# 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 3α-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 seconds, 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.

*Keywords: Boar taint, screen-printing, biosensor, sensor, differential pulse voltammetry, chronoamperometry, GC-FID, GC-NPD*

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26 **1. Introduction**

27 Boar taint is the term given to the unfavourable flavour and aroma sometimes experienced with
28 meat and meat products derived from entire (uncastrated) male *Sus scrofa domesticus*, commonly
29 termed the domestic pig. Decades of research indicates that boar taint is due to the excessive
30 accumulation of several naturally occurring compounds in porcine adipose tissue with the main
31 compounds being skatole and androstenone. Currently there are no EU-approved rapid
32 instrumental methods to detect and quantify the boar taint compounds at the point of test and, in
33 particular, on the abattoir line (Haugen *et al*., 2012). A commonly used and effective approach for
34 reducing boar taint has been castration. However, an increasing number of countries are moving
35 towards an entire male pig production system because of animal welfare concerns. An entire male
36 pig production system will ultimately lead to a higher incidence of boar taint unless the alternative
37 methods for reduction of boar taint have been implemented. A number of approaches to the
38 reduction of boar taint compounds have been considered or developed. This includes genetic
39 selection, using immunocastration, manipulation of pigs' diet and management and sperm sorting
40 (Squires and Bonneau, 2014). However, some of these approaches are only effective for the
41 reduction of skatole level (e.g. diet and management), others are either expensive (sperm sorting),
42 under development (genetic tests) or have not been widely accepted yet (immunocastration).

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

50 of the effectiveness of the methods for boar taint reduction such as genetic selection,
51 immunocastration, and dietary manipulation and other.

52 Studies into the threshold levels for perception of boar taint have shown substantial disparity, this
53 has been linked to human olfactory genetic variation and differences in the processing of meat
54 products (Font-i-Furnols, 2012). A review by Walstra *et al*. (1999) identified several published
55 studies reporting the associations between concentrations of androstenone and skatole and the
56 unpleasant aroma or taste of pork. These studies were used to determine general threshold levels
57 for boar taint compounds at which consumers would negatively react to meat from entire male pigs.
58 The thresholds were between 0.5 and 1.0 ppm for 5α-androst-16-en-3-one (androstenone)
59 (Desmoulin *et al*., 1982; Mortensen *et al*., 1986) and between 0.2 and 0.25 ppm for 3-methylindole
60 (skatole) (Armstrong, 1993; Mortensen *et al*., 1986).

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

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

70 Recently, various strategies for the electroanalytical measurement of a wide variety of compounds
71 in food products and beverages have been developed (Hughes *et al*., 2016). One example is the
72 successful development of a voltammetric assay for the quantification of trace heavy metals in
73 various food products and vegetables (Mendoza *et al*., 2015). In another approach, amperometry

74 has been employed in conjunction with enzyme-based biosensors to quantify a range of
75 organophosphate pesticides, organochlorine pesticides, and insecticides in food products (Bollella
76 et al., 2016; Crew et al., 2011; Palchetti and Mascini, 2015; Tortolini et al., 2016). In addition, a
77 screen-printed carbon electrode (SPCE) modified with the redox mediator cobalt phthalocyanine,
78 was successfully applied to the direct measurement of citric acid in lime fruits (Honeychurch *et al*.,
79 2010). This latter example of a successful food quality-monitoring sensor for *in-situ* measurements
80 demonstrated the potential for direct analysis of food quality.

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

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

92 **2. Experimental**

93 *2.1. Chemicals and reagents*

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

97 *2.1.1. Electroanalysis*

98 Biosensors for androstenone detection were prepared as previously described by the authors (Hart
99 *et al*., 2016).

100 A phosphate buffer solution (pH 7.0) was prepared by titrating stock solutions of monosodium
101 dihydrogen phosphate and disodium monohydrogen phosphate to the desired pH at a
102 concentration of 0.5 M. Buffer solutions were further diluted with deionised water to a final working
103 concentration of 0.1 M phosphate buffer. Sodium chloride stock solutions were prepared at 1.0 M
104 and similarly diluted to a working solution concentration of 0.1 M sodium chloride. Sodium
105 hydroxide stock solutions were prepared at 0.1 M in deionised water.

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

112 A 1 µg/ml stock solution of androstenone was prepared in HPLC grade methanol. Working standards
113 were prepared by diluting the androstenone stock solution with phosphate buffer and methanol
114 (5% final volume). A stock standard for chronoamperometric adipose tissue fortification studies was
115 prepared at a concentration of 1 mg/ml in methanol and working standard concentrations were
116 calculated in relation to the weight of individual sections of adipose tissue sample.

117 A selection of potentially interfering compounds was prepared at concentrations typically occurring
118 in adipose tissue or associated tissues. Unsaturated and saturated fatty acids (linoleic, palmitic and
119 oleic acids) were dissolved to saturation in 0.1M phosphate buffer (pH 7.0) with 0.1M NaCl, and

120 evaluated directly. The hormones testosterone, estradiol and estrone, were dissolved in methanol
121 at a concentration of 2.5 ppm and diluted to 2 ppb, 0.078 ppb and 0.138 ppb, respectively in 0.1M
122 pH7 phosphate buffer with 0.1M NaCl. Solutions of the amino acids alanine, glycine and taurine
123 were prepared in 0.1M phosphate buffer (pH 7.0) with 0.1M NaCl at concentrations of 590 ppm,
124 110 ppm and 240 ppm respectively for analysis. Stock solutions (50 mM) of thiamine hydrochloride,
125 pantothenic acid, nicotinamide, and pyridoxine hydrochloride were prepared in deionised water.
126 Whereas, stock solutions (20 mM) of riboflavin and folic acid were prepared with a sodium
127 hydroxide stock solution. Working standards (5 mM) were prepared by diluting the stock standards
128 in phosphate buffer and sodium chloride stock solutions.

