

# 1 **Past, present, and future trends in boar taint detection**

2 Clément Burgeon<sup>a,\*</sup>, Marc Debliquy<sup>b</sup>, Driss Lahem<sup>c</sup>, Justine Rodriguez<sup>b</sup>, Ahmadou Ly<sup>c</sup>, Marie-Laure  
3 Fauconnier<sup>a</sup>

4 <sup>a</sup> Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, Université de Liège, Passage  
5 des Déportés 2, 5030 Gembloux, Belgium

6 <sup>b</sup> Service de Science des Matériaux, Faculté Polytechnique, Université de Mons, Rue de l'Épargne 56,  
7 7000 Mons, Belgium

8 <sup>c</sup> Materia Nova ASBL, Materials R&D Centre, Parc Initialis, Avenue Nicolas Copernic 3, 7000 Mons,  
9 Belgium

## 10 **Abstract**

11 *Background:* Boar taint is an unpleasant smell found in the meat of some uncastrated male pigs. This taint  
12 is often prevented by surgical castration without anesthesia or analgesia. However, this practice is an  
13 animal welfare concern. Production of entire males and immunocastration were suggested as alternatives.  
14 Ensuring that meat is untainted remains a priority for slaughterhouses. This has initiated research about  
15 the development of new boar taint detection methods. Most focus on detecting skatole and androstenone,  
16 two major contributors to boar taint.

17 *Scope and approach:* This review aims to describe past methods and recent advances made in rapid boar  
18 taint detection, and provide leads for future research. The main findings of past methods such as the use of  
19 insect behavior-based sensors, e-noses, and gas chromatography–mass spectrometry, are presented.  
20 Recently developed methods based on mass spectrometry, Raman spectroscopy, and sensors are also  
21 discussed. Finally, biosensors showing promising results and potential for boar taint detection are  
22 presented. The advantages and drawbacks of these techniques, cost analysis, and possible challenges  
23 encountered during their application to on-line detection are addressed.

24 *Key findings and conclusions:* This review presents numerous techniques that were developed for boar  
25 taint detection. Some methods, such as laser diode thermal desorption combined with tandem mass  
26 spectrometry, proved their on-line/at-line efficiency as they are fast and accurate. However, initial  
27 investment and difficulty of implementation could lead to reluctance in applying these. Further research  
28 could focus on testing new sensor materials whereas sensory evaluation remains the most practical method  
29 used in slaughterhouses.

30 **Keywords:** Androstenone, skatole, boar taint detection, slaughterhouse, biosensor

31 \* Corresponding author: *E-mail address:* [cburgeon@uliege.be](mailto:cburgeon@uliege.be) (Clément Burgeon)

## 32 **1. Introduction**

33 Boar taint is a strong, unpleasant smell found in the meat of some uncastrated male pigs. This smell,  
34 caused by a complex mixture of molecules, is released upon cooking of the meat. The major molecules  
35 responsible for this smell are androstenone ( $5\alpha$ -androst-16-en-3-one) and skatole (3-methylindole), which  
36 are known more commonly for their urine and fecal smell, respectively (Patterson, 1968; Vold, 1970).  
37 Surgical castration of male piglets without pain relief is a common practice worldwide. This castration is a  
38 fast and cheap way for farmers to ensure that the meat they sell to slaughterhouses is exempt from boar  
39 taint.

40 Surgical castration without anesthesia or analgesia is often criticized for the pain caused to the piglet. In  
41 2010, many European stakeholders had pledged to stop surgical castration practices by 2018 (European  
42 Commission, 2010). Although the 2018 objectives were not successfully met, actions to promote  
43 alternatives to surgical castration are under way (Backus et al., 2018). As listed by the European Food  
44 Safety Authority in a report, these alternatives are the raising of entire (i.e., uncastrated) males,  
45 immunocastration, sperm sexing for production of females only, chemical castration, and administration  
46 of hormones to inhibit the hypothalamic–pituitary–gonadal axis (EFSA, 2004). In practice, the last three  
47 are considered unrealistic because sperm sexing is too expensive for large-scale applications, chemical  
48 castration is painful for the animal, and lastly, even though castration by injection of exogenous hormones  
49 is possible, its administration is prohibited in the EU (Bonneau & Weiler, 2019). Such substances are  
50 well-known for their growth-promotional effects and have been prohibited by the EU in 1981 for  
51 administration to farm animals (European Communities, 1981).

52 Therefore, the remaining alternatives are immunocastration, and the production of entire males.  
53 Immunocastration has been a very reliable technique, and non-responders accounted for only 0-3% of  
54 vaccinated pigs. The reason for the occurrence of non-responders is uncertain, but is said to originate  
55 either from health issues in the pig or simply missing the pig during vaccination in group-housing systems  
56 (Čandek-Potokar et al., 2017). Even though all pigs were found to be correctly immunocastrated in a  
57 recent study by Kress et al. (2020), and particular attention was paid to the piglets' health and vaccine  
58 administration, ensuring that the meat produced is taint-free remains a top priority.

59 The practice of rearing of entire males is currently increasing (Backus et al., 2018). Despite research into  
60 reducing boar taint in several fields, such as genetics (van Son et al., 2017; Zadinová et al., 2017), breed  
61 selection (Aluwé et al., 2011), and selection of boar slaughter weight and boar feed (Heyrman et al., 2018;  
62 Wesoly & Weiler, 2012), 4% and 25% of carcasses in slaughterhouses are strongly and moderately  
63 tainted, respectively (Aluwé et al., 2009). Hence, such carcasses must be distinguished from the untainted  
64 ones to satisfy consumers. These distinguished carcasses are then used in a variety of products where boar  
65 taint can be reduced or simply where masking strategies can be applied. Example of these strategies  
66 include the use of spices, smoking the meat and diluting it with untainted one (Škrlep et al., 2020).

67 Several analytical procedures have been suggested as reference methods for the quantification of skatole  
68 and androstenone. These methods have shown good criteria during in-house validation (Bekaert et al.,  
69 2012; Fischer et al., 2011; Hansen-Møller, 1994; Verplanken et al., 2016), and in-house validation  
70 followed by an inter-laboratory collaborative study (Buttinger & Wenzl, 2014, 2020). Except for the  
71 portable gas chromatography–mass spectrometry (GC-MS) method proposed by Verplanken et al. (2016),  
72 all the above-mentioned methods are time-consuming (sample preparation and analysis) and cannot be  
73 used for detection in slaughterhouses.

74 Although sensory evaluation and colorimetric methods for the detection of boar taint are well-  
75 implemented in slaughterhouses now, research into new detection methods has been ongoing for decades.  
76 The classification method for carcasses used should meet standards such as low cost (less than 1.30  
77 euro/analysis), speed (less than 10 s), automation, and 100% sensitivity and specificity (no false positives  
78 and no false negatives) (Haugen et al., 2012).

79 A recent study by Font-i-Furnols et al. (2020) has described and compared currently used boar taint  
80 detection methods, and identified those that are practically implementable in slaughterhouses. The  
81 methods that have been described in this study analyze boar taint odor as a whole, or the androstenone and  
82 skatole independently found in adipose tissue.

83 This current review presents advances in boar taint detection in a chronological manner. Recent (i.e., after  
84 2015) and innovative research performed on boar taint detection is supplemented with older research on  
85 boar taint, and suggestions are provided on aspects that are worth further investigation. Some technologies  
86 have already been tested for the detection of boar taint compounds, but they require further development.  
87 Others, such as odorant-binding proteins (OBPs), have found applications for odor detection in other  
88 domains and have hence been suggested as promising leads for boar taint detection. This review presents  
89 several biological materials that could have a leading edge in boar taint detection methods based on  
90 bioelectronic noses. Finally, this review investigates potential challenges encountered during on-line boar  
91 taint detection, by considering the range of elements involved at various levels, which could interfere with  
92 the correct detection of tainted carcasses.

93 All methods described in this review are summarized in Table 1 and presented according to their  
94 appearance in the text. It is to be noted that the type of information given in the method sensitivity column  
95 in the table, may vary from one article to another. Further, limits of detection and quantification are given  
96 when they are available. A careful interpretation of these limits must be performed, as the way in which  
97 they were determined varies. For example, some articles determined these for standards diluted in solvent,  
98 some in fat and finally others in melted fat. When these limits are not available, indications as to whether  
99 measurements could be performed at or below the cut-off limits are given. These commonly accepted  
100 thresholds generally range from 0.2 to 0.25  $\mu\text{g g}^{-1}$  of fat for skatole and 0.5 to 1.0  $\mu\text{g g}^{-1}$  of fat for  
101 androstenone (Bonneau, 1998). However, the exact threshold values may vary between studies and are  
102 hence given in the sensitivity column.

## 103 **2. Present boar taint detection methods in slaughterhouses**

104 Boar taint detection at the slaughterhouse is performed in two different environments, either at-line or on-  
105 line. At-line detection is performed in the slaughterhouse but not on the slaughter line, while on-line  
106 detection refers to measurements performed directly on the slaughter line (Font-i-Furnols et al., 2020;  
107 Lundström et al., 2009). Both detection environments have advantages and disadvantages.

108 On-line detection does not require fat sampling, and the carcass can hence be directly excluded from the  
109 slaughtering line, if tainted. However, on-line detection must not hamper the speed at which carcasses are  
110 slaughtered. The slaughtering speed is approximately 360 carcasses/h in medium-sized slaughterhouses,  
111 but can reach up to 600 carcasses/h in large slaughterhouses (Borggaard et al., 2017; Font-i-Furnols et al.,  
112 2020). One must remember that boar taint evaluation can be performed exclusively on entire and  
113 immunocastrated males, which account for only 39% of the total male population (De Briyne et al., 2016).  
114 A slaughterhouse must however be prepared in the eventual case of long slaughtering sequences made up  
115 solely of entire and immunocastrated males. In this case, if a single measuring device is used, it must be

116 capable of operating at such speeds, i.e., less than 10 s. More than one measuring device should be used in  
117 alternation, if the detection speed is lower than the slaughtering speed.

118 On the other hand, at-line detection does not necessarily need to function at slaughtering speed, but  
119 requires fat sampling, which could result in the need of an additional operator in some slaughterhouses  
120 and hence generate extra costs. Additionally, a carcass traceability system must be implemented to  
121 associate the measurement performed on a sample to the corresponding carcass.

