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2-Aminoacetophenone — A hepatic skatole metabolite as a potential contributor to boar taint

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ABSTRACT

The major objective of the presented study was to evaluate whether the hepatic skatole metabolite 2-aminoacetophenone (2-AAP) is a potential contributor to boar taint, which is an undesired off-flavor in pork. Therefore, backfat samples were screened by HS-SPME–GC/MS revealing a significant accumulation of the hepatic skatole metabolite 2-AAP in boar fat. Subsequently, a stable-isotope dilution assay (SIDA) was elaborated to precisely quantitate 2-AAP in a set of 130 backfat samples. The observed concentrations ranged between 34 ng/g and 1178 ng/g, resulting in a mean value of 100 ng/g. In addition, the odor detection threshold of 2-AAP was evaluated by a trained sensory panel using a single-staircase, triple forced choice paradigm. The determined 2-AAP odor detection threshold is similar to the thresholds of the major boar taint compounds androstenone and skatole. Finally, a sensory evaluation of backfat samples spiked with 2-AAP was performed in a triangle test with untrained testers. Here, the 2-AAP spiked samples were frequently identified as the odd sample independent of their respective androstenone and skatole levels. In conclusion, the hepatic skatole metabolite 2-AAP was identified as a potential contributor to boar taint.

1. Introduction

Boar taint is known as an unpleasant and offensive off-flavor that impairs the quality of pork. Sensory descriptors such as sweaty, urine- or fecal-like are frequently used to characterize boar taint (Brooks & Pearson, 1989; Dijksterhuis et al., 2000). According to the current state of knowledge, there are two major compounds responsible for boar taint: the boar pheromone androstenone (5α-androst-16-en-3-one) and the indole-related compound skatole (3-methylindole) (Patterson, 1968; Vold, 1970). While androstenone is endogenously synthesized in the boar's testes with the onset of puberty, skatole originates from microbial breakdown of the amino acid tryptophan and is formed in the pig's intestine. Once absorbed and distributed via the blood stream, androstenone and skatole are especially enriched in the fat tissue (Claus, Weiler, & Herzog, 1994). Due to their steady accumulation, androstenone and skatole may reach perceptible concentrations and cause boar taint when sensed during heating or consumption. Backfat concentrations of 1000 ng/g androstenone and 200 ng/g skatole are frequently reported as consumer acceptance thresholds to distinguish between tainted and untainted carcasses (Lundström, Matthews, & Haugen, 2009; Walstra et al., 1999).

As boar taint leads to product rejections and consumer dissatisfaction (Bonneau, Walstra, et al., 2000), the sensory evaluation and chemical analysis of boar taint as well as its formation and reduction in pork have become important tasks for scientists and the pork industry (Frieden, 2013; Haugen, Brunius, & Zamaratskaia, 2012; Meier-Dinkel et al., 2013). Especially, the relationship of a boar taint compound's concentration and the resulting odor impression is of special interest. Several sensory studies revealed a strong positive correlation ($R^2 = 0.53$–0.66) between the concentration of androstenone and skatole and the resulting boar taint perception in backfat samples (Annor-Frempong, 1997; Bejerholm & Barton-Gade, 1992; Lundström et al., 1988). However, trained panelists do frequently judge samples as “tainted” while chemical analysis attests low androstenone and skatole concentrations and vice versa (Bonneau, Kempster, et al., 2000; Bonneau et al., 1992; Mathur et al., 2012; Xue et al., 1996). This discrepancy has led to the assumption that other unknown compounds might contribute to boar taint (Babol, Squires, & Gullett, 1995; Bonneau et al., 1992; Lundström

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et al., 2009; Rius, Hortós, & García Regueiro, 2005; Rius Solé & García Regueiro, 2001). The musky smelling boar pheromones 3α-androstenol and 3β-androstenol as well as the fecal smelling indole, for instance, are known to have minor contribution to the overall perception of boar taint (Bonneau, Kempster, et al., 2000; Xue & Dial, 1997). Moreover, odorants derived from lipid oxidation (e.g. short chain fatty acids, ketones or aldehydes) or intestinal digestion processes (e.g. phenolic compounds) are also suggested by some authors to contribute to boar taint (Rius Solé & García Regueiro, 2001; Rius et al., 2005).

Interestingly, another group of potential boar taint compounds has not yet been investigated: the skatole metabolites. After microbial formation in the intestine, skatole is partly absorbed by the intestinal mucosa. While circulating in the blood stream, the majority of skatole is subject to hepatic CYP2E1 metabolism, resulting in seven known phase-I metabolites (Fig. 1A) (Diaz et al., 1999). Among these, 2-AAP (2) especially attracts immediate attention as a potential boar taint compound, because 2-AAP has already been identified as an off-flavor compound in white wines (Fan, Tsai, & Qian, 2007; Rapp, Versini, & Ullemeyer, 1993; Schmarr, Ganß, Sang, & Potouridis, 2007). Depending on its concentration, aroma descriptors such as "mothballs", "naphthalene", "animal" or "floral" are used to describe the odor of 2-AAP (Fan et al., 2007). These descriptors, in turn, are very similar to those that are frequently used for skatole. Moreover, in vitro studies have revealed that 2-AAP does not underlie any sulfation or glucuronidation in phase-II metabolism (Diaz & Squires, 2003), thus limiting the renal excretion of 2-AAP. In consequence, a certain amount of free 2-AAP will circulate in the blood stream and may accumulate in the fat tissue. To estimate the in vivo partitioning of a boar taint compound between aqueous and lipophilic compartments, the octanol–water partitioning coefficient (K_{ow}) can be used (Fischer, Brinkmann, Elsinghorst, & Wüst, 2012). As skatole and 2-AAP share comparable K_{ow} values (400 vs. 40), one can expect a somewhat lower but still significant tendency for fat accumulation. When accumulated in sufficient concentrations, 2-AAP may then trigger product rejections whenever sensed by the consumer during preparation and consumption of pork.