129 *2.1.2. Gas chromatography*

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

133 Stock solutions of androstenone and 3α-hydroxy-5α-androstan-17-one (androsterone) were
134 prepared with HPLC grade methanol and hexane (Fisher Scientific; Loughborough, UK) at a ratio of
135 9:1 methanol:hexane. An internal standard, androsterone, was used at the concentration of 2 µg/ml
136 with sample extracts.

137 *2.2. Apparatus and instrumentation*

138 *2.2.1. Electroanalysis*

139 All voltammetric and chronoamperometric measurements were carried out with a µAutolab III
140 (Metrohm, Netherlands) potentiostat interfaced to a PC for data acquisition via the proprietary
141 software (NOVA v1.1). Screen-printed electrodes were supplied by Gwent Electronic Materials Ltd
142 (Pontypool, UK); the working electrode used for the measurement of skatole was fabricated using a

143 carbon ink (C2030519P4), whereas the working electrode for the biosensor used a carbon ink
144 modified with Meldola's blue (MB) (BE2031028D1/247) and the enzyme 3α-hydroxysteroid
145 dehydrogenase (3α-HSD) was drop-coated onto the surface. The enzyme was immobilised onto the
146 MB-SPCE using glutaraldehyde by drying in a vacuum at $4^{o}$C. The reference electrode for both the
147 sensor and biosensor was fabricated using a Ag/AgCl ink (C61003P7).

148 The surface temperature of the adipose tissue was measured prior to electrochemical
149 measurements using an infrared thermometer with laser targeting (Maplin Electronics; Rotherham,
150 UK). Measurements of internal temperature and pH were obtained with a Testo 205 temperature-
151 pH probe (Alton, UK).

152 *2.2.2. Gas chromatography analysis*

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

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

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

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

172 *2.3. Procedures*

173 *2.3.1. Androstenone biosensor*

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

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

185 *2.3.2. Skatole sensor*

186 The cyclic voltammetric study of skatole was performed using the following conditions: initial/final
187 potential -0.1 V, switching potential +1.0 V, scan rate 50 mV/s. The measurement was made with a

188 SPCE (vs. Ag/AgCl) in an electrochemical cell containing 0.1 mM skatole in 0.1 mM phosphate buffer
189 and 0.1 mM sodium chloride (pH 7.0). Cyclic voltammograms were obtained at room temperature.

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

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

197 *2.3.3. Interference studies*

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

203 *2.4. Analytical application*

204 2.4.1. *Electroanalysis*

205 Samples of subcutaneous porcine adipose tissue were obtained from both a local retailer (fresh)
206 and a pig producer (frozen). Frozen samples were thawed before being warmed to 35°C for analysis.
207 An incision was made in the superficial backfat layer for the insertion of the (bio)sensors; Fig. S1
208 shows these sensors inserted into the adipose tissue sample for interrogation with the
209 instrumentation shown. To determine the endogenous levels of skatole and androstenone, the

210 current measurements were compared to the slope of the calibration plots obtained from
211 subcutaneous adipose tissue samples spiked with known amounts of the compounds.

212 Fresh samples were used to calibrate the two measurement platforms, the two calibration studies
213 were carried out independently and in both studies a section of adipose tissue was cut into sections
214 and fortified relative to the weight of sample (µg/g). All additions were achieved by adding 10 µl
215 aliquots to a tissue incision made in the superficial backfat layer to standardise liquid additions and
216 the samples were stored in sealed containers at $4^{o}$C for 24 hours before analysis to allow diffusion
217 through the tissue whilst minimising sample degradation. Control samples were prepared by adding
218 a 10 µl aliquot of methanol.

219 2.4.2. *Gas chromatography*

220 Samples analysed by electrochemical techniques for endogenous levels of skatole and
221 androstenone were also prepared for gas chromatography analysis using a solvent extraction
222 procedure. Frozen samples were thawed prior to extraction. The skin, hypodermal layer and lower
223 deep back-fat layer were removed leaving the remaining superficial back fat layer for analysis.
224 Analysis on each sample were performed in duplicate. The variation between duplicates was below
225 10% and the average values were used in the final correlation plots.

226 The procedure for extracting androstenone from adipose tissue was modified from the method
227 described by Verheyden *et al*. (2007). Small pieces of adipose tissue (10 g total) were microwaved
228 at 480 W for 1 min. The melted adipose was centrifuged (5000 x g for 5 min), then 0.25 g (in
229 duplicate) added to a tube containing 1 ml methanol and hexane (9:1). The sample underwent a
230 mixing procedure three times (30 s vortex then 5 min ultrasonication). The tube was centrifuged for
231 a second time (700 x g for 5 min), then allowed to cool block for 1 hour. The supernatant was
232 transferred to an autosampler vial where the internal standard (androsterone) was added to give a
233 final concentration of 2.0 µg/ml.