122 Currently, two methods are widely used for boar taint detection in slaughterhouses. The first consists of a  
123 sensory evaluation performed by a trained expert after heating fat from the neck region to release the low-  
124 volatility boar taint compounds (skatole and androstenone have a vapor pressure of  $7.3 \times 10^{-4}$  kPa and  $1.3$   
125  $\times 10^{-6}$  kPa at 25 °C, respectively). Selection and training of assessors for boar taint detection in  
126 slaughterhouses is a well-established practice, given that inter- (and intra-) individual variability in  
127 olfactory acuity exists for androstenone and skatole (Trautmann et al., 2014). Individuals possess varying  
128 perception thresholds and some even present anosmia, i.e., a lack of odor perception, for androstenone.  
129 Hence, assessors are selected according to their olfaction sensitivity for androstenone and skatole. They  
130 follow a well-structured training program that consists of training with skatole and androstenone  
131 standards. Further, they practice with fat samples in the laboratory and, finally, practice on-line to get  
132 accustomed to the working conditions. Once the training is completed, the assessor can perform the  
133 evaluation on-line, where fat is heated and smelled right off the carcass or at-line, on a fat sample (Font-i-  
134 Furnols et al., 2020). Through the use of this technique, it is assumed that if trained assessors cannot  
135 detect boar taint compounds in fat samples under controlled conditions, it is unlikely that an untrained  
136 consumer will detect the taint in less controlled conditions (Trautmann, 2016).

137 Sensory evaluation by trained experts is preferred by many slaughterhouses (compared to the colorimetric  
138 assay described later), as it does not require substantial initial investment. Apart from selecting and  
139 training the assessor, the main cost is the salary of the assessor. Additionally, sensory evaluation of the  
140 taint is the only method that assesses boar taint as a whole. It has been found that 33% of the variation in  
141 boar taint is due to skatole only, 36% to androstenone only, and 50% due to the combination of the two  
142 molecules (Hansson et al., 1980). Perceiving all volatile organic compounds (VOCs) responsible for the  
143 taint allows for not only the perception of the odor of each of these, but also for the perception of the odor  
144 resulting from potential synergistic effects.

145 The second method is a colorimetric assay (Mortensen & Sørensen, 1984) often used at-line in Danish  
146 slaughterhouses. This method analyzes only indolic compounds, and provides results as “skatole  
147 equivalents.” The contribution of other molecules such as androstenone is not accounted for, resulting in a  
148 partially complete result, used as a basis for classification of carcasses. This method is already  
149 implemented in slaughterhouses and is hence cost-effective (lower than 1.30 euro/ analysis). However, a  
150 high initial investment must be considered (Font-i-Furnols et al., 2020), which may partly explain the  
151 decision of many slaughterhouses to currently use sensory evaluation.

### 152 **3. Past research in boar taint detection**

#### 153 **3.1. Insect behavior-based sensing**

154 Classical Pavlovian conditioning has been used in several species of insects. This learning procedure is  
155 defined as the association of a conditioned stimulus with an unconditioned reward, to analyze novel  
156 chemical cues (Wäckers et al., 2011). Pavlovian conditioning has been used for a variety of applications in  
157 different insects.

158 Parasitic species, such as the wasp *Microplitis croceipes* (Hymenoptera: Braconidae), have been used  
159 extensively for insect-learning experiments. *M. croceipes* have been shown to memorize and react to a  
160 broad range of molecules, including some that are not found in their natural environment (Olson et al.,  
161 2003). Additionally, these wasps have been shown to differentiate conditioned odors of similar molecules,  
162 based on molecular chain length and the position of functional groups (Meiners et al., 2002). Further, *M.*  
163 *croceipes* show specific conditionable behaviors depending on the resource: seeking behavior for food  
164 resource and coiling behavior for host resource (Olson et al., 2003).

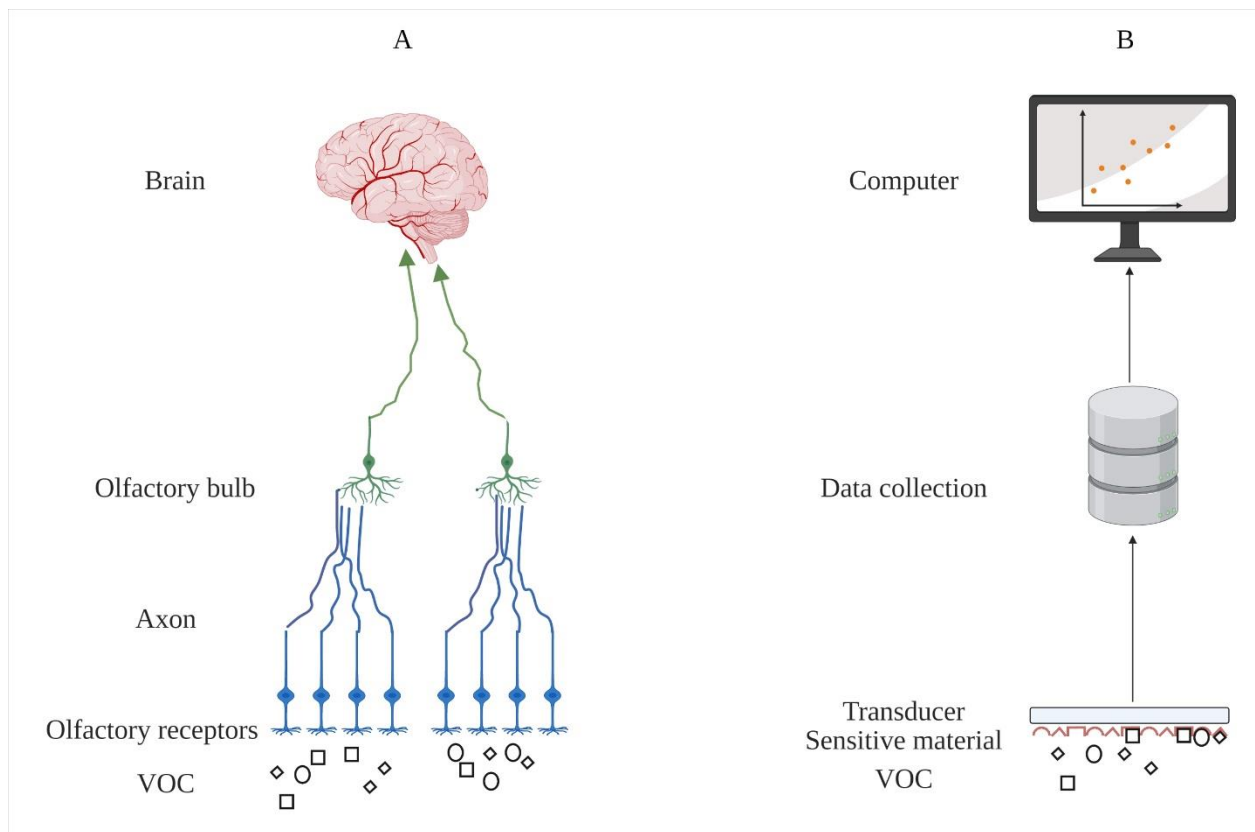
165 These properties have led to the use of *M. croceipes* in a variety of applications, such as the detection of  
166 methyl benzoate, the major VOC of cocaine (Olson & Rains, 2014); and indole, skatole, and  
167 androstenone, the major molecules responsible for boar taint (Olson et al., 2012; Wäckers et al., 2011).  
168 Both tests were performed using a “wasp hound.” This device is a cylinder equipped with a camera at the  
169 top to record the movements of the wasps, and a small hole at the bottom to allow the entrance of VOCs  
170 for possible detection (Wäckers et al., 2011). If no recognized VOC is present, the wasps move freely. If a  
171 VOC is present, they will tend to aggregate in front of the opening, and this will be recorded by a camera  
172 (Schott et al., 2014).

173 Wäckers et al. (2011) found that, after conditioning, the wasps were able to recognize indole, skatole, and  
174 androstenone separately, as well as in a 1:1:1 mixture. The concentrations perceived by the wasps in this  
175 experiment were within the range of the compounds found in boar fat (0.1 to 0.4  $\mu\text{g g}^{-1}$ ). Olson et al.  
176 (2012) performed further research into boar taint detection by *M. croceipes*. They found that, as for other  
177 insects, the olfactory learning of this species is concentration dependent. Additionally, the direction of  
178 concentration generalization (i.e., learning a concentration and being able to report others) was found to be  
179 odor-dependent. Finally, it was shown that these parasitic wasps can report low, medium, and high  
180 concentrations of the above-mentioned three molecules in boar fat at 25 °C (Olson et al., 2012).

181 No recent research has been conducted on this sensing method, and many aspects must still be accounted  
182 for, before considering such a method for use in slaughterhouses. First, the wasps’ minimum detection  
183 thresholds for these molecules should be determined (Olson et al., 2012). Additionally, the wasps may  
184 react to natural unconditioned stimuli (Schott et al., 2014), which would give false positives. This could be  
185 a potential drawback. More importantly, a facility must be created at the slaughterhouse, and personnel  
186 must be mobilized to rear, keep, and train the insects before use (Haugen et al., 2012). Ensuring that the  
187 wasps are confined to the rearing chambers and wasp hound is primordial, as having freed wasps in the  
188 slaughterhouse could present some risks for the operators and additionally bring up issues in terms of food  
189 hygiene. Animal needs and habits (e. g., eating and resting) also need to be addressed before considering  
190 the use of wasps as biosensors. Although such a method is considered low-investment (500 to 3000 euros)  
191 (Haugen et al., 2012), operational cost should be well-studied to determine whether analysis falls below  
192 the estimated 1.30 euro/analysis, mentioned earlier.

### 193 3.2. Electronic noses (e-noses)

194 The e-nose is an artificial device composed of an array of sensors, whose purpose is to imitate the human  
195 nose, both in terms of functioning and results (Haugen & Kvaal, 1998). In the human nose, odorants bind  
196 to receptors on olfactory neurons (Figure 1a). This creates an action potential in the receptor and induces  
197 depolarization of the axon. Once at the axon terminal, this signal is passed along to mitral cells, which  
198 make up the olfactory bulb, along with axon terminals and several glomeruli. The olfactory bulb is the  
199 region where the signal is transformed into an electric signal and transferred to the brain, allowing it to  
200 process the information (Zhang et al., 2018).



201  
 202 **Figure 1.** Comparison of odor perception by the human nose and an e-nose. (A) Human olfaction (B) VOC detection  
 203 by e-nose.

