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A Novel ECL Method for the Determination of Skatole in Porcine Adipose Tissue

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ABSTRACT: A new method for the determination of skatole present in porcine adipose tissue samples utilising the electrochemiluminescence of skatole is presented. It has been observed that oxygen radicals produced at a high cathodic voltage can react with oxidised skatole to create an excited intermediate molecule that then relaxes, generating a peak photon emission at around 480 nm. A strong ECL signal using BDD electrodes was observed optimally when a reduction potential of -1.8 V was applied, held for 40 s, before holding an oxidation potential of 0.8 V for 10 s. Using this principle, a calibration curve using known concentrations of skatole showed good linearity (range 0.025 – 2 μ M) and very low detection limit (LOD), 0.7 nM. A method that demonstrates for the first time an approach that utilises this ECL reaction, and has the potential to be developed into an analytical device for use in the slaughterhouse, has been developed. This was achieved by extracting skatole out of the porcine adipose tissues into acetonitrile - giving an extraction efficiency of 67.6%. This method was then validated by analysing the skatole content of 33 pig fat samples that had been previously tested using a standard technique, HPLC, containing a range of concentrations (0.02 – 2.58 μ g/g). This ECL method exhibited excellent reliability and correlation with HPLC, giving a R² coefficient of 0.911, thus demonstrating the potential for this method to be developed for an on-line skatole detector.

Boar taint is an undesired odour found in around 10% of the meat of uncastrated adult male pigs, which is frequently described as being faecal or urine-like, especially once the meat has been heated before consumption.¹⁻³ To avoid the occurrence of this off-taste, many countries routinely surgically castrate their male piglets without any anaesthesia.⁴ Recently there has been an increased desire, and pressure from many regulatory bodies, to find alternatives to surgical castration - due to concerns of the animal welfare. Furthermore, uncastrated pigs will exhibit improved growth rate, and decreased food consumption thus increasing the productivity and sustainability of pork production.⁵⁻⁷ This has led to a commitment of the pig industry stakeholders under the invitation of the European Commission to find a solution to stop the castration of pigs in Europe by January 1st 2018 - provided that a viable alternative could be found.⁸ The main contributing factors to boar taint are androstenone, a pheromone produced in the testes of boars and skatole (3-methylindole), an indolic compound which is the product of the enzymatic cleavage of tryptophan in the large intestine.^{1,9,10} Both of these two compounds display highly lipophilic properties and thus are found to accumulate in pork fat, hence the possibility the odour could be released while cooking the meat.¹

Suitable and practical alternatives to surgical castration are highly sought after. This means that either a different method of sterilization, like immunocastration, is used, or the male pigs can even be left uncastrated.⁶ In order to expand the production of uncastrated male pigs, either the risk of these two compounds occurring in pork fat must be reduced or a

technique to identify which carcasses are tainted must be developed. Since we will never achieve full certainty that a carcasses contains no boar taint, an effective method of determining the presence of boar taint so that the tainted meat can be sorted in the abattoirs is desirable. Currently, carcasses are evaluated using a sensory evaluation (also referred to as Human Nose Scoring) that suffers from several weaknesses.³ For example, the reproducibility of the human nose scoring method is low^{2,11} and a good sensitivity of detection of tainted carcasses is greatly at the expense of a good specificity. Therefore, there is a need for new on/at line objective methods that require minimal sample preparation, do not have to be performed by a specialist (preferably with a high degree of automation), are rapid and are cheap – so that they can be readily utilised in an industrial setting.³

Previously, a strategy for detecting one of the key molecules at the origin of boar taint, skatole, was presented using ECL (electrochemiluminescence or electrogenerated chemiluminescence).¹² This analytical technique utilises the phenomenon where chemical species generated at the surface of an electrode undergo electron transfer reactions to form excited states that then emit a measurable light signal, which is proportional to the concentration of the target species.¹³⁻¹⁵ The advantage of ECL sensors over other chemical sensors is that they inherit the merits of both electrochemical and spectroscopic methods. Specifically, the sensitivity of chemiluminescence with the stability and simplicity of electrochemical methods, leading to it become increasing popular in many industries including medical and food analysis. Okajima and Oshaka demonstrated that through the

