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ORIGINAL PAPER

Screening of fungi from the phylum Basidiomycota for degradation of boar taint aroma compounds

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Abstract

Wood-degrading fungi and enzyme preparations derived thereof were identified to degrade boar taint compounds. Fungal strains ($n = 27$) were analytically and sensorially screened for skatole (SK) and androstenone (AEON) degradation in head space (HS) vials and agar plates, respectively. Eight strains were able to reduce > 80% of AEON and > 80% of SK intensities. Three enzyme fractions (extracellular, intracellular, and mycelial) obtained from submerged cultures of *Cerrena zonata*, *Irpex lacteus*, *Marasmius cohortalis* (MCO), and *Trametes hirsuta* were used for SK and AEON bioconversion. Several enzyme fractions were able to reduce 90–100% of SK/AEON concentrations in the reaction mixtures based on the volatile analysis. MCO extracellular enzyme fractions (EEF) and mycelial enzyme fractions (MEF) were able to completely abolish both compounds and were therefore used for a sensory 2-alternative forced choice discrimination test in parallel to quantitative analysis. HS-SPME–GC–MS results demonstrated that active EEF and active MEF reduced > 98% SK and AEON intensities individually and > 92% in the SK/AEON mixture. Discrimination tests with trained panelists revealed that samples were perceived significantly less intense regarding SK, AEON, and SK/AEON than the control samples with *d'* values (a discrimination value) of 1.19, 0.74, and 0.72 for active EEF and 2.12, 0.88, and 2.12 for active MEF respectively, which were well in line with the analytical results. This indicates that both fractions (EEF and MEF) were effective in the boar taint reduction in aqueous solutions. The study revealed that Basidiomycota or enzymes derived thereof may become interesting tools to remove boar taint compounds.

Keywords Boar taint compounds · Skatole · Androstenone · Basidiomycota · Biodegradation

Introduction

Boar taint is described as having a sweaty, urine-like and/or fecal odor (orthonasal and retronasal), which may cause discomfort to pork meat consumers [1]. According to several authors, skatole (SK) and androstenone (AEON) are considered to represent the main drivers of boar taint odor, although the occurrence and perception of boar taint compounds (BTCs) are rather complex and not fully understood to date [2, 3]. If male piglets are not castrated, AEON is produced in the testicles at the onset of sexual maturity and, if not metabolized, accumulates in adipose tissue [4]. SK is not sex specific, i.e., it can also occur in female pigs, and is caused by microbial degradation of tryptophan in the large intestine [5, 6]. There is an evidence that elevated AEON level inhibits the metabolism of SK (and thus the excretion), so that in general higher levels of SK are observed in boars [7]. In addition to AEON and SK, further substances have been identified to contribute to odor abnormalities in boar meat such as indole, 2′-aminoacetophenone and 3α- and 3β-androstenol [8, 9]. A significant interaction of AEON and SK in the perception of odor abnormalities in boar meat was demonstrated, but it is necessary to determine the relationship between the level of boar odor compounds and

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consumer acceptance [10]. Boar taint elicits negative consumer responses, which can lead to severe economic losses in the pork industry [11–13]. According to the current legislation, pork with a "pronounced sexual odor" is classified as unfit for human consumption by the European Union regulation (EC 854/2004) [14]. To avoid losses in the meat processing industry due to boar taint, various approaches have been tested, such as breeding programs, feeding strategies, and physical or chemical castration [5, 15]. So far, the most common method of preventing boar taint is surgical castration, which is, however, considered critical in light of animal welfare, especially when applied without pain relief. Castration without anesthesia is no longer allowed in Germany since 01/01/2021 [16]. In addition, other strategies have been identified as effective for masking boar taint, such as dilution, smoking, masking, fermentation, or curing and drying. However, these approaches are only suitable for certain cuts and it was therefore suggested to combine them. For example, a combination of smoking and spicing was able to eliminate the perception of androstenone in Frankfurters manufactured from entire pigs with high levels of androstenone [17]. Nevertheless, there are limitations to such masking strategies to ensure that the products still meet consumers' sensory expectations. Thus, it is a major challenge to find alternative solutions that meet the current needs of the pork industry [18].

Basidiomycota are known to be extremely diverse and have interesting metabolic and physiological properties that could be well suited for biodegradation or transformation of low molecular mass molecules such as SK and AEON [19, 20]. Basidiomycota produce a plethora of extra- and intracellular enzymes, which have a broad range of biotechnological applications. The majority of enzymes capable of degradation of complex molecules, such as polycyclic aromatic hydrocarbons, dyes, active pharmaceutical ingredients (APIs), or pesticides, are involved in lignin degradation (manganese peroxidases [MnP], lignin peroxidases [LiP], versatile peroxidases [VP], aryl alcohol oxidases, peroxygenases, laccases, and dye decolorizing peroxidases [DyP]) [20–23]. Several white-rot fungi, such as *Phanerochaete chrysosporium*, *Phlebia ochraceofulva*, *Pycnoporus sanguineus*, *Pleurotus ostreatus*, and *Trametes versicolor,* have been used to degrade a variety of xenobiotic compounds [21, 24]. More recently, Liu et al. [25] demonstrated an efficient removal of β-estradiol (up to 80%) by laccases from *Pycnoporus* sp., *T. versicolor*, and *Hymenochaete spreta* from solid medium (poultry litter). Moreover, an intracellular system has been described for transforming different xenobiotics, e.g., by the cytochrome P450 family [20, 26]. Moreover, Baeyer–Villiger monooxygenases (BVMOs) have been found to oxidize substrates such as steroids, alkanes, and cyclic ketones [27]. Furthermore, fungi are able to produce extracellular biosurfactants (sophorolipids, protein–lipid/polysaccharide complexes, glycolipids, and glycolipoproteins) with hydrophilic and hydrophobic moieties that facilitate the interaction between hydrophobic substrates and rather hydrophilic extracellular fungal enzymes [28, 29]. Apart from that, the removal of compounds may also be triggered by adsorption phenomena mediated by the specific composition of the fungal cell wall [20, 30].

