Past, present, and future trends in boar taint detection

Clément Burgeon[a,*], Marc Debliquy[b], Driss Lahem[c], Justine Rodriguez[b], Ahmadou Ly[c], Marie-Laure Fauconnier[a]

[a] Laboratory of Chemistry of Natural Molecules, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

[b] Service de Science des Matériaux, Faculté Polytechnique, Université de Mons, Rue de l'Epargne 56, 7000 Mons, Belgium

[c] Materia Nova ASBL, Materials R&D Centre, Parc Initialis, Avenue Nicolas Copernic 3, 7000 Mons, Belgium

Abstract

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

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

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

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

* Corresponding author: *E-mail address:* cburgeon@uliege.be (Clément Burgeon)

1. Introduction

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

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

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

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

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

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

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

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

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

2. Present boar taint detection methods in slaughterhouses

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

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

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

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

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

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

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

3. Past research in boar taint detection

3.1. Insect behavior-based sensing

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

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

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

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

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

3.2. Electronic noses (e-noses)

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

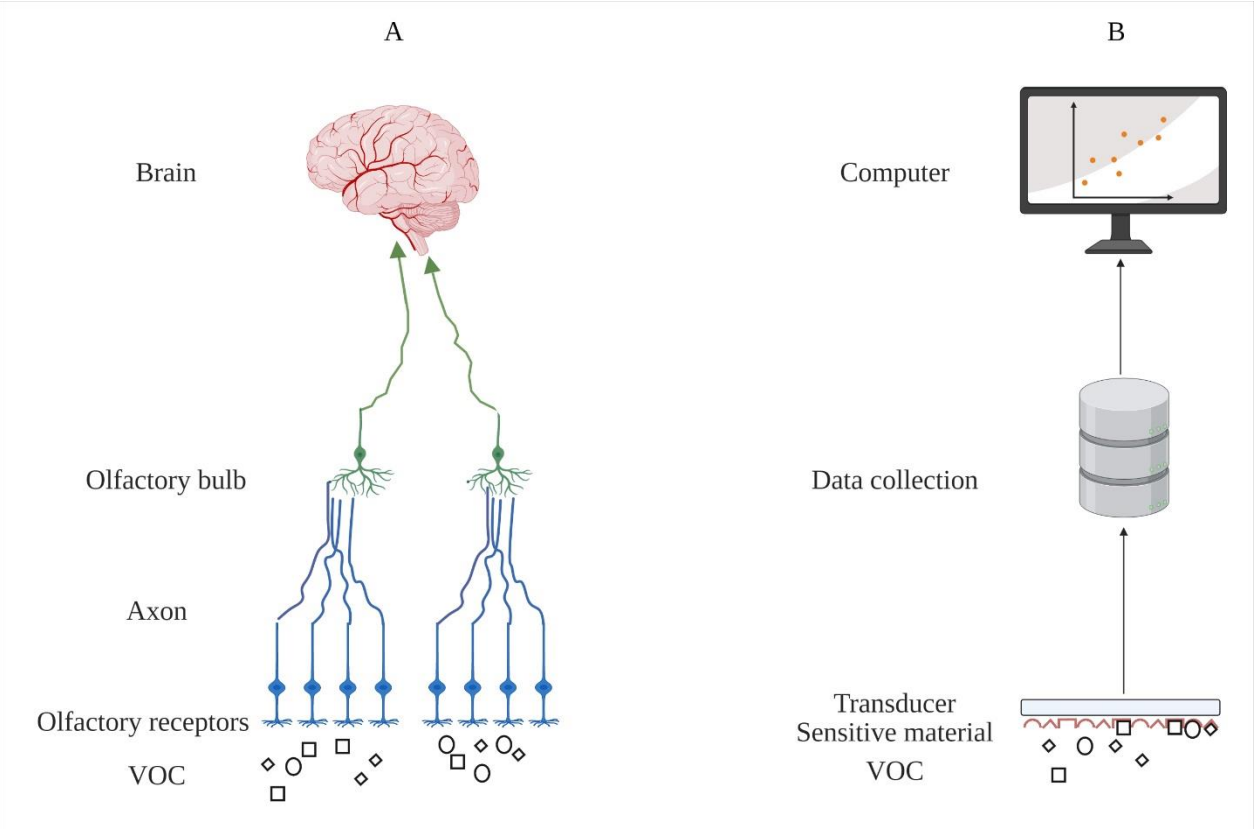

Figure 1. Comparison of odor perception by the human nose and an e-nose. (A) Human olfaction (B) VOC detection by e-nose.

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Recent advances in boar taint detection