generation of a superoxide ion ($O_2^{\cdot-}$), an excited electrogenerated derivative of skatole (*N*-(2-acetylphenyl)formamide) is produced, becoming an ECL emitter upon its relaxation, thus allowing the determination of skatole in acetonitrile. It was demonstrated that cycling from a cathodic potential then back to an anodic potential (i.e. $0\text{ V} \searrow -1.2\text{ V} \nearrow 0.2\text{ V}$, at 50 mVs^{-1}) caused the electrogeneration of the oxygen radical and oxidation of skatole, generating an ECL signal. Despite this promising development in determining skatole for various applications, the method presented only investigated the use of pure acetonitrile as the ECL solvent without assessing more complex matrixes (such as pork fat). Additionally, before now, a method based on the ECL of skatole has never been implemented in any applied study, including boar taint analysis.

The overall objective of this research is to investigate the ECL of skatole for the development of a chemical sensor that then can be implemented for its detection and quantification in the fat of uncastrated male pigs. Specific aims were: to explore the mechanisms for producing ECL signal, to optimize the extraction procedure and applied potentials, and to look for any interferences and finally to validate the method using a blind test on real boar fat samples, benchmarked against a 'gold standard' laboratory method: HPLC.

EXPERIMENTAL SECTION

Chemicals and reagents. Skatole (3-methylindole), androstenone, L-tryptophan, indole, tetrabutylammonium hexafluorophosphate (TBAHFP), sodium sulphate, and acetonitrile (anhydrous, 99.8%), sodium hydride (60% in mineral oil) were all purchased from Sigma-Aldrich and were of analytical grade. Deionised water was obtained from a Direct-Q UV 3 (Millipore).

Sampling and HPLC analysis. On the day after slaughter, a piece of back-fat was sampled on the carcass of pigs in the neck region (between cervical and first dorsal ribs), vacuum-packed and stored at $-20\text{ }^\circ\text{C}$ for further analysis.

After thawing, a piece of fat weighing 10 to 20 g was heated and centrifuged at $11\ 200\times g$ for 20 min at $+4\text{ }^\circ\text{C}$, and a 2 ml sample of the supernatant was used to extract androstenone, indole and skatole with methanol. These compounds were measured by HPLC.¹⁶ Concentrations are expressed per gram of the lipid fraction from adipose tissue. The limit of detection of the method was 0.24, 0.03 and 0.03 $\mu\text{g/g}$, respectively, for androstenone, skatole and indole.¹⁶

Sample preparation and extraction for ECL. Extraction of skatole was achieved using a liquid-liquid extraction with acetonitrile. Samples were prepared by, first, cutting 5 g of frozen pig fat into 50 ml glass beaker which was then heated in a microwave oven (800 W) for 3 minutes. This insured that fat was melted and some of the water content evaporated. 1 ml of liquid fat was then added to a 15 ml plastic tube along with 10 ml of acetonitrile and reheated in a water bath to $80\text{ }^\circ\text{C}$ to prevent the fat from solidifying during extraction. Samples were then placed in a shaker (Multi Reax, Heidolph) and agitated at 2000 rpm for 10 minutes. They were then centrifuged (Sigma 2-16P) at 4000 rpm for 5 minutes, until two distinct layers were formed. In order to easily extract the upper layer, the sample was then placed in a freezer at $-20\text{ }^\circ\text{C}$ for 1 hour before 10 ml of acetonitrile was decanted into

another 15 ml tube. To remove any traces of water, which can disrupt the ECL measurement, 960 mg of sodium sulphate were added to each sample and 387.4 mg TBAHFP were added to make a 0.1 M salt solution. Finally, samples were then alkaline buffered with the addition of 120 mg NaH and left to sit for 15 minutes before analysis.

Standard solutions were prepared from a stock solution of 10 μM skatole diluted to seven solutions of 0, 0.05, 0.1, 0.25, 0.5, 1, 2 μM , with 0.1 M TBAHFP in acetonitrile. To check for any possible endogenous interferences with other boar taint compounds or close-related molecules, standard solutions containing 2 μM of androstenone, indole and tryptophan were prepared in 0.1 M TBAHFP acetonitrile solution, and tested under the same conditions as skatole.