Taken together, the application of wood-degrading fungi or their enzymes to remove or reduce the impact of boar odor compounds during the production of meat products may become an innovative tool. Therefore, this study focused on (1) the screening of 27 fungal strains for degradation of SK and AEON, (2) the testing of different enzyme fractions (extracellular, intracellular, and mycelial), and (3) the analytical quantification of compounds, alongside sensory evaluation by a trained sensory panel. More specifically, the following hypothesis was addressed: blending of enzymes or biomass (mycelia) derived from Basidiomycota may significantly mask and/or reduce the perception of skatole and androstenone in an aqueous reaction mixture.

Materials and methods

Microorganisms

The Basidiomycota used in this work were supplied by the Centraalbureau voor Schimmelcultures (CBS, Baarn, Netherlands), the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Braunschweig, Germany), Yoichi Honda of the Kyoto University, Han Wösten of the University of Utrecht, the Friedrich Schiller University Jena (FSU), the company Mycelia NV (Deinze, Belgium), and the culture collection of the Justus Liebig University Giessen (LCB). Table 1 summarizes the fungal strains used in this work.

The stock cultures of the fungi were maintained on solid malt extract–peptone agar (MPA) medium containing 30 g/L malt extract (Fluka, Neu-Ulm, Germany), 3 g/L soy peptone (Fluka), and 15 g/L agar–agar (Roth, Karlsruhe, Germany). Strains were incubated at 24 °C for 6 days in darkness and then kept at 4 °C for further use.

Screening for skatole and androstenone degradation: analytics and sensory evaluation

A total of 27 mushrooms (Table 1) were used to identify suitable candidate strains degrading the key BTCs SK and AEON. Screening experiments were performed in headspace (HS) vials (Th. Geyer, Renningen, Germany) and on agar plates (Greiner Bio-One, Frickenhausen, Germany) containing a reduced malt extract–peptone (MEPR) culture medium supplemented with a mixture of SK (98% purity,

Table 1 List of screened Basidiomycota strains

Basidiomycota	Short name	Origin	Collection number
Bjerkandera adusta	BAD	DSMZ	4708
Phanerochaete chrysosporium	PCH	FSU	L 13–2
Lentinula edodes	LED	CBS	389.89
Laetiporus sulphureus	LSU	DSMZ	1014
Fomitopsis pinicola	FPI-D	DSMZ	3521
Marasmius cohortalis	MCO	DSMZ	8257
Hypsizygus elongatipes	HEL	DSMZ	8292
Trametes hirsuta	THI	DSMZ	5241
Ganoderma anullaris	GAN	DSMZ	9943
Pleurotus eryngii	PER	DSMZ	8264
Cerrena zonata (formerly known as *Irpex consors*)	CZO	DSMZ	7382
Hericium coralloides	HCO	CBS	107,488
Polyporus umbellatus	PUM	CBS	483.72
Punctularia atropurpurascens	PAT	CBS	388.66
Psathyrella conopilus	PSCO	CBS	164.72
Armillaria mellea	AMEL	DSMZ	2941
Pleurotus ostreatus	POS	Yoichi Honda	
Fomitopsis pinicola	FPI-F	LCB	
Gymnopilus penetrans	GPE	LCB	
Stropharia rugosoannulata	M5012	Mycelia	M 5012
Peniophora lycii	PLY-FP	LCB	
Stropharia rugosoannulata	SRU	Mycelia	M 5012
Morchella esculenta	MES	DSMZ	10,370
Ganoderma lucidum	GLU	DSMZ	3515
Irpex lacteus	ILA	CBS	431.48
Flammulina velutipes	FVE-D	DSMZ	1658
Schizophyllum commune H4-8	SCH	Han Wösten	

Acros Organics, Geel, Belgien) and AEON (100%, Santa Cruz Biotechnology Inc., Heidelberg, Germany). MEPR medium composition: 3 g/L malt extract, 0.3 g/L soy peptone, 2.37 g/L NH_4NO_3, 1.5 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7\ H_2O$, and 1 mL/L trace elements solution (0.08 g/L $FeCl_3 \cdot 6\ H_2O$, 0.09 g/L $ZnSO_4 \cdot 7\ H_2O$, 0.03 g/L $MnSO_4 \cdot H_2O$, 0.005 g/L $CuSO_4 \cdot 5\ H_2O$, and 0.4 g/L EDTA), pH 6.0. The chemicals were purchased as follows: potassium dihydrogenphosphate (99.5%) by Th. Geyer, magnesium sulfate heptahydrate by Roth, ferric chloride hexahydrate, zinc sulfate heptahydrate, manganese(II) sulfate monohydrate, Ethylenediaminetetraacetic acid by AppliChem (Darmstadt, Germany), and copper sulfate pentahydrate by Acros Organics.

Reduction of SK/AEON concentrations was measured in the gas phase by solid-phase microextraction-(SPME)–GC–MS after 7 days of cultivation (as described below) in HS vials. First, HS vials were filled with MEPR (4 mL) and supplemented with SK/AEON solutions (mixture of either both compounds or individual, 40 µL of 0.3 µg/mL SK and 4.5 µg/mL AEON in ethanol with min. 99.8% by Roth). After solidification, the HS vials were inoculated with a 0.5 mm^2 agar plug of the respective fungus of a 7-day-old pre-culture agar plate (Table 1). Two types of control samples were prepared as follows: MEPR supplemented with SK/AEON mixture (without fungus) and MEPR inoculated with the respective fungus (without SK/AEON). Samples and controls were statically incubated for 7 days at 24 °C. Afterward, the HS vials were placed directly on the autosampler of the HS-SPME–GC–MS and analyzed. All experiments were performed in triplicate.