4.1. MS-based methods

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

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

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

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

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

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

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

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

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

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

4.2. Raman spectroscopy-based methods

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

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

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

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

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

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

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

5. Biosensors – a path to be further investigated for boar taint detection

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

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

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

5.1. OR-based bioelectronic nose

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

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

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

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

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

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

5.2. OBP-based bioelectronic nose

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

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

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

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

5.3. Aptamer-based biosensors

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

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

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

5.4. Production cost of biosensors

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

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

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

6. Challenges and solutions for sensor-based detection in slaughterhouses

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

6.1. Environment-specific noise challenges

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

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

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

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

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

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

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

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

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

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

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

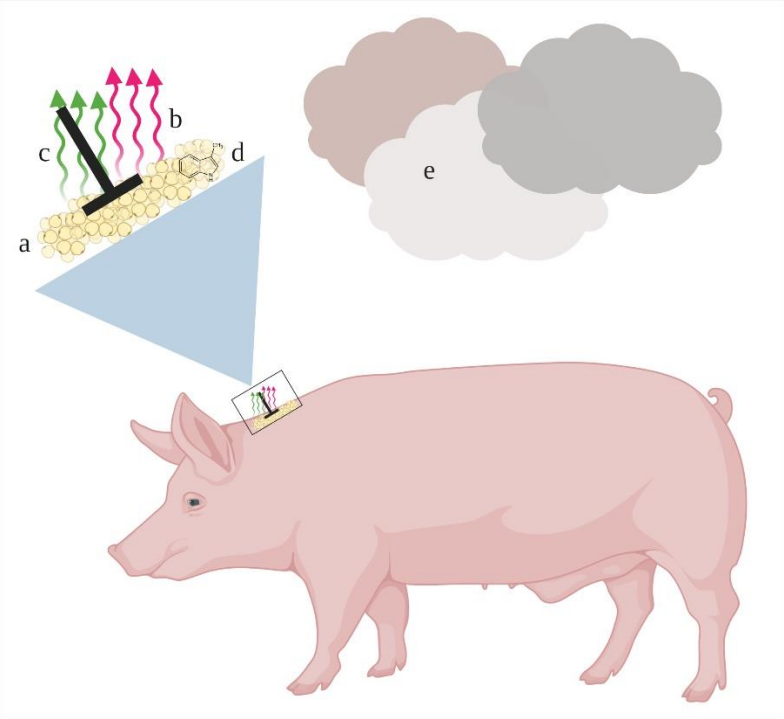

Figure 2. Factors affecting sensitivity of detection. (a) complex fat matrix, (b) lipid oxidation products, (c) Maillard reaction products, (d) low skatole and androstenone content in fat, and (e) slaughterhouse's VOCs background noise

1.1. Drifts and corrections

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

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

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

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

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

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

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

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

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

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

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

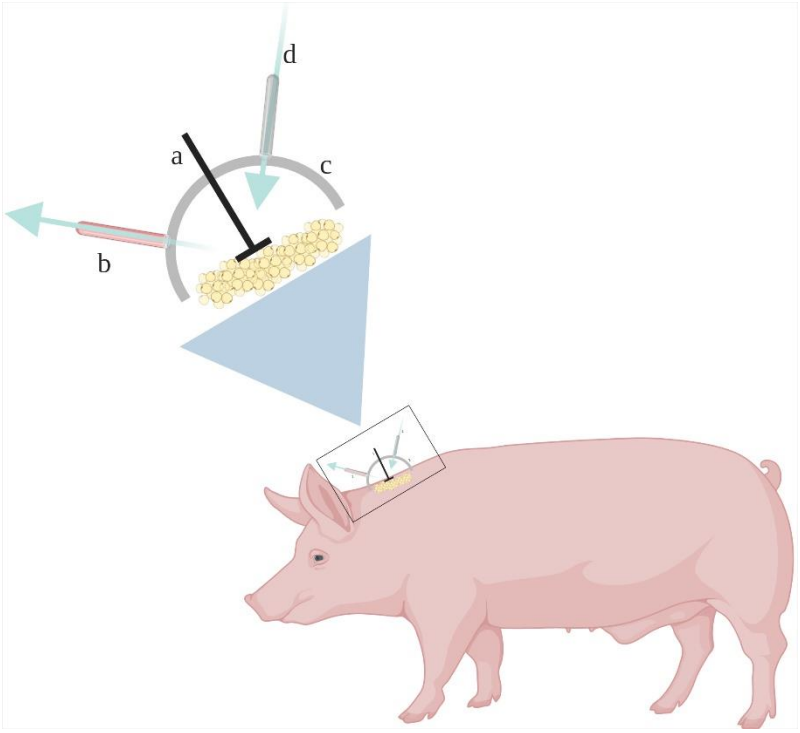

Figure 3. Sampling and detection of boar taint in a closed environment. (a) heating device, (b) sensor, (c) closed environment, and (d) inert gas.

7. Conclusion

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

Additionally, this method focuses particularly on the detection of skatole and androstenone. As highlighted by this review, such analysis does not represent the real sensory perception of boar taint, but 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.

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, + and – represent positive and negative findings, respectively. In the "method sensitivity" column, indications of limits of detection (LODs) and limits of quantification (LOQs) are given when possible. Indications of acceptance thresholds or lowest concentrations tested are given when possible. EC_{50} is the concentration that yields a half-maximal response. N/A indicates that the information is not available.

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

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
	1. Analytical methods used for laboratory purposes			
Porcine adipose tissue	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^{-1}, LOQ = 30 ng g^{-1}	
	High performance liquid chromatography – fluorescence detection (HPLC-FD)	- Time-consuming and expensive	Skatole: LOD <3 ng ml^{-1}, LOQ = 30 ng g^{-1}	
		- Off-line detection method	Androstenone: LOD = 20 ng ml^{-1}, LOQ= 200 ng g^{-1}	
Porcine adipose tissue	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^{-1}	
	Isotope dilution - Gas Chromatography - Mass Spectrometry	+ Robust and free of matrix interferences	Skatole: 210 - 1150 ng g^{-1}	
	Isotope Dilution - Liquid Chromatography - Mass Spectrometry	- Off-line detection method	Androstenone: 320 - 3850 ng g^{-1}	

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
		2. Present boar taint detection methods in slaughterhouses		
Porcine adipose tissue	Heating of the fat Detection with human nose by sensory evaluation	+ Selection and training of the assessors + 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	LOD variable from one assessor to another. Selection and training of assessors performed to ensure that the assessor detects (LOD) the taint below rejection thresholds	(Trautmann et al., 2014)
Porcine adipose tissue	Solvent extraction of indolic compounds Addition of color reagent Spectrophotometric detection (580 nm)	+ Cost-effective + Robust method - High initial investment - Result in "skatole equivalents", contribution of androstenone not considered	LOD determined in back-fat. LOD for skatole equivalents in the range 0.02 - 0.04 ng g^{-1}	(Mortensen & Sørensen, 1984)

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

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
		3.2 Electronic noses (e-noses)		
Sunflower oil with vegetable fat, fortified with varying levels of skatole or androstenone **Porcine adipose tissue**	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^{-1} Androstenone: 0.5 µg g^{-1}	(Annor-Frempong et al., 1998)
Porcine adipose tissue	Heated at 35 °C, 30 min. Quartz microbalances	+ Limit of detection below androstenone accepted threshold of 0.5 µg g^{-1} - Expensive, time consuming	LOD for androstenone in back-fat < 0.5 µg g^{-1}	(Di Natale et al., 2003)
Porcine adipose tissue	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^{-1} Androstenone: 0.5 µg g^{-1}	(Vestergaard et al., 2006)