Apparatus and ECL Measurements. ECL measurements were made using lab-grown boron-doped diamond (BDD) electrodes on top of a silicon substrate as previously described and characterised.^{17,18} Additionally, the potential window of the electrodes were determined (2.69 V in 0.1 M lithium perchlorate, 0.5 mV/s scan rate) and SEM images were taken (supplementary material). Electrodes were fabricated using a 10 x 10 mm (worker electrode, WE) or 15 x 15 mm (counter electrode, CE) section of BDD. Electrical contact was taken through the silicon from the back side, achieved with a copper tape. Electrodes from cut sheets of 0.128 mm foil gold (Sigma-Aldrich) and 1 mm foil glassy carbon (Sigma-Aldrich) were fabricated for comparison with BDD. The two electrodes (worker and counter), along with a Pt quasi-reference wire electrode were then placed inside a 10 ml custom 3D-printed cell fitted with optical glass window. When measuring total light emission, electrochemical measurements were made using an Autolab PGSTAT128N (Autolab) and the photon emission was measured with a PDM03-9107-USB PMT (ET enterprises, UK). When obtaining spectral data, electrochemical measurements were made using a portable PalmSens potentiostat (PalmSens) and the photon emission was measured with a Fluoromax 4P spectrofluorometer (Horiba Jobin Yvon).

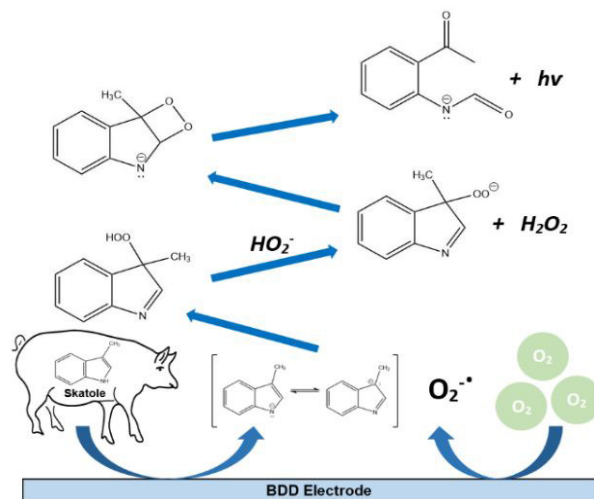


Figure 1. Schematic showing the electrogenerated reaction of skatole on the surface of an electrode in acetonitrile, assisted by dissolved oxygen to produce the excited molecule that emits a photon.

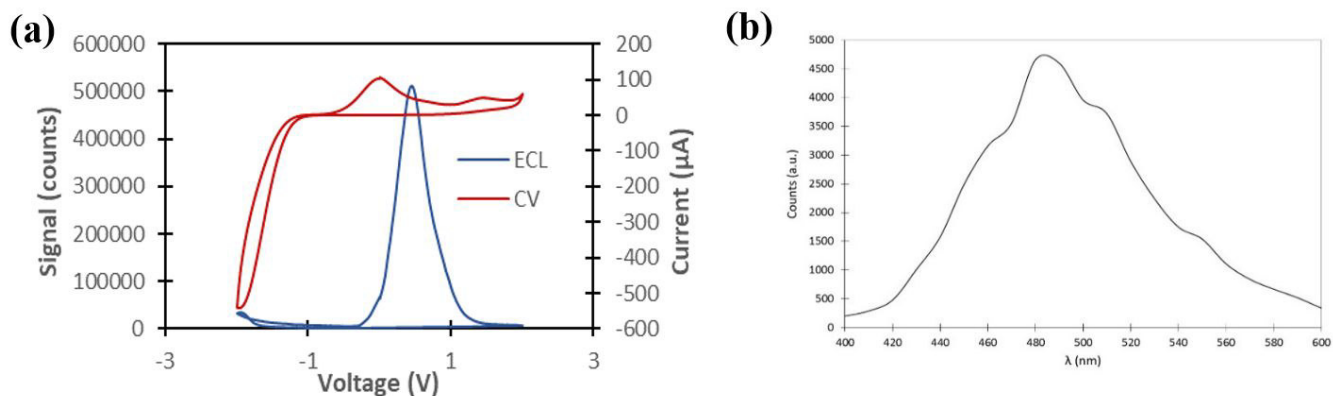


Figure 2. (a) Cyclic voltammetry response of 1 μM of skatole in 0.1 M TBAHFP/acetonitrile (red) along with corresponding ECL signal (blue). (b) Spectroscopic response of 1 μM skatole along a range of wavelength (400-600 nm) obtained by pulsing the voltage from 1 to -2 V at holding each for 0.2 s, showing a peak intensity at 480 nm.