204 Similarly, for e-noses, when gases (in this case, VOCs) reach the surface of a sensor (i.e., the sensitive  
 205 layer), a change occurs in the surface's properties (e.g. conductivity change and absorbance change). This  
 206 change is transformed into an electrical signal by the transducer (Figure 1b). These signals are then  
 207 gathered and processed by a computer, where a pattern is identified and a response is delivered to the user  
 208 (Wojnowski et al., 2017). In the case of carcass sorting, the response should simply be whether the carcass  
 209 is considered tainted or not, i.e., above or below a defined threshold (for example, the threshold described  
 210 for skatole and androstenone in the previous section).

211 Sensors used in the e-nose operate according to different principles. The conductivity variations of the  
 212 sensitive layer are monitored for some sensors. These include metal–oxide–semiconductor (MOS)  
 213 sensors, metal–oxide–semiconductor field-effect transistor (MOSFET) sensors, conducting polymer  
 214 composites, and intrinsically conducting polymers (CPs). Electrochemical (e.g., potentiometric sensors),  
 215 optical (e.g., absorbance-based sensors), and piezoelectric properties (e.g., quartz crystal microbalances)  
 216 are monitored for other sensors (Guo et al., 2015; Loutfi et al., 2015; Wojnowski et al., 2017). E-noses can  
 217 operate with one type or a combination of various gas sensors. Studies on boar taint detection using such  
 218 sensors are discussed hereafter and are summarized in Table 1.

219 The first sensor arrays used non-specific gas sensors, i.e., they detect and respond to a variety of  
 220 molecules present in the gas phase. The molecules modify the sensor's property (mentioned above), the  
 221 signals recorded by each sensor in the array are then combined, and complex data processing allows the  
 222 classification and recognition of odors (Peris & Escuder-Gilabert, 2016).

223 Berdague and Talou (1993) tested a prototype MOS array system on heated fat samples originating from  
224 entire and castrated male pigs, as well as from female pigs. Bourrounet et al. (1995) developed a system  
225 based on the use of five commercial MOS sensors to analyze the headspace of heated (150 °C, 30 s) entire  
226 male pig fat and classify the samples according to their androstenone content (previously determined by  
227 enzyme-linked immunosorbent assay, ELISA). Although a classification accuracy of 84.2% was reported,  
228 one of the main conclusions of this work was that the device had to be miniaturized before further use  
229 (Bourrounet et al., 1995). Annor-Frempong et al. (1998) used an e-nose composed of a 12-conducting-  
230 polymer-type (polypyrrole) sensor array to discriminate lipid and fat samples with varying amounts of  
231 skatole and androstenone (at 22-23 °C). A correlation coefficient of 0.78 was found between the results  
232 obtained with this array and the assessment performed by a sensory panel (Annor-Frempong et al., 1998).  
233 Di Natale et al. (2003) used a quartz crystal microbalance coated with various types of metalloporphyrins  
234 (a type of piezoelectric sensor) to measure the presence of androstenone in the headspace of heated (35  
235 °C, 30 min) pork fat. The interaction occurring at the surface of the sensor was specific, through the  
236 interaction of androstenone with porphyrin rings, and non-specific through cavity interactions with alkylic  
237 chains. This research led to the finding that the correlation coefficient between the added androstenone  
238 concentration in fat and the values determined with the sensor array was 0.98. This method is too time-  
239 consuming for wide-scale applications in slaughterhouses and requires expensive materials (quartz  
240 microbalances). Additionally, it was found that the sensor's limit of detection of androstenone was below  
241 the human olfaction threshold of 0.5 µg g<sup>-1</sup>. Such a result is helpful in detecting carcasses for which boar  
242 taint is primarily caused by androstenone. Tainted carcasses presenting high skatole and low androstenone  
243 concentrations cannot be classified as tainted with the exclusive use of this androstenone-sensitive sensor.  
244 Additionally, it was found that skatole is the major compound responsible for consumer dissatisfaction  
245 with smelling tainted carcasses (Bonneau et al., 2000). Therefore, skatole-sensitive sensors should be  
246 developed to complement the information obtained with the androstenone-sensitive sensors.

247 Vestergaard et al. (2006) evaluated the use of an ion mobility spectrometry-based electronic nose (MGD-1  
248 system) for boar taint analysis. It comprised of headspace analysis of samples incubated at 40 °C for 10  
249 min. This equipment was proven effective in sorting fat samples in terms of high and low levels of skatole  
250 and androstenone (after multivariate analyses). The author of the study reminds, however, that even if a  
251 high correlation is found between the androstenone content and the results obtained with the e-nose, an  
252 on-line sampling and detection device must still be developed, raw data pre-processing must be  
253 automated, and the subsequent multivariate methods must be optimized.

254 Although many e-noses are already available in the market (with prices ranging from 10000 to 40000  
255 euros) (Haugen et al., 2012), none of the commercially available e-noses, nor the prototypes presented in  
256 the aforementioned studies appear to have been tested for on-line/at-line slaughterhouse applications. On-  
257 line/at-line testing should be undertaken because good correlations were observed between the results  
258 obtained with the sensors and the actual taint, which was either evaluated by a sensory panel, or by  
259 determining the fat's skatole and androstenone content.

260 Promising new sensor materials that could be further considered for boar taint detection, the challenges  
261 with them, and how to tackle these challenges, is presented later in this review (sections 5 and 6).

### 262 3.3. Gas chromatography–mass spectrometry (GC-MS) based methods

263 Mass spectrometry (MS) is a well-known technology that has been widely used for its reproducibility,  
264 stability, and sensitivity. Hence, MS-based techniques have been the focus of many research studies on  
265 boar taint detection.

266 MS has been used in combination with gas chromatography (GC-MS) to analyze VOC profiles found in  
267 the headspace of heated fat. As boar taint compounds such as skatole and androstenone are highly  
268 hydrophobic and hard to volatilize, fat must be heated at high temperatures to detect these compounds in  
269 its headspace.

270 Sørensen & Engelsen (2014), have used a dynamic headspace sampling–gas chromatography–mass  
271 spectrometry (DHS-GC-MS) technique (fat incubated at 150 °C for 12 min) for rapid screening for the  
272 presence of indole, skatole, and androstenone in pig adipose tissue. Target ions of  $m/z$  117 (indole), 130  
273 (skatole), and 257 and 272 (androstenone) were monitored to allow proper quantification of these  
274 molecules. Limits of detection of 0.082  $\mu\text{g g}^{-1}$ , 0.097  $\mu\text{g g}^{-1}$ , and 0.623  $\mu\text{g g}^{-1}$ ; and prediction errors of  
275 0.096  $\mu\text{g g}^{-1}$ , 0.094  $\mu\text{g g}^{-1}$ , and 0.331  $\mu\text{g g}^{-1}$  were obtained for indole, skatole, and androstenone,  
276 respectively. Hence, this method should be adequately sensitive for boar taint detection, if the commonly  
277 accepted thresholds of 0.2  $\mu\text{g g}^{-1}$  for skatole and indole, and 1  $\mu\text{g g}^{-1}$  for androstenone are used. However,  
278 effort to reduce the time of analysis is still needed, as the first result was issued in 24 min and the  
279 following in 6 min, i.e., a maximum of ten analyses were performed per hour, compared to several  
280 hundred carcasses analyzed with the current human nose technique (Sørensen & Engelsen, 2014).  
281 Verplanken et al. (2016) used a solid phase microextraction–gas chromatography–mass spectrometry  
282 (SPME-GC-MS) technique for boar taint detection. By optimizing fat heating, the extraction time was  
283 drastically reduced to 45 s (heating at 400 °C), allowing the total run time for one sample to be 3.5 min,  
284 when coupled to an analysis by portable GC-MS. Even though the portable GC-MS method showed good  
285 validation results, this method lacked sensitivity. It was unable to detect boar taint compounds at threshold  
286 levels, leading to possible false results (Verplanken et al., 2016).

287 Finally, these methods are known to be expensive, representing a high initial investment ranging from  
288 100000 euros to 600000 euros, depending on the resolution of the MS (Haugen et al., 2012). However,  
289 providing an exact running cost is difficult, because many costs, such as the technician's salary, cost of  
290 solvents and gases used, and cost of maintenance add up to the depreciation of the initial investment.

291 Additionally, the analysis time remains very important for methods in which molecules are separated by  
292 GC prior to MS-detection. Recent studies have therefore turned towards the use of MS without upstream  
293 GC separation.

## 294 **4. Recent advances in boar taint detection**

### 295 **4.1. MS-based methods**

296 Verplanken et al. (2017) tested rapid evaporative ionization mass spectrometry (REIMS) for the rapid  
297 detection of boar taint. REIMS is based on the formation of gaseous molecular ions by thermal  
298 evaporation of biological tissues, with the help of an electrosurgical electrode as an ion source. These ions  
299 are carried by a Venturi air jet pump to an MS for detection and establishment of a mass spectrum  
300 (Schäfer et al., 2009). Compared to the aforementioned techniques, REIMS has the advantage of  
301 providing a heating source and sampler of molecular ions in a single, hand-held tool. Additionally, this  
302 method does not require any sampling before analysis. These criteria make this method easy to be used by  
303 the operator and could be used on-line in slaughterhouses (the MS part of the device is in a separate room  
304 but is connected to the sampling tool by a long tubing). In their work, Verplanken et al. (2017) sampled  
305 neck fat from 50 sow, 50 tainted boar, and 50 untainted boar carcasses to perform in-lab tests. The mass  
306 spectra analyzed are hence mainly composed of ions produced by ionization of lipids. Chemometrics



307 (orthogonal partial least-square discriminant analysis models in this case) was then applied to the obtained  
308 mass spectra. The model provided a highly accurate classification (99% correct classification) and  
309 discrimination between the samples seem to have originated mainly from differences in the fatty acid and  
310 phospholipid region of the mass spectra. Additionally, although high initial investments are expected, the  
311 cost of analysis in this method was estimated to be lower than 1.0 euro/analysis, and the analysis speed  
312 was 3-5 s/sample (Verplanken et al., 2017).

313 Although fast analysis was achieved, cleaning of the equipment must also be considered as it slows down  
314 the hourly analysis speed. Verplanken et al. (2017) cleaned the equipment after every 10 samples. Thus, if  
315 an analysis time of 5 s/sample is considered, the cleaning procedure should not last longer than 52 s, for  
316 this method to be used in medium-sized slaughterhouses (350 carcasses/h). Hemeryck et al. (2019)  
317 developed a statistical model on 1097 fat samples in the laboratory and later tested this in a  
318 slaughterhouse. The analysis took less than 10 s/sample and the study concluded that this approach  
319 allowed for correct classification of the carcasses (no indication of the classification accuracy was given).  
320 Further validation is needed about the potential use of REIMS for slaughterhouse applications, as the  
321 effectiveness of this method in more heterogenous conditions (different carcasses in different  
322 slaughterhouses) is not guaranteed. Several factors such as genetics, diets, and rearing conditions affect  
323 the molecular profiles analyzed in untargeted approaches (Font-i-Furnols et al., 2020).

