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Novel, rapid, low-cost screen-printed (bio)sensors for the direct analysis of boar taint compounds androstenone and skatole in porcine adipose tissue: Comparison with a high-resolution gas chromatographic method

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ABSTRACT

This is the first report on the fabrication, characterisation and application of an electrochemical (bio)sensor system for the simultaneous measurement of skatole and androstenone. A biosensor for androstenone was fabricated using a Meldola's Blue modified SPCE (MB-SPCE) by depositing NADH and the enzyme 3 α -hydroxysteroid dehydrogenase onto the MB-SPCE surface; samples of adipose tissue were analysed using the biosensors in conjunction with chronoamperometry. Cyclic voltammetry was used to investigate the electrochemical behaviour of skatole at a screen-printed carbon electrode (SPCE vs. Ag/AgCl). An oxidation peak was observed around +0.55 V (vs. Ag/AgCl) and differential pulse voltammetry was applied for quantification of skatole in adipose tissue (*in-situ*). Quantitative analysis was achieved using calibration plots obtained from fortified meat samples. The concentrations obtained by the electrochemical and gas chromatographic (GC) methods demonstrated a good positive correlation. The (bio)sensor system completed both measurements within 60 s, as compared to several hours for GC, and at a considerably reduced cost and complexity. Consequently, the novel (bio)sensor system should have applications for analysis of carcasses on the abattoir processing line.

1. Introduction

Boar taint is the term given to the unfavourable flavour and aroma sometimes experienced with meat and meat products derived from entire (uncastrated) male *Sus scrofa domesticus*, commonly termed the domestic pig. Decades of research indicates that boar taint is due to the excessive accumulation of several naturally occurring compounds in porcine adipose tissue with the main compounds being skatole and androstenone. Currently there are no EU-approved rapid instrumental methods to detect and quantify the boar taint compounds at the point of test and, in particular, on the abattoir line (Haugen et al., 2012). A commonly used and effective approach for reducing boar taint has been castration. However, an increasing number of countries are moving towards an entire male pig production system because of animal welfare concerns. An entire male pig production system will ultimately lead to a higher incidence of boar taint unless the alternative methods for reduction of boar taint have been implemented. A number of approaches to the reduction of boar taint compounds have been considered or developed. This includes genetic selection, using immunocastration, manipulation of pigs' diet and management and sperm sorting (Squires

and Bonneau, 2014). However, some of these approaches are only effective for the reduction of skatole level (e.g. diet and management), others are either expensive (sperm sorting), under development (genetic tests) or have not been widely accepted yet (immunocastration).

If left undetected, tainted carcasses entering the food chain may result in consumer dissatisfaction leading to a decrease in repeat purchasing of pork and even the total rejection of pork by some consumers, which would lead to economic losses across the pig industry. Furthermore, European legislation (European Parliament, 2004) states that carcasses with a pronounced sexual odour are unfit for human consumption. Therefore, having an effective technology for rapid, sensitive, accurate and cost-effective detection of boar taint compounds on-line without sample preparation is of great importance to the pig industry. Such a technology would also be beneficial for evaluation of the effectiveness of the methods for boar taint reduction such as genetic selection, immunocastration, and dietary manipulation and other.

Studies into the threshold levels for perception of boar taint have shown substantial disparity, this has been linked to human olfactory genetic variation and differences in the processing of meat products (Font-i-Furnols, 2012). A review by Walstra et al. (1999) identified

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several published studies reporting the associations between concentrations of androstenedione and skatole and the unpleasant aroma or taste of pork. These studies were used to determine general threshold levels for boar taint compounds at which consumers would negatively react to meat from entire male pigs. The thresholds were between 0.5 and 1.0 ppm for 5 α -androst-16-en-3-one (androstenedione) (Desmoulin et al., 1982; Mortensen et al., 1986) and between 0.2 and 0.25 ppm for 3-methylindole (skatole) (Armstrong, 1993; Mortensen et al., 1986).

The most common method for boar taint detection in the European pig industry is the use of an organoleptic judge to assess the volatile profile of heated adipose tissue samples. Although this is the most popular quality control practice, it is fraught with scientific discrepancies and its reliability is poorly documented (Trautmann et al., 2014).

Analytical methods for quantification of boar taint compounds are limited to laboratory chromatographic methods, e.g. gas chromatography. Although the analytical methods are accurate and sensitive, their disadvantages are the lack of portability and high cost. They also require a sample preparation step, specialised training staff and are unsuitable for on-line measurement due to long analysis times.

Recently, various strategies for the electroanalytical measurement of a wide variety of compounds in food products and beverages have been developed (Hughes et al., 2016). One example is the successful development of a voltammetric assay for the quantification of trace heavy metals in various food products and vegetables (Mendoza et al., 2015). In another approach, amperometry has been employed in conjunction with enzyme-based biosensors to quantify a range of organophosphate pesticides, organochlorine pesticides, and insecticides in food products (Bollella et al., 2016; Crew et al., 2011; Palchetti and Mascini, 2015; Tortolini et al., 2016). In addition, a screen-printed carbon electrode (SPCE) modified with the redox mediator cobalt phthalocyanine, was successfully applied to the direct measurement of citric acid in lime fruits (Honeychurch et al., 2010). This latter example of a successful food quality-monitoring sensor for *in-situ* measurements demonstrated the potential for direct analysis of food quality.