At the beginning of each set of measurements, BDD electrodes were thoroughly cleaned using electrochemical activation by submerging them in a clean solution of 0.1 M TBAHFP acetonitrile and applying 0.5 s pulses of 2 mA and -2 mA for 200 cycles to remove any fouling on the surface of the electrode, similarly to what has been described previously.^{18,19} Each measurement was performed using 8 ml of the sample pipetted into the electrochemical cell, enclosed in a light-tight box to remove any background signal. In-between measurements, chemical cell was rinsed twice with acetonitrile to remove any sample residue.

RESULTS AND DISCUSSION

Electrochemical and electrochemiluminescent behaviour of skatole. As previously described by Okajima and Oshaka, **Figure 1** shows the mechanism for ECL of skatole as understood until now. The electrogenerated $\text{O}_2^{\cdot-}$ acts as a proton acceptor to the skatole and leads to the anion of skatole (3-MI^-) and the HO_2^{\cdot} radical. This radical further reacts to form a 1,2-dioxetane-like intermediate which self-degrades to a *N*-(2-acetylphenyl)acetamide in its excited state, relaxing with a photon emission. This mechanism has been described using observations made from skatole ECL with a glassy carbon (GC) electrode.

In this study, BDD electrodes have been selected due to preliminary comparative experiments using gold, GC and BDD electrodes which demonstrated that while the target ECL reaction was observed for each, the BDD electrode displayed the best signal/blank ratio and so would be expected to achieve the lowest LOD. Additionally biological media (such as fat

tissue) can often lead to fouling of the electrode material. Cycling the electrodes (0 V to 1 V to -2 V, 50 mV/s) in the pork fat extracts, it was found that after more than 25 cycles, there was a significant reduction in the electrodes reactivity and resulting ECL signal (supplementary material). This electrode fouling was likely related to the electrochemical polymerisation of indole, as what has been reported previously.¹⁷ BDD holds an advantage here due to its resistance to fouling and its ability to be electrochemically cleaned *in-situ*.¹⁹

The material of the electrode can have a large effect on the mechanisms and efficiency of ECL reactions and so the effect of a new electrode must be investigated.²⁰ **Figure 2 (a)** shows the Cyclic Voltammetry (CV) used to investigate the electrochemical and ECL behaviour of skatole on BDD electrodes ($0\text{ V} \rightarrow 2\text{ V} \rightarrow -2\text{ V}$, scan rate 50 mVs^{-1}) and found a cathodic current peak at -1.85 V corresponding to the reduction oxygen ($\text{O}_2 \rightarrow \text{O}_2^{\cdot-}$) and the anodic current peak occurring at 0.05 V , representing the reoxidation of $\text{O}_2^{\cdot-}$. The total light emission during the CV recorded using the PMT which is displayed in **Figure 2 (a)** shows the ECL emission of skatole occurring at 0.72 V , only after the initial oxidation of oxygen had occurred. Compared to GC, BDD displayed slightly shifted voltage peaks, however, the overall mechanism leading to ECL emission of skatole is presumed to be same as previously proposed.

To obtain spectroscopic data on the light emission from the ECL of skatole, a constant and consistent light emission, observed with the spectrofluorometer from the surface of the electrode, was achieved by quick pulsing from high to low