Moreover, reduction of SK/AEON perception was sensory evaluated on agar plates by a trained panel after 7 days of culture. Petri dishes were filled with MEPR (5 mL) and supplemented with SK/AEON solution mixture (50 µL of 0.3 µg/mL SK and 4.5 µg/mL AEON in ethanol). The plates were inoculated with a 0.5 mm^2 agar plug of the respective fungus of a 7-day-old pre-culture (Table 1). The respective control samples were prepared as follows: MEPR plus SK/AEON mixture and MEPR plus fungus. Samples and controls were incubated for 7 days at 24 °C. Thereafter, the cultures were evaluated for odor intensity by a trained panel (as described in the "Sensory evaluation on agar plates").

All sensory evaluation experiments using the fungal strains on agar plates were performed in triplicate.

Strains with profound reduction of SK/AEON perception based on the analytical (> 80%) and the sensory evaluation (< 7.5) on HS vials and agar plates, respectively, were selected for further experiments.

Headspace solid phase microextraction (HS-SPME)–GC–MS

SK and AEON degradation was investigated by means of HS-SPME–GC–MS, using a 65 µm polydimethylsiloxane/divinylbenzene fiber (PDMS/DVB; Supelco, Bellefonte, PA, USA). Sealed HS vials (samples and controls) after 7 days of culture were directly placed on an MPS 2XL multipurpose sampler (GERSTEL, Mülheim an der Ruhr, Germany), operating with a heated agitator and an SPME assembly. Compounds were desorbed in the inlet (SPME liner, 0.75 mm i.d.; Supelco) at 250 °C for 1 min of an Agilent Technologies 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with an Agilent VF-WAXms column (Agilent Technologies; 30 m × 0.25 mm i.d., 0.25 µm film thickness) and connected to an Agilent 5975C MSD Triple-Axis mass spectrometer. Helium [5.0, purchased from Praxair (Düsseldorf, Germany)] was used as a carrier gas with a constant flow rate of 1.2 mL × min^{-1}.

The conditions for HS-SPME sampling were as follows: the fiber was equilibrated for 5 min at 60 °C followed by extraction for 20 min at 60 °C with agitation. Then, the SPME fiber was inserted into the injector of the gas chromatograph for thermal desorption in splitless mode for 7 min, with the injector temperature held at 250 °C. The GC oven temperature was programmed to ramp from 80 °C (3 min) to 240 °C (9 min) at 5 °C × min^{-1}. Mass spectra were acquired in scan mode with a range of *m/z* 33–300. Ionization was performed by electron impact at 70 eV with an ion source temperature set to 230 °C. SK and AEON were identified initially by comparing mass spectra obtained with data from the NIST14 database (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the use of authentic reference compounds as standards. Furthermore, retention indices were determined with a C_7–C_{30} alkane mix and were compared to the published data and to those of the authentic reference compounds.

Sensory evaluation on agar plates

Sensory analyses were conducted by five panelists from the Institute of Food Chemistry and Food Biotechnology of Justus Liebig University Giessen. Prior to the sensory analyses, the panel was trained for the sensory intensity description of SK and AEON using serial dilutions (1:100, 1:1000, and 1:10,000) of SK (0.3 µg/mL) and AEON (4.5 µg/mL) on plates containing MEPR–agar (individual and in combination); five replicates were used for each compound and dilution step. The panelists were asked to rate the odor intensity of the compounds on a ten-point scale (0 = not perceptible to 10 = very intense) [31]. A plate containing the highest concentration of the compound/s (10) and a plate containing MEPR (0) were used as references. Experiments were conducted in an adequately designed, ventilated, and lighted room. A trained panel evaluated the intensity of the SK/AEON odor on plates after 7 days of culture in control and samples (in triplicates). These sensory analyses revealed whether the strains were able to decrease the odor intensities of SK/AEON in the samples and to compare their performance with the results of GC analyses.

Bioconversion of skatole and androstenone in HS vials by different enzyme fractions

Based on the screening experiments, strains able to degrade SK and AEON were used for the next steps: submerged cultivation, preparation of enzyme fractions, bioconversion tests, and sensory evaluation of bioconversion in HS vials.

Preparation of enzyme fractions

Strains were cultivated submerged to produce sufficient amounts of biomass and enzymes. The culture medium (MP) was prepared by dissolving malt extract (30 g) and soy peptone (3 g) in 1 L of deionized water. The pH was adjusted to 6.0 with 1.0 M KOH (Roth) prior to autoclaving (Laboklav 160, SHP, Helmbrechts, Germany). For preparation of the precultures, a 1 cm^2 agar plug from the leading mycelial edge from a MPA plate was transferred into 100 mL MP medium and then homogenized with a T 25 digital Ultra-Turrax homogenizer (IKA, Staufen, Germany; 30 s, 10,000 rpm). The precultures were grown on an incubation shaker (Orbitron, Infors HAT, Bottmingen, Switzerland; 150 rpm, deflection 25 mm) in darkness at 24 °C for 7 days. Afterward, the precultures were homogenized, and 10% (*v*/*v*) of the homogenate were inoculated for submerged cultivation into a 2-L Erlenmeyer flask containing 800 mL medium (main culture). Cultures were incubated and grown for 7 days as mentioned before. Then, the mycelia (pellets) were separated from the supernatant by centrifugation (3300*g*, 10 min, Allegra X-15R, Beckman Coulter, Brea, CA, USA), washed twice with deionized water, freeze-dried (freeze dryer christ alpha 1–2, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany), and kept at − 20 °C. The separated culture supernatants were lyophilized and represent the extracellular enzyme fractions (EEF) containing secreted enzymes. The lyophilized mycelia (lyo-myc) represent the intracellular and mycelium-bound enzyme fraction (MEF) as well as water-insoluble cell wall