In the current study, this principle was used for the development of (bio)sensors for the direct detection and quantification of boar taint compounds, androstenedione and skatole, directly in adipose tissue. These sensors were produced using screen-printing technology, which allows for mass production at low cost. The development of these electrochemical (bio)sensors for boar taint analysis is now protected by the European Patent EP 2966441 B1 (Hart et al., 2017).

The aims of the present study were: (i) to conduct a detailed characterisation of the electrochemical performance of the (bio)sensors for the measurement of skatole and androstenedione. This includes the study on potential interference of some compounds commonly found in pig adipose tissue with the (bio)sensors performance; (ii) to evaluate the (bio)sensors using samples of porcine adipose tissue and (iii) to validate the (bio)sensors performance against the gas chromatography-based method as a 'gold standard' for skatole and androstenedione analysis.

2. Experimental

2.1. Chemicals and reagents

All reagents used were of analytical grade and purchased from Sigma Aldrich (Poole, UK) unless specified otherwise. Individual reagents used in the electroanalytical and gas chromatographic analyses are detailed in the following sections.

2.1.1. Electroanalysis

Biosensors for androstenedione detection were prepared as previously described by the authors (Hart et al., 2017).

A phosphate buffer solution (pH 7.0) was prepared by titrating stock solutions of monosodium dihydrogen phosphate and disodium monohydrogen phosphate to the desired pH at a concentration of 0.5 M. Buffer solutions were further diluted with deionised water to a final

working concentration of 0.1 M phosphate buffer. Sodium chloride stock solutions were prepared at 1.0 M and similarly diluted to a working solution concentration of 0.1 M sodium chloride. Sodium hydroxide stock solutions were prepared at 0.1 M in deionised water.

A 10 mM stock solution of skatole was prepared by dissolving a known mass in HPLC grade methanol (Fisher Scientific; Loughborough, UK). Working standards were prepared by diluting the stock skatole solution with phosphate buffer, sodium chloride stock solution and methanol (5% final volume). A stock standard for voltammetric adipose tissue fortification studies was prepared in methanol at a concentration of 1 mg/ml and working standard concentrations were calculated taking into account the weight of individual sections of adipose tissue samples.

A 1 μ g/ml stock solution of androstenedione was prepared in HPLC grade methanol. Working standards were prepared by diluting the androstenedione stock solution with phosphate buffer and methanol (5% final volume). A stock standard for chronoamperometric adipose tissue fortification studies was prepared at a concentration of 1 mg/ml in methanol and working standard concentrations were calculated in relation to the weight of individual sections of adipose tissue sample.

A selection of potentially interfering compounds was prepared at concentrations typically occurring in adipose tissue or associated tissues. Unsaturated and saturated fatty acids (linoleic, palmitic and oleic acids) were dissolved to saturation in 0.1 M phosphate buffer (pH 7.0) with 0.1 M NaCl, and evaluated directly. The hormones testosterone, estradiol and estrone, were dissolved in methanol at a concentration of 2.5 ppm and diluted to 2 ppb, 0.078 ppb and 0.138 ppb, respectively in 0.1 M pH7 phosphate buffer with 0.1 M NaCl. Solutions of the amino acids alanine, glycine and taurine were prepared in 0.1 M phosphate buffer (pH 7.0) with 0.1 M NaCl at concentrations of 590 ppm, 110 ppm and 240 ppm respectively for analysis. Stock solutions (50 mM) of thiamine hydrochloride, pantothenic acid, nicotinamide, and pyridoxine hydrochloride were prepared in deionised water. Whereas, stock solutions (20 mM) of riboflavin and folic acid were prepared with a sodium hydroxide stock solution. Working standards (5 mM) were prepared by diluting the stock standards in phosphate buffer and sodium chloride stock solutions.

2.1.2. Gas chromatography

Stock solutions of skatole and 5-methylindole were prepared with HPLC grade ethyl acetate (Fisher Scientific; Loughborough, UK). An internal standard, 5-methylindole, concentration of 0.5 μ g/ml was used with samples extracts.

Stock solutions of androstenedione and 3 α -hydroxy-5 α -androst-17-one (androsterone) were prepared with HPLC grade methanol and hexane (Fisher Scientific; Loughborough, UK) at a ratio of 9:1 methanol: hexane. An internal standard, androsterone, was used at the concentration of 2 μ g/ml with sample extracts.

2.2. Apparatus and instrumentation

2.2.1. Electroanalysis

All voltammetric and chronoamperometric measurements were carried out with a μ Autolab III (Metrohm, Netherlands) potentiostat interfaced to a PC for data acquisition via the proprietary software (NOVA v1.1). Screen-printed electrodes were supplied by Gwent Electronic Materials Ltd (Pontypool, UK); the working electrode used for the measurement of skatole was fabricated using a carbon ink (C2030519P4), whereas the working electrode for the biosensor used a carbon ink modified with Meldola's blue (MB) (BE2031028D1/247) and the enzyme 3 α -hydroxysteroid dehydrogenase (3 α -HSD) was drop-coated onto the surface. The enzyme was immobilised onto the MB-SPCE using glutaraldehyde by drying in a vacuum at 4 °C. The reference electrode for both the sensor and biosensor was fabricated using a Ag/AgCl ink (C61003P7).