324 Another MS-based detection method that has recently been used for at-line boar taint detection is laser  
325 diode thermal desorption–tandem mass spectrometry (LDTD-MS/MS). In this method, a small amount of  
326 liquid sample is inserted into a well plate and left to dry before an infrared laser diode heats up the bottom  
327 of the plate, allowing complete sublimation of the sample. The vaporized sample then undergoes  
328 atmospheric pressure chemical ionization (APCI), an ionization method that does not break down the  
329 molecules and produces monocharged ions. These ions are then detected by tandem mass spectrometry  
330 (Bynum et al., 2014). In the case of boar taint detection, a liquid-liquid extraction step must be performed  
331 before injection into the well plate. This step allows a separation of indole, skatole, androstenone, and  
332 other molecules with similar characteristics from other more polar molecules. This solvent, containing the  
333 molecules of interest, is injected into the well plate.

334 Two teams have been working on LDTD-MS/MS boar taint detection during the same period of time: the  
335 Danish Technological Institute (DTI) (Borggaard et al., 2017) and Phytronix Technologies, Inc., in  
336 collaboration with Shimadzu Corporation (Auger et al., 2018). Both developed similar methods and  
337 analyzed similar results, except that Borggaard et al. (2017) quantified skatole and androstenone only,  
338 while Auger et al. (2018) quantified skatole, androstenone, and indole.

339 Both LDTD-MS/MS methods achieved good validation criteria. The correlation coefficients for their  
340 calibration curves were greater than 0.99, and the limits of quantification were lower than the commonly  
341 accepted thresholds. Although  $0.2 \mu\text{g g}^{-1}$  for indole and skatole, and  $1 \mu\text{g g}^{-1}$  for androstenone are  
342 commonly accepted thresholds, the exact sorting threshold for androstenone is still under investigation by  
343 the DTI, and should range between 0.5 to  $2 \mu\text{g g}^{-1}$  androstenone in fat (Borggaard et al., 2017; Støier,  
344 2019). Additionally, both LDTD-MS/MS methods were precise, with a maximum relative coefficient of  
345 variation (% CV) of 5% in the work by Borggaard et al. (2017) and 15% in the work by Auger et al.  
346 (2018). As stated by Font-i-Furnols et al. (2020), sample preparation in the second study needs further  
347 optimization, which might be the reason behind the higher % CV.

348 Although sample preparation before injection into the well plate lasts several minutes, the LDTD-MS/MS  
349 analysis in itself takes less than 10 seconds per sample to accurately quantify boar taint compounds. Using

350 such method in slaughterhouses is hence feasible provided that a carcass traceability system is put in  
351 place. Both teams have applied for a patent for boar taint detection by LDTD-MS/MS (WO2016139291  
352 for the DTI application and WO2017147709 for the application by Phytronix Technologies, Inc.).

353 The studies performed by the DTI appear to be more advanced. An economical study concluded that  
354 although this method requires high initial investment, the estimated overall price of analysis is 0.70  
355 euro/carcass (Borggaard et al., 2017). Additionally, the method has also been accredited by the Danish  
356 Accreditation Fund (DANAK) and is now being tested in a Danish slaughterhouse with a fully automated  
357 system, from fat sampling to detection of the compounds (Støier, 2019).

358 Given the recent advances in LDTD-MS/MS, it appears to be promising and may soon replace the  
359 colorimetric method currently used in Danish slaughterhouses (Font-i-Furnols et al., 2020).

## 360 4.2. Raman spectroscopy-based methods

361 In recent years, Raman spectroscopy has been efficiently used in the food industry for protein and lipid  
362 analysis. Raman spectroscopy is based on the Raman effect, which is a process by which a portion of  
363 photons are scattered from a sample irradiated by a laser beam. An inelastic collision occurs as a result,  
364 thus changing the vibrational or rotational energy of the molecules. The scattered radiation is  
365 characterized by a different wavelength. A Raman spectrum can be seen as a “fingerprint” of the  
366 scattering material, thus giving quantitative and qualitative information on the irradiated sample (Yaseen  
367 et al., 2017). Raman spectra are influenced by the composition of fatty acids in lipids, as well as by their  
368 degree of saturation (Herrero, 2008). Recent studies have shown a correlation between the variability in  
369 the fatty acid composition of boars and varying levels of indole, skatole, and androstenone. Mörlein and  
370 Tholen (2014), found that the concentrations of polyunsaturated fatty acids were significantly higher in  
371 boars with low indole, skatole, and androstenone levels, as compared to highly tainted boars. Liu et al.  
372 (2016) used a portable Raman device to analyze and classify fat tissues with varying levels of boar taint  
373 compounds. The fat was not diluted with a solution but was thawed and used directly for analysis in this  
374 experiment. After selecting specific ranges of signals from the spectra and analyzing the results by partial  
375 least squares discriminant analysis (PLS-DA), a classification accuracy of 81% was obtained. Although  
376 such a result is encouraging and implies that the fatty acid composition of boar fat could be used as a  
377 proxy to detect tainted carcasses, the accuracy of this method should be verified in slaughterhouses. The  
378 pigs being slaughtered may vary in terms of breed and diets, which could have repercussions on the  
379 accuracy of the proposed model (Font-i-Furnols et al., 2020).

380 Sørensen et al. (2015) also used Raman spectroscopy for boar taint analysis. In contrast to the above-  
381 mentioned study, which used normal Raman scattering to detect variations in fatty acid composition,  
382 Sørensen et al. (2015) used surface-enhanced Raman scattering (SERS) to directly quantify skatole and  
383 androstenone. SERS increases the method’s sensitivity by several orders of magnitude and should allow  
384 the quantification of molecules, such as skatole and androstenone, present at low concentrations in the  
385 matrix. Low limits of detection were found for skatole and androstenone in solution ( $2.1 \times 10^{-11}$  M and  $1.8$   
386  $\times 10^{-10}$  M, respectively). However, high prediction errors were obtained when quantifying skatole and  
387 androstenone in fat samples ( $0.17 \mu\text{g g}^{-1}$  and  $1.5 \mu\text{g g}^{-1}$ , respectively).

388 Although high prediction errors have been found in this work, further optimization of such techniques  
389 should be encouraged. Raman spectroscopy has potential on-line applications because of its relatively low  
390 investment cost (20000 to 50000 euros) (CBRNE Tech Index, 2018), no need for sampling (portable hand-  
391 held-tool), and having multiple uses (also true for LDTD-MS/MS and REIMS). It not only detects tainted  
392 carcasses, but can also provide information on other aspects of meat quality (Font-i-Furnols et al., 2020).

### 393 4.3. Specific sensors based on the intrinsic properties of target molecules

394 Hart et al. (2016) filed for a patent for a new electrochemical sensor system capable of detecting and  
395 quantifying boar taint. This sensor system is composed of two parts, both based on the intrinsic  
396 (reduction-oxidation) properties of the target molecules (i.e., androstenone and skatole), and detected by  
397 means of carbon electrodes deposited by screen-printing. Skatole is detected based on its electrochemical  
398 behavior using cyclic voltammetry (direction oxidation at the surface of the electrode). The enzymatic  
399 activity of androstenone is analyzed using an enzyme electrode where the reduction of androstenone to  
400 androstanol occurs in the presence of the enzyme 3 $\alpha$ -hydroxysteroid dehydrogenase, NADPH, and  
401 Meldola's blue as a reduction mediator (Hart et al., 2016).

402 The efficiency of this new sensor system was tested by Westmacott et al. (2020) and compared to results  
403 obtained by gas chromatography for both molecules. Good correlation coefficients ( $R^2=0.801$  for skatole  
404 and  $R^2=0.932$  for androstenone), substantial recoveries (114.5% for skatole and 95.9% for androstenone),  
405 and a relatively fast analysis (within 60 s) was obtained.

406 This technology presents many favorable aspects, beyond results in preliminary tests. It is considered very  
407 easy to produce on a large scale and at low cost (Westmacott et al., 2020). Carbon is a cheap material, and  
408 screen-printing is a reliable technology for mass production of low-cost disposable sensors. As these  
409 sensors are disposable, any cross-contamination is avoided. Lastly, this technology can, in theory, be  
410 easily used for on-line measurements with an automated or manual portable device (Font-i-Furnols et al.,  
411 2020). The feasibility of on-line detection must be tested before considering mass production and use in  
412 slaughterhouses.

## 413 **5. Biosensors – a path to be further investigated for boar taint** 414 **detection**

415 This section will discuss biological materials that have not yet been used for boar taint detection in meat  
416 samples; however, they are worth being investigated further for their affinity towards molecules  
417 responsible for boar taint (e.g., skatole), or they have shown encouraging results for the detection of these  
418 molecules in other applications. Hence, these biological materials could be used to develop biosensors.

419 Biosensors are “measuring devices that trace chemical compounds, organisms, or physical measurands by  
420 spatially and functionally combining a biological component with a physical or chemical transducer”  
421 (Paczkowski et al., 2011). The definition of a “biological component” is very vast, and it could be an  
422 enzyme, antibody, organelle, cell, organ, or complete organism (the last one has been explained in section  
423 3.1. “Insect behavior-based sensing”). The transducer simply converts the response occurring after the  
424 reaction of the bio-component and analyte into a measurable output (Paczkowski et al., 2011).

425 Biosensors are often based on the use of specific receptors or proteins of the sensory system, which are  
426 coupled to electronic transducers. These are often referred to as bioelectronic noses.

### 427 5.1. OR-based bioelectronic nose

428 These bioelectronic noses are based on the use of olfactory receptor (OR) proteins, or cells which express  
429 olfactory receptors on their membrane. ORs act as odorant-recognition elements and are combined with  
430 transducers, which allows the conversion of the detected biological signal into an electrical signal  
431 processable by a computer (Zhang et al., 2018).

432 In contrast to chemical sensors, bioelectronic noses based on the use of ORs benefit from the “naturally  
433 optimized molecular recognition and sensitivity of the ORs” (Manai et al., 2017). Their sensitivity is also  
434 greater to that of gas-sensor array systems. Sensitivity up to the femtomolar can be achieved for odorants  
435 found in liquid conditions and up to the parts per trillion for odorants in gaseous conditions (Manai et al.,  
436 2017; Zhang et al., 2018). The downside of the use of ORs is that they must remain in hydrophobic  
437 conditions to ensure their functionality (Guo et al., 2015; Manai et al., 2017), which is challenging for  
438 practical applications.