234 The procedure for extracting skatole from adipose tissue was modified from Dehnhard *et al*. (1993).
235 Small pieces of adipose tissue (10 g in total) were microwaved at 320 W for 4 min. The melted
236 adipose was centrifuged (5000 x g for 5 min), then 1 g (in duplicate) added to a tube containing 15
237 ml hexane (9:1), this sample underwent a mixing procedure three times (30 s vortex then 1 min
238 shaking). Then 5 ml acetonitrile was added to the tube and the mixing procedure repeated. The
239 extract in acetonitrile (4 ml) was added to a small round bottom flask and taken to dryness using a
240 rotary evaporator, the residue was then reconstituted with ethyl acetate (500 µl), ultrasonicated (1
241 min), and then vortexed (1 min). The supernatant was transferred to an autosampler vial where the
242 internal standard (5-methylindole) was added to give a final concentration of 0.5 µg/ml.

243 **3. Results and discussion**

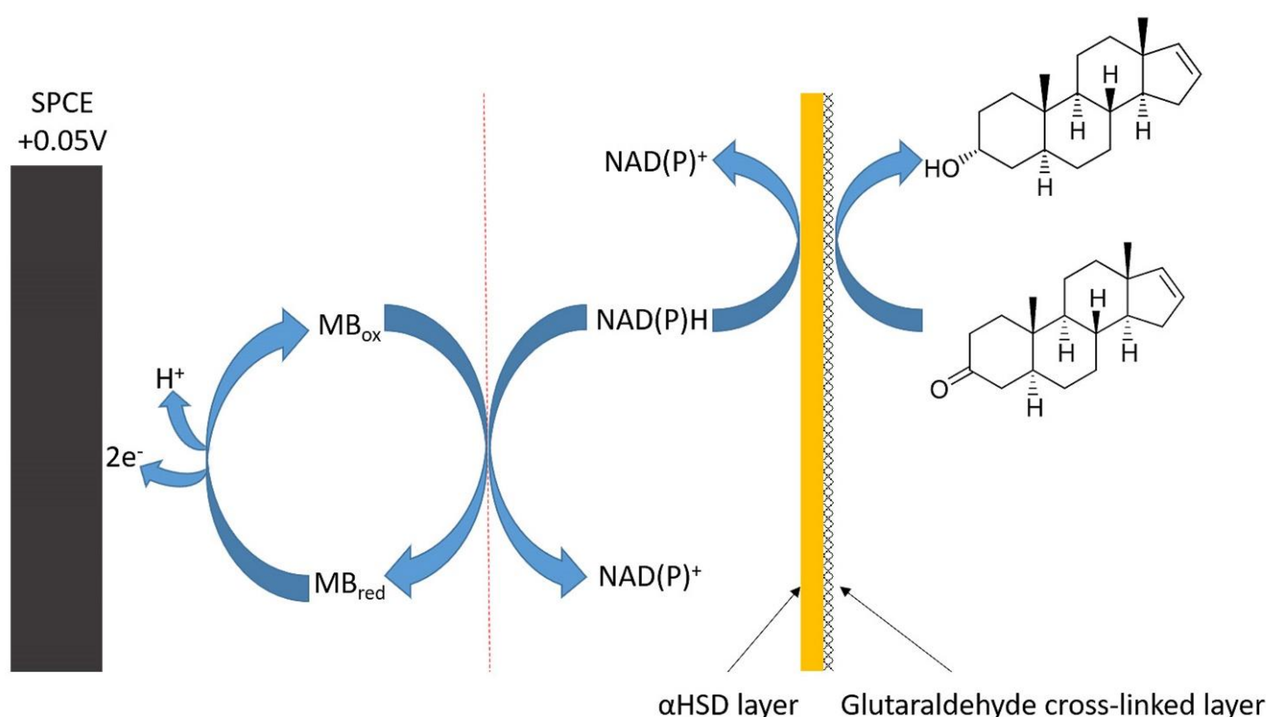
244

245 *3.1. Measurement of porcine adipose tissue pH*

246 Initially we determined the pH range of porcine adipose tissue in order to fully characterise the
247 (bio)sensors for operation on real samples. From this information, it was possible to deduce
248 whether there would be a significant effect on the (bio)sensor response due to pH differences. The
249 pH measurements were carried out in an abattoir on ten random carcasses. The mean pH of adipose
250 tissue after the splitting of the carcass was calculated to be pH 7.0 (n=10) over the range pH 6.71-
251 7.54 (temperature range of 31.7-38.6°C). In preliminary studies, it was shown that the magnitudes
252 of the responses for both skatole and androstenone were constant over this range of pH and
253 temperature values; this indicated that no correction would be required to the results.
254 Subsequently all aqueous characterisation studies were carried out at pH 7 using phosphate buffer
255 solutions.

256 *3.2. Chronoamperometric behaviour of androstenone using a screen-printed biosensor*

257 Fig. 1 shows the sequence of reactions involved in the operation of the androstenone biosensor. An
258 electrocatalytic reaction occurs by the interaction of NADH with $MB_{ox}$ to form $MB_{red}$; this is followed
259 by the electrochemical oxidation of the latter at the SPCE surface and constitutes the analytical
260 signal. Consequently, in the absence of androstenone a maximum response is obtained. In the
261 presence of androstenone an enzymatic reaction occurs between the αHSD and androstenone,
262 which requires the cofactor NADH, resulting in the conversion of NADH on the electrode surface to
263 $NAD^+$, which results in a decrease in current response. The decrease in current is directly
264 proportional to the concentration of androstenone.