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
		3.3 Gas chromatography–mass spectrometry (GC-MS) based methods		
Indole, skatole and androstenone diluted in methanol **Porcine adipose tissue**	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^{-1} Skatole: 97 ng g^{-1} Androstenone: 623 ng g^{-1}	(Sørensen & Engelsen, 2014)
Skatole and androstenone diluted in corn oil **Porcine adipose tissue**	Optimal extraction at heating parameters 400 °C, 45 s Solid phase microextraction - Gas Chromatography – Mass Spectrometry Poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber selected after optimization for solid-phase microextraction	+ Results in 3.5 min + Good validation criteria - Lack of sensitivity	Lack of sensitivity with portable GCMS for androstenone: no detection even at 10 µg g^{-1}	(Verplanken et al., 2016)
		4. Recent advances in boar taint detection		
		4.1 MS-based methods		
Porcine adipose tissue	REIMS	+ Results in less than 10s	Cut-off limits used: Indole: 0.1 µg g^{-1} Skatole: 0.2 µg g^{-1} Androstenone: 0.5 µg g^{-1}	(Verplanken et al., 2017)

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
		4.1 MS-based methods		
Porcine adipose tissue	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 µg g^{-1}, LOQ = 0,1 µg g^{-1}	
	Laser Diode Thermal Desorption Ion Source Tandem Mass Spectrometry	+ Sampling can be fully automated (currently tested in slaughterhouse)	Androstenone: LOD = 0.2 µg g^{-1}, LOQ = 0,05 µg g^{-1}	
Porcine adipose tissue	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)
		-Requires fat sampling and traceability system	Indole: 0,0165 µg g^{-1} to 0,132 µg g^{-1}	
	Supernatant left to dry for 1 min	+ Sampling can be fully automated	Skatole: 0,0413 µg g^{-1} to 0,660 µg g^{-1}	
	Laser Diode Thermal Desorption Ion Source Tandem Mass Spectrometry		Androstenone: 0,3325 µg g^{-1} to 2,660 µg g^{-1}	

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
		4.2 Raman spectroscopy-based methods		
Porcine adipose tissue	Sample thawed at 4°c overnight, equilibrated for 1h Raman spectroscopy from 300 to 2100 cm^{-1} with 8 cm^{-1} 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^{-1} Androstenone: 1.5 µg g^{-1}	(Liu et al., 2016)
Porcine adipose tissue	Fat extraction. Surface-enhanced Raman scattering, spectra acquisition for 20s from 200 to 3400 cm^{-1} with a 10 cm^{-1} spectral resolution	- High prediction errors	LOD determined in melted fat: Skatole: 2.4 x 10^{-6} M Androstenone: 1.2 x 10^{-7} M	(Sørensen et al., 2015)
		4.3 Specific sensors based on the intrinsic properties of target molecules		
Skatole and androstenone diluted in methanol	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
	5. Biosensors - a path to be further investigated for boar taint detection			
	5.1 OR-based bioelectronic noses			
423 human odorant receptors 66 odors at high and low concentrations	Cell-based assay technique Olfactory psychophysical study	+ Response of OR7D4 specific to androstenone and androstadienone	N/A	(Keller et al., 2007)
Androstenone diluted in dimethyl sulfoxide (DMSO)	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. OR7D4s anchored to a gold electrode, response monitored by square wave voltammetry.	+ Very low limit of detection (10^{-14} M)	LOD for androstenone in solution 10^{-14} M	(Guo et al., 2015)
Odorants diluted in ND96 (in mM: 96 NaCl, 2 KCl, 1 CaCl2, 1 $MgCl_2$, 5 HEPES, pH 7.5)	Diluted odorants applied for 20 s at a flow rate of 1.65 ml/min Recording of odorant-induced currents from oocytes expressing CquiORs	+ CquiOR2 very selective for indole, CquiOR10 very selective and highly sensitive for skatole	Skatole EC_{50} for CquiOR10 + CquiOR7 of 90 nM	(Hughes et al., 2010; Pelletier, Hughes, et al., 2010)
50 AgamORs 110 odorants diluted in either water, ethanol or paraffin oil	Amplification of coding regions of AgOR and expression of these in the "empty-neuron" system Functional characterization of AgamORs Odorant tuning curves	+ AgamOR2 narrowly tuned and highly active by indole	Indole response threshold between 10^{-7} and 10^{-6} dilution	(Carey et al., 2010)

Matrix analyzed	Sample preparation and detection method	Main findings	Method sensitivity	Reference
		5.2 OBP-based bioelectronic noses		
Indole diluted in water	*Attenu* fluorescence-quenching assay system, detection in less than 30 min (emission wavelength shift from 460 nm to 416 nm) Lateral flow biosensor, in less than 20 min	+ AgamOBP1 highly specific and sensitive to indole	In fluorescence quenching assay, detection of indole at less than 100 nM	(Dimitratos et al., 2019)
		5.3 Aptamer-based biosensors		
Skatole and androstenone diluted in water	Gold nanoparticle aptasensors Absorbance shift from 524 nm to 660 nm in the presence of skatole and androstenone	+ Aptamer selected specific to skatole and androstenone - Tests must be performed with molecules in gaseous phase	Significant color change for skatole and androstenone at concentrations as low as 10^{-13} M	(Frimpong et al., 2017)

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References

Aluwé, M., Millet, S., Bekaert, K. M., Tuyttens, F. A. M., Vanhaecke, L., De Smet, S., & De Brabander, D. L. (2011). Influence of breed and slaughter weight on boar taint prevalence in entire male pigs. *Animal*, *5*(8), 1283–1289. https://doi.org/10.1017/S1751731111000164

Aluwé, M., Millet, S., Nijs, G., Tuyttens, F. A. M., Verheyden, K., De Brabander, H. F., De Brabander, D. L., & Van Oeckel, M. J. (2009). Absence of an effect of dietary fibre or clinoptilolite on boar taint in entire male pigs fed practical diets. *Meat Science*, *82*(3), 346–352. https://doi.org/10.1016/j.meatsci.2009.02.001