439 Keller et al. (2007) investigated the differences in sensory perception from one human to another. To  
440 perform this, they focused on androstenone, since the perception of steroids varies greatly (i.e., the  
441 perception of androstenone varies from urine smell to floral smell from one person to another). To  
442 determine which OR was stimulated in the presence of androstenone, a luciferase assay was performed.  
443 The OR7D4 olfactory receptor appeared not only highly stimulated by androstenone, but was also very  
444 specific to it. In a second test where the response of OR7D4 was tested in the presence of 66 odors, the  
445 receptor responded only to androstenone and androstadienone (Keller et al., 2007). This finding agrees  
446 with the absence of differentiation of these two molecules during sensory assessments made by panelists  
447 in similar studies (Brooks & Pearson, 1989).

448 Based on the use of OR7D4, Guo et al. (2015) developed a bioelectronic nose in which these receptors  
449 were anchored to a gold electrode to ensure signal transmission, and square wave voltammetry was used  
450 to monitor the response of the electrode to varying concentrations of androstenone in the solution. The  
451 limit of detection of  $10^{-14}$  M seen in this study is far below the accepted threshold value for androstenone  
452 and shows the potential of OR7D4 for the development of bioelectronic noses for androstenone detection.  
453 Developing systems with ORs specific to several boar taint molecules should increase the strength of  
454 carcass classification in slaughterhouses. Thus, OR-based bioelectronic noses should be investigated  
455 further with the other molecules responsible for boar taint: skatole and indole.

456 These two molecules have been identified as oviposition attractants for the southern house mosquito,  
457 *Culex quinquefasciatus* (Diptera: Culicidae), which is known to be a pathogen vector (Du & Millar, 1999).  
458 An understanding of *C. quinquefasciatus* olfactory receptors (CquiORs) involved in the perception of such  
459 molecules appears to be an important step in the improvement of “attract-and-kill” strategies that use  
460 oviposition attractants. CquiOR2 was found to be 10 to 70 times more selective for indole, as compared to  
461 other indole derivatives. Further, CquiOR10 was found to be very sensitive and narrowly tuned to skatole  
462 (Hughes et al., 2010; Pelletier, Hughes, et al., 2010). Olfactory receptors of *Anopheles gambiae* (Diptera:  
463 Culicidae) have also been investigated. *A. gambiae* is the major vector of malaria in sub-Saharan  
464 countries. This insect locates human hosts through olfaction, but not much is known about its molecular  
465 recognition. Carey et al. (2010) investigated the response of 50 AgamORs (*A. gambiae* olfactory  
466 receptors) to 110 odorants. It appears that AgamOR2 is narrowly tuned and strongly activated by indole,  
467 which is found in human breath and sweat, at up to 30% in the headspace of the latter (Carey et al., 2010)

468 As Guo et al. (2015) performed studies with OR7D4 for the detection of androstenone, bioelectronic noses  
469 could be tested with CquiOR2, CquiOR10, from *C. quinquefasciatus*, and AgamOR2 from *A. gambiae*,  
470 for detection and quantification of skatole and indole.

## 471 5.2. OBP-based bioelectronic nose

472 Odorant-binding proteins (OBPs) refer to a class of proteins found in vertebrates and insects. Although  
473 their structures are very different in these two organisms, their function remains similar. OBPs are  
474 responsible for the initial step of molecule recognition and odor perception and are found in high

475 concentrations in the nasal mucus of vertebrates and lymph of the insects' sensilla (Dimitratos et al., 2019;  
476 Pelosi et al., 2014). The OBPs of both vertebrates and insects possess thermal stability. They can  
477 withstand high temperatures, which is interesting, because boar fat must be heated at very high  
478 temperatures to volatilize skatole and androstenone. If denatured as a result of overheating, restoring the  
479 OBPs to their initial condition will reverse the damage, which is economically attractive as it increases the  
480 number of detections that can be potentially performed by an OBP-based sensor (Pelosi et al., 2014).

481 Being thermally stable makes OBPs ideal for the development of bioelectronic noses. In such sensors, the  
482 binding of the molecule of interest to the protein can have several impacts, such as modification of  
483 protein's mass and refractive index. This allows OBPs to be used with various transducers (Pelosi et al.,  
484 2014). OBP-based bioelectronic noses for boar taint detection could be developed with the use of the  
485 appropriate OBP.

486 Dimitratos et al. (2019) have worked on the development of biosensors for the rapid detection of water  
487 contamination by harmful coliform bacteria. To achieve this, the research team proposed the development  
488 of rapid tests to detect and quantify indole, a characteristic metabolite. The OBP, AgamOBP1, from the  
489 insect *A. gambiae*, was used as the detector. The results of the two tests, based on competitive binding for  
490 AgamOBP1's binding pocket, appeared to be highly specific and sensitive to indole, with a limit of  
491 detection in water lower than 100 nM (Dimitratos et al., 2019). OBPs from other species could also be  
492 used for sensor applications. Pelletier, Guidolin, et al. (2010) found that an OBP from *C. quinquefasciatus*,  
493 CquiOBP1 was involved in the reception of oviposition attractants such as mosquito oviposition  
494 pheromones, skatole, and indole. As for OR-based bioelectronics noses, considering the variability in  
495 sensors and their specificity to various VOCs of interest, an interesting outcome would be to combine  
496 these sensors into a common bioelectronic nose.

### 497 5.3. Aptamer-based biosensors

498 Aptamers, often referred to as "chemical antibodies," are single-stranded DNA or RNA (ss-DNA or ss-  
499 RNA) oligonucleotides that are produced *in vitro* based on systematic evolution of ligands by exponential  
500 enrichment (SELEX). Aptamers may be used for a large variety of applications, and are able to detect a  
501 wide range of compounds, from metal ions to whole organisms (Jayan et al., 2020). These applications  
502 include clinical therapy (Ng & Adamis, 2006), drug delivery systems (Min et al., 2011), and aptasensors,  
503 i.e., a type of biosensor where the receptors are aptamers. Several types of aptasensors have been  
504 developed. These include electrochemical, mass-sensitive, and optical aptasensors (fluorescence-based  
505 and colorimetric-based).

506 Frimpong et al. (2017) investigated the feasibility of detecting skatole and androstenone with gold  
507 nanoparticle (AuNP) aptasensors. Based on capture SELEX, two aptamers with high affinity and  
508 selectivity for skatole and androstenone were selected and electrostatically absorbed to citrate-capped  
509 AuNPs. In an environment favorable for AuNP aggregation and in the absence of the molecules of  
510 interest, the aptamers prevent the aggregation of AuNPs, i.e., the aptamer-AuNP complexes are dispersed  
511 in the solution. When the molecules of interest are also present in the solution, the aptamers that have a  
512 stronger affinity for them tend to unbind from the AuNP surface, and bind to skatole and androstenone.  
513 Under saline conditions, the NPs aggregate, leading to an absorbance shift in the UV-VIS region from 524  
514 nm to 660 nm (a color change from pink to blue). Frimpong et al. (2017) reported a significant color  
515 change when AuNPs in saline conditions, were placed in contact with skatole and androstenone in  
516 aqueous solutions, with concentrations ranging from  $1.0 \times 10^{-13}$  M to  $1.0 \times 10^{-4}$  M. Additionally,  
517 absorbance measurements were also performed in the presence of only tryptophan or indole. In this case,  
518 no significant color change was reported, thus proving the specificity of the aptamer considered.

519 Although aptasensors seem to be a promising solution for boar taint detection, based on the specific  
520 detection of skatole and androstenone, more research must be performed to allow on-line use of such  
521 technology. First, research on the potential use of such aptamers for the detection of skatole and  
522 androstenone in the gaseous phase should be undertaken. Second, the speed of measurement must be  
523 optimized (currently 30 min for the incubation of aptamers and AuNPs before detection). Lastly, time  
524 consuming fat extraction would be avoided in the case of gaseous phase sampling, resulting in faster  
525 detection.

#### 526 5.4. Production cost of biosensors

527 In contrast to the methods described in section 4, the biosensors discussed in this section must either be  
528 developed further or tested for boar taint detection (tested with boar fat samples). It seems premature to  
529 provide an idea of investment or operational cost at this stage of development.

530 Several aspects must be considered in order to establish the investment cost of such sensors. The  
531 production of the biological component must be considered. This includes not only amplification, but also  
532 purification of the biological material. Second, the transducer's production must be considered. Limiting  
533 the costs of production appears to have been part of the analysis by Guo et al. (2015), when developing the  
534 sensors. Guo et al. (2015) used square wave voltammetry as the transduction technique, as it is considered  
535 more rapid, efficient, and low-cost, when compared to electrochemical impedance spectroscopy. In their  
536 work, Frimpong et al. (2017) mentioned the use of aptamers as they are cost-effective solutions.

537 The economic feasibility of such biosensors must be analyzed in greater depth before considering  
538 potential industrial use. Two economic scenarios must be considered: one for medium-sized  
539 slaughterhouses (approximately 360 carcasses/h) and another for large-sized slaughterhouses  
540 (approximately 600 carcasses/h). As is the case for many instrumental methods, the operational cost will  
541 decrease for bigger slaughterhouses. As mentioned earlier, each analysis should ideally cost less than 1.30  
542 euro. Whether disposable or non-disposable biosensors are created must also be considered as this could  
543 affect the final price of each analysis.

## 544 **6. Challenges and solutions for sensor-based detection in** 545 **slaughterhouses**

546 Although biosensors are promising new solutions for boar taint detection, they face many challenges when  
547 used in slaughterhouses. Some of these are specific to the environment in which boar taint is detected and  
548 others are general to any sensor. The environment referred to in this case is not only the slaughterhouse  
549 but also the fat's headspace in which the VOCs are detected.

### 550 6.1. Environment-specific noise challenges

551 The detection of boar taint by analysis of the fat's headspace can be strongly impacted by the large variety  
552 of VOCs present. These VOCs can impact the selectivity and sensitivity of the sensor used. Hence, the  
553 sensor should be robust against potential fouling. A better understanding of the VOCs found in the  
554 headspace, including their origin, is important to tackle such fouling.

555 As mentioned earlier, for skatole and androstenone to be detected, fat must be heated (Figure 2) at high  
556 temperatures. As a result, most of the VOCs found in the headspace of heated fat originate from the  
557 degradation of lipids (Figure 2b), more specifically the oxidation of fatty acids, starting at around 70 °C

558 (Ladikos & Lougovois, 1990). The compounds resulting from heating the fat include alcohols, aliphatic  
559 hydrocarbons, aldehydes, ketones, esters, carboxylic acids, aromatic compounds, and oxygenated cyclic  
560 compounds such as lactones and alkylfurans (Mottram, 1998).

