Food Chemistry 135 (2012) 2128–2132

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Quantitative determination of the boar taint compounds androstenone, skatole, indole, 3α-androstenol and 3β-androstenol in wild boars (*Sus scrofa*) reveals extremely low levels of the tryptophan-related degradation products

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ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 26 June 2012
Accepted 28 June 2012
Available online 14 July 2012

Keywords:
Wild boar (*Sus scrofa*)
Boar taint
Skatole
Androstenone
Androstenol
Indole

ABSTRACT

The major boar taint compounds androstenone and skatole as well as the minor compounds indole, 3α-androstenol and 3β-androstenol were determined in back fat samples of 23 male wild boars by applying a recently published SIDA–HS-SPME–GC/MS method. The boar pheromones androstenone, 3α-androstenol and 3β-androstenol were found in extraordinary high concentrations, resulting in mean values of 3329 ng/g androstenone, 1273 ng/g 3α-androstenol and 545 ng/g 3β-androstenol. Interestingly, skatole was not detectable in about 50% of the boars and negligibly low in all other samples as expressed by a mean skatole value of only 14 ng/g. Indole was also found in every sample, but again in low concentrations with a mean value of 40 ng/g. Possible factors explaining this remarkably low skatole deposition in wild boars such as intestinal flora and anatomy, dietary composition, housing or genetic predisposition are discussed in this paper.

1. Introduction

Boar taint is known as the offensive off-odour in meat of non-castrated male pigs that negatively affects the quality of pork. It is often described as a sweaty, musky, faecal or urine-like odour and lowers the consumer acceptance of pork when perceived by sensitive consumers. In consequence, boar taint is a major drawback in the production of entire male pigs. The occurrence of boar taint is mainly associated with high levels of the boar pheromone androstenone (ANON) and the heterocyclic aromatic amine skatole (SK) (Patterson, 1968; Vold, 1970). Back fat concentrations of 1000 ng/g ANON and 200 ng/g SK are frequently reported as threshold values to distinguish between tainting and non-tainting carcasses (Prusa et al., 2011). In addition to ANON and SK, other compounds, such as indole (IND), 3α-androstenol (3α-OL) and 3β-androstenol (3β-OL) are also mentioned as minor contributors (Bonneau et al., 2000; Xue & Dial, 1997).

Once synthesised in the testes of sexually mature boars, ANON is released into the bloodstream via the testicular vein and subsequently accumulated in adipose tissue as a result of its lipophilic nature. It is perceived as a urine-like and sweaty odour. SK, in contrast, originates from microbial breakdown of tryptophan in the intestine of the pigs. Although skatole is only partly absorbed by the intestinal mucosa and subject to CYP2E1 metabolism in the liver, the unmetabolised residue enriches in adipose tissue and thereby contributes to boar taint as well (Chen, Zamaratskaia, Andersson, & Lundstrom, 2007; Doran, 2002). The odour of skatole is often described as faecal-like (Claus, Weiler, & Herzog, 1994).

The incidence of boar taint in pork has not been a serious concern for centuries, as surgical castration of male piglets was practised routinely in Europe (Fabrega et al., 2011). This practise effectively prevents the testicular release of androstenone and thereby also reduces the accumulation of skatole, as the hepatic clearance of skatole is impaired by the presence of androstenone (Doran, 2002; Font i Furnols et al., 2008). However, boar taint currently gains in importance throughout Europe, as surgical castration without anesthaesia will be voluntarily banned for animal welfare reasons by 2018 (Haugen, Brunius, & Zamaratskaia, 2012). In consequence, a considerable number of tainted carcasses is expected and the pork-producing industry fears serious economic losses (Weiler et al., 2000). Therefore, great efforts are undertaken to find minimisation concepts for the major boar taint compounds SK and ANON, as well as reliable detection methods to sort out tainted carcasses before retail.

The European wild boar (*Sus scrofa*) is the genuine form of our domestic pig breeds (Macchi et al., 2010). Wild boars are seasonal breeding animals, whereas domestic pigs are known to be fertile throughout the year (Kozdrowski, 2004). Hence, wild boars have a

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http://dx.doi.org/10.1016/j.foodchem.2012.06.105

clearly marked rutting season, mainly falling into late autumn and early winter (Mauget & Boissin, 1987). Initiated and regulated by decreasing day length in wintertime, male wild boars show a deviating steroid composition during rutting season resulting in up to ten-fold increased testosterone and androstenone levels in seminal plasma (Macchi et al., 2010; Weiler, Claus, Dehnhard, & Hofäcker, 1996). In this context, wild boar meat should be highly susceptible to boar taint, especially when shot during rutting season in winter.