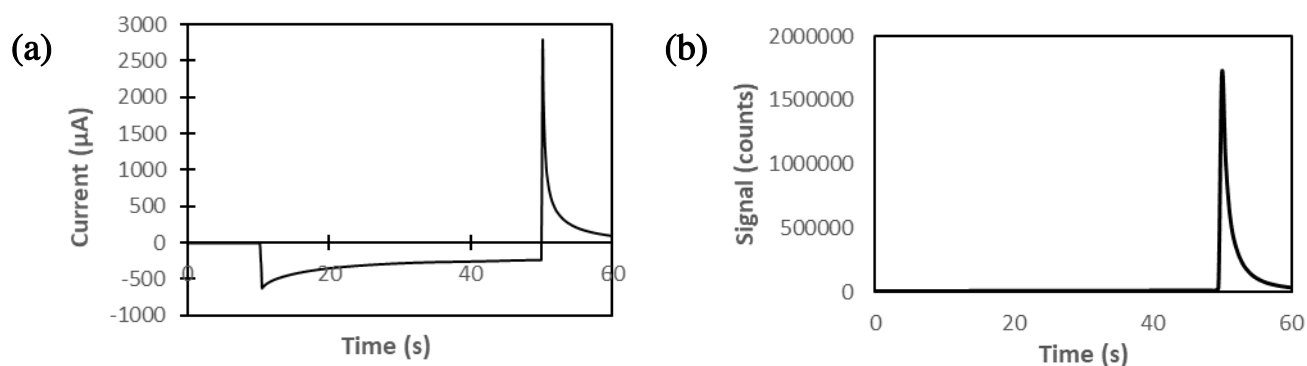


Figure 3.(a) Multistep amperometry showing optimised pulse for creating skatole ECL signal by 0 V (10 s) to -1.8 V (40 s) to 0.8 V (10 s) (b) corresponding ECL signal detected using a PMT from 1 μM skatole in 0.1 M of TBAHFP in acetonitrile.

voltages (1 V to -2 V, 0.5 s, 100 cycles) (supplementary material). This allows both the reduction of oxygen and oxidation of skatole to happen near simultaneously at the surface of the working electrode. The spectrofluorometer, with an integration time set longer than the pulse time can then create a smooth emission spectrum showing a peak light intensity of 480 nm (**Figure 2 (b)**), similarly to what has been reported previously.¹²

Optimisation As with most ECL co-reactant systems, both the excited derivative of the target molecule and co-reactant ($O^{\cdot -}$) are generated from the surface of the working electrode. It was found that the optimal signal intensity occurred when using chronoamperometry to, first, hold the voltage at a very negative potential (≈ -2 V) to form build-up of $O^{\cdot -}$ radicals at the surface of the working electrode and then to pulse to a positive potential (≈ 1 V) to oxidise the skatole, which can then react with the oxygen radicals. This reaction produced a strong sharp light signal precisely at the time of skatole reduction. Several variables for this procedure were investigated, using a 1 μ M skatole, to optimise the reliability and LOD, including the upper and lower applied potentials and the hold time at the negative potential (supplementary material). The optimal procedure of: 0 V (5 s hold) \rightarrow -1.8 V (40 s hold) \rightarrow 0.8 V (10 s hold) was found where a 1 μ M solution of skatole gives a signal of about 1800000 counts (**Figure 3(a) and (b)**). The blank solution (acetonitrile with 0.1 M TBAHFP) also displayed a significant luminescence using the same pulse procedure, of around 15000 counts. This occurred regardless of the electrode material (BDD, GC or Gold), and thus this blank emission is likely to have arisen from the ECL of the salt or solvent itself. However, due to the stability and reliability of this background signal and the high sensitivity of skatole using this method, it does not have a significant impact on the overall efficiency and LOD of this analytical method.

Selectivity The selectivity was tested on several compounds that are found in relatively high abundance in porcine adipose tissue and have the potential to be ECL active. These included androstrenone and indole, the two compounds most commonly ascribed to contributing to boar taint^{10,21} and tryptophan to which skatole is derived.^{22,23} 2 μ M solutions of each compound were tested in acetonitrile using the same procedure as skatole. The results are summarized in **Table 1**.

Table 1. Overview of each possible interference endogenous compounds and the corresponding ECL response using 2 μ M of analyte along with the response of blank acetonitrile and 2 μ M skatole

Analyte	Signal (counts)	Standard Deviation (%)	Signal/blank ratio
Blank	15429	2.41	-
Skatole	3051572	2.39	197.8
Androstrenone	14325	7.06	1
Tryptophan	14162	9.10	1
Indole	17776	9.08	1.2

It was observed that neither androstrenone nor L-Tryptophan displayed a significant signal over the standard blank. The 2 μ M indole spiked sample did however produce a significant, if very small, light emission. Comparing the indole ECL signal (signal/blank = 1.2) and the skatole ECL signal (signal/blank = 197.8) skatole has almost a 200-fold stronger signal than indole. As indole has been found to occur in pig fat sample within the same orders of magnitude than skatole, and these concentrations rarely exceed 2 μ M,²⁴ it can be assumed that indole presence in fat samples will not significantly affect the detection of skatole, and thus it was determined that no interference would be contributed from these compounds.

Calibration. The skatole is extracted from the fat medium using a high ratio of acetonitrile over fat (10:1). Initial experiments using extracts of un-spiked skatole free fat samples found a background blank signal, comparable to that of standard blank acetonitrile (≈ 15000 counts) thus demonstrating further the lack of interfering compounds or matrix effect. Thus, the calibration was made from clean acetonitrile solutions without having to go through the procedure of spiking actual pig fat samples with skatole.

