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Rapid method for the simultaneous detection of boar taint compounds by means of solid phase microextraction coupled to gas chromatography/mass spectrometry☆

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ABSTRACT

Because of animal welfare issues, the voluntary ban on surgical castration of male piglets, starting January 2018 was announced in a European Treaty. One viable alternative is the fattening of entire male pigs. However, this can cause negative consumer reactions due to the occurrence of boar taint and possibly lead to severe economic losses in pig husbandry. In this study, headspace solid phase microextraction (HS-SPME) coupled to GC–MS was used in the development and optimization of a candidate method for fast and accurate detection of the boar taint compounds. Remarkably fast extraction (45 s) of the boar taint compounds from adipose tissue was achieved by singeing the fat with a soldering iron while released volatiles were extracted in-situ using HS-SPME. The obtained method showed good performance characteristics after validation according to CD 2002/657/EC and ISO/IEC 17025 guidelines. Moreover, cross-validation with an in-house UHPLC-HR-Orbitrap-MS method showed good agreement between an in-laboratory method and the new candidate method for the fast extraction and detection of skatole and androstenone, which emphasizes the accuracy of this new SPME-GC–MS method. Threshold detection of the boar taint compounds on a portable GC–MS could not be achieved. However, despite the lack of sensitivity obtained on the latter instrument, a very fast method with run-to-run time of 3.5 min for the detection of the boar taint compounds was developed.

1. Introduction

During the past decades, the awareness on animal welfare has significantly increased. As a consequence, in 2010 a voluntary abandonment on surgical castration of pigs, beginning January 2018, was announced in a European treaty [1]. One viable alternative for the surgical castration of pigs is the production of entire males. However, the main set-back in the raise of entire males is the possible occurrence of boar taint, an unpleasant odour caused by the release of androstenone (AEON), skatole (SK), and indole (IND) when meat of boars is heated [2]. As a consequence boar taint provokes negative consumer reactions [3,4], which can cause severe economic losses in pig husbandry [5]. Since currently, apart from castration, no strategies for complete elimination of boar taint are available, there is a need for detection of boar taint at the slaughter line. Indeed, adequate at-line detection of the boar taint compounds makes it possible to identify tainted carcasses and thus prevent negative consumer reactions [6].

A large number of in-laboratory methods for the simultaneous detection of IND, SK and AEON have already been developed [7–12]. These methods require sampling and are characterized by excessive sample pre-treatment followed by relatively long analysis times. Consequently, these methods cannot achieve the high-throughput needed at the slaughter line. Over the past years research has been devoted to the development of boar taint detection methods applicable at the slaughter line. Sensory methods such as the

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soldering iron method, directly applicable at-line [13,14], are widely practiced and provide a fast and holistic detection of boar taint. However, results provided with these methods are relying on the sensory score of one trained assessor [15]. Moreover, other bottlenecks such as habituation, fatigue, and inter-individual variation were recently revealed [14].

The use of other techniques such as parasitic biosensors [16,17], chemical sensor array technology [18,19], colorimetric analysis [20], direct MS [21], and gas-phase spectrometry [21] show great potential as applications in a slaughterhouse environment. However, sensitivity and specificity obtained with these techniques remain questionable [22]. Moreover, these methods are often poorly validated, lack at- line but more importantly do not meet the industrial necessities with regard to analysis time and automation capability [22].

Recently, Sørensen et al. used surface-enhanced Raman scattering combined with multivariate data analysis for targeted quantification of the boar taint compounds [23]. This method shows great potential for optimization into an on-line application with regard to low equipment cost, acquisition time and portability. However, extensive extraction taking at least 60 min per sample remains necessary, which limits its possibilities for at-line implementation. Moreover, the observed prediction errors amounted to 87% and 352% around the odour threshold for SK and AEON, respectively, which hampers accurate quantification of the boar taint compounds.

The use of liquid or gas chromatography combined with mass spectrometry has proven to be a powerful tool for highly accurate in-laboratory detection of boar taint in adipose tissue [8–10,12]. More recently, a high-throughput gas chromatography-mass spectrometry protocol with the possibility for automation was developed [24]. This method provides a quantitative result from the first carcass within 24 min followed by results from sequential carcasses every 6 min. Although the method requires sampling of back fat and as a consequence cannot be applied at-line, GC–MS has proven to be a powerful tool for fast and accurate analysis of the boar taint compounds. Use of a portable GC–MS instrument combined with headspace solid phase microextraction (HS-SPME) would allow an even higher throughput and would eliminate the current time-consuming sampling procedures. In this study a HS-SPME extraction protocol was developed and was tested on a person-portable GC–MS for the rapid detection of the three known boar taint compounds in neck fat. Sample extraction was optimized using a D-optimal and central composite face-centred (CCF) design. Afterwards, the method was validated according to the CD 2002/657/EC and ISO/IEC 17025 guidelines [25,26]. Finally, the fast HS-SPME-GC–MS method was cross-validated with an in-laboratory UHPLC-HR-Orbitrap-MS method [10] and transferred to a portable GC–MS instrument.