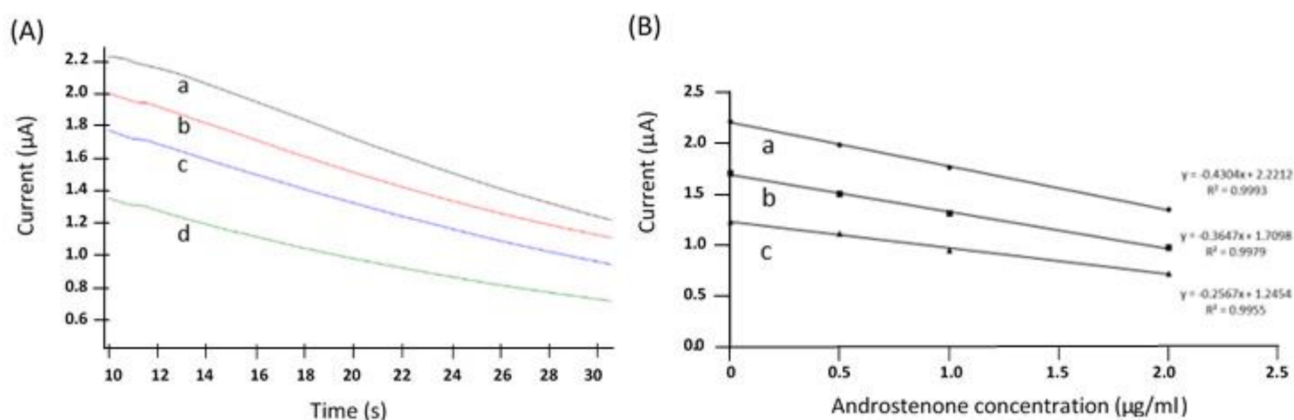

265

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

269

270 Fig. 2A shows the chronoamperograms obtained for androstenone over the concentration range
271 0.5-2.0 ppm. The current response is highest when androstenone is not present at the electrode

272 surface and then decreases as the concentration of androstenone increases. The current
273 measurements were taken at 10, 20, and 30 s after the application of the potential (+0.05 V vs.
274 Ag/AgCl). Fig. 2B shows the resulting calibration plots, from which it is evident that a linear
275 relationship exists between the current at three different measurement times and androstenone
276 concentration over the concentration range studied in aqueous buffer solution. This demonstrates
277 the feasibility of measuring this boar taint compound in porcine adipose tissue. From our calibration
278 data, we deduced that the linear range was from 0.3 ppm to at least 4 ppm with a detection limit of
279 0.3 ppm (signal to noise ratio of 3:1).




280 Fig. 2. (A) Chronoamperograms obtained with the androstenone biosensors at a range of concentrations (a)
281 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
282 containing 0.1 M sodium chloride and 5 % methanol. Applied potential +0.05 V. Solution volume 100 µl,
283 coverage represented by inset. Biosensors operated at 30°C. (B) Corresponding calibration graph obtained
284 from current measurements at 10s (a) 20s (b) and 30s (c).

285

286 A temperature study was carried out over the temperature range 25$^{o}$C-40$^{o}$C. It was shown that
287 there was no significant difference in biosensor response between 25$^{o}$C-35$^{o}$C; however, at 40$^{o}$C the
288 response indicated that the enzyme had denatured (Fig. S2). It should be mentioned that the

289 temperature of 35°C is likely to be the maximum temperature of carcasses on the abattoir

290 production line at the point of measurement for the desired final application.

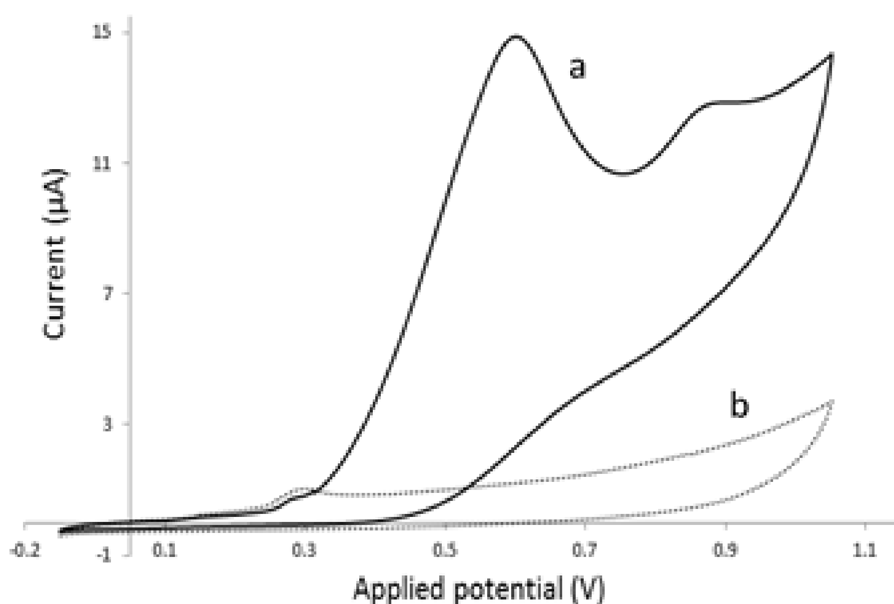
291 3.3. *Voltammetric behaviour of skatole using a screen-printed carbon electrode*

292 The redox behaviour of skatole at screen-printed carbon electrodes (vs. Ag/AgCl) was investigated

293 using cyclic voltammetry. The cyclic voltammetric response (Fig. 3) demonstrated a well-defined

294 oxidation peak with an $E_p$ value of +0.6 V vs Ag/AgCl with phosphate buffer pH 7.0, this is followed

295 by a smaller peak at +0.9 V applied potential.