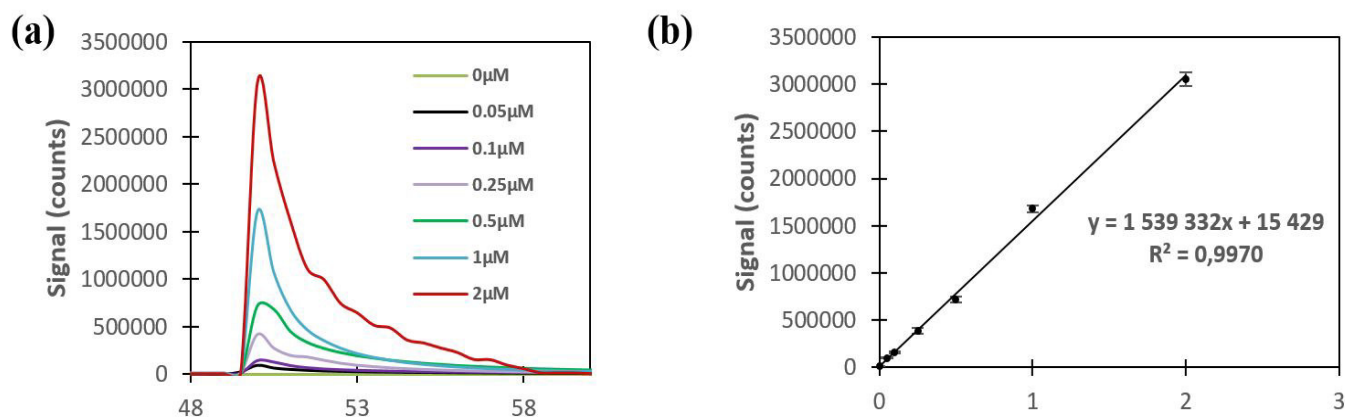


Figure 4. (a) ECL response obtained using BDD electrodes and optimised voltage pulse of solutions containing skatole over a concentration range (0, 0.05, 0.01, 0.25, 0.5, 1, 2 μ M). The procedure used was 0 V (10 s) \rightarrow -1.8 V (40 s) \rightarrow 0.8 V. (b) The corresponding calibration plot for skatole using ECL peak height vs concentration ($n = 5$).