Thus, the objective of the presented work was the evaluation of 2-AAP as a potential boar taint compound. After a first qualitative analysis of 2-AAP in backfat samples, a novel SIDA–HS-SPME–GC/MS method was developed to precisely quantitate 2-AAP in backfat samples. Finally, the odor detection threshold of 2-AAP was determined and sensory evaluation of backfat samples spiked with 2-AAP was performed.

2. Materials and methods

2.1. Chemicals

All chemicals were obtained from Sigma-Aldrich (Steinheim, Germany), Alfa Aesar (Karlsruhe, Germany) or Steraloids (Newport, USA) in analytical grade. The deuterium labeled internal standards d_3-2-aminoacetophenone (d_3-2-AAP) and d_3-skatole (d_3-SK) were obtained from Dienstleistungszentrum ländlicher Raum (Neustadt/Weinstraße, Germany) or ELFI Analytik (Neufahrn, Germany), respectively.

2.2. Animals and samples

Backfat samples from the neck region were collected at slaughter from a group of conventionally fattened intact boars of type Piétrain × Baden-Württemberg Hybrid. The boars were group penned and fed ad libitum until reaching a slaughter weight between 85 kg and 95 kg (around 140 days of age). The fat layer was separated from the skin and meat residues and a piece of approximately 3 × 5 cm pure fat (corresponding to 20–70 g fat, depending on the thickness of the fat layer) was wrapped in aluminum foil, vacuum packed and stored at −20 °C until analysis.

2.3. Screening for and quantitation of 2-AAP

2.3.1. Sample preparation and HS-SPME conditions

The screening for 2-AAP in boar backfat samples as well as the subsequent quantitation of 2-AAP were achieved by adapting a previously published SIDA–HS-SPME–GC/MS procedure (Fischer et al., 2011). For sample preparation, backfat samples were thawed, diced and heated for 1.5 min at 700 W in a common household microwave. The remaining connective tissue was separated from the liquid fat by decanting and an aliquot of 500 mg of the warm liquid fat was transferred into a 2 mL plastic cap and filled with 1 mL of methanol for extraction. The sealed cap was immediately mixed for 30 s to give a cloudy emulsion. In order to separate the fat from the methanolic phase, a freezing step was carried out by centrifuging the samples at −10 °C (10 min,

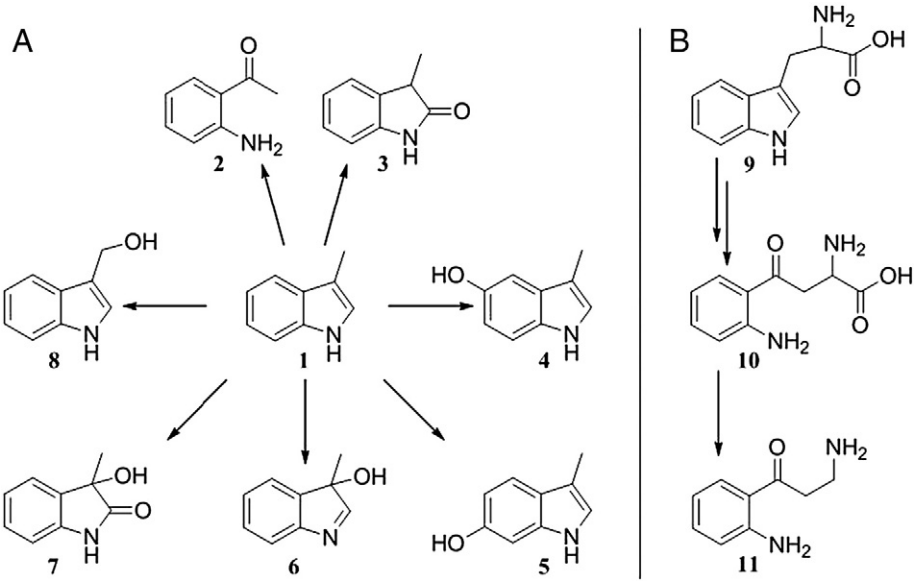

Fig. 1. Hepatic skatole metabolites build during phase-I-metabolism in pigs (A); skatole (1), 2-AAP (2), 3-methyloxindole (3), 5-hydroxyskatole (4), 6-hydroxyskatole (5), 3-hydroxy-3-methylindolenine (6), 3-hydroxy-3-methyloxindole (7), and indole-3-carbinole (8) (Diaz, Skordos, Yost, & Squires, 1999). Intermediates build during hepatic tryptophan metabolism in rats (B); tryptophan (9), kynurenine (10), and kynurenamine (11) (Kaseda, Noguchi, & Kido, 1973).