296 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

297 0.1 M phosphate buffer with 0.1 M sodium chloride. Method parameters: 50 mV/s scan rate; -0.1 V start/end potential;

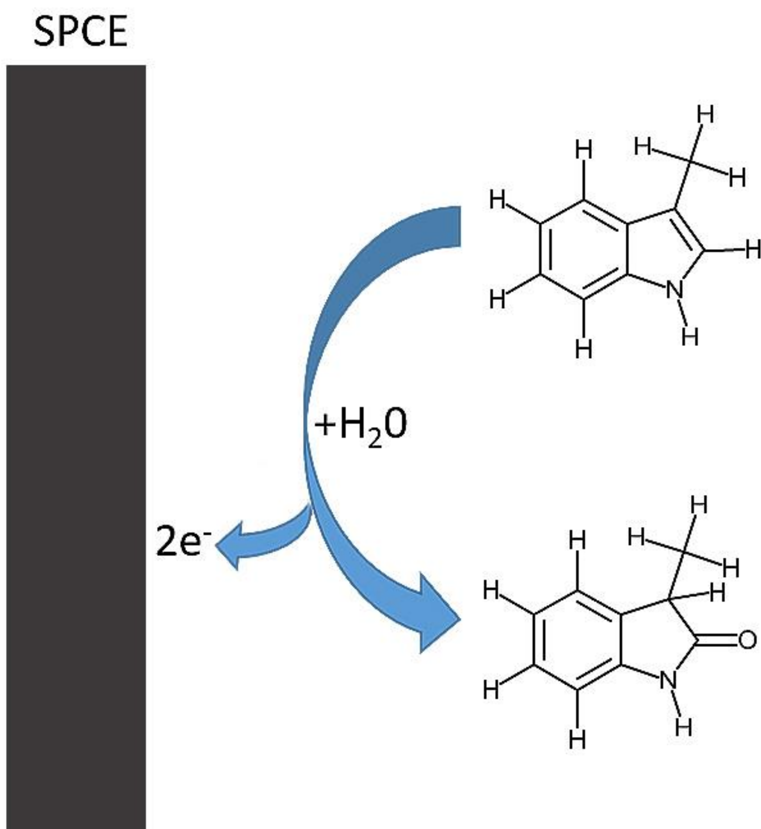
298 +1.0 V switching potential.

299

300 The proposed mechanism for the initial irreversible oxidation of skatole at the screen-printed

301 carbon electrode to 3-methyl-2-oxindole is shown in Fig. 4. The mechanism responsible for the

302 production of the latter peak is most likely due to the oxidation of the product 3-methyl-2-oxindole.



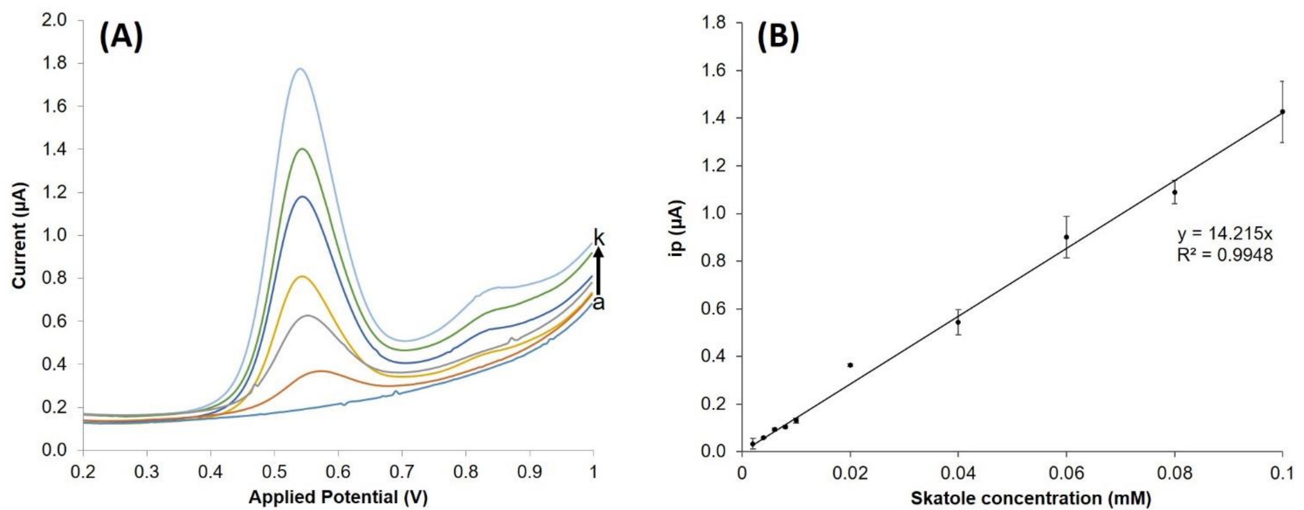

303 Fig. 4. Schematic displaying the initial oxidation of 3-methylindole to 3-methyl-2-oxindole resulting in the
304 generation of the analytical response at the screen-printed carbon electrode.

305

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

309 Fig 5A shows that skatole exhibits well-defined differential pulse voltammetric peaks over the
310 concentration range 2-100 µM (0.26-13.11 ppm), with a peak potential (Ep) of +0.55 V at pH 7. Fig
311 5B shows that the magnitude of the peak current ($i_p$) is linear over this concentration range and the
312 calculated coefficient of variation is below 10 % (n=3). This performance data suggests that the
313 differential pulse voltammetry method should be suitable for the measurement of skatole in
314 adipose tissue. From our calibration data, we deduced that the linear range was from 0.052 ppm to
315 at least 4 ppm with a detection limit of 0.052 ppm (signal to noise ratio of 3:1).