The surface temperature of the adipose tissue was measured prior to electrochemical measurements using an infrared thermometer with

laser targeting (Maplin Electronics; Rotherham, UK). Measurements of internal temperature and pH were obtained with a Testo 205 temperature-pH probe (Alton, UK).

2.2.2. Gas chromatography analysis

A PerkinElmer Clarus 580 Gas Chromatograph with autosampler interfaced to a PC for data acquisition with the TotalChrom Navigator software package (v6.3.2) was used for gas chromatographic studies. The GC was equipped with both a flame ionisation detector (FID) and a nitrogen-phosphorous detector (NPD). A sample injection volume of 1 μ L was introduced to the GC via the autosampler with a Hamilton 5 μ L syringe (Merck, Darmstadt). A glass inlet liner with quartz wool (Merck, Darmstadt) was employed to trap non-volatile residues.

The gas chromatographic parameters and apparatus for skatole measurement were the same as those described by [Whittington et al. \(2004\)](#). This set-up used a CP-Wax 57 CB capillary column [25m $\times$ 0.32 mm i.d.] (Agilent, Santa Clara, USA), helium carrier gas, and NPD.

The gas chromatographic parameters and apparatus for androstene measurement were similar to those described by [Nicolau-Solano et al. \(2007\)](#). A CP-Sil 8 CB [25 m $\times$ 0.25 mm (i.d.)] (Agilent, Santa Clara USA) capillary column, hydrogen carrier gas, and FID were employed.

Adipose tissue sample extracts were centrifuged using a Micro Centaur Plus Micro Centrifuge (East Sussex, UK) and a Allegra X-22R Benchtop Centrifuge from Beckman Coulter (California, USA). Sample mixing was carried out with a Denley Vibromix by Thermo Electron Corporation (Warwickshire, UK) and a Vibrax-VXR shaker by IKA (Staufen, Germany). Ultrasonication was performed with a Sonomatic Ultrasonic Bath by Langford Electronics Ltd (Hunningham Hill, UK). To assist the exchange of solvents for the GC measurement of skatole a Rotovapor, Waterbath B-4841 and Vac V-500 by Buchi (Flawil, Switzerland) were employed for rotary evaporation.

2.3. Procedures

2.3.1. Androstene biosensor

The amperometric androstene biosensor was fabricated as described above (2.2.1). Chronoamperometry was carried out using the biosensor controlled at 35 °C. Aliquots (100 μ L) of each standard solution of androstene were deposited on the biosensor surface in to ensure complete coverage. The concentration range of the androstene standards used for the calibration study were in the range 0.5–2.0 μ g/ml. A potential of +0.05 V was applied to the working electrode, using 10 s deposition time at open circuit, and the current response was monitored for 2 min. Temperature studies for androstene were performed by adjusting the heater plate to study the chronoamperometric responses over the temperature range 25 °C–40 °C.

In order to deduce the average pH of adipose tissue, 10 carcasses were investigated on an abattoir processing line. The probe was inserted into the subcutaneous adipose tissue of pig carcasses in the upper back region. The average pH was then used in the characterisation studies for the biosensors.

2.3.2. Skatole sensor

The cyclic voltammetric study of skatole was performed using the following conditions: initial/final potential –0.1 V, switching potential +1.0 V, scan rate 50 mV/s. The measurement was made with a SPCE (vs. Ag/AgCl) in an electrochemical cell containing 0.1 mM skatole in 0.1 mM phosphate buffer and 0.1 mM sodium chloride (pH 7.0). Cyclic voltammograms were obtained at room temperature.

Calibration studies for skatole were performed using differential pulse voltammetry (DPV) with skatole concentrations in the range of 2–100 mM. The following DPV parameters were used: start potential +0.1 V, final potential +1.0 V, scan rate 50 mV/s, step potential 0.005 V, modulation amplitude 0.005 V, modulation time 0.05 s. Differential pulse voltammograms were obtained at room temperature.

Temperature studies for skatole were performed by adjusting the heater plate to study the chronoamperometric responses over the temperature range 25 °C–40 °C.

2.3.3. Interference studies

Investigations into possible endogenous interference compounds were performed with the two measurement platforms: one for skatole and another for androstene. Several water-soluble vitamins were investigated using the plain SPCE (vs. Ag/AgCl) under the conditions described for skatole measurement. Hormones and fatty acids were investigated using the androstene (bio)sensor under the conditions described for androstene measurement.

2.4. Analytical application

2.4.1. Electroanalysis

Samples of subcutaneous porcine adipose tissue were obtained from both a local retailer (fresh) and a pig producer (frozen). Frozen samples were thawed before being warmed to 35 °C for analysis. An incision was made in the superficial backfat layer for the insertion of the (bio)sensors; [Fig. S1](#) shows these sensors inserted into the adipose tissue sample for interrogation with the instrumentation shown. To determine the endogenous levels of skatole and androstene, the current measurements were compared to the slope of the calibration plots obtained from subcutaneous adipose tissue samples spiked with known amounts of the compounds.