Annor-Frempong, I. E., Nute, G. R., Wood, J. D., Whittington, F. W., & West, A. (1998). The measurement of the responses to different odour intensities of "boar taint" using a sensory panel and an electronic nose. *Meat Science*, *50*(2), 139–151. https://doi.org/10.1016/S0309-1740(98)00001-1

Armstrong, P., Nizio, K. D., Perrault, K. A., & Forbes, S. L. (2016). Establishing the volatile profile of pig carcasses as analogues for human decomposition during the early postmortem period. *Heliyon*, *2*(2), 1–24. https://doi.org/10.1016/j.heliyon.2016.e00070

Auger, S., Lacoursière, J., & Picard, P. (2018). *High-throughput analysis of Indole , Skatole and Androstenone in pork fat by LDTD-MS / MS Positive MRM transition LDTD-LC-MS / MS System*. 3–4.

Backus, G., Higuera, M., Juul, N., Nalon, E., & de Briyne, N. (2018). *Second progress report 2015 – 2017 on the European declaration on alternatives to surgical castration of pigs*. 1–18.

Bekaert, K. M., Vanden Bussche, J., François, S., Tuyttens, F. A. M., De Brabander, H. F., Vandendriessche, F., & Vanhaecke, L. (2012). A validated ultra-high performance liquid chromatography coupled to high resolution mass spectrometry analysis for the simultaneous quantification of the three known boar taint compounds. *Journal of Chromatography A*, *1239*(January 2012), 49–55. https://doi.org/10.1016/j.chroma.2012.03.060

Berdague, J. ., & Talou, T. (1993). Examples of semiconductor gas sensors applied to meat products. *Science Des Aliments*, *13*, 141–148.

Bonneau, M. (1998). Use of entire males for pig meat in the European union. *Meat Science*, *49*(SUPPL. 1), 257–272. https://doi.org/10.1016/S0309-1740(98)00089-8

Bonneau, M., Walstra, P., Claudi-Magnussen, C., Kempster, A. J., Tornberg, E., Fischer, K., Diestre, A., Siret, F., Chevillon, P., Claus, R., Dijksterhuis, G., Punter, P., Matthews, K. R., Agerhem,

759 H., Béague, M. P., Oliver, M. A., Gispert, M., Weiler, U., Von Seth, G., … Cook, G. L. (2000).
760 An international study on the importance of androstenone and skatole for boar taint: IV.
761 Simulation studies on consumer dissatisfaction with entire male pork and the effect of sorting
762 carcasses on the slaughter line, main conclusions and recommendations. *Meat Science*, *54*(3),
763 285–295. https://doi.org/10.1016/S0309-1740(99)00105-9

764 Bonneau, M., & Weiler, U. (2019). Pros and cons of alternatives to piglet castration: Welfare, boar
765 taint, and other meat quality traits. In *Animals* (Vol. 9, Issue 11, pp. 1–12).
766 https://doi.org/10.3390/ani9110884

767 Borggaard, C., Birkler, R., Meinert, L., & Støier, S. (2017). At-line rapid instrumental method for
768 measuring the boar taint components androstenone and skatole in pork fat. *Icomst 2017*, 279–
769 280. http://boars2018.com/wp-content/uploads/2017/12/Borggaard-et-al-2017_ICoMST_DMRI-
770 at-line-rapid-measure-for-boar-taint.pdf

771 Bossi, A., Bonini, F., Turner, A. P. F., & Piletsky, S. A. (2007). Molecularly imprinted polymers for
772 the recognition of proteins: The state of the art. *Biosensors and Bioelectronics*, *22*(6), 1131–
773 1137. https://doi.org/10.1016/j.bios.2006.06.023

774 Bourrounet, B., Talou, T., & Gaset, A. (1995). Application of a multi-gas-sensor device in the meat
775 industry for boar-taint detection. *Sensors and Actuators: B. Chemical*, *27*(1–3), 250–254.
776 https://doi.org/10.1016/0925-4005(94)01596-A

777 Brooks, R. I., & Pearson, A. M. (1989). Odor thresholds of the C19-Δ16-steroids responsible for boar
778 odor in pork. *Meat Science*, *24*(1), 11–19. https://doi.org/10.1016/0309-1740(89)90062-4

779 Buttinger, G., & Wenzl, T. (2014). Development of Reference Methods for the Detection and the
780 Measurement of the main compounds responsible for Boar Taint. In *Publications Office of the*
781 *European Union: Geel, Belgium*. https://doi.org/10.2787/96937

782 Buttinger, G., & Wenzl, T. (2020). Validation by collaborative trial of a method for the determination
783 by GC–MS and LC–MS/MS of boar taint marker compounds in pork tissue. *Food Chemistry: X*,
784 *6*(March), 100083. https://doi.org/10.1016/j.fochx.2020.100083

785 Bynum, N. D., Moore, K. N., & Grabenauer, M. (2014). Evaluation of laser diode thermal desorption-
786 tandem mass spectrometry (LDTD-MS-MS) in forensic toxicology. *Journal of Analytical*
787 *Toxicology*, *38*(8), 528–535. https://doi.org/10.1093/jat/bku084

788 Čandek-Potokar, M., Škrlep, M., & Zamaratskaia, G. (2017). Immunocastration as Alternative to
789 Surgical Castration. In *Theriogenology* (pp. 109–126).
790 https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-
791 biometrics

792 Carey, A. F., Wang, G., Su, C. Y., Zwiebel, L. J., & Carlson, J. R. (2010). Odorant reception in the
793 malaria mosquito Anopheles gambiae. *Nature*, *464*(7285), 66–71.
794 https://doi.org/10.1038/nature08834

795 CBRN Tech Index. (2018). *Surface Enhanced Raman Spectroscopy (SERS)*. Retrieved from
796 https://www.cbrnetechindex.com/Biological-Detection/Technology-BD/Molecular-
797 Spectroscopy-BD-T/Surface-Enhanced-Raman-BD-MS. Accessed January 4, 2021