561 Optimization of the extraction temperature and time is necessary, because lipid oxidation increases as  
562 temperature rises, and skatole and androstenone are difficult to volatilize. This should result in maximal  
563 skatole and androstenone concentrations in the headspace, with minimal lipid degradation products.

564 Other VOCs typically found in the headspace of heated meat originate from the Maillard reaction  
565 occurring between a reducing sugar and an amino acid (Figure 2c), as well as the reaction between the  
566 lipid-degradation products and the Maillard reaction products, which can result in several compounds  
567 (Imafidon & Spanier, 1994). Further information about the interaction between the Maillard reaction and  
568 lipid oxidation was provided by Zamora & Hidalgo (2011). Although these reactions are not as important  
569 as the lipid degradation, they still need to be considered, given the presence of collagen fibers and the  
570 hydrosoluble molecules found in water.

571 The slaughterhouse's VOCs background noise may also add to the difficulty of detecting boar taint  
572 (Figure 2e). To the best of our knowledge, numerous studies have been performed to analyze VOCs  
573 originating from swine operations, including Feilberg et al. (2010) and Schiffman et al. (2001). However,  
574 none have analyzed the ambient air in slaughterhouses as a source of background noise.

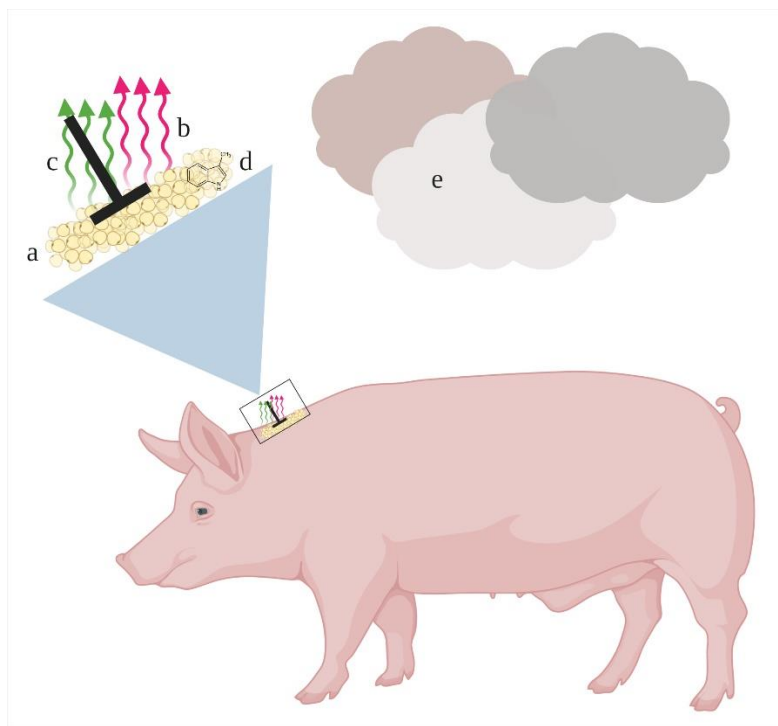
575 Schiffman et al. (2001) identified more than 300 volatile compounds (VOCs and other gases) in air  
576 samples from swine operations. These include molecules from a wide variety of classes, including acids,  
577 phenolic compounds, and aldehydes present at high concentrations, as well as nitrogen- and sulfur-  
578 containing VOCs. Most of these VOCs are derived from undigested proteins that decompose in manure  
579 (Hobbs et al., 2004). However, VOCs originating from manure are unlikely to contribute much to the  
580 VOC profile of slaughterhouses, as the pigs are washed and checked for cleanness at various stages,  
581 including prior to transportation from the farm and at the slaughterhouse before the scalding step (Food  
582 and Agriculture Organization of the United Nations, 1991).

583 Some of the VOCs found in the global environment of the slaughterhouse originate in part from the blood,  
584 as the steps performed before sorting of the carcass include evisceration and splitting of the carcass.  
585 Forbes et al. (2014) analyzed the effect of aging and storage conditions on human blood and reported that  
586 fresh blood presented a simple VOC profile, mainly including 2-heptanone, 4-heptanone, 2-octen-1-ol,  
587 and 1-octen-3-ol. 1-octen-3-ol makes up more than 95% of the profile. Some of the above-mentioned  
588 molecules could make up part of the slaughterhouse's "background noise," as domestic pigs and humans  
589 resemble each other in terms of organs and chemical composition of tissues (Paczkowski et al., 2014),  
590 Similarly, pig carcasses have been used widely in forensic science as an analogue to human cadavers. The  
591 studies in this field that analyzed early post-mortem intervals could provide an estimation of the VOC  
592 profile of carcasses in slaughterhouses. Armstrong et al. (2016), who analyzed early post-mortem intervals  
593 (0-72 h), found that the VOC profile of a pig carcass at 1 h post-mortem was composed of a variety of  
594 molecules, including sulfur-containing compounds, alcohols, and carboxylic acids. However, the most  
595 abundant class of compounds was esters, with molecules such as cis-3-hexenyl acetate, ethyl acetate, and  
596 methyl acetate.

597 The slaughterhouse's VOCs background noise probably has a stronger impact on on-line detection than on  
598 at-line detection, as the latter is performed in a laboratory where air quality can be more easily controlled  
599 (e.g., by filtering the incoming air). Whether these VOCs are found in the air of the slaughterhouse, and  
600 their extent, should be verified. Many factors, such as temperature, affect the decomposition rate of a

601 carcass (Dekeirsschieter et al., 2009). Hence the VOC profile originating from it may vary significantly  
602 within and between slaughterhouses.

603 As previously mentioned, the unpleasant smell of boar taint is perceived at an odor threshold of 0.2 to 0.25  
604  $\mu\text{g g}^{-1}$  fat for skatole and 0.5 to 1  $\mu\text{g g}^{-1}$  fat for androstenone. The maximum concentrations found in  
605 tainted fat are as high as 0.8  $\mu\text{g g}^{-1}$  for skatole and 5  $\mu\text{g g}^{-1}$  for androstenone (Fischer et al., 2011). The  
606 concentration levels at which these molecules are found in the fat's headspace could affect the sensitivity  
607 of both specific and non-specific methods. In case of on-line detection, there is limited time available for  
608 heating of the carcass and detection of the taint. Early heating of the fat on a larger surface could be a part  
609 of the solution to this problem. As addressed previously, these molecules are very hard to volatilize; thus,  
610 early heating should be performed at very high temperatures.



611  
612 **Figure 2.** Factors affecting sensitivity of detection. (a) complex fat matrix, (b) lipid oxidation products, (c) Maillard  
613 reaction products, (d) low skatole and androstenone content in fat, and (e) slaughterhouse's VOCs background noise

### 614 1.1. Drifts and corrections

615 Another challenge encountered in sensor-based detection of boar taint is temporal sensor drift. It is  
616 defined as the gradual variation in the sensor response when exposed to the same analyte under the same  
617 conditions. The reasons for such a drift are classified into two main categories: first- and second-order  
618 drift.

619 First-order drift is due to interaction occurring at the surface of the sensor. This includes aging of the  
620 sensor causing the reactive phase to reorganize itself, and sensor poisoning due to the binding of  
621 contaminants to the reactive surface. Second-order drift is caused by variations in experimental conditions,  
622 such as humidity variations (Vergara et al., 2012).



623 Data processing using mathematical analysis can be used to detect and correct the errors in case of first-  
624 order drifts. These methods are either univariate or multivariate, depending on whether drift compensation  
625 is performed on the sensors individually or on the sensor array.

626 An example of such a univariate method is the multiplicative drift correction method proposed by Haugen  
627 et al. (2000). They suggested a calibration method that considers the temporal drift in sequence and in  
628 between sequences. The suggested methodology consisted of recalibrating the sensor with a reference  
629 sample after a given number of analyses. In the case of boar taint, the reference sample could be a sow fat  
630 sample with known low amounts of skatole and indole, to which analytes of interest are added. VOCs  
631 could be sampled under the same conditions. However, such methods require complicated and time-  
632 consuming experimental set-ups that are not suitable for rapid on-line sorting of carcasses.

633 Several multivariate methods have also been developed, which are either supervised or unsupervised. In  
634 supervised methods, the training samples are labeled to group them in a set of classes. Thus, in the case of  
635 boar taint detection, tainted samples could be grouped together in advance. Unsupervised methods, on the  
636 other hand, do not use labeling prior to statistical analysis (Di Carlo & Falasconi, 2012). Examples of  
637 supervised and unsupervised methods include the ensemble method introduced by Vergara et al. (2012)  
638 and the drift correction method based on common principal component analysis (CPCA) proposed by  
639 Ziyatdinov et al. (2010), respectively.

640 A more practical solution to reduce first-order drifts related to sensor poisoning could be to clean the  
641 sensor after a fixed number of analyses, using organic solvents. Sensors could also be replaced after a  
642 fixed number of analyses.

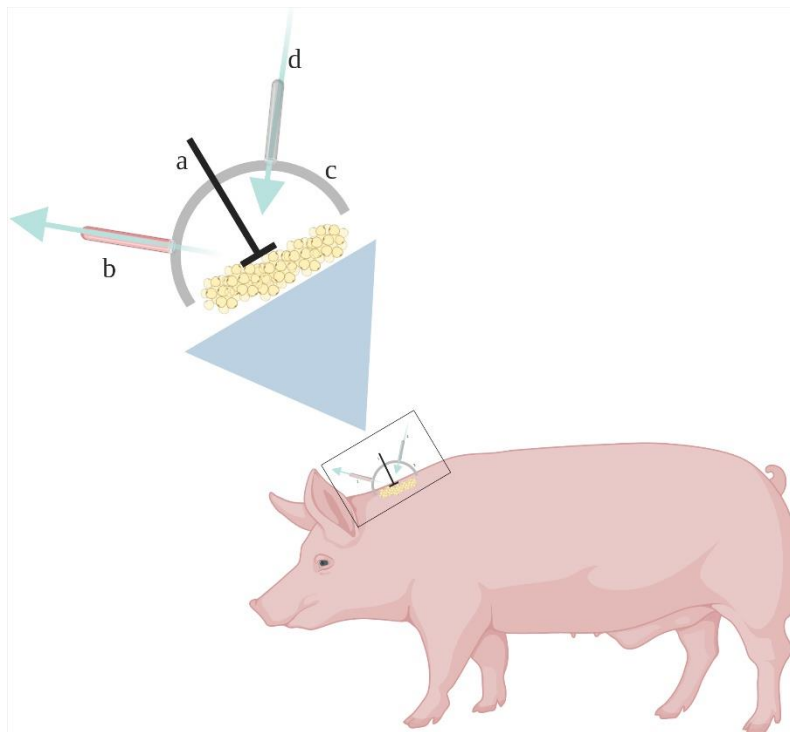
643 The solvents used during the cleaning process, and the replaced sensors must be correctly disposed of.  
644 Thus, it needs to be determined when a sensor is to be cleaned, and when it is to be replaced. Low-cost  
645 sensors developed on substrates such as carbon or plastic, can be discarded after a single use.