316




317 Fig. 5. (A) Differential pulse voltammograms obtained using SPCEs (vs. Ag/AgCl) with solutions containing
318 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)
319 100. The following experimental conditions were used: initial potential +0.1 V, final potential +1.0 V, scan
320 rate 50 mV/s, step potential 0.005 V, modulation amplitude 0.005 V, modulation time 0.05 s. (B)
321 Corresponding calibration plot for skatole using peak height measurements vs. concentration (n=3).

322

323 A set of four calibration studies were performed to determine the effect of temperature on the
324 magnitude of skatole peak response using differential pulse voltammetry (25°C, 30°C, 35°C and
325 40°C). Peak current measurements were taken from triplicate differential pulse voltammograms
326 and plotted against concentration. The calibration plots exhibited similar slopes, and the sensitivity
327 increased very slightly with decreasing temperature. Therefore, this study demonstrates that this
328 method is suitable for the measurement of skatole over the expected carcass temperature range
329 (25°C-35°C).

330 *3.4. Investigations into possible endogenous interference compounds*

331 A selection of water-soluble vitamins was identified from previous studies (e.g. Greenfield *et al*.,
332 2009) to be present at concentrations of voltammetric relevance to the measurement of skatole. A
333 selection of these vitamins has been reported to exhibit electroactivity at carbon electrodes;
334 nicotinamide (Hu *et al*., 2001), riboflavin (Revin and John, 2012; Safavi *et al*., 2010), thiamine (Hart

335 *et al*., 1995; Oni *et al*., 2002), pyridoxine (Hu *et al*., 2001; Qu *et al*., 2004; Zhang and Wang, 2011),
336 and folic acid (Kalimuthu and John, 2009; Revin and John, 2012). Only folic acid and pyridoxine
337 exhibited oxidation responses at SPCEs (vs. Ag/AgCl) at pH 7.0; however, the peak potentials were
338 more than 250 mV and 200 mV higher than that of skatole respectively. Thus, these compounds
339 were not seen as possible interferences for the analysis of subcutaneous porcine adipose tissue.

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

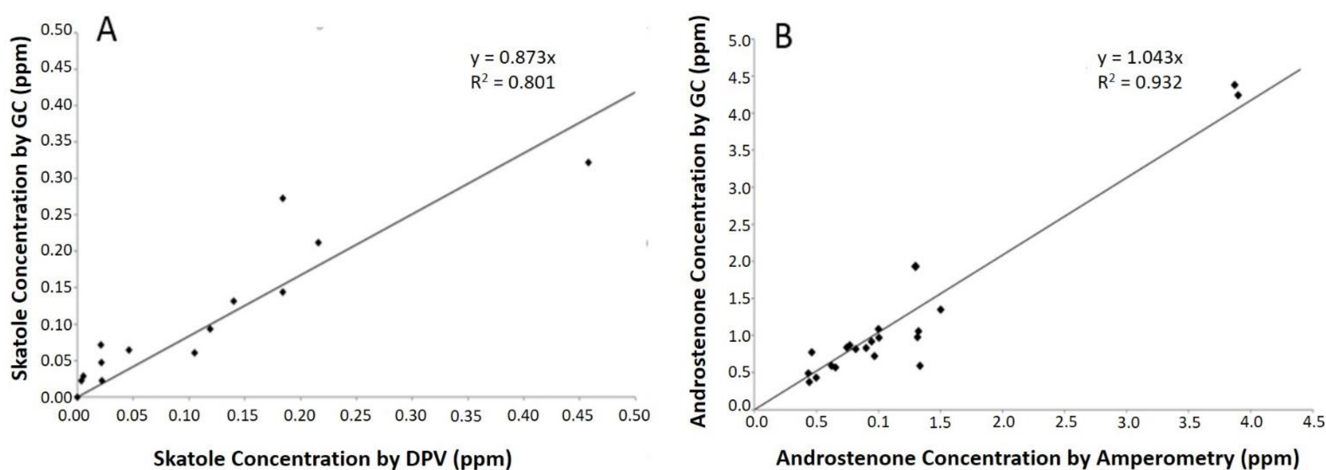
345 *3.5. Application of electrochemical (bio)sensors to adipose tissue and correlation with gas-*
346 *chromatographic method*

347 Differential pulse voltammograms (Fig. S3) were obtained using the skatole sensor with a sample of
348 subcutaneous porcine adipose tissue. Scan (a) represents the endogenous voltammetric profile
349 from the tissue, whereas scan (b) was obtained after fortification with 2 μg/g skatole. An increase
350 in current at a peak potential of 0.8 V indicates the presence of endogenous levels of skatole, this
351 was confirmed by GC-NPD analysis. It is important to mention that the peak potential for skatole in
352 an aqueous solution is 0.25 V lower than that observed in adipose tissue.

353 The traditional methodologies for boar taint determination chosen to validate the novel sensor
354 technology use gas chromatographic separation techniques coupled with ionisation detectors. A
355 series of calibration studies were performed using known concentrations of the analytes to
356 determine the magnitude of peak responses for each methodology (Fig. S4). The standards for gas
357 chromatographic studies were prepared using the solvents required for sample extract analysis.

358 Whereas the standards for electrochemical studies were prepared by fortifying sections of adipose
359 tissue for in-situ analysis with the (bio)sensors (Hart *et al*., 2017).

