Detection of Boar Taint
Need for harmonised methods and rapid methods

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Abstract— **Boar taint occurs in meat from some entire male pigs which makes it unfit for human consumption and is basically caused by the presence of the substances skatole and androstenone. A number of methods have been and are still in use for quantiative determination of these substances in adipose tissue from entire male pigs. Until date, no harmonised analytical protocols exist for analysing skatole and androstenone in boar fat tissue which makes it difficult to compare results from different laboratories. This is essential in order to set sound sensory tresholds for perception of boar taint and proper sorting criteria for boar taint detection at the slaughterline. Accordingly, there is a great need for a harmonised analytical protocol for the analysis of skatole and androstenone. Since the production of entire male pigs is highly desirable due to animal welfare issues related to surgical castration, there will be a need for future cost-effective rapid on/at-line detection methods in slaughterhouses for identifying carcasses with unacceptable levels of boar taint compounds. The paper gives a short review on existing analysis methodology for skatole and androstenone and addresses the need for standardisation and harmonisation in this field. In addition, emerging technologies with potential for rapid boar taint detection for slaughterline application will be reviewed.**

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***Index Terms*— androstenone, boar taint, detection, skatole.**

I. Introduction

Male piglets are still surgically castrated in many countries in order to prevent consumers from getting boar tainted meat. Boar taint represents a complex sensory issue that relates to a few malodourous compounds present in pork meat from entire, uncastrated male pigs. The two substances skatole and androstenone have been ascribed to contribute most to the sensory perception of boar odour and flavour [1-2]. The reported sensory perception levels of the boar taint compounds androstenone and skatole are 0.5–1 ppm and 0.20–0.25 ppm, respectively on a fat basis [3], and which have also been suggested as sorting criteria for entire male pig carcasses. But also some other substances have been suggested to play a role in the overall perception of boar taint, i.e. indole [4], 4-phenyl-3-buten-2-one and short-chain fatty acids [5-6]

Due to aninal welfare issues, ban of castration of male pigs is desirable. So far there is a limited legislation with regard to off-flavours in food and food products.

Analysis of boar taint has traditionally been performed by a trained sensory panel and been related to the levels of skatole and androstenone on a fat basis. A number of methodologies have been published over the years on the analysis of skatole and androstenone in boar fat, comprising colorimetric, HPLC, GC and immunolgical methods. However, there is still no standardised protocol for these analyses.

In addition, rapid high throughput on/at line methods to detect boar tainted male pig carcasses at the slaughterline will be needed in future. Recently, several emerging technologies with a potential for rapid boar taint detection have been investigated. These methods represent basically two strategies that may be applied either to solid phase (fat samples) or gas-phase; So-called "fingerprinting" methods aim at indirect measurement of boar odour by applying non-specific methods. Typical techniques are based on chemical gas sensor arrays, spectroscopy and direct mass spectrometry where the results are related either to sensory data or chemical levels of skatole and androstenone. The other alternative aims at measuring the boar odour substances with high selectivity, i.e. specific measurement techniques where the single substances are analysed and quantified. These are typically based on fast gas chromatography, spectroscopy, colorimetry, and biosensors.

II. Material and Methods

A. *Skatole and androstenone analysis methodology*

There exist a great number of analysis methods for quantitative determination of skatole and androstenone in boar adipose tissue, which have been published over the last 30 years. They are based on the extraction and cleanup of boar back fat tissue combined with analytical methods based on chromatography (LC, GC) combined with different detecting principles (LC: UV, Fluorescense; GC: MS. GC, FID, TSD, ECD) or immunological methods (EIA, RIA, TR-FIA).

B. Need for standardisation and harmonisation

The legislation related to off-flavours in food and food products is rather vague; The EU Regulation (854/2004) contains the general provision that "meat is to be declared unfit (for human consumption) if it indicates…organoleptic

anomalies, in particular a pronounced sexual odour. Member States may establish their acceptability criteria and recognise a test method to ensure that carcasses with pronounced sexual odour will be detected" [7].

Since different method protocols are being used at different laboratories, this has made it difficult to compare results, and differences between laboratories may occur. In addition to published methods, there are also in-house validated methods and modified published methods in use. So far, there have been carried out 2-3 inter-laboratory comparison studies but which have not been published. There is not yet any accepted official reference method available for the analysis of skatole and androstenone.