646 Another solution to reduce the drift of sensors is to develop new sensor materials that possess greater  
647 selectivity and specificity towards the analytes of interest, leading to an increased lifespan of such sensors.  
648 Such materials include molecularly imprinted polymers (MIPs). These are “synthetic materials with  
649 artificially generated recognition sites able to specifically rebind a target molecule in preference to other  
650 closely related compounds” (Turiel & Martín-Esteban, 2010). MIPs are resistant to a wide range of  
651 temperatures and pH, and their synthesis is cheap and easy (Turiel & Martín-Esteban, 2010). They have  
652 already been used for many applications, including drug delivery, protein separation, and for making  
653 sensors (Bossi et al., 2007; Zang et al., 2020). MIP-based sensors have been developed for various  
654 purposes, such as acetaldehyde detection (Debliquy et al., 2016), L-nicotine detection (Thoelen et al.,  
655 2008), and penicillin G detection (Weber et al., 2018). Only a few studies have investigated the use of  
656 MIPs for the detection of boar taint, thus offering research possibilities.

657 Verplanken (2018) attempted to develop MIPs through a non-covalent approach for the detection of  
658 skatole and androstenone. MIPs with sufficient specificity and selectivity for use in screening assays could  
659 not be obtained through non-covalent imprinting of androstenone. This may be attributed to the lack of  
660 anchoring chemical functional groups on the androstenone molecule. However, when various MIPs were  
661 combined in an array and tested on boar neck fat samples, a classification accuracy of 82.7% was obtained  
662 for skatole detection. Further research should be performed on developing MIPs for androstenone  
663 detection. Such attempts could focus on binding of the template and the functional monomer through a  
664 semi-covalent or covalent approach. If successful, integrating such MIPs in an array could increase the  
665 classification accuracy. Even if these MIPs were deposited on a quartz crystal microbalance to widen its  
666 range to nonconductive polymer-based MIP, the electronic nose would be cumbersome because additional

667 equipment is needed for monitoring the frequency variation with analytes. Another alternative is  
668 monitoring the resistance change of sensors based on conductive polymer MIPs, such as polyaniline and  
669 polypyrrole. The resulting electronic nose would be smaller, cheaper, and easier to use. Debliquy et al.  
670 (2016) developed an acetaldehyde-based MIP using a pyrrole monomer as a functional monomer. The  
671 MIP-based sensors showed a rapid response to acetaldehyde in the parts per million range.

672 Finally, a potential solution to reduce both first- and second-order drifts is to work under extremely  
673 controlled conditions. The environmental factors in the sampling procedure could be minimized by  
674 heating the carcass fat and sampling its VOCs in a closed environment where the air is replaced by a dry  
675 inert gas (Figure 3). Working in an oxygen-free environment would also help in preventing the creation of  
676 lipid-oxidation products, thus simplifying the detection process.



677  
678 **Figure 3.** Sampling and detection of boar taint in a closed environment. (a) heating device, (b) sensor, (c) closed  
679 environment, and (d) inert gas.

## 680 7. Conclusion

681 The large amount of research addressed in this review demonstrates that boar taint detection has been a  
682 major concern for the meat industry for decades. This review highlights that the at-line LDTD-MS/MS  
683 method is currently the most promising method for the rapid detection of boar taint in slaughterhouses.  
684 Given its good validation criteria and its potential to perform fast analysis at a low operational cost, this  
685 method is currently being tested in slaughterhouses. However, high initial investment, as well as the need  
686 for significant modifications in the slaughter line layout, could lead to a certain reluctance towards its  
687 implementation particularly in small infrastructures.

688 Additionally, this method focuses particularly on the detection of skatole and androstenone. As  
689 highlighted by this review, such analysis does not represent the real sensory perception of boar taint, but  
690 serves as an indicator for the detection of tainted carcasses. The exact and complete odor of boar taint

691 caused by a variety of molecules potentially acting in synergy can only be fully perceived by the human  
692 nose, making this detection technique perennial amongst all others being developed.

693 Compared to LDTD-MS/MS, REIMS and Raman spectroscopy should also allow to better encompass this  
694 complex odor given that they are untargeted methods. Additionally, these methods can be used for on-line  
695 detection as Raman spectroscopy can be portable and REIMS possesses a hand-held measuring tool.

696 Being an on-line method could be seen as a strong asset for techniques being developed. As a matter of  
697 fact, the growing meat demand goes with an increase in the number of carcasses slaughtered daily. This  
698 will either lead to the creation of bigger slaughterhouses or to an acceleration of the slaughtering pace with  
699 a “just-in-time” management of the carcasses needed. Hence, an on-line detection method seems more  
700 suited for the latter.

701 Sensor-based methods might be another solution for on-line detection provided that it is able to tackle the  
702 major challenge of detecting low headspace concentrations of boar taint compounds in a VOC-rich  
703 environment. Early heating of the fat and sampling in a closed and controlled environment, were presented  
704 as solutions to tackle this issue. These suggestions will help in accelerating the validation of sensor-based  
705 methods in real slaughterhouse conditions provided they have, just as any other developed method,  
706 previously been validated in laboratory conditions and proved to be economically viable.

707 In the future, several rapid and reliable detection methods might co-exist in the market. The chosen  
708 method will vary between slaughterhouses depending on the size of the installation, the slaughtering speed  
709 and the financial means available for purchasing the system, adapting the slaughter lines and finally to  
710 operate (i.e. operational costs). In any case, research in the field of rapid boar taint detection still has a  
711 bright future ahead of it.

712 **Table 1.** Summary of detection techniques described in the review. Note that the methods are presented in the same order as they occur in the text. In the “main findings” column,  
 713 + and – represent positive and negative findings, respectively. In the “method sensitivity” column, indications of limits of detection (LODs) and limits of quantification (LOQs) are  
 714 given when possible. Indications of acceptance thresholds or lowest concentrations tested are given when possible. EC<sub>50</sub> is the concentration that yields a half-maximal response.  
 715 N/A indicates that the information is not available.

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<i>1. Analytical methods used for laboratory purposes</i>				
Porcine adipose tissue	Melting of fat, extraction with methanol in water bath (60°C, 60 min), freezing, centrifugation and solid-phase extraction	+ Good validation criteria  + LOD and LOQ below rejection thresholds indicated in literature	LOD and LOQ determined in melted fat:  Indole: LOD = 2.5 ng g <sup>-1</sup> , LOQ = 5 ng g <sup>-1</sup>	(Bekaert et al., 2012)
	Ultra-high performance liquid chromatography – High resolution mass spectrometry	- Time-consuming sample preparation  - Off-line detection method	Skatole: LOD = 2.5 ng g <sup>-1</sup> , LOQ = 5 ng g <sup>-1</sup>  Androstenone: LOD = 7 ng g <sup>-1</sup> , LOQ = 10 ng g <sup>-1</sup>	
Porcine adipose tissue	Thawing of fat, melting, extraction with methanol (55°C, 10 min), freezing, centrifugation and solvent evaporation	+ Good validation criteria  + LOD and LOQ below rejection thresholds indicated in literature	LOD and LOQ determined in melted fat:  Indole: LOD = 0.5 ng g <sup>-1</sup> , LOQ = 1 ng g <sup>-1</sup>	(Fischer et al., 2011)
	Poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber used for solid-phase microextraction	- Off-line detection method	Skatole: LOD = 0.1 ng g <sup>-1</sup> , LOQ = 0.5 ng g <sup>-1</sup>	
	Stable Isotope Dilution Analysis - Headspace Solid-Phase Microextraction - Gas Chromatography - Mass spectrometry	- Deuterated compounds as internal standards are expensive or time-consuming to produce	Androstenone: LOD = 35 ng g <sup>-1</sup> , LOQ = 60 ng g <sup>-1</sup>	

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<i>1. Analytical methods used for laboratory purposes</i>				
<b>Porcine adipose tissue</b>	Homogenization with methanol, 5 min sonication, 15 min cooling in ice bath, centrifugation for 5 min at 4000g, 5 min cooling in ice bath	+ Good validation criteria	LOD determined with standards in solution, LOQ determined as ten times the LOD.	(Hansen-Møller, 1994)
	Androstenone derivatization with dansylhydrazine	+ Quantification of indole, skatole and androstenone	Indole: LOD <3 ng ml <sup>-1</sup> , LOQ = 30 ng g <sup>-1</sup>	
	High performance liquid chromatography – fluorescence detection (HPLC-FD)	- Time-consuming and expensive	Skatole: LOD <3 ng ml <sup>-1</sup> , LOQ = 30 ng g <sup>-1</sup>	
		- Off-line detection method	Androstenone: LOD = 20 ng ml <sup>-1</sup> , LOQ= 200 ng g <sup>-1</sup>	
<b>Porcine adipose tissue</b>	Two methods tested and validated by collaborative trails	+ Validated by inter-lab collaborative study (ISO 5725-2:1994)	Method validated with melted fat in the following range:	(Buttinger & Wenzl, 2014, 2020)
	Freezing of fat, grinding, melting, centrifugation, size exclusion chromatography, solvent evaporation	+ Performances compliant with requirements	Indole: 90 - 970 ng g <sup>-1</sup>	
	Isotope dilution - Gas Chromatography - Mass Spectrometry	+ Robust and free of matrix interferences	Skatole: 210 - 1150 ng g <sup>-1</sup>	
	Isotope Dilution - Liquid Chromatography - Mass Spectrometry	- Off-line detection method	Androstenone: 320 - 3850 ng g <sup>-1</sup>	