2. Materials and methods

2.1. Reagents and chemicals

The reference standards IND (2,3-benzopyrrole, CAS 120-72-9), SK (3-methylindole, CAS 83-34-1) and AEON (5α-androst-16-ene-3-one, CAS 18339-16-7) and the internal standards 2-methylindole (2-MID, CAS 95-20-5) and androstadienedione (1,4-androstadiene-3,17-dione, ADD, CAS 897-06-3) were obtained from Sigma Aldrich (St. Louis, MO, USA). For each compound a stock solution was prepared in methanol at a concentration of 1 mg ml^{-1}. Solutions were stored in dark glass bottles at −20 °C. Reagents were of analytical grade and were obtained from VWR International (Merck, Darmstadt, Germany). SPME fibres were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Samples

Nine boar taint positive carcasses were selected at the slaughter line by means of the soldering iron method optimized by Bekaert et al. and a neck fat sample was taken [14]. All samples were cooled during transport to the lab and were immediately stored upon arrival at −20 °C until analysis.

2.3. Sample pre-treatment optimization

2.3.1. SPME fibre selection

In this study four different SPME fibres (polydimethylsiloxane (PDMS) 100 μm, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm, carboxen/polydimethylsiloxane (CAR/PDMS) 75 μm, polyacrylate (PA) 85 μm) for the extraction of IND, SK and AEON from fat were compared by building equilibration curves for each fibre. In order to imitate adipose tissue as a sample matrix, corn oil was used during SPME fibre selection. To this end, 2 g of corn oil was spiked with IND, SK, and AEON at 1 μg g^{-1}, 2 μg g^{-1}, and 10 μg g^{-1}, respectively. Prior to extraction, corn oil solutions were equilibrated at 80 °C for 2 min. Extraction of the boar taint compounds on the SPME fibres was carried out in a headspace volume of 20 ml at 80 °C for 1, 5, 15, 30 and 60 min. Repeatability (n = 3) of each fibre was evaluated at an extraction time of 15 min. All analysis were conducted with an MPS® autosampler with headspace-solid phase microextraction unit.

2.3.2. D-optimal and central composite face-centered (CCF) design

In order to select the best matrix for optimization of the HS-SPME protocol, a comparison between corn oil solutions, blank neck fat of sow carcasses containing little or no background levels of the boar taint compounds, and boar neck fat samples was made. To this end 2 g of corn oil or blank neck fat (minced or not-minced) were fortified at 100 μg kg^{-1}, 200 μg kg^{-1}, and 500 μg kg^{-1} by addition of 100 μl of a standard solution containing 2, 4 and 10 ng μl^{-1} of IND, SK, and AEON, respectively. After fortification, the samples were left at room temperature for 30 min to allow distribution of the added compounds into the matrix. Boar neck fat with comparable levels of boar taint compounds (IND: 88.4 μg kg^{-1}, SK: 108 μg kg^{-1}; AEON: 588 μg kg^{-1}) was selected and quantified with a validated in-house method [10]. All samples were then subjected to HS-SPME extraction. Extraction occurred in a headspace volume of 20 ml and a DVB/PDMS 65 μm fibre was used. The samples (2 g) were not equilibrated and were extracted for 2 min at 200 °C. Afterwards, the SPME fibre was immediately injected into the GC–MS. All analyses were conducted in triplicate with an MPS® autosampler with headspace-solid phase microextraction unit.

Because of the complex nature of extraction of IND, SK and AEON from adipose tissue due to the relatively low volatility of the compounds, further optimization occurred by using an experimental design. Based on literature, different variables (extraction time, extraction temperature, sample size, headspace volume, desorption time and fibre type) that may significantly influence the extraction were selected, taking into account the relevance of each variable in an at-line environment. All variables were screened in a D-optimal design including 3 centre points and a total of 19 runs. The obtained areas of the chromatographic peaks for IND, SK, and AEON were used to generate response surface plots. During execution of the D-optimal design, extraction of the samples was carried out in a confined environment using headspace vials. The samples were not equilibrated prior to extraction and were heated using an oven tray, while exposed to the SPME fibre, in order to achieve the required extraction temperature. Afterwards, the SPME fibre was immediately injected into the GC–MS for analysis. Ranges of all