B. Rapid methods for boar taint detection

So far, the only method that has been taken into use on an industrial scale at-line for the purpose of sorting boar-tainted carcasses, is a colorimetric method that measures the sum of both skatole and indole, i.e. the so-called "skatole equivalent"-method [8], and which was introduced in Danish slaughter plants from 1991. The method is based on solvent extraction of fat followed by addition of reagent and spectrophotometric fluorescence measurement of skatole and indole.

Chemical gas sensor arrays, so-called electronic noses, have been suggested to have a potential for rapid objective sorting of boar tainted pig carcasses [9-14]. The sensing principles may be based on heat generation, conductivity, electrical polarisation, electrochemical activity, ionisation, optical, dielectric, and magnetic properties. This technology has been reviewed recently [15] and particularly for the boar taint detection application [16]. Recently, direct mass spectrometry (MS) has also been applied to measure boar taint in combination with pyrolysis of adipose tissue samples. [17].

Recently, commercial ultra-fast gas chromatographic instruments have become available, enabling significantly shorter analysis times than conventional gas chromatographs and which may have a potential for the analysis of the boar substances.

Another technique that is under development for boar taint detection is gas phase Fourier Transform Infra Red (FTIR) combined with photo acoustic spectroscopy (PAS) [18-19].

Biosensing, using trained insects is another interesting method that has been investigated as a potential future rapid method for sorting boar carcassess [19-20].

III. RESULTS AND DISCUSSION

A. Skatole and androstenone analysis methodology

Due to a great variability in analysis protocols used for quantitative analysis of skatole and androstenone, which applies to both sampling, extraction, cleanup, detection and quantification, this may lead to a significant variation in results between laboratories when analysing identical samples. In some cases also the sampling location of the carcass may vary which may influence the result. It has been shown that the levels of skatole and androstenone may vary depending on where on the carcass the sample has been taken [21-24]. Most laboratories analyse backfat samples, however the sample location of backfat is not well defined, some take the backfat at the 6th rib (T6) and some analyse belly fat. In addition, some laboratories analyse the adipose tissue and report the results in relation to amount adipose tissue extracted, whereas other laboratories analyse the pure fat phase that has been extracted from the adipose tissue and the levels are expressed in relation to amount pure fat analysed. This may make a difference of 30-70 % in the reported results [25-26]. For the extraction also different procedures are applied, some use solvent extraction and others use melting/freezing of the fat tissue in combination with solvent extraction to isolate the boar substances from the fat phase. In addition, different solvents are being used, either methanol, ethyl acetate or hexane. For the fat cleanup, different methods are used, column cleanup using solid phase extraction (C18, SiOH, polymers) or immunoaffinity columns. When using immunological methods for determining androstenone, there may also occure deviations in the results compared to other methods which may relate to the lack of specificity of the antibody applied and possible cross-interferences with other related steroids.

B. Need for standardisation and harmonisation

At present in-house developed, modified published and published methods are in use, which have not been fully validated. Information of performance characteristics of the methods used for the quantification of skatole and androstenone in boar fat samples is scarce and this also applies for the verification of the methodological parameters (trueness, ruggedness, recovery, uncertainty etc.). The great differences in protocols may also explain the differences observed between laboratories, and which makes it difficult to compare results between the laboratories. For example the levels of androstenone determined on identical samples using different methods may vary with a factor 2-4 [27].

To date there exist still no official analysis protocol for the analysis of the boar substances skatole and androstenone. Accordingly, there is in the EU, no harmonised method for detecting the boar taint compounds skatole and androstenone. In order to establish a harmonised analysis protocol, this standardisation process should start off with an inter-laboratory comparison study that should be carried out according to internationally accepted guidelines for proficiency testing [28]. In addition it would also be necessary to establish certified reference material for method performance testing.

It is therefore a great need for standardised and harmonised methods in this field. This will be essential for defining sound sensory tresholds and carcass sorting criteria for entire male pigs [29].

C. Rapid methods for boar taint detection

Basically, the colorimetric method applied in Denmark, is an at-line method, since back fat samples are physically removed from the carcass and taken to an automated analyser at the slaughter line and the results are used for sorting

carcasses later down the production line, with a capacity of 200 samples per hour [8]. For the big slaughter plants in Europe this capacity is still not sufficient, where up to 1000 carcasses may need to be analysed in an hour. Besides, there is also a need for analysing androstenone.