798 De Briyne, N., Berg, C., Blaha, T., & Temple, D. (2016). Pig castration: Will the EU manage to ban
799 pig castration by 2018? *Porcine Health Management*, *2*(29), 1–11.
800 https://doi.org/10.1186/s40813-016-0046-x

801 Debliquy, M., Dony, N., Lahem, D., Tang, X., Zhang, C., Raskin, J. P., & Olivier, M. G. (2016).
802 Acetaldehyde Chemical Sensor based on Molecularly Imprinted Polypyrrole. *Procedia*
803 *Engineering*, *168*, 569–573. https://doi.org/10.1016/j.proeng.2016.11.527

804 Dekeirsschieter, J., Verheggen, F. J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., &

Haubruge, E. (2009). Cadaveric volatile organic compounds released by decaying pig carcasses (Sus domesticus L.) in different biotopes. *Forensic Science International*, *189*(1–3), 46–53. https://doi.org/10.1016/j.forsciint.2009.03.034

Di Carlo, S., & Falasconi, M. (2012). Drift Correction Methods for Gas Chemical Sensors in Artificial Olfaction Systems: Techniques and Challenges. In *Advances in Chemical Sensors*. https://doi.org/10.5772/33411

Di Natale, C., Pennazza, G., Macagnano, A., Martinelli, E., Paolesse, R., & D'Amico, A. (2003). Thickness shear mode resonator sensors for the detection of androstenone in pork fat. *Sensors and Actuators, B: Chemical*, *91*(1–3), 169–174. https://doi.org/10.1016/S0925-4005(03)00084-4

Dimitratos, S. D., Hommel, A. S., Konrad, K. D., Simpson, L. M., Wu-Woods, J. J., & Woods, D. F. (2019). Biosensors to monitor water quality utilizing insect odorant-binding proteins as detector elements. *Biosensors*, *9*(2), 1–15. https://doi.org/10.3390/bios9020062

Du, Y. J., & Millar, J. G. (1999). Electroantennogram and oviposition bioassay responses of Culex quinquefasciatus and Culex tarsalis (Diptera: Culicidae) to chemicals in odors from Bermuda grass infusions. *Journal of Medical Entomology*, *36*(2), 158–166. https://doi.org/10.1093/jmedent/36.2.158

EFSA. (2004). Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the castration of piglets 1 (Question N ° EFSA-Q-2003 - 091) Adopted on the 12 th and 13 th July 2004. *The EFSA Journal*, *1*(July), 1–18.

European Commission. (2010). *European Declaration on alternatives to surgical castration of pigs*. Retrieved from https://ec.europa.eu/food/animals/welfare/practice/farm/pigs/castration_alternatives_en. Accessed December 18, 2020

European Communities. (1981). *Council Directive 81/602/EC*. *L*, 32–33. Retrieved from https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A31981L0602. Accessed December 18, 2020

Feilberg, A., Liu, D., Adamsen, A. P. S., Hansen, M. J., & Jonassen, K. E. N. (2010). Odorant emissions from intensive pig production measured by online proton-transfer-reaction mass spectrometry. *Environmental Science and Technology*, *44*(15), 5894–5900. https://doi.org/10.1021/es100483s

Fischer, J., Elsinghorst, P. W., Bücking, M., Tholen, E., Petersen, B., & Wüst, M. (2011). Development of a candidate reference method for the simultaneous quantitation of the boar taint compounds androstenone, 3α-androstenol, 3β-androstenol, skatole, and indole in pig fat by means of stable isotope dilution analysis-headspace solid-phase micro. *Analytical Chemistry*, *83*(17), 6785–6791. https://doi.org/10.1021/ac201465q

Font-i-Furnols, M., Martín-Bernal, R., Aluwé, M., Bonneau, M., Haugen, J. E., Mörlein, D., Mörlein, J., Panella-Riera, N., & Škrlep, M. (2020). Feasibility of on/at line methods to determine boar taint and boar taint compounds: An overview. *Animals*, *10*(10), 1–26. https://doi.org/10.3390/ani10101886

Food and Agriculture Organization of the United Nations. (1991). *Guidelines for slaughtering meat cutting and further processing*. Retrieved from http://www.fao.org/3/t0279e/T0279E00.htm. Accessed December 14, 2020

Forbes, S. L., Rust, L. T., Trebilcock, K., Perrault, K. A., & McGrath, L. T. (2014). Effect of age and storage conditions on the volatile organic compound profile of blood. *Forensic Science, Medicine, and Pathology*, *10*(4), 570–582. https://doi.org/10.1007/s12024-014-9610-3

Frimpong, N., Derosa, M. C., Ranganathan, V., & Callahan, J. (2017). Optimized Gold Nanoparticle

Aptamer-Based Sensor To Detect Boar Taint. *Proceedings of the National Conference On Undergraduate Research (NCUR) 2017*.

Guo, Z., Zine, N., Lagarde, F., Daligault, J., Persuy, M. A., Pajot-Augy, E., Zhang, A., & Jaffrezic-Renault, N. (2015). A novel platform based on immobilized histidine tagged olfactory receptors, for the amperometric detection of an odorant molecule characteristic of boar taint. *Food Chemistry*, *184*, 1–6. https://doi.org/10.1016/j.foodchem.2015.03.066

Hansen-Møller, J. (1994). Rapid high-performance liquid chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. *Journal of Chromatography B: Biomedical Sciences and Applications*, *661*(2), 219–230. https://doi.org/10.1016/S0378-4347(94)80049-9

Hansson, K.-E., Lundström, K., Fjelkner-Modif, S., & Persson, J. (1980). The importance of androstenone and skatole for boar taint. *Swedish Journal of Agricultural Research*, *10*, 167–173.