constituents (like chitin, β-glucans) and fungal lipids [32]. Moreover, the lyo-myc was used to isolate the intracellular enzyme fraction (IEF) by the use of a laboratory bead mill (Dyno Mill Research Lab, Wab Group, Switzerland). Therefore, 5 g of lyo-myc was mixed with 200 mL of sodium tartrate buffer (100 mM, pH 4.5 from potassium sodium tartrate, Roth) and glass beads (Ø 0.25–0.5 mm, 55 mL, Roth) followed by grinding for 10 min at 4 °C. Afterward, the insoluble parts were removed by centrifugation (3300*g*, 10 min) and the supernatant was lyophilized.

Bioconversion experiments

Bioconversion experiments were carried out by using the fractions separately. Therefore, either up to 160 mg EEF, 100 mg IEF, or up to 100 mg MEF of selected strains were placed into HS vials containing 4 mL of sodium tartrate buffer (100 mM, pH 4.5) supplemented with 40 µL of SK/AEON solutions (individual or as mixture 0.3 µg/mL SK and 0.45 µg/mL AEON in ethanol). The HS vials were placed in a rotor (Stuart SB3, Antylia Scientific, Vernon Hills, IL, USA, 20 rpm) overnight at 24 °C in darkness. An HS vial containing only buffer supplemented with SK/AEON solution was used as control. In addition, a set of deactivated enzyme fractions was prepared by heating (95 °C for 30 min, water bath WB20, P-D Industriegesellschaft, Dresden, Germany) the HS vials containing the enzyme fractions and the buffer. After cooling to room temperature, the samples were supplemented with the SK/AEON solution mixture and placed in a rotor (20 rpm, 24 °C in darkness) overnight. All experiments were performed in triplicate. The controls, active, and deactivated (D) samples were analyzed by HS-SPME–GC–MS as described above. The degradation of SK/AEON was calculated based on the peak areas of the control sample chromatograms (100%) compared to the peak area of SK or AEON of the samples treated with the enzyme fractions (active or deactivated) and expressed as relative percentages (Supplementary information S1). The best performing set of enzyme fractions were used for sensory evaluation by a trained panel.

Sensory evaluation of bioconversion reactions in HS vials

A sensory analysis was carried out using the best combination of strain and enzyme fractions to compare the effect on the boar taint odor degradation (individual (SK, AEON) or SK/AEON mixture) using a trained panel at the Department of Animal Sciences from the University of Göttingen. The panel consisted of overall seven assessors (five female and two male) sensitive to AEON and SK except one assessor who was partially anosmic to AEON. The panelists had background training and experience ranging between 7 months to 2 years and 4 months working with AEON and SK. Panelist screening and training was done using various dilutions of AEON and SK based on [33]. The training and evaluation sessions took place twice a week, Tuesday and Thursday, for an average time of 1.5–2 h per session (a day). The number of panelists varies throughout the paper as the experiments were conducted over several months so occasionally individual panel members were absent due to, e.g., sickness. Plus, when only androstenone was to be evaluated, one partially anosmic panelist was excluded from the data analysis.

For sensory evaluation, 20 µL of bioconversion sample after undergoing centrifugation (3300*g*, 10 min, Allegra X-15R) was pipetted on paper smell strips, which were placed in plastic reaction tubes labeled with a random three-digit code. Tubes were sealed immediately and stored at room temperature until analysis on the next day. A 2-alternative forced choice (2-AFC) discrimination testing procedure was applied [34]. Each panelist was served 10 sets of 2-AFC test in a rack, which included both enzyme fractions either as active or deactivated samples in a session for AEON or SK. Individual sensory sessions was dedicated to evaluate AEON and SK degradation, respectively. At a session, five replicates of the 2-AFC tests were served per panelist and type of comparison for MEF and EEF enzyme fractions (e.g., active MEF vs. control; deactivated MEF vs. control). For sensory evaluation of boar taint samples (SK/AEON), each panelist was served a total of 18 sets of 2-AFC tests for each enzyme fraction either in active or deactivated form in two sessions. The panelists were required to take up to 40 s to assess (sniff) each pair and indicate the sample that they perceived to be more intense in AEON and/or SK odor. Panelists were provided coffee beans for in-between sample neutralization. Both, the p value (significance level under the assumption of the null hypothesis, i.e., there is no sensory difference between control and bioconversion sample) and d-prime (d'), as a universal measure of the degree of sensory difference, were considered when analyzing the results. A $d' = 1$ can be considered a threshold at which samples can be discriminated [35].

Statistical analysis of sensory data

Peak areas of SK and AEON determined by HS-SPME (Figs. 1, 2 and 2S) and perceived odor intensities of SK and AEON on agar plates (Fig. 1) were analyzed using SPSS Statistics software version 17.0 (International Business Machines Corporation, Armonk, NY, USA). Data were subjected to an analysis of variance (ANOVA) and the differences were studied with Duncan's test, with a significance level of $p \leq 0.05$.