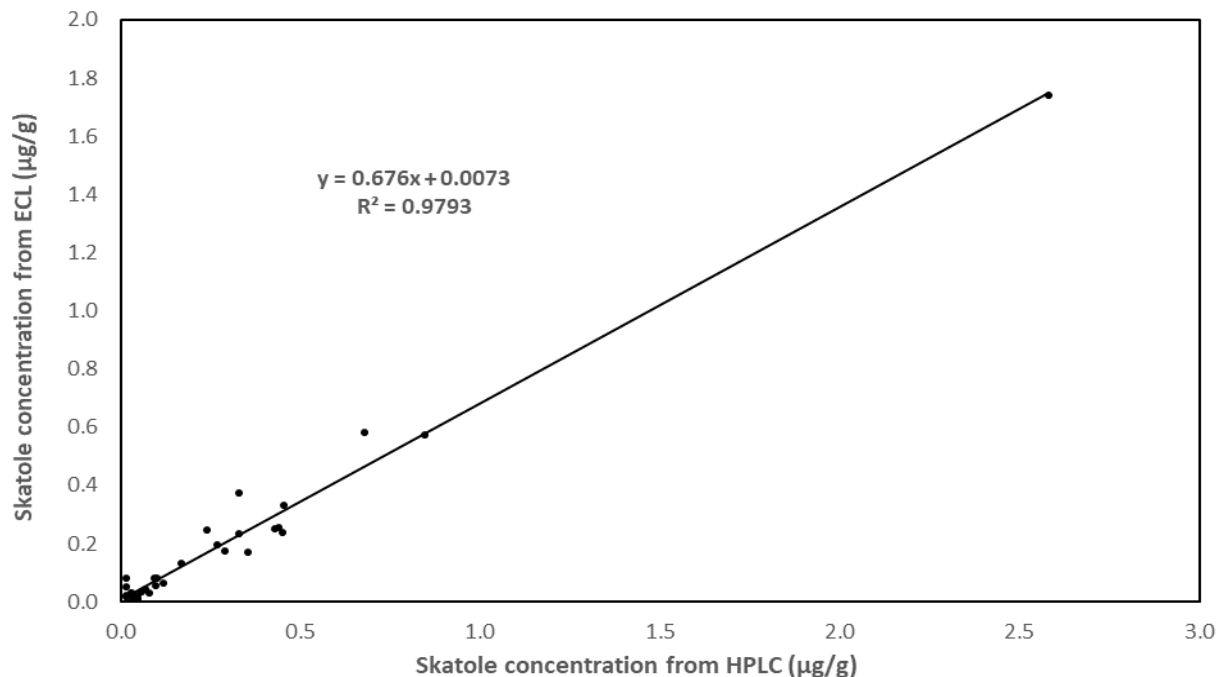


Figure 5. Correlation plot for the determination of skatole in porcine adipose tissue measured by ECL measurements and the corresponding HPLC method (n = 33).

A range of solutions was chosen (0.05 – 2 µM) based on the reported concentration likely to be found in uncastrated boars and each of these solutions were analysed in five replicates. Strong signals were obtained from each calibration standard solutions from ≈ 15000 counts (0 µM) to ≈ 3000000 counts (2 µM, **Figure 4(a)**). ECL measurements over the replicates demonstrated sufficient reproducibility with the percentage random standard deviation (%RSD) ranging from 2 – 7%. Calibration graph showed excellent linearity across the whole range (0 -2 µM) and gave a linear fit coefficient of $R^2 = 0.997$ (**Figure 4(b)**). Despite the high background signal obtained from unspiked acetonitrile solutions, very low LOD (Blank + 3*SD) and LOQ (Blank + 10*SD) of 0.7 and 2.4 nM respectively in acetonitrile solution were achieved, as can be expected from photoluminescent techniques. As the extraction of skatole requires a ratio of 10:1 acetonitrile to liquid fat, this means that the LOD and LOQ of native skatole in fat samples are around 7.3 and 24.2 nM (0.92 and 3.16 ng/g respectively). Looking at the expected skatole concentrations (0.02 – 2.58 µg/g) and the skatole threshold contribution for boar taint (0.20 - 0.25 µg/g), clearly establishing the sufficient sensitivity of this technique for the determination of skatole in pig fat.

Validation with HPLC. Due to the lipophilic nature of skatole its analysis is almost always performed in fat tissue samples as it presents the highest concentration. Thus, samples usually have to go through an extraction process to remove the difficult lipid-matrix.²⁵ Methods include liquid-liquid extraction, steam distillation and solid phase extraction. As the described ECL process requires the final matrix to be in acetonitrile, which is fat-insoluble – thus making this method an excellent candidate for skatole analysis.

One factor that makes the skatole determination in fat samples particularly difficult is the high content of water

present ($\approx 15\%$). For the cathodic electrogeneration of the superoxide ion to be possible, the analyte has to be anhydrous. Side experiments showed that with the addition of just 1% water to the analyte resulted in a 50% loss of signal and addition of 2% water caused a complete extinction of the skatole luminescence. In order to mitigate this issue, samples had to be processed to remove as much water as possible. This was done in three steps: first by the use of the microwave to facilitate the evaporation of water content and then by the addition of a hygroscopic salt (sodium sulphate) into the final solution to remove trace molecules.

Furthermore, during the ECL reaction, skatole becomes, at several stages, a proton acceptor. However, the solution extracted from the pig fat contains a large quantity of proton-donating compounds, in particular fatty acids. It is therefore necessary, to prevent the protonation of these intermediate skatole species by the addition of a strong base, in this case NaH. This also has the additional benefit of reacting with some of the water traces.

For validation of this method, 33 samples of adipose tissue were obtained and were subsequently analysed using the benchmark technique, HPLC (determining the concentrations of skatole, androstenone and indole).¹⁶ Each of these 33 samples were then received by the ECL lab in a blind test omitting the results of the HPLC measurements. ECL measurements were performed on each sample, with 5 of the adipose tissues sub-sampled 3 times to test for error in the extraction and sample preparation and the reliability of these measurements. Like with the calibration solutions, each measurement was performed 5 times and averaged for instrumental error.