Fresh samples were used to calibrate the two measurement platforms, the two calibration studies were carried out independently and in both studies a section of adipose tissue was cut into sections and fortified relative to the weight of sample (μ g/g). All additions were achieved by adding 10 μ L aliquots to a tissue incision made in the superficial backfat layer to standardise liquid additions and the samples were stored in sealed containers at 4 °C for 24 h before analysis to allow diffusion through the tissue whilst minimising sample degradation. Control samples were prepared by adding a 10 μ L aliquot of methanol.

2.4.2. Gas chromatography

Samples analysed by electrochemical techniques for endogenous levels of skatole and androstene were also prepared for gas chromatography analysis using a solvent extraction procedure. Frozen samples were thawed prior to extraction. The skin, hypodermal layer and lower deep back-fat layer were removed leaving the remaining superficial back fat layer for analysis. Analysis on each sample were performed in duplicate. The variation between duplicates was below 10% and the average values were used in the final correlation plots.

The procedure for extracting androstene from adipose tissue was modified from the method described by [Verheyden et al. \(2007\)](#). Small pieces of adipose tissue (10 g total) were microwaved at 480 W for 1 min. The melted adipose was centrifuged (5000 $\times$ g for 5 min), then 0.25 g (in duplicate) added to a tube containing 1 ml methanol and hexane (9:1). The sample underwent a mixing procedure three times (30 s vortex then 5 min ultrasonication). The tube was centrifuged for a second time (700 $\times$ g for 5 min), then allowed to cool block for 1 h. The supernatant was transferred to an autosampler vial where the internal standard (androsterone) was added to give a final concentration of 2.0 μ g/ml.

The procedure for extracting skatole from adipose tissue was modified from [Dehnhard et al. \(1993\)](#). Small pieces of adipose tissue (10 g in total) were microwaved at 320 W for 4 min. The melted adipose was centrifuged (5000 $\times$ g for 5 min), then 1 g (in duplicate) added to a tube containing 15 ml hexane (9:1), this sample underwent a mixing procedure three times (30 s vortex then 1 min shaking). Then 5 ml acetonitrile was added to the tube and the mixing procedure repeated. The extract in acetonitrile (4 ml) was added to a small round bottom flask and taken to dryness using a rotary evaporator, the residue was then reconstituted with ethyl acetate (500 μ L), ultrasonicated (1 min), and

then vortexed (1 min). The supernatant was transferred to an autosampler vial where the internal standard (5-methylindole) was added to give a final concentration of 0.5 µg/ml.

3. Results and discussion

3.1. Measurement of porcine adipose tissue pH

Initially we determined the pH range of porcine adipose tissue in order to fully characterise the (bio)sensors for operation on real samples. From this information, it was possible to deduce whether there would be a significant effect on the (bio)sensor response due to pH differences. The pH measurements were carried out in an abattoir on ten random carcasses. The mean pH of adipose tissue after the splitting of the carcass was calculated to be pH 7.0 ($n=10$) over the range pH 6.71–7.54 (temperature range of 31.7–38.6 °C). In preliminary studies, it was shown that the magnitudes of the responses for both skatole and androstenone were constant over this range of pH and temperature values; this indicated that no correction would be required to the results. Subsequently all aqueous characterisation studies were carried out at pH 7 using phosphate buffer solutions.

3.2. Chronoamperometric behaviour of androstenone using a screen-printed biosensor

Fig. 1 shows the sequence of reactions involved in the operation of the androstenone biosensor. An electrocatalytic reaction occurs by the interaction of NADH with MB_{ox} to form MB_{red}; this is followed by the electrochemical oxidation of the latter at the SPCE surface and constitutes the analytical signal. Consequently, in the absence of androstenone a maximum response is obtained. In the presence of androstenone an enzymatic reaction occurs between the α HSD and androstenone, which requires the cofactor NADH, resulting in the conversion of NADH on the electrode surface to NAD⁺, which results in a decrease in current response. The decrease in current is directly proportional to the concentration of androstenone.

Fig. 2A shows the chronoamperograms obtained for androstenone over the concentration range 0.5–2.0 ppm. The current response is highest when androstenone is not present at the electrode surface and then decreases as the concentration of androstenone increases. The current measurements were taken at 10, 20, and 30 s after the application of the potential (+0.05 V vs. Ag/AgCl). Fig. 2B shows the resulting calibration plots, from which it is evident that a linear

relationship exists between the current at three different measurement times and androstenone concentration over the concentration range studied in aqueous buffer solution. This demonstrates the feasibility of measuring this boar taint compound in porcine adipose tissue. From our calibration data, we deduced that the linear range was from 0.3 ppm to at least 4 ppm with a detection limit of 0.3 ppm (signal to noise ratio of 3:1).