Hart, J., Crew, A., McGuire, N., & Doran, O. (2016). *Sensor and method for detecting androstenone or skatole in boar taint* (Patent No. EP2966441A1). European Patent Application. https://patents.google.com/patent/EP2966441A1/en

Haugen, J. E., & Kvaal, K. (1998). Electronic nose and artificial neural network. *Meat Science*, *49*(SUPPL. 1). https://doi.org/10.1016/S0309-1740(98)90054-7

Haugen, J. E., Tomic, O., & Kvaal, K. (2000). A calibration method for handling the temporal drift of solid state gas-sensors. *Analytica Chimica Acta*, *407*(1–2), 23–39. https://doi.org/10.1016/S0003-2670(99)00784-9

Haugen, J. E., van Wagenberg, C., Backus, G., Nielsen, B. E., Borgaard, C., Bonneau, M., Panella-Riera, N., & Aluwé, M. (2012). *BoarCheck: A study on rapid methods for boar taint used or being developed at slaughter plants in the European Union*. Retrieved from https://ec.europa.eu/food/sites/food/files/animals/docs/aw_prac_farm_pigs_cast-alt_research_boarcheck_20140901.pdf. Accessed January 5, 2021

Hemeryck, L. Y., Stead, S. L., Decloedt, A., Huysman, S., Balog, J., DeSpiegeleer, M., Pringle, S. D., Boland, A., & Vanhaecke, L. (2019). First implementation of Rapid Evaporative Ionisation Mass Spectrometry (REIMS) for the at-line screening of boar carcasses in the slaughterhouse. *67th Conference of Mass Spectrometry and Allied Topics (ASMS)*.

Herrero, A. M. (2008). Raman spectroscopy a promising technique for quality assessment of meat and fish: A review. *Food Chemistry*, *107*(4), 1642–1651. https://doi.org/10.1016/j.foodchem.2007.10.014

Heyrman, E., Millet, S., Tuyttens, F. A. M., Ampe, B., Janssens, S., Buys, N., Wauters, J., Vanhaecke, L., & Aluwé, M. (2018). On farm intervention studies on reduction of boar taint prevalence: Feeding strategies, presence of gilts and time in lairage. *Research in Veterinary Science*, *118*(February), 508–516. https://doi.org/10.1016/j.rvsc.2018.05.008

Hobbs, P. J., Webb, J., Mottram, T. T., Grant, B., & Misselbrook, T. M. (2004). Emissions of volatile organic compounds originating from UK livestock agriculture. *Journal of the Science of Food and Agriculture*, *84*(11), 1414–1420. https://doi.org/10.1002/jsfa.1810

Hughes, D. T., Pelletier, J., Luetje, C. W., & Leal, W. S. (2010). Odorant receptor from the Southern House mosquito narrowly tuned to the oviposition attractant skatole. *Journal of Chemical Ecology*, *36*(8), 797–800. https://doi.org/10.1007/s10886-010-9828-9

Imafidon, G. I., & Spanier, A. M. (1994). Unraveling the secret of meat flavor. *Trends in Food Science and Technology*, *5*(10), 315–321. https://doi.org/10.1016/0924-2244(94)90182-1

Jayan, H., Pu, H., & Sun, D. W. (2020). Recent development in rapid detection techniques for microorganism activities in food matrices using bio-recognition: A review. *Trends in Food*

Science and Technology, *95*, 233–246. https://doi.org/10.1016/j.tifs.2019.11.007

Keller, A., Zhuang, H., Chi, Q., Vosshall, L. B., & Matsunami, H. (2007). Genetic variation in a human odorant receptor alters odour perception. *Nature*, *449*(7161), 468–472. https://doi.org/10.1038/nature06162

Kress, K., Weiler, U., Schmucker, S., Čandek-Potokar, M., Vrecl, M., Fazarinc, G., Škrlep, M., Batorek-Lukač, N., & Stefanski, V. (2020). Influence of housing conditions on reliability of immunocastration and consequences for growth performance of male pigs. *Animals*, *10*(1). https://doi.org/10.3390/ani10010027

Ladikos, D., & Lougovois, V. (1990). Lipid Oxidation in Muscle Foods: A Review. *Food Chemistry*, *35*, 295–314.

Liu, X., Schmidt, H., & Mörlein, D. (2016). Feasibility of boar taint classification using a portable Raman device. *Meat Science*, *116*, 133–139. https://doi.org/10.1016/j.meatsci.2016.02.015

Loutfi, A., Coradeschi, S., Mani, G. K., Shankar, P., & Rayappan, J. B. B. (2015). Electronic noses for food quality: A review. In *Journal of Food Engineering* (Vol. 144, pp. 103–111). Elsevier Ltd. https://doi.org/10.1016/j.jfoodeng.2014.07.019

Lundström, K., Matthews, K. R., & Haugen, J. E. (2009). Pig meat quality from entire males. *Animal*, *3*(11), 1497–1507. https://doi.org/10.1017/S1751731109990693

Manai, R., Habchi, M., Kamouni-Belghiti, D., Persuy, M. A., Rousseau, L., Possas Abreu, M., Grebert, D., Badonnel, K., Bergonzo, P., Pajot-Augy, E., Sanz, G., & Scorsone, E. (2017). Diamond micro-cantilevers as transducers for olfactory receptors - based biosensors: Application to the receptors M71 and OR7D4. *Sensors and Actuators, B: Chemical*, *238*, 1199–1206. https://doi.org/10.1016/j.snb.2016.07.013

Meiners, T., Wäckers, F., & Lewis, W. J. (2002). The effect of molecular structure on olfactory discrimination by the parasitoid Microplitis croceipes. In *Chemical Senses* (Vol. 27, Issue 9, pp. 811–816). https://doi.org/10.1093/chemse/27.9.811

Min, K., Jo, H., Song, K., Cho, M., Chun, Y. S., Jon, S., Kim, W. J., & Ban, C. (2011). Dual-aptamer-based delivery vehicle of doxorubicin to both PSMA (+) and PSMA (-) prostate cancers. *Biomaterials*, *32*(8), 2124–2132. https://doi.org/10.1016/j.biomaterials.2010.11.035

Mörlein, D., & Tholen, E. (2014). Fatty acid composition of subcutaneous adipose tissue from entire male pigs with extremely divergent levels of boar taint compounds - An exploratory study. *Meat Science*, *99*, 1–7. https://doi.org/10.1016/j.meatsci.2014.08.002