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<i>2. Present boar taint detection methods in slaughterhouses</i>				
<b>Porcine adipose tissue</b>	Heating of the fat	+ Selection and training of the assessors	LOD variable from one assessor to another.	(Trautmann et al., 2014)
	Detection with human nose by sensory evaluation	+ Detection of taint based on global VOC profile generated by heating  + Small investment  - Evaluation of assessors affected by several factors (e.g. fatigue)  - Long training of assessors to decrease subjectivity of assessor's evaluation	Selection and training of assessors performed to ensure that the assessor detects (LOD) the taint below rejection thresholds	
<b>Porcine adipose tissue</b>	Solvent extraction of indolic compounds	+ Cost-effective	LOD determined in back-fat.	(Mortensen & Sørensen, 1984)
	Addition of color reagent	+ Robust method	LOD for skatole equivalents in the range 0.02 - 0.04 ng g <sup>-1</sup>	
	Spectrophotometric detection (580 nm)	- High initial investment  - Result in "skatole equivalents", contribution of androstenone not considered		

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<b>3. Past research in boar taint detection</b>				
<b>3.1 Insect behavior-based sensing</b>				
<b>Skatole and androstenone diluted in dichloromethane (DCM)</b>	<i>M. croceipes</i> placed in arena	+ Recognition of indole, skatole and androstenone separately and in a mixture		
	Wasp hound with sugar water and odor source each time	+ Insect can report various concentrations found in boar fat	N/A	(Olson et al., 2012; Wäckers et al., 2011)
<b>Porcine adipose tissue</b>		- Insect response to natural unconditioned stimulus		
<b>3.2 Electronic noses (e-noses)</b>				
<b>Porcine adipose tissue</b>	Prototype MOS array system	N/A	N/A	(Berdague & Talou, 1993)
<b>Porcine adipose tissue</b>	5 commercial MOS array system	+ Classification accuracy of 84.2%  - Miniaturization required	Classification in two classes based on androstenone content: < 0.7 µg g <sup>-1</sup> and > 1.7 µg g <sup>-1</sup>	(Bourrounet et al., 1995)

<b>Matrix analyzed</b>	<b>Sample preparation and detection method</b>	<b>Main findings</b>	<b>Method sensitivity</b>	<b>Reference</b>
<i>3.2 Electronic noses (e-noses)</i>				
<b>Sunflower oil with vegetable fat, fortified with varying levels of skatole or androstenone</b>	Ambient temperature (22-23 °C), acquisition for 60 s.  12 conducting-polymer array system	+ Correlation of 0.78 between results obtained with sensory panel and sensor array system	Cut-off limits used:  Skatole: 0.2 µg g <sup>-1</sup>  Androstenone: 0.5 µg g <sup>-1</sup>	(Annor-Frempong et al., 1998)
<b>Porcine adipose tissue</b>	Heated at 35 °C, 30 min.  Quartz microbalances	+ Limit of detection below androstenone accepted threshold of 0.5 µg g <sup>-1</sup>  - Expensive, time consuming	LOD for androstenone in back-fat < 0.5 µg g <sup>-1</sup>	(Di Natale et al., 2003)
<b>Porcine adipose tissue</b>	Incubation at 40 °C, 10 min  Ion mobility spectrometry based electronic nose	+ Sorting of carcasses into high and low levels of skatole and androstenone  - Sensitivity to be determined	Cut-off limits used:  Skatole: 0.21 µg g <sup>-1</sup>  Androstenone: 0.5 µg g <sup>-1</sup>	(Vestergaard et al., 2006)



Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<i>3.3 Gas chromatography–mass spectrometry (GC-MS) based methods</i>				
<b>Indole, skatole and androstenone diluted in methanol</b>	Incubation at 150°C, 12 minutes Dynamic Headspace Sampling – Gas Chromatography – Mass Spectrometry	+ Results in only 6 minutes - Expensive, fat sampling required	LOD determined in back-fat: Indole: 82 ng g <sup>-1</sup> Skatole: 97 ng g <sup>-1</sup> Androstenone: 623 ng g <sup>-1</sup>	(Sørensen & Engelsen, 2014)
<b>Porcine adipose tissue</b>				

<b>Skatole and androstenone diluted in corn oil</b>	Optimal extraction at heating parameters 400 °C, 45 s	+ Results in 3.5 min		
<b>Porcine adipose tissue</b>	Solid phase microextraction - Gas Chromatography – Mass Spectrometry Poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber selected after optimization for solid-phase microextraction	+ Good validation criteria - Lack of sensitivity	Lack of sensitivity with portable GCMS for androstenone: no detection even at 10 µg g <sup>-1</sup>	(Verplanken et al., 2016)

#### **4. Recent advances in boar taint detection**

##### *4.1 MS-based methods*

<b>Porcine adipose tissue</b>	REIMS	+ Results in less than 10s	Cut-off limits used: Indole: 0.1 µg g <sup>-1</sup> Skatole: 0.2 µg g <sup>-1</sup> Androstenone: 0.5 µg g <sup>-1</sup>	(Verplanken et al., 2017)
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Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<i>4.1 MS-based methods</i>				
<b>Porcine adipose tissue</b>	1.5 mL brine and 1.5 mL acetonitrile added to sample (0,3 to 0,8 g). Homogenization for 30 s, followed by centrifugation for 5 min at 5000 g	+ Accurate measurements	LOD and LOQ determined in back-fat:	(Borggaard et al., 2017)
	Supernatant left to dry for 2 min	-Requires fat sampling and traceability system	Skatole: LOD = 0,05 $\mu\text{g g}^{-1}$ , LOQ = 0,1 $\mu\text{g g}^{-1}$	
	Laser Diode Thermal Desorption Ion Source Tandem Mass Spectrometry	+ Sampling can be fully automated (currently tested in slaughterhouse)	Androstenone: LOD = 0.2 $\mu\text{g g}^{-1}$ , LOQ = 0,05 $\mu\text{g g}^{-1}$	
<b>Porcine adipose tissue</b>	3.0 mL NaOH (1N in water) + methyl-ter-butyl ether (MTBE). Vortexing for 1 min. Decantation for 2 min	+ Accurate measurements	Calibration ranges:	(Auger et al., 2018)
	Supernatant left to dry for 1 min	-Requires fat sampling and traceability system	Indole: 0,0165 $\mu\text{g g}^{-1}$ to 0,132 $\mu\text{g g}^{-1}$	
	Laser Diode Thermal Desorption Ion Source Tandem Mass Spectrometry	+ Sampling can be fully automated	Skatole: 0,0413 $\mu\text{g g}^{-1}$ to 0,660 $\mu\text{g g}^{-1}$  Androstenone: 0,3325 $\mu\text{g g}^{-1}$ to 2,660 $\mu\text{g g}^{-1}$	

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<i>4.2 Raman spectroscopy-based methods</i>				
<b>Porcine adipose tissue</b>	Sample thawed at 4°C overnight, equilibrated for 1h Raman spectroscopy from 300 to 2100 cm <sup>-1</sup> with 8 cm <sup>-1</sup> resolution, data acquisition about 20 min per sample	+ Classification accuracy of 81% after partial least square regression discriminant analysis (PLS-DA)	Cut-off limits used: Skatole: 0.2 µg g <sup>-1</sup> Androstenone: 1.5 µg g <sup>-1</sup>	(Liu et al., 2016)
<b>Porcine adipose tissue</b>	Fat extraction. Surface-enhanced Raman scattering, spectra acquisition for 20s from 200 to 3400 cm <sup>-1</sup> with a 10 cm <sup>-1</sup> spectral resolution	- High prediction errors	LOD determined in melted fat: Skatole: 2.4 x 10 <sup>-6</sup> M Androstenone: 1.2 x 10 <sup>-7</sup> M	(Sørensen et al., 2015)
<i>4.3 Specific sensors based on the intrinsic properties of target molecules</i>				
<b>Skatole and androstenone diluted in methanol</b>	Voltammetric detection for skatole, enzyme electrode for androstenone	+ Correlation of 0.801 for skatole and 0.932 for androstenone when compared to GC-MS results + Measurements within 60 s - Must be tested with slaughterhouse conditions	LOD in solution: Androstenone 0.3 ppm Skatole 0.052 ppm	(Hart et al., 2016; Westmacott et al., 2020)

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
<b>5. Biosensors - a path to be further investigated for boar taint detection</b>				
<i>5.1 OR-based bioelectronic noses</i>				
<b>423 human odorant receptors 66 odors at high and low concentrations</b>	Cell-based assay technique	+ Response of OR7D4 specific to androstenone and androstadienone	N/A	(Keller et al., 2007)
	Olfactory psychophysical study			
<b>Androstenone diluted in dimethyl sulfoxide (DMSO)</b>	Measurement performed in the range of -400 mV to 600 mV. Scan rate, duration and amplitude of 100 mV/s, 0.05 s and 5 mV respectively.	+ Very low limit of detection ( $10^{-14}$ M)	LOD for androstenone in solution $10^{-14}$ M	(Guo et al., 2015)
	OR7D4s anchored to a gold electrode, response monitored by square wave voltammetry.			
<b>Odorants diluted in ND96 (in mM: 96 NaCl, 2 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 5 HEPES, pH 7.5)</b>	Diluted odorants applied for 20 s at a flow rate of 1.65 ml/min	+ CquiOR2 very selective for indole, CquiOR10 very selective and highly sensitive for skatole	Skatole EC <sub>50</sub> for CquiOR10 + CquiOR7 of 90 nM	(Hughes et al., 2010; Pelletier, Hughes, et al., 2010)
	Recording of odorant-induced currents from oocytes expressing CquiORs			
<b>50 AgamORs 110 odorants diluted in either water, ethanol or paraffin oil</b>	Amplification of coding regions of AgOR and expression of these in the “empty-neuron” system	+ AgamOR2 narrowly tuned and highly active by indole	Indole response threshold between $10^{-7}$ and $10^{-6}$ dilution	(Carey et al., 2010)
	Functional characterization of AgamORs  Odorant tuning curves			

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
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*5.2 OBP-based bioelectronic noses*

<b>Indole diluted in water</b>	<i>Attenu</i> fluorescence-quenching assay system, detection in less than 30 min (emission wavelength shift from 460 nm to 416 nm)	+ AgamOBP1 highly specific and sensitive to indole	In fluorescence quenching assay, detection of indole at less than 100 nM	(Dimitratos et al., 2019)
	Lateral flow biosensor, in less than 20 min			

*5.3 Aptamer-based biosensors*

<b>Skatole and androstenone diluted in water</b>	Gold nanoparticle aptasensors	+ Aptamer selected specific to skatole and androstenone	Significant color change for skatole and androstenone at concentrations as low as $10^{-13}$ M	(Frimpong et al., 2017)
	Absorbance shift from 524 nm to 660 nm in the presence of skatole and androstenone	- Tests must be performed with molecules in gaseous phase		

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