A temperature study was carried out over the temperature range 25 °C–40 °C. It was shown that there was no significant difference in biosensor response between 25 °C–35 °C; however, at 40 °C the response indicated that the enzyme had denatured (Fig. S2). It should be mentioned that the temperature of 35 °C is likely to be the maximum temperature of carcasses on the abattoir production line at the point of measurement for the desired final application.

3.3. Voltammetric behaviour of skatole using a screen-printed carbon electrode

The redox behaviour of skatole at screen-printed carbon electrodes (vs. Ag/AgCl) was investigated using cyclic voltammetry. The cyclic voltammetric response (Fig. 3) demonstrated a well-defined oxidation peak with an E_p value of +0.6 V vs Ag/AgCl with phosphate buffer pH 7.0, this is followed by a smaller peak at +0.9 V applied potential.

The proposed mechanism for the initial irreversible oxidation of skatole at the screen-printed carbon electrode to 3-methyl-2-oxindole is shown in Fig. 4. The mechanism responsible for the production of the latter peak is most likely due to the oxidation of the product 3-methyl-2-oxindole.

Having demonstrated that skatole was electroactive at a screen-printed carbon electrode (vs. Ag/AgCl); we investigated the more sensitive and selective differential pulse voltammetric method for the proposed application.

Fig. 5A shows that skatole exhibits well-defined differential pulse voltammetric peaks over the concentration range 2–100 µM (0.26–13.11 ppm), with a peak potential (E_p) of +0.55 V at pH 7. Fig. 5B shows that the magnitude of the peak current (i_p) is linear over this concentration range and the calculated coefficient of variation is below 10% ($n=3$). This performance data suggests that the differential pulse voltammetry method should be suitable for the measurement of skatole in adipose tissue. From our calibration data, we deduced that the linear range was from 0.052 ppm to at least 4 ppm with a detection limit of 0.052 ppm (signal to noise ratio of 3:1).

A set of four calibration studies were performed to determine the

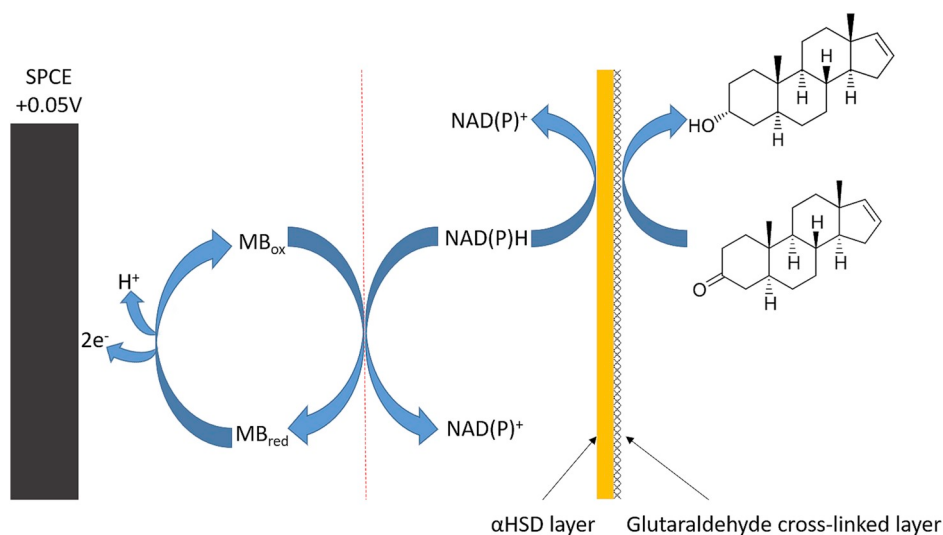


Fig. 1. Schematic displaying the interaction between hydroxysteroid dehydrogenase, cofactor NAD(P)H and the analyte androstenone at the surface of the electrode, resulting in the subsequent decrease in the analytical response.