Mortensen, A. B., & Sørensen, S. E. (1984). *Relationship between boar taint and skatole determined with a new analysis method.* Proceedings of the 30th European meeting of meat research workers.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry*, *62*(4), 415–424. https://doi.org/10.1016/S0308-8146(98)00076-4

Ng, E. W. M., & Adamis, A. P. (2006). Anti-VEGF aptamer (pegaptanib) therapy for ocular vascular diseases. *Annals of the New York Academy of Sciences*, *1082*, 151–171. https://doi.org/10.1196/annals.1348.062

Olson, D., & Rains, G. (2014). Use of a parasitic wasp as a biosensor. *Biosensors*, *4*(2), 150–160. https://doi.org/10.3390/bios4020150

Olson, D., Rains, G. C., Meiners, T., Takasu, K., Tertuliano, M., Tumlinson, J. H., Wäckers, F. L., & Lewis, W. J. (2003). Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors. In *Chemical Senses* (Vol. 28, Issue 8, pp. 545–549). https://doi.org/10.1093/chemse/bjg071

Olson, D., Wäckers, F., & Haugen, J. E. (2012). Threshold Detection of Boar Taint Chemicals Using Parasitic Wasps. *Journal of Food Science*, *77*(10), 1–6. https://doi.org/10.1111/j.1750-3841.2012.02883.x

Paczkowski, S., Nicke, S., Ziegenhagen, H., & Schütz, S. (2014). Volatile emission of decomposing pig carcasses (Sus scrofa domesticus L.) as an indicator for the postmortem interval. *Journal of Forensic Sciences*, *60*(s1), S130–S137. https://doi.org/10.1111/1556-4029.12638

Paczkowski, S., WeiBbecker, B., Schöning, M. J., & Schütz, S. (2011). *Biologically-Inspired Systems - Chapter 12. Biosensors on the Basis of Insect Olfaction* (A. Vilcinskas (ed.)). Springer.

Patterson, R. L. S. (1968). 5α-androst-16-ene-3-one:—Compound responsible for taint in boar fat. *Journal of the Science of Food and Agriculture*, *19*(1), 31–38. https://doi.org/10.1002/jsfa.2740190107

Pelletier, J., Guidolin, A., Syed, Z., Cornel, A. J., & Leal, W. S. (2010). Knockdown of a mosquito odorant-binding protein involved in the sensitive detection of oviposition attractants. *Journal of Chemical Ecology*, *36*(3), 245–248. https://doi.org/10.1007/s10886-010-9762-x

Pelletier, J., Hughes, D. T., Luetje, C. W., & Leal, W. S. (2010). An odorant receptor from the Southern house mosquito Culex pipiens quinquefasciatus sensitive to oviposition attractants. *PLoS ONE*, *5*(4), 1–8. https://doi.org/10.1371/journal.pone.0010090

Pelosi, P., Mastrogiacomo, R., Iovinella, I., Tuccori, E., & Persaud, K. C. (2014). Structure and biotechnological applications of odorant-binding proteins. In *Applied Microbiology and Biotechnology* (Vol. 98, Issue 1, pp. 61–70). https://doi.org/10.1007/s00253-013-5383-y

Peris, M., & Escuder-Gilabert, L. (2016). Electronic noses and tongues to assess food authenticity and adulteration. *Trends in Food Science and Technology*, *58*, 40–54. https://doi.org/10.1016/j.tifs.2016.10.014

Schäfer, K. C., Dénes, J., Albrecht, K., Szaniszló, T., Balogh, J., Skoumal, R., Katona, M., Tóth, M., Balogh, L., & Takáts, Z. (2009). In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. *Angewandte Chemie - International Edition*, *48*(44), 8240–8242. https://doi.org/10.1002/anie.200902546

Schiffman, S. S., Bennett, J. L., & Raymer, J. H. (2001). Quantification of odors and odorants from swine operations in North Carolina. *Agricultural and Forest Meteorology*, *108*(3), 213–240. https://doi.org/10.1016/S0168-1923(01)00239-8

Schott, M., Wehrenfennig, C., Gasch, T., & Vilcinskas, A. (2014). Insect Antenna-Based Biosensors for In Situ Detection of Volatiles. *Advances in Biochemical Engineering/Biotechnology*. https://doi.org/10.1007/10

Škrlep, M., Tomašević, I., Mörlein, D., Novaković, S., Egea, M., Garrido, M. D., Linares, M. B., Peñaranda, I., Aluwé, M., & Font-i-Furnols, M. (2020). The Use of Pork from Entire Male and Immunocastrated Pigs for Meat Products—An Overview with Recommendations. *Animals*, *10*(10), 1–26. https://doi.org/10.3390/ani10101754

Sørensen, K. M., & Engelsen, S. B. (2014). Measurement of boar taint in porcine fat using a high-throughput gas chromatography-mass spectrometry protocol. *Journal of Agricultural and Food Chemistry*, *62*(39), 9420–9427. https://doi.org/10.1021/jf5022785

Sørensen, K. M., Westley, C., Goodacre, R., & Engelsen, S. B. (2015). Simultaneous quantification of the boar-taint compounds skatole and androstenone by surface-enhanced Raman scattering (SERS) and multivariate data analysis. *Analytical and Bioanalytical Chemistry*, *407*(25), 7787–7795. https://doi.org/10.1007/s00216-015-8945-2

Støier, S. (2019). *A new instrumental boar taint detection method*. *October*. https://www.boarsontheway.com/wp-content/uploads/2019/11/Boar-taint-detection-02102019-

aangepast.pdf

Thoelen, R., Vansweevelt, R., Duchateau, J., Horemans, F., D'Haen, J., Lutsen, L., Vanderzande, D., Ameloot, M., vandeVen, M., Cleij, T. J., & Wagner, P. (2008). A MIP-based impedimetric sensor for the detection of low-MW molecules. *Biosensors and Bioelectronics*, *23*(6), 913–918. https://doi.org/10.1016/j.bios.2007.08.020

Trautmann, J. (2016). *Sensory Quality Control of Boar Taint*. Ph.D. Thesis, Georg-August-University Göttingen, Göttingen, Germany.