Gas-sensor array technology is a gas- phase fingerprinting technique that not measures the boar compounds specifically when applied to the vapour phase of boar fat samples. The technique requires training and calibration against sensory data or content of skatole and androstenone in the samples analysed. The results may show significant correlation between the sensor readings and levels of skatole and androstenone or sensory attributes related to boar odour and flavour. This indicates that the technology may have a potential for rapid sorting of boars at the slaughter line. Results show 10-20 % false classification rates based on the suggested sorting criteria, and there is still a need for research and development in this field in order to end up with a successful application.

The direct MS technique is based on transfer of the gas-phase of a sample into the ion-source of the mass spectrometer, followed by ionisation and mass fragmentation of the molecules. Since all the volatile compounds will be fragmented, the output data represent the accumulated fragment masses over the scanned mass range, and therefore chemometrics is required to interpret the data. This technique can be combined with different sampling methods and most used are headspace or pyrolysis. Recent results on the use of pyrolysis of boar fat tissue combined with direct mass spectrometry have shown that high classification rates can be obtained when applying suggested sensory treshold levels of androstenone and skatole as classification criteria in combination with sensory data and skatole and androstenone concentrations [17].

Recent work has demonstrated that androstenone, skatole and indole in principle can be separated and detected within 10 seconds by use of ultra-fast GC [19]. However, sampling is the critical stage of the analysis. Applying headspace sampling is not sufficiently sensitive and selective to allow direct gas-phase measurement of the boar taint substances. It is therefore necessary to carry out a classical cleanup step to isolate the boar taint compounds prior to GC analysis. Normally, this requires different protocols for isolating the androstenone and indoles and would therefore be the time consuming step of the analysis. However, recent work suggests that it is possible by the use of novel solid phase extraction (SPE) phases to isolate the indole and steroid compounds simultaneously in one SPE procedure [19]. This methodology would enable quantification of all three compounds in one analysis, and could also be automated by using commercial GC-interfaced automatic SPE systems. However, this would still be an at-line method, since samples would need to be taken from the carcasses and transferred to the automated analysis system.

It has been shown that the boar taint compounds have distinguishable gas phase IR spectra that would enable direct detection in the vapour phase [19] By using photoacoustic spectroscopy, the water issue is also overcome, since the photo acoustic signal is highly linear and the interfering water background spectrum can therefore be subtracted from the spectra to obtain the pure spectrum of the individual boar taint substances and allow specific detection of each compound. Since it is a very fast technique, it could have a potential for on-line use. However, gas sampling is still a critical point that needs to be standardised to establish a complete prototype method.

The use of trained insects is based on classical conditioning and the feeding behavioural response is applied as a positive recognition of the learned odour. Trials carried out with solitary parasitic wasps have shown that they are able to recognise skatole, indole and androstenone in the gas phase at low levels individually and also in mixtures of all the three substances [19]. However, it still remains to be demonstrated whether they can be applied for quantitative analysis of boar fat samples applying sensory odour tresholds as sorting criteria. This methodology would also require a standardised boar fat gas phase sampling system.

IV. Conclusion

At presents there is still a significant variability in method protocols among laboratories which analyse skatole and androstenone in boar carcasses, and which makes it difficult to compare results between laboratories. It is therefore a need for establishing fully validated, standardised and harmonised methods in this field. This will be essential for defining proper sensory treshold levels that may be used for establishing objective quantitative criteria for sorting out boar tainted carcasses at the slaughterline.

So far, several of the rapid boar detection methods that have been investigated show that there is still too high a percentage of false negatives ranging from 5-20 % based on the suggested sorting criteria. In addition, these methods do not yet seem to fulfil all the industrial method specifications with regard to cost efficiency, simplicity and analysis time. The analysis time should be very short (seconds-minutes) to obtain a fast result and in order to handle the high number of samples per hour. In most cases it is the sampling that is the time consuming part of the analysis. Also, methods are still too costly, since at the end it is the cost efficiency that is the driver for industrial implementation of new measurement technology.

The chosen method should ideally have a performance that allows 100 % correct classification of both acceptable and not-acceptable samples with regard to boar taint. A low percentage of false positives may be acceptable, depending on the national market conditions, but the number of false negatives should be zero.

It can be concluded that so far, there is still no dedicated rapid measurement technology available for high throughput on/at-line detection of boar tainted carcasses that measure both androstenone and the indoles.

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