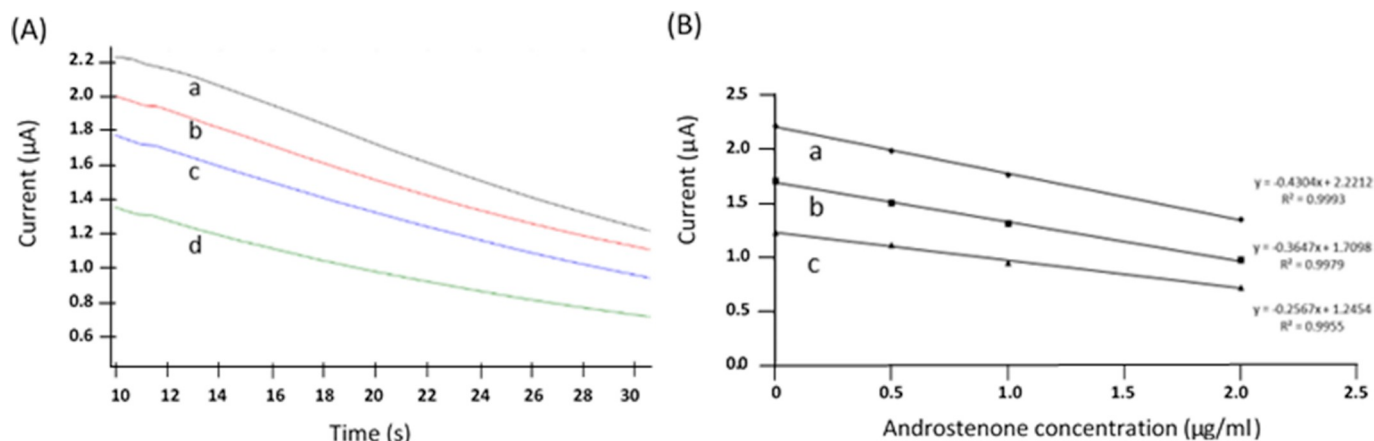


Fig. 2. (A) Chronoamperograms obtained with the androstenedione biosensors at a range of concentrations (a) 0.0 μg/ml (b) 0.5 μg/ml (c) 1.0 μg/ml (d) 2.0 μg/ml. Solutions prepared with pH 7 0.1 M phosphate buffer containing 0.1 M sodium chloride and 5% methanol. Applied potential +0.05 V. Solution volume 100 μl, coverage represented by inset. Biosensors operated at 30 °C. (B) Corresponding calibration graph obtained from current measurements at 10s (a) 20s (b) and 30s (c).

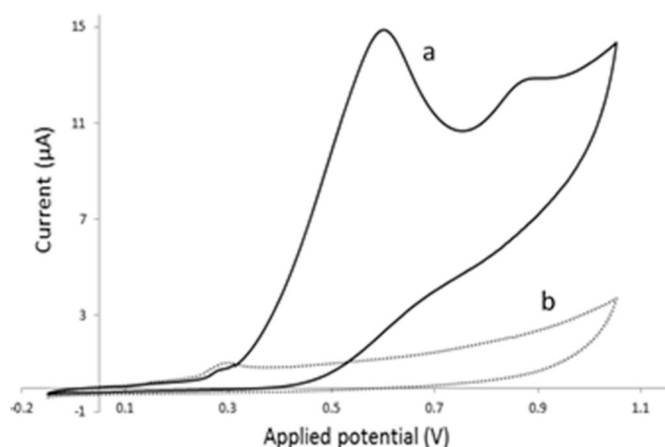


Fig. 3. Cyclic voltammograms obtained with a SPCE in a solution containing (a) 0.1 mM skatole and (b) 0.0 mM in pH 7 0.1 M phosphate buffer with 0.1 M sodium chloride. Method parameters: 50 mV/s scan rate; -0.1 V start/end potential; +1.0 V switching potential.

effect of temperature on the magnitude of skatole peak response using differential pulse voltammetry (25 °C, 30 °C, 35 °C and 40 °C). Peak current measurements were taken from triplicate differential pulse voltammograms and plotted against concentration. The calibration plots exhibited similar slopes, and the sensitivity increased very slightly with decreasing temperature. Therefore, this study demonstrates that this method is suitable for the measurement of skatole over the expected carcass temperature range (25 °C–35 °C).

3.4. Investigations into possible endogenous interference compounds

A selection of water-soluble vitamins was identified from previous studies (e.g. Greenfield et al., 2009) to be present at concentrations of voltammetric relevance to the measurement of skatole. A selection of these vitamins has been reported to exhibit electroactivity at carbon electrodes; nicotinamide (Hu et al., 2001), riboflavin (Revin and John, 2012; Safavi et al., 2010), thiamine (Hart et al., 1995; Oni et al., 2002), pyridoxine (Hu et al., 2001; Qu et al., 2004; Zhang and Wang, 2011), and folic acid (Kalimuthu and John, 2009; Revin and John, 2012). Only folic acid and pyridoxine exhibited oxidation responses at SPCEs (vs. Ag/AgCl) at pH 7.0; however, the peak potentials were more than 250 mV and 200 mV higher than that of skatole respectively. Thus, these compounds were not seen as possible interferences for the analysis of

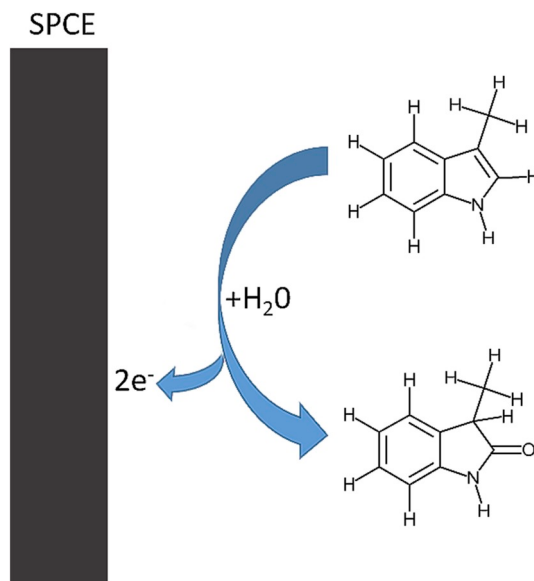


Fig. 4. Schematic displaying the initial oxidation of 3-methylindole to 3-methyl-2-oxindole resulting in the generation of the analytical response at the screen-printed carbon electrode.

subcutaneous porcine adipose tissue.