Trautmann, J., Gertheiss, J., Wicke, M., & Mörlein, D. (2014). How olfactory acuity affects the sensory assessment of boar fat: A proposal for quantification. *Meat Science*, *98*(2), 255–262. https://doi.org/10.1016/j.meatsci.2014.05.037

Turiel, E., & Martín-Esteban, A. (2010). Molecularly imprinted polymers for sample preparation: A review. *Analytica Chimica Acta*, *668*(2), 87–99. https://doi.org/10.1016/j.aca.2010.04.019

van Son, M., Kent, M. P., Grove, H., Agarwal, R., Hamland, H., Lien, S., & Grindflek, E. (2017). Fine mapping of a QTL affecting levels of skatole on pig chromosome 7. *BMC Genetics*, *18*(1), 1–9. https://doi.org/10.1186/s12863-017-0549-8

Vergara, A., Vembu, S., Ayhan, T., Ryan, M. A., Homer, M. L., & Huerta, R. (2012). Chemical gas sensor drift compensation using classifier ensembles. *Sensors and Actuators, B: Chemical*, *166–167*, 320–329. https://doi.org/10.1016/j.snb.2012.01.074

Verplanken, K. (2018). *Valorisation of boar meat and analytical approaches for the fast detection of boar taint at the slaughter line*. Ph.D. Thesis, Ghent University, Ghent, Belgium.

Verplanken, K., Stead, S., Jandova, R., Poucke, C. Van, Claereboudt, J., Bussche, J. Vanden, Saeger, S. De, Takats, Z., Wauters, J., & Vanhaecke, L. (2017). Rapid evaporative ionization mass spectrometry for high-throughput screening in food analysis: The case of boar taint. *Talanta*, *169*(February), 30–36. https://doi.org/10.1016/j.talanta.2017.03.056

Verplanken, K., Wauters, J., Van Durme, J., Claus, D., Vercammen, J., De Saeger, S., & Vanhaecke, L. (2016). Rapid method for the simultaneous detection of boar taint compounds by means of solid phase microextraction coupled to gas chromatography/mass spectrometry. *Journal of Chromatography A*, *1462*, 124–133. https://doi.org/10.1016/j.chroma.2016.07.077

Vestergaard, J. S., Haugen, J. E., & Byrne, D. V. (2006). Application of an electronic nose for measurements of boar taint in entire male pigs. *Meat Science*, *74*(3), 564–577. https://doi.org/10.1016/j.meatsci.2006.05.005

Vold. (1970). Fleischproduktionseigenschaftenbei Ebernund Kastraten IV: Organoleptische und gaschromatographische Untersuchungen wasserdampfflüch- tiger Stoffe des Rücken-speckes von Ebern. *Meldinger Fra Norges Landbrugshøgskole*, *49*, 1–25.

Wäckers, F., Olson, D., Rains, G., Lundby, F., & Haugen, J. E. (2011). Boar Taint Detection Using Parasitoid Biosensors. *Journal of Food Science*, *76*(1). https://doi.org/10.1111/j.1750-3841.2010.01887.x

Weber, P., Riegger, B. R., Niedergall, K., Tovar, G. E. M., Bach, M., & Gauglitz, G. (2018). Nano-MIP based sensor for penicillin G: Sensitive layer and analytical validation. *Sensors and Actuators, B: Chemical*, *267*, 26–33. https://doi.org/10.1016/j.snb.2018.03.142

Wesoly, R., & Weiler, U. (2012). Nutritional influences on skatole formation and skatole metabolism in the pig. *Animals*, *2*(2), 221–242. https://doi.org/10.3390/ani2020221

Westmacott, K. L., Crew, A. P., Doran, O., & Hart, J. P. (2020). 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. *Biosensors and*

Bioelectronics, *150*(August), 111837. https://doi.org/10.1016/j.bios.2019.111837

Wojnowski, W., Majchrzak, T., Dymerski, T., & Jacek, G. (2017). Electronic noses : Powerful tools in meat quality assessment. *Meat Science*, *131*(April), 119–131. https://doi.org/10.1016/j.meatsci.2017.04.240

Yaseen, T., Sun, D. W., & Cheng, J. H. (2017). Raman imaging for food quality and safety evaluation: Fundamentals and applications. *Trends in Food Science and Technology*, *62*, 177–189. https://doi.org/10.1016/j.tifs.2017.01.012

Zadinová, K., Stupka, R., Stratil, A., Čítek, J., Vehovský, K., Lebedová, N., Šprysl, M., & Okrouhlá, M. (2017). Association analysis of SNPs in the porcine CYP2E1 gene with skatole, indole, and androstenone levels in backfat of a crossbred pig population. *Meat Science*, *131*(November 2016), 68–73. https://doi.org/10.1016/j.meatsci.2017.04.236

Zamora, R., & Hidalgo, F. J. (2011). The Maillard reaction and lipid oxidation. *Lipid Technology*, *23*(3), 59–62. https://doi.org/10.1002/lite.201100094

Zang, M., Chen, H., Wang, L., Zhang, Z., Zhang, K., Li, D., Li, X., & Wang, S. (2020). *Original article Changes in flavour compound profiles of precooked pork after reheating (warmed-over flavour) using gas chromatography – olfactometry – mass spectrometry with chromatographic feature extraction*. 978–987. https://doi.org/10.1111/ijfs.14306

Zhang, X., Cheng, J., Wu, L., Mei, Y., Jaffrezic-Renault, N., & Guo, Z. (2018). An overview of an artificial nose system. In *Talanta* (Vol. 184, Issue February, pp. 93–102). https://doi.org/10.1016/j.talanta.2018.02.113

Ziyatdinov, A., Marco, S., Chaudry, A., Persaud, K., Caminal, P., & Perera, A. (2010). Drift compensation of gas sensor array data by common principal component analysis. *Sensors and Actuators, B: Chemical*, *146*(2), 460–465. https://doi.org/10.1016/j.snb.2009.11.034