Sensory data of the bioconversion experiments were analyzed using R (v4.1.0) [36] and the SensR package [37]. The *discrim* function was used to analyze the results of the

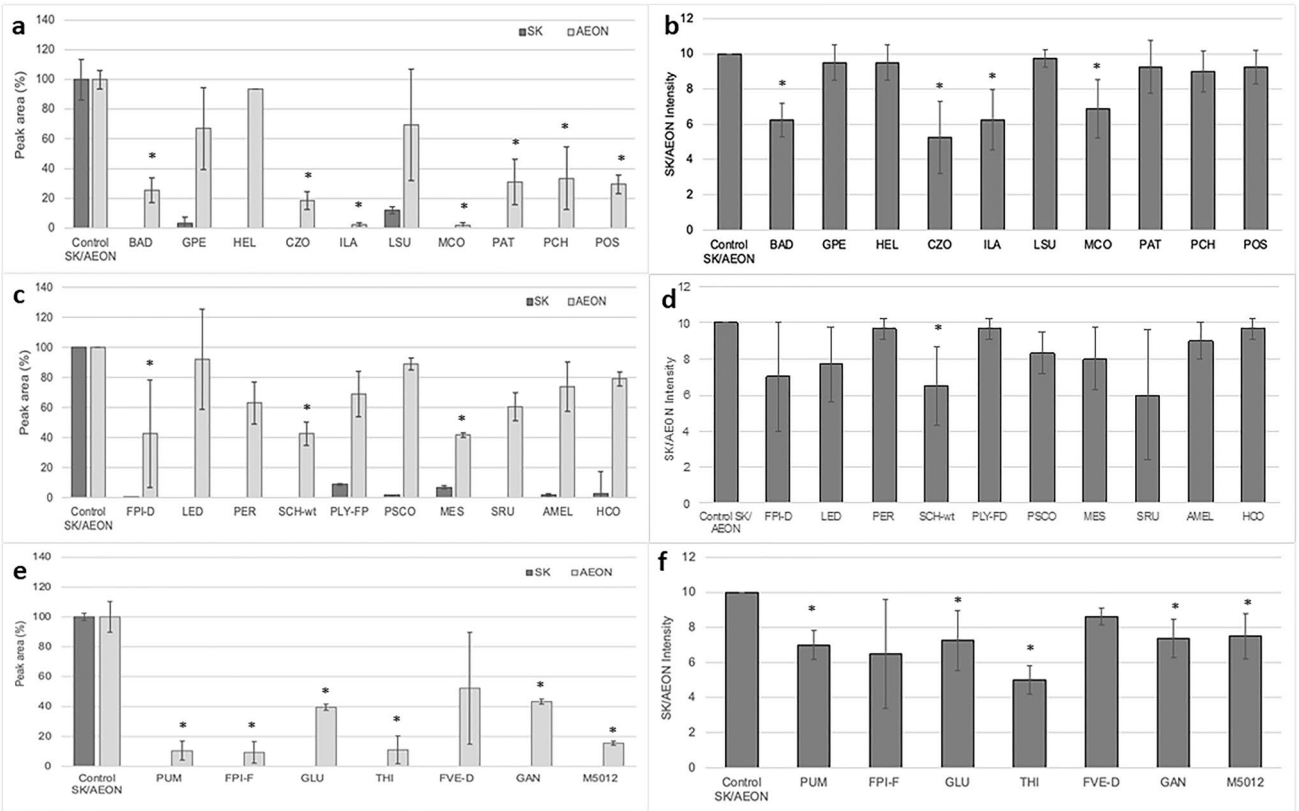

Fig. 1 **a**, **c** and **e** Relative peak areas of skatole (SK) and androstenone (AEON) in % determined by HS-SPME–GC–MS in HS vials after 7 days of culture with 27 basidiomycetous strains in three successive experiments. **b**, **d** and **f** Perceived odor intensities of SK and AEON on agar plates after 7 days of culture by the trained panel in three successive experiments. The intensities of the odor substances were described on a 0–10 scale (0 = not perceptible, 10 = very intensely perceptible), and the controls without fungus were also analyzed on day 7 and had an intensity of 10. The total number of observations was $n = 15$ (5 panelists × 1 replicate in three sessions). The error bars indicate the standard deviation of all observations. In Fig a, c, and e, all the treatments showed significant differences with control for SK; the asterisk indicates significant differences with the control for AEON ($p < 0.05$). **b**, **d** and **f** The asterisk indicates significant differences with the control ($p < 0.05$)

2-AFC. In result, the p value ($p < 0.05$) indicated the level of significance for a sensory difference. The d' value including 95% confidence intervals (CI) was used as a measure for the magnitude of the sensory difference [34].

Results and discussion

Screening of Basidiomycota for degradation of SK and AEON

Twenty-seven different species of Basidiomycota from the orders Agaricales (10), Polyporales (10), Russulales (2), Corticiales (1), and Pezizales (1) were screened analytically and sensorially for reduced peak areas or perception of the main BTCs SK and AEON, respectively.

The screening results indicated that at least eight strains were able to degrade SK and AEON simultaneously in HS vials (< 20% left of both compounds), and these strains showed a reduction in the intensity of the compounds on plates in the sensory evaluation (Fig. 1). The sensory compound intensities were lower than 7.5 (using 0–10 hedonistic scale), whereas the blank with the SK and AEON had the value 10. Among them, six strains belonged to Polyporales such as *Bjerkandera adusta* (BAD), *Cerrena zonata* (CZO), *Irpex lacteus* (ILA), *Fomitopsis pinicola* (FPI-F), *Polyporus umbellatus* (PUM), and *Trametes hirsuta* (THI), and two belonged to Agaricales, namely *Stropharia rugosoannulata* (M5012) and *Marasmius cohortalis* (MCO).