360 Samples of adipose tissue obtained from retailers and a pig producer were analysed using the
361 amperometric (bio)sensor and voltammetric sensor for the measurement of androstenone and
362 skatole respectively. The endogenous concentration of skatole was calculated by referring the
363 current measurements of peak height for the individual samples to the calibration graph obtained
364 from *in-situ* measurements of fortified adipose tissue; the *in-situ* calibration gave a slope of 60 nA
365 per µg skatole per g of porcine adipose tissue (Hart *et al*., 2017). A similar approach was used to
366 determine the endogenous concentration of androstenone, by referring the current value, at 20 s,
367 for the individual samples to the chronoamperometric calibration graph; the in-situ calibration gave
368 a negative slope of -1.9 µA per µg androstenone per g of porcine adipose tissue (Hart *et al*., 2017).
369 These adipose tissue samples were then prepared for gas chromatographic separation and analysed
370 by the respective ionisation detector. Skatole was determined with a thermionic detector whereas
371 androstenone was determined with a flame ionisation detector, concentrations were calculated by
372 referring to the calibration graphs (Fig. S4). Samples were analysed in duplicate by each method and
373 the average values obtained by the novel and traditional methods were plotted.




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

376 concentration of androstenone in subcutaneous porcine adipose tissue measured by both the amperometric
377 biosensors and the corresponding GC-FID method (n=21).

378

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

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

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

394 **4. Conclusions**

395 The laboratory-based studies presented in this paper have demonstrated the successful application
396 of electroanalytical approaches using (bio)sensors for the measurement of boar taint compounds in
397 pig adipose tissue. The detection limits and linear ranges for the androstenone biosensor and
398 skatole sensor encompass the concentration ranges relevant to the boar taint. The evaluation of

399 the androstenone biosensor and skatole sensor was carried out carried out using a GC reference
400 method and the results obtained confirms the potential for this electrochemical approach for the
401 direct analysis of adipose tissue. The focus of our future work will be to adapt this (bio)sensor
402 system for applications in an abattoir environment for on-line analysis.

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410

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516 Supporting Information

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

521

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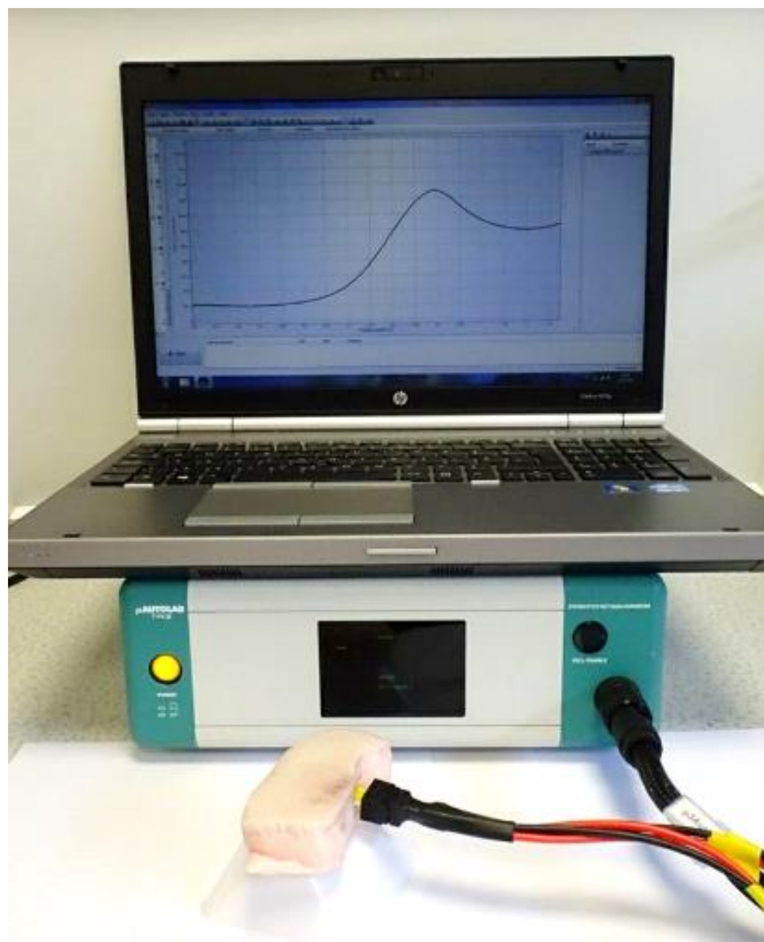

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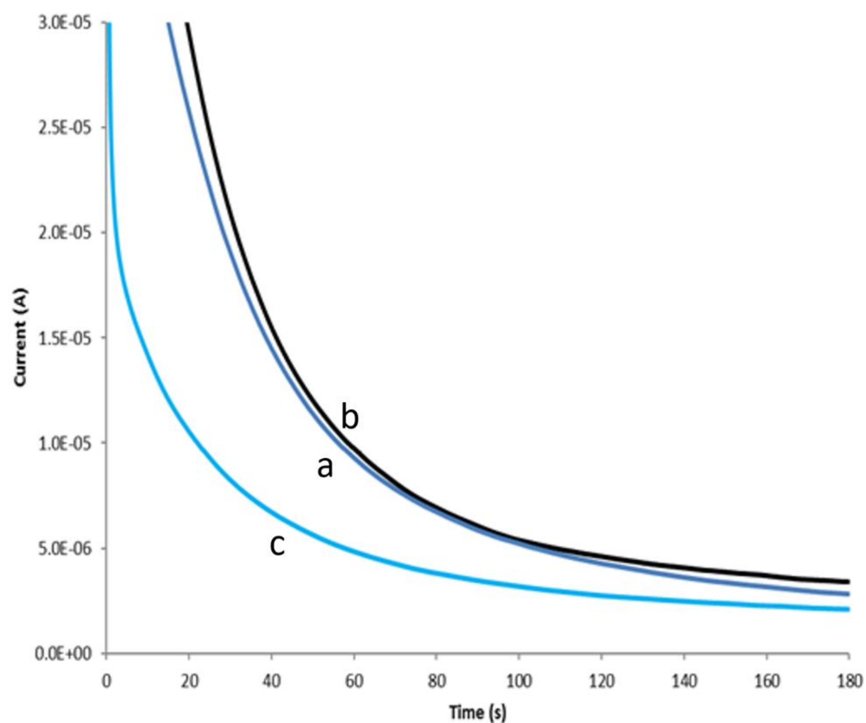
532 Fig. S1. Screen-printed (bio)sensors integrated with a commercial potentiostat (µAutolab III) for the direct
533 measurement of androstenone and skatole in adipose tissue.