A similar study was carried out with the amperometric (bio)sensor for a series of relevant naturally occurring compounds. The effect of several hormones and fatty acids on the measurement of androstenedione was investigated. Estrone, estradiol, testosterone, linoleic acid, oleic acid and palmitic acid were subjected to the same analytical procedure as the androstenedione standard solutions. None of these compounds changed the magnitude of the biosensor response.

3.5. Application of electrochemical (bio)sensors to adipose tissue and correlation with gas-chromatographic method

Differential pulse voltammograms (Fig. S3) were obtained using the skatole sensor with a sample of subcutaneous porcine adipose tissue. Scan (a) represents the endogenous voltammetric profile from the tissue, whereas scan (b) was obtained after fortification with 2 μg/g skatole. An increase in current at a peak potential of 0.8 V indicates the presence of endogenous levels of skatole, this was confirmed by GC-NPD analysis. It is important to mention that the peak potential for skatole in an aqueous

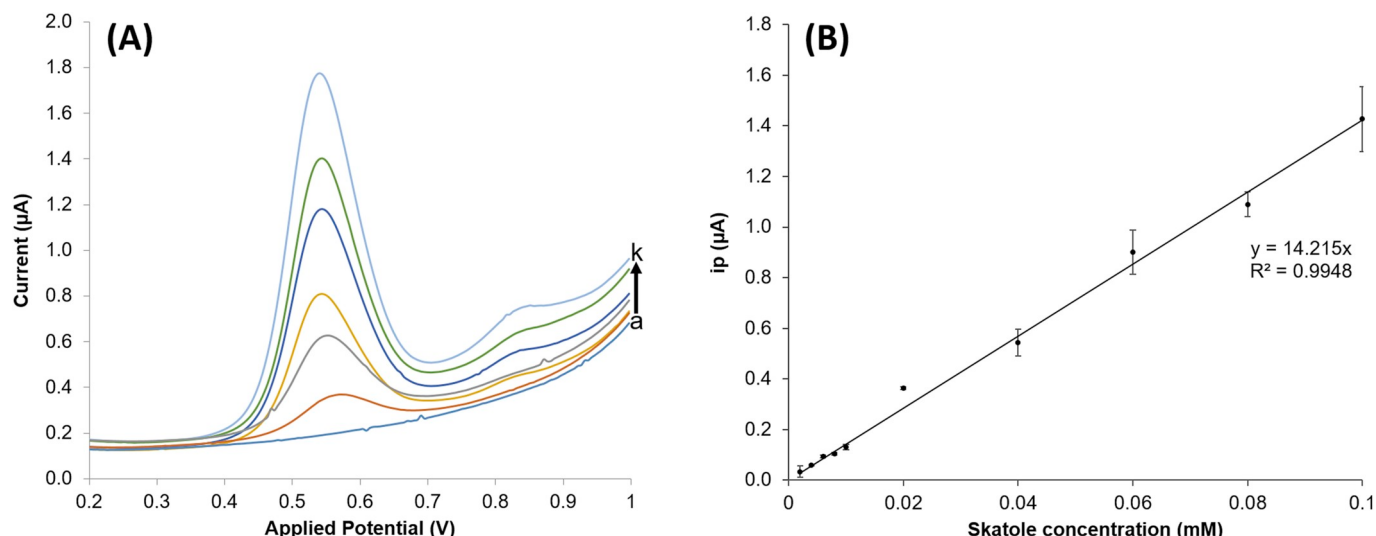


Fig. 5. (A) Differential pulse voltammograms obtained using SPCEs (vs. Ag/AgCl) with solutions containing skatole over the concentration range (μM): (a) 0; (b) 2; (c) 4; (d) 6; (e) 8; (f) 10; (g) 20; (h) 40; (i) 60; (j) 80; (k) 100. The following experimental conditions were used: initial potential +0.1 V, final potential +1.0 V, scan rate 50 mV/s, step potential 0.005 V, modulation amplitude 0.005 V, modulation time 0.05 s. (B) Corresponding calibration plot for skatole using peak height measurements vs. concentration ($n = 3$).

solution is 0.25 V lower than that observed in adipose tissue.

The traditional methodologies for boar taint determination chosen to validate the novel sensor technology use gas chromatographic separation techniques coupled with ionisation detectors. A series of calibration studies were performed using known concentrations of the analytes to determine the magnitude of peak responses for each methodology (Fig. S4). The standards for gas chromatographic studies were prepared using the solvents required for sample extract analysis. Whereas the standards for electrochemical studies were prepared by fortifying sections of adipose tissue for *in-situ* analysis with the (bio)sensors (Hart et al., 2017).

Samples of adipose tissue obtained from retailers and a pig producer were analysed using the amperometric (bio)sensor and voltammetric sensor for the measurement of androstenone and skatole respectively. The endogenous concentration of skatole was calculated by referring the current measurements of peak height for the individual samples to the calibration graph obtained from *in-situ* measurements of fortified adipose tissue; the *in-situ* calibration gave a slope of 60 nA per μg skatole per g of porcine adipose tissue (Hart et al., 2017). A similar approach was

used to determine the endogenous concentration of androstenone, by referring the current value, at 20 s, for the individual samples to the chronoamperometric calibration graph; the *in-situ* calibration gave a negative slope of $-1.9 \mu\text{A}$ per μg androstenone per g of porcine adipose tissue (Hart et al., 2017). These adipose tissue samples were then prepared for gas chromatographic separation and analysed by the respective ionisation detector. Skatole was determined with a thermionic detector whereas androstenone was determined with a flame ionisation detector, concentrations were calculated by referring to the calibration graphs (Fig. S4). Samples were analysed in duplicate by each method and the average values obtained by the novel and traditional methods were plotted.