Examining the sub-sample replicates performed in triplicate it was found that there was a %RSD between the samples of 1-

4%. The instrument replicates performed 5 times has a %RSD of 2 – 9% showing that instrument error was significantly higher than sampling error, and thus the sampling and extraction procedures were sufficiently reliable and did not introduce any significant sources of error to this analysis.

After analysis, the concentrations determined from the traditional HPLC method were revealed and plotted against the novel ECL measurement. It should be noted that the sensitivity of the ECL method (LOD = 0.92 ng/g) was actually better than the HPLC method (LOD = 30 ng/g), meaning that a total of 4 of the samples examined in this study were above the limit of detection in the ECL measurement but below the limit of detection in the HPLC measurement. A correlation plot of the two different methods to determine skatole concentration in porcine tissue is presented (**Figure 5**) with a coefficient of correlation of $R^2 = 0.9793$. However, one sample contained a significantly higher concentration of skatole than the others and could lead to an overestimation of the coefficient. Removing this upper value from the graph, a high correlation coefficient of 0.911 was still obtained. No correlation was found between ECL signal and the androstenone or indole content of the fat. The results show that there is a very good agreement between the more conventional liquid chromatography and the new electrochemiluminescent technique. Adding to that, this method is very simple, instrumentation is inexpensive and in theory, does not require the need of a specialist to operate, making it particularly attractive to the on-line detection of boar taint analysis.

Determining the exact efficiency of extraction is difficult for skatole in porcine adipose tissue as there exists no matrix-specific certified reference material (CRM). However from the HPLC result, it can be inferred (assuming that the result given by the HPLC is the exact content) that from the gradient from the correlation slope, the recovery of this process is 67.6%, clearly showing room for even further improvement of the extraction efficiency and thus the sensitivity of this novel technique.

This method displays high sensitivity, a trait that is associated with luminescence techniques²⁶ with an instrument detection limit of 0.92 ng/g. Even taking into account the extraction dilution and recovery that gives a method LOD of 1.35 ng/g, comparable to the lower end of the state of the art in much more expensive and complex instruments with both GC-MS^{27–29} and HPLC^{30,31} and with a comparable sample mass.

For the systematic determination of skatole for commercial purposes every pig in each slaughterhouse would have to be tested. The limitations of the ‘gold standard’ chromatographic techniques (expensive and very slow) makes them unviable for this type of continuous analysis. However, ECL analysis can be performed fast and at a very low cost (both in continuous costs and instrumentation). Furthermore, it has a much higher sensitivity than what is being considered at the moment for on-line detection of boar taint in abattoirs, performed by electrochemical sensors (52 ng/g),³² colorimetric methods (20 ng/g),³³ Laser Diode Thermal Desorption Tandem Mass Spectrometry (LDTD-MS/MS) (20 ng/g)³⁴ or even the human nose panels.³

Currently, the aspects of the method that need to be improved for the development of an in-situ analytical device lay in the sample preparation and extraction. Although actual time of analysis can be very fast, with each pulse cycle only

taking 1 minute to perform, the extraction process of fat into the acetonitrile can be time consumptive. Sample size (5 g) is also something that can be improved as lowering it would simplify and increase the speed of sample preparation. ECL has the advantage of being easily downsized, with assays commonly performed using very small droplets which could be further investigated for a commercial device.

CONCLUSION

There has been an increasing interest to find a suitable method for identifying tainted pig carcasses by quantifying active compounds, an important one being skatole. Here is presented a novel method exploiting the ECL properties of skatole to produce a sensitive, selective and reliable signal enabling its quantification in porcine adipose tissue. Calibration showed a very good linear range of this method, along with an unrepresented low detection limit for a method not using a chromatographic separation phase. Comparative blind study using pork fat that had been already tested using the reference method, HPLC, also demonstrated the accuracy and reliability of this technique. This along with the inherent simplicity of the method, demonstrates the potential for developing an ECL approach. Focus for future work will be in the creation of a downscaled device which requires smaller sample sizes.

DECLARATION OF COMPETING INTERESTS

The authors declare that there is no known competing personal or financial interests which could have an influence on the content of the work which is presented in this paper

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Optimization of upper and lower potential applied for ECL voltage pulse; potential window and SEM image of BDD; example of voltage pulse used for emission spectrum acquisition; ECL response on different electrode materials; Effect of electrode fouling from pork fat extracts (PDF)

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The manuscript was written through contributions of all authors.

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