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535 Fig. S2. Chronoamperograms obtained at a potential of +0.05V with the biosensors (vs. Ag/AgCl) at 40°C (a
536 & b) and 35°C (c) in the presence of androstenone (a & c) and in the absence of androstenone (b).

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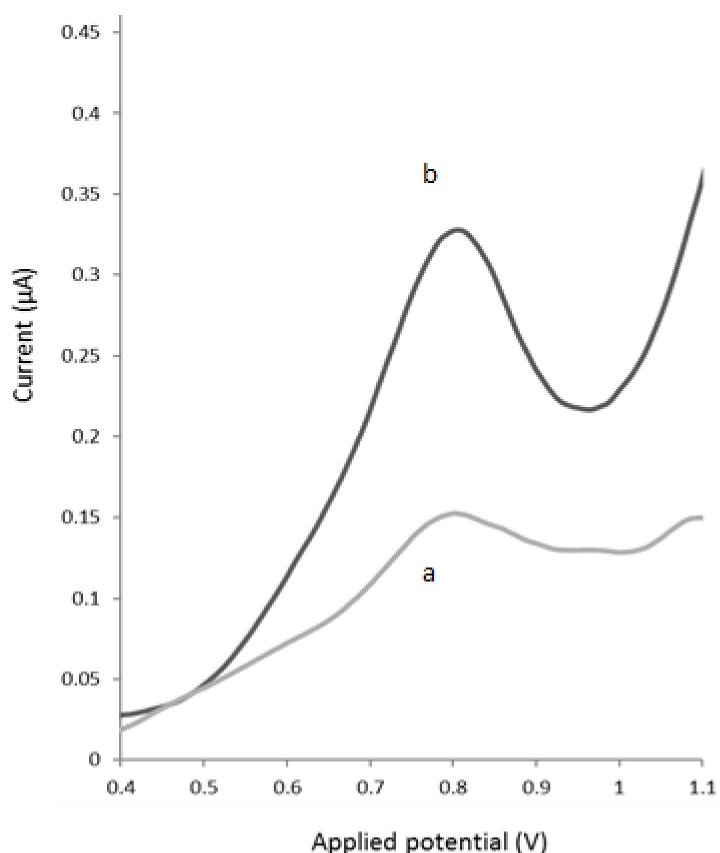


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542 Fig. S3. Differential pulse voltammograms obtained with SPCEs (vs. Ag/AgCl) in (a) subcutaneous porcine
543 adipose tissue and (b) subcutaneous porcine adipose tissue fortified with a 2 μg/g skatole standard. The
544 following experimental conditions were used: initial potential +0.1 V, final potential +1.0 V, scan rate 50
545 mV/s, step potential 0.005 V, modulation amplitude 0.005 V, modulation time 0.05 s.

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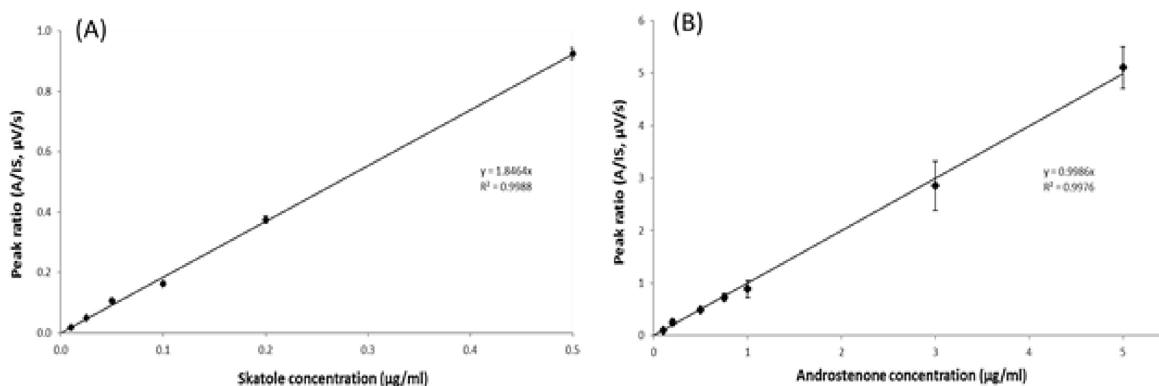


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549 Fig. S4. (A) GCNPD calibration plot of peak ratio (analyte area/internal standard area) vs. concentration of
550 skatole (n=3). (B) GCFID calibration plot of peak ratio vs. androstenone concentration (n=3).

551