For the first time, fungi of the phylum Basidiomycota and their enzyme systems were shown to reduce the contents of the main BTCs, SK and AEON. All strains that showed efficient BTC degradation belonged to the class of white-rot fungi, which are known to secrete various oxidoreductases that have been reported to degrade complex molecules like, e.g., dyes, sterols, steroids, or APIs

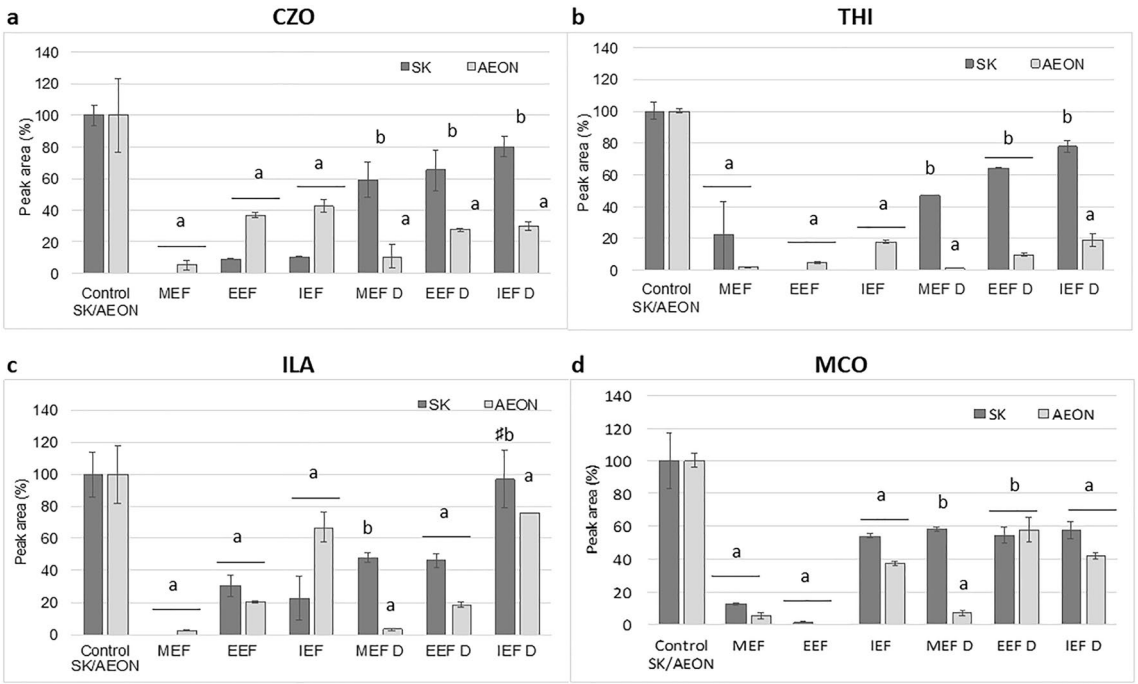

Fig. 2 Relative peak areas of skatole (SK) and androstenone (AEON) in % determined by SPME–GC–MS in HS vials after bioconversion overnight with enzyme fractions prepared from four selected strains: extracellular enzyme fraction (EEF), intracellular enzyme fraction (IEF), and mycelial enzyme fraction (MEF). Buffer inoculated with SK/AEON mixture was used as control (blank). The error bars indicate standard deviations of the measurements ($n=3$). Treatment with enzyme fractions from all strains showed significant differences with controls (SK/AEON) using a Duncan's test ($p<0.05$), except for SK treated with IEF from ILA, Fig. 2**c** (#; $p>0.05$). Different letters mean significant differences between active and deactivated samples ($p<0.05$)

[20–23, 38]. On the basis of the screening results, four strains (CZO, ILA, MCO, and THI) were selected for the bioconversion experiments.

Bioconversion of SK/AEON in HS vials by enzyme fractions of selected strains

Bioconversion experiments were carried out with enzyme fractions prepared from mycelia or supernatants collected from the four selected strains (CZO, THI, ILA, and MCO). After bioconversion of SK/ AEON overnight, the samples (controls, treatments, and deactivated (D)) were analyzed by HS-SPME–GC–MS. Several enzyme fractions were found to reduce 90–100% of the mixture of SK/AEON in the reaction mixtures (Fig. 2). EEF from MCO completely abolished both compounds and THI EEF reduced more than 90% of both compounds. For IEF, only the samples from the THI showed promising results as SK and AEON were reduced by 90% and 80%, respectively. Application of deactivated EEF resulted in a partial reduction of SK and AEON (shown for MCO, Supplementary information S2) and the mixture of SK/AEON (Fig. 2d). This surprising result may be explained by adsorption of the BTCs to solid particles present in the highly concentrated enzyme fraction (Supplementary information S3). For EEF and IEF, the main mechanism is thought to be biodegradation through the action of modifying enzymes, such as laccases, peroxidases (DyP, MnP, LiP), and BVMOs among others [20–22, 38–49]. MEF from MCO, CZO, and ILA reduced up to 100% of SK and more than 90% of AEON. For MEF, which represent lyophilized mycelia, an adsorption mechanism could play an important role in the reduction of BTCs intensities in addition to possible enzymatic degradation. Not much is known about the interactions between microbial biomass and BTCs, but it can be speculated that the reduction of BTCs by MEF involves, in addition to the enzymatic degradation, a mechanism of surface adsorption [29, 32]. These hypotheses are underlined by the fact that deactivated MEF samples (D) also showed a partial removal of both compounds (individual or in combination), being more evident for AEON. Fungi have complex cell wall structures consisting of polysaccharides, proteins, and lipids that provide many functional groups for the binding of molecules in different ways [28, 29]. In this sense, several authors pointed out that fungal biomass may be used as bio-adsorbent to concentrate and remove dyes or other complex molecules such as steroids or APIs from solutions [20, 50, 51]. For example, Fu and Viraraghavan [50] demonstrated that functional

groups such as carboxyl, amino, phosphate groups as well as lipid fractions present in dead fungal biomass from *Aspergillus niger* played an important role in biosorption of different types of dyes. These functional groups may not only act as binding sites but also in electrostatic attraction [51]. Recently, Liu et al. [52] showed that living and dead biomass of *Aspergillus fumigatus* achieved significant biosorption and removal efficiencies for sterols in aqueous solution; the total removal efficiency of sterols was 97% and 84%, respectively. The adsorption properties might be associated to the protein part on the cell surface, which is closely related with the sorption of hydrophobic compounds. This could also explain our results related to SK and AEON adsorption observed in the bioconversion experiments using deactivated enzyme fractions. Different responses to the compounds (SK and AEON) on the surfaces of active and deactivated mycelia might be explained by differences in adsorption capacities [53]. EEF and MEF of MCO represent attractive options for industrial applications, as they are simple to produce and efficiently degrade BTCs. Therefore, the next step was to evaluate the biodegradation/adsorption capabilities of active and deactivated MEF and EEF from MCO by performing a sensory 2-alternative forced choice (2-AFC) discrimination testing.