However, wild boar meat as game meat, in general, is a highly valuable product and a welcome diversion for many German households due to its high nutritional quality, *e.g.*, low fat content and superior taste (Hoffman & Wiklund, 2006). About 500,000 wild boars are annually shot by German hunters (Deslandes, Gariépy, & Houde, 2012). Besides direct marketing from hunters to consumers or restaurants, the obtained meat is also retailed by game cutting companies (Paulsen, Vali, & Bauer, 2011). The overall German wild boar meat consumption in 2011 was reported at 13,000 tonnes (Deutscher Jagdschutzverband, 2012). Interestingly, no or only negligible complaints about boar taint are known with respect to the meat of entire male wild boars, despite the following facts: none of the male wild boars is castrated and approximately 40% of the shot male boars are older than 10 month. As sexual maturity on average is attained at this age, a considerable amount of the obtained carcasses should be prone to boar taint (Macchi et al., 2010; Mauget & Boissin, 1987). However, only a few carcasses of perennial male boars are discarded due to their extraordinarily offensive smell and this exclusively during rutting season in wintertime. In this context, the investigation of the major boar taint compounds androstenone and skatole in back fat of male wild boars is of great interest. As there are a lack of data concerning both major and minor boar taint compounds in the adipose tissue of adult male wild boars, the aim of the present work was to determine these compounds in a set of 23 animals, shot during hunting season in 2011/12. Quantitation of ANON, SK, 3α-OL, 3β-OL and IND was performed by applying a recently developed stable isotope dilution analysis–headspace solid-phase microextraction–gas chromatography/mass spectrometry (SIDA–HS-SPME–GC/MS) procedure (Fischer et al., 2011).

2. Materials and methods

2.1. Animals and samples

A total of 20 back fat samples from the neck region of mature male wild boars were collected during hunting season in two different forest districts (Hessen, Central Germany). The animals of interest, namely male adolescent boars older than 10 month and perennial male adult boars, were selected by their tooth eruption and tooth wear, where the tooth eruption allows for narrow age estimation in the period between the 5th and 24th month of life (Kierdorf et al., 2004; Stubbe, 2009). Whenever a shot boar was older than 24 month, the age was estimated due to the extent of tooth wear. In addition to the age, the weight of each animal after evisceration as well as the date of shooting was documented. Moreover, three samples of male wild boar piglets between the 6th and 8th month of life were taken to confirm the reported age related variation in pheromone concentrations. All collected back fat samples were wrapped in aluminium foil, vacuum-packaged and stored at –20 °C until analysis.

2.2. Boar taint analysis

The determination of ANON, SK, 3α-OL, 3β-OL and IND was achieved by applying a previously published SIDA–HS-SPME–GC/MS procedure. Therefore, back fat samples were skinned, diced and subsequently melted in a microwave. After separation of the connective tissue and addition of the internal standards, the analytes were extracted from liquid fat with methanol. The obtained methanolic supernatant, containing the analytes of interest, was then transferred into a headspace vial and evaporated to dryness by compressed air. Finally the headspace vial was sealed and passed to the automated HS-SPME–GC/MS measurement (Fischer et al., 2011).

3. Results and discussion

A set of 20 back fat samples of mature male wild boars and three premature male wild boar piglets were collected during hunting season 2011/12 and measured by applying a recently published SIDA–HS-SPME–GC/MS procedure. All determined concentrations of ANON, SK, 3α-OL, 3β-OL and IND are presented in Table 1.

3.1. Boar Pheromones

The determined ANON concentrations reveal that most of the investigated mature boars show very high ANON values, resulting in a mean ANON value of 3329 ng/g (a maximum ANON value of 6700 ng/g was observed). Moreover, remarkable individual differences of the ANON concentrations were observed, as the ANON values varied up to a factor of 10, even in the same age group. As 3α-OL and 3β-OL originate from enzymatic reduction of ANON in the boar's testes and were therefore found in considerable concentrations as well (Brophy & Gower, 1972). The mean values for 3α-OL and 3β-OL were 1273 ng/g and 545 ng/g with maximum values of 3524 ng/g 3α-OL and 1783 ng/g 3β-OL. Considering the pheromone ratios in detail, the determined ANON levels exceed the 3α-OL and 3β-OL levels in all samples. Comparison of the determined androstenol concentrations reveals that 3α-OL occurs in higher concentrations than 3β-OL in every sample. The mean concentration ratio of 3α-OL/3β-OL is 2.5 and in fair agreement with an earlier published ratio of 1.9 determined for domestic boars (Fischer, Brinkmann, Elsinghorst, & Wüst, 2012).

Only two of the supplied adult boars were shot in August before the onset of rutting. Hence, the monitoring of the reported pheromone boost during rutting was rather restricted: the five year old boar shot on 19th of August already showed high pheromone concentrations (2120 ng/g ANON, 555 ng/g 3α-OL, 177 ng/g 3β-OL), whereas low pheromone concentrations (143 ng/g ANON, 71 ng/g 3α-OL, n.d. 3β-OL) were observed for a 16 month old boar shot on 5th of August. All other boars shot between end of October and end of January showed high pheromone quantities according to the onset and progress of rutting. Extraordinary high ANON values above 5000 ng/g were observed in six boars. Those extremely high ANON levels seem to peak at the end of December, as four out of six boars pass the 5000 ng/g level at that time. This peaking is probably observed for two reasons. First, the steady ANON production and deposition throughout rutting leads to a continuously increased back fat concentration and, second, the high weight loss of the boars during rutting additionally concentrates ANON in the remaining fat tissue. Same peaking behaviour was observed in a recent study investigating the annual concentration pattern of ANON in male wild boars (Treyer et al., 2012). The investigated control group of three premature piglets (<8 month) in contrast showed no or comparatively low pheromone concentrations (see also Table 1). Within the group of mature boars, however, no age- or weight-dependent alteration of the ANON concentrations were found.

3.2. Skatole and indole

Unexpectedly, no or negligibly low quantities of SK were found in the adult wild boars as well as in the control group of premature