The correlation plot for endogenous skatole concentration in subcutaneous porcine adipose tissue (Fig. 6A) shows a strong positive correlation for the two analytical methodologies, with a calculated coefficient of determination (R^2) of 0.80. Therefore, the results of these studies show that the voltammetric sensor is in good agreement with the conventional gas chromatographic method. This demonstrates that the sensor approach shows promise for measurements of skatole in pig

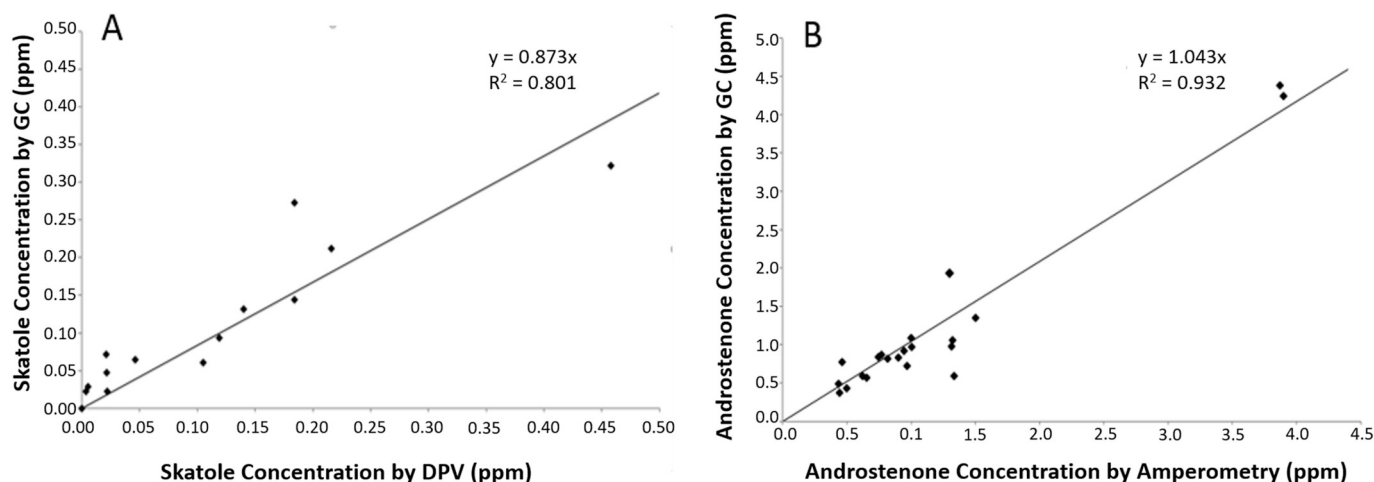


Fig. 6. (A) Correlation plot for the concentration of skatole in subcutaneous porcine adipose tissue measured by both the voltammetric sensors and the corresponding GC-NPD method ($n = 14$); (B) correlation plot for the concentration of androstenone in subcutaneous porcine adipose tissue measured by both the amperometric biosensors and the corresponding GC-FID method ($n = 21$).

carcasses on the abattoir processing line.

Fig. 6B also shows that a strong positive correlation was obtained for the calculated concentrations of androstenone in subcutaneous adipose tissue samples measured by both the novel method and traditional method, with a calculated R^2 of 0.93. Therefore, the results of this study show that the results of androstenone analysis obtained by the (bio)sensors are in good agreement with the results obtained by conventional gas chromatographic method on the same samples. This demonstrates that the (bio)sensors offer a promising approach for measuring androstenone and skatole on the abattoir processing line.

It should be mentioned that the mean recovery, from the above data, by the (bio)sensors compared to the GC method for skatole was 114.5%, and for androstenone was 95.9%.

4. Conclusions

The laboratory-based studies presented in this paper have demonstrated the successful application of electroanalytical approaches using (bio)sensors for the measurement of boar taint compounds in pig adipose tissue. The detection limits and linear ranges for the androstenone biosensor and skatole sensor encompass the concentration ranges relevant to the boar taint. The evaluation of the androstenone biosensor and skatole sensor was carried out using a GC reference method and the results obtained confirms the potential for this electrochemical approach for the direct analysis of adipose tissue. The focus of our future work will be to adapt this (bio)sensor system for applications in an abattoir environment for on-line analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

K.L. Westmacott: Investigation, Writing - original draft. **A.P. Crew:** Investigation, Writing - original draft, Supervision. **O. Doran:** Funding acquisition, Conceptualization, Writing - original draft, Supervision. **J. P. Hart:** Supervision, Conceptualization, Writing - original draft, Investigation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2019.111837>.

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