Sensory evaluation of the bioconversion of SK, AEON, and SK/AEON by enzyme preparations from MCO

EEF fraction

For SK, the samples obtained with the active EEF fraction were perceived less intense than the control sample containing only buffer and SK, but no enzyme fraction ($p < 0.05$; $d' = 1.19$; 95% CI = 0.47, 1.95) (Fig. 3). The samples obtained with deactivated EEF was perceived less intense than the control as well, but the difference was apparently smaller ($p < 0.05$; $d' = 0.80$; 95% CI = 0.13, 1.49) (Fig. 3).

For AEON, the sample produced with the active EEF fraction was perceived to be less intense than the control sample containing only AEON and buffer (Fig. 4) ($p < 0.05$; $d' = 0.74$; 95% CI = 0.02, 1.49). By trend, also the sample generated with deactivated EEF fraction was perceived to be less intense than the control ($p > 0.05$; $d' = 0.36$; 95%; CI = 0.00, 1.06), but the difference was far less pronounced as compared to SK. When both AEON and SK were added, the reduction of odor intensities was lower in the samples obtained with deactivated EEF.

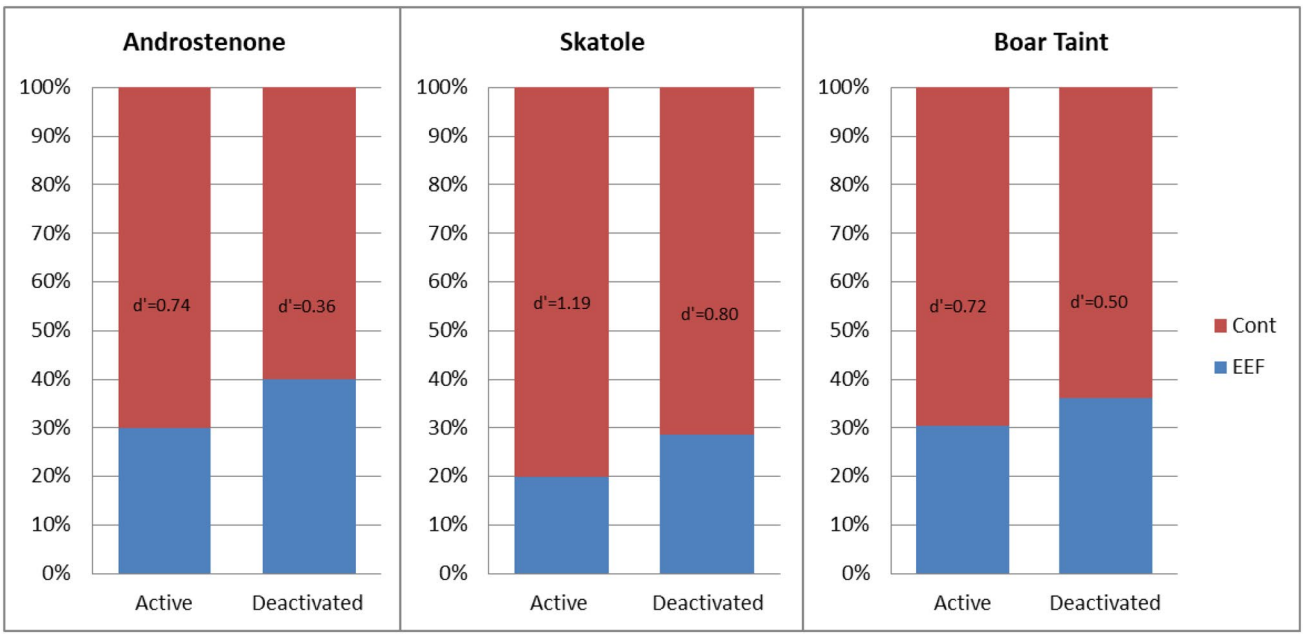

Fig. 3 Aggregated results of 2-AFC tests for bioconversions with extracellular enzyme fraction (EEF) in active and deactivated form vs. control regarding the odor perception of AEON, SK, and a mixture of AEON/SK (Boar Taint). The Y-axis shows the relative frequencies of which sample was chosen to be more intense in odorant perception. The total number of observations was $n = 35$ (7 panelists × 5 replicates divided in two sessions) for AEON and SK and $n = 30$ for AEON/SK (boar taint) (6 panelists × 5 replicates in two sessions). Cont = Control

