a pure crystal of androstenone placed in a vial and heated to 60°C.

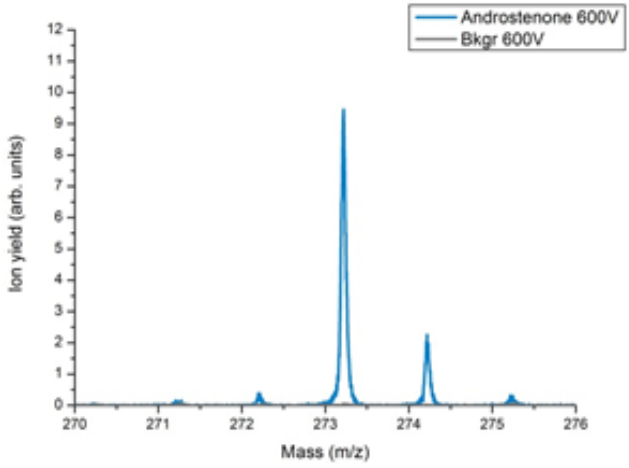

Figure 3. Mass spectrum using the PTR-TOF-MS to sample androstenone.

Notice that the crystal is not pure. The androstenone derivative androstenol can be seen 2 mass units above the main component. The other three components are most likely also related to androstanone. The above measurement proves that androstenone can be detected by the PTR-TOF-MS.

In the smaller experiment involving adsorption of fat headspace on SPME fibres, 6 fat samples were analyzed.
Results of measuring androstenone on these fibres are shown in figure 4.

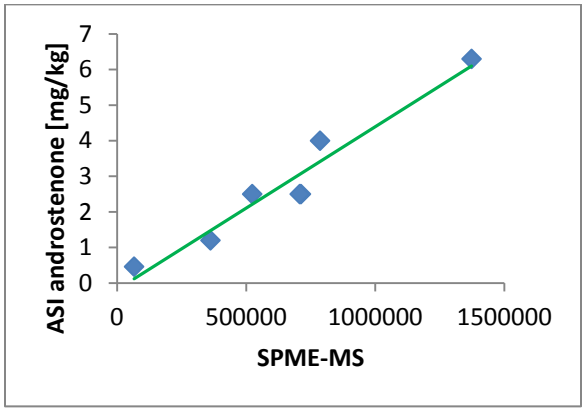

Figure 4. HPLC androstenone versus mass spectrum ion yield from androstenone.

IV. CONCLUSION

The results from these very coarse experiments demonstrate that there is good potential in using the PTR-TOF-MS for detecting boar taint. However, the conditioning of the samples prior to measurement requires a good deal of refinement before the method can be implemented as an at-line method at an abattoir.

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