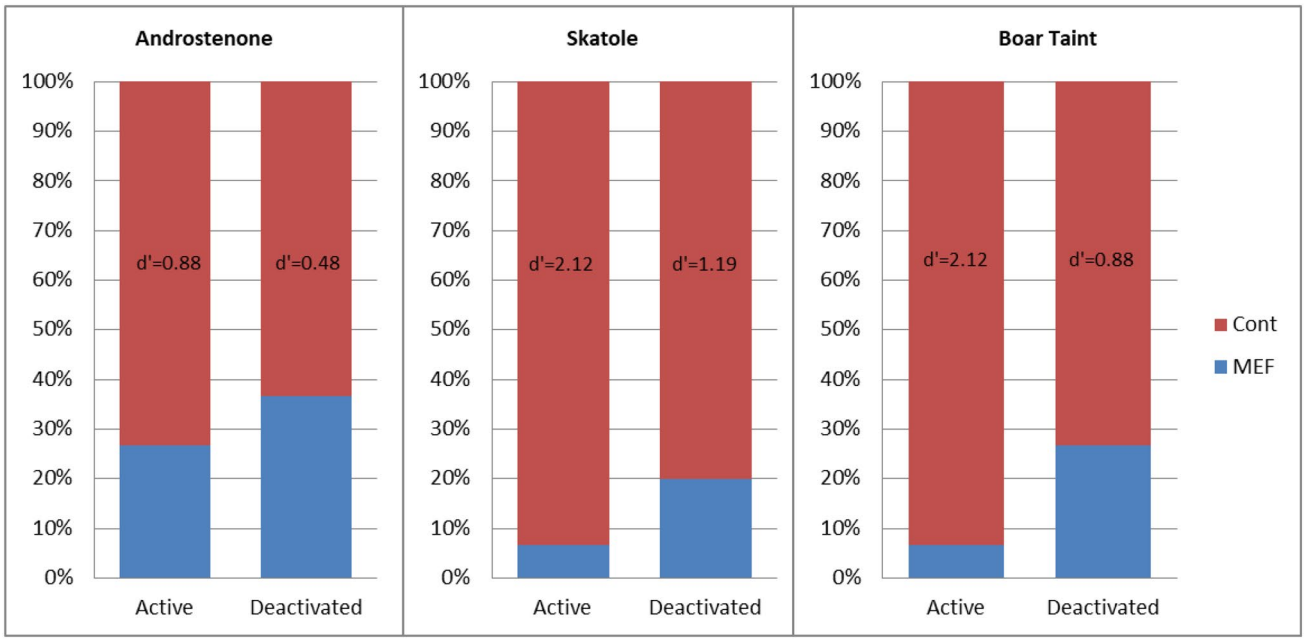

Fig. 4 Aggregated results of 2-AFC tests for bioconversions with enzyme fraction mycelial enzyme fraction (MEF) in active and deactivated form vs. control regarding the odor perception of AEON, SK and mixture of AEON/SK (Boar Taint). Y-axis shows relative frequencies of which sample was chosen to be more intense in odorant perception. Total number of observations was 30 (5 panelists × 6 replicates divided in two sessions) for all samples AEON, SK and AEON/SK (boar taint). Cont = Control

MEF fraction

For AEON, the odor of the samples generated with active MEF fraction was less intense than that of the control samples containing only buffer and AEON ($p < 0.05$; $d' = 0.88$; 95% CI = 0.15, 1.64) (Fig. 4). However, the samples containing the deactivated MEF fraction only tended ($p > 0.05$) to be perceived less intense than the control ($d' = 0.48$; 95% CI = 0, 1.19) (Fig. 4). For SK, the bioconversion samples containing the active and deactivated MEF fractions were perceived significantly less intense than the control samples containing only buffer and SK, but no enzyme fraction ($p < 0.05$; $d' = 2.12$; 95% CI = 1.09, 3.4) and ($p < 0.05$; $d' = 1.19$; 95% CI = 0.41, 2.01) (Fig. 4), respectively.

For the mixture of AEON/SK (boar taint) (Fig. 4), the samples containing the active and deactivated MEF were perceived less intense than the control (buffer/AEON/SK) ($p < 0.05$; $d' = 2.12$; 95% CI = 1.09; 3.4) and ($p < 0.05$; $d' = 0.88$; 95% CI = 0.15, 1.64), respectively. However, the degree of sensory difference was by far larger for the active MEF samples as compared to the deactivated MEF samples.

The findings of the sensory analyses overall well matched those of the HS-SPME analyses (Figs. 2d and S2). As per the sensory evaluation, the deactivated EEF fraction was less effective, especially for androstenone. Also the samples with deactivated EEF were perceived less intense regarding SK when compared to the control though the magnitude of the sensory difference to buffer sample was lower ($d' = 0.80$) than for active EEF ($d' = 1.19$). Overall, sensory differences between treatment and control samples appear to be larger for the MEF than for the EEF fraction.

We used two criterions to evaluate the sensory findings: (i) the significance level (p) and (ii) the effect size (d'). In some cases, $p < 0.05$ indicates significance, i.e., the treatment sample is considered less intense than the control, while under the null hypothesis each sample would be chosen with 50% probability though d' is below 1. In addition, a d' below 1 does not necessarily mean that samples cannot be discriminated, this rather is a rule of the thumb. Based on the significance criterion, both MEF and EEF showed significant differences for SK and SK/AEON (boar taint) intensity when compared with the control ($p = 0.01$) and ($p = 0.01$) though d' did not always exceeded a value of 1. In general, these sensory results were in line with those of the chemical analyses, as with both methods reduced amounts of BTCs were found.

Conclusions

In this study, we demonstrate for the first time that several strains of white-rot fungi were able to reduce the quantity (relative percentage) and the sensory intensities of skatole and androstenone, the main compounds responsible for

boar taint. A series of screening and bioconversion experiments using three different enzyme fractions prepared from selected strains showed that extracellular and mycelial fractions of *M. cohortalis* reduced up to 100% SK and AEON individually or in a mixture of both compounds. SPME-GC–MS results of the bioconversion samples with both fungal fractions were in line with the results obtained by trained sensory panelists, who overall indicated lower odor intensities of the bioconversion samples compared to the control samples. Results also demonstrated that fungi possess adsorption mechanisms in combination with the extracellular enzymes, which have a broad range of biotechnological applications. The present findings are promising as they could open new options for the processing of boar tainted meat avoiding economic (and unethical) losses.

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Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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