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Distribution of skatole and androstenone in the pig carcass correlated to sensory characteristics

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ABSTRACT

Reliable sorting and optimised use of boar-tainted pig carcasses are dependent on knowledge of the distribution of skatole and androstenone and the corresponding sensory characteristics. In this study, skatole and androstenone were measured in the neck fat and in six different meat cuts from 14 entire male pigs. There was a strong correlation between the content of skatole in the neck fat and in the meat cuts, though the concentration of skatole in the meat cuts was much lower than in the neck fat. Furthermore, clear correlations were found between the intensity of boar taint flavours and the concentration of skatole in both the neck fat and the meat. The concentration of androstenone was below the limit of quantification (LOQ) in a large number of meat cuts, and a correlation could therefore not be established. Despite low concentrations of skatole and/or androstenone in the meat cuts, distinct boar taint flavours were detected in the cooked lean meat.

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1. Introduction

In Europe, the potential ban on surgical castration by 2018 is an area of focus. Many issues related to male pig production are therefore at the centre of research. One of these issues concerns fully understanding the significance of the different concentration levels of skatole and androstenone in the neck fat of entire males and the boar taint in different meat cuts in relation to the sorting of tainted carcasses. The establishment of correlations between chemically measured concentrations of boar taint compounds in the neck fat and the perceived eating quality of the whole carcass is relevant not just in order to establish reliable sorting criteria, but also to optimise the use of the carcasses, as different sorting limits might be present for different parts of the carcass.

There is a general consensus that two compounds are primarily responsible for boar taint: skatole (3-Methyl-Indole) and androstenone (5α-androst-16-en-3-one) (Patterson, 1968; Vold, 1970; Bonneau, 1982; Bonneau & Squires, 2004; Byrne, Thamsborg, & Hansen, 2008), even though other compounds have also been proposed, such as p-cresol and 4-ethylphenol (Patterson, 1967) and indole and androstenol (Fischer et al., 2011). Current research is therefore focused on skatole and androstenone, even though indole is analysed simultaneously with the two other compounds. Methodologies for assessing the boar-tainted carcasses consist of sampling the neck fat and evaluating the odour (human nose) (Trautmann, Meier-Dinkel, Gertheiss, & Mörlein, 2016) or performing chemical analyses of the content of androstenone and skatole in the fat sample (Meinert, Claudi-Magnussen, & Støier, 2013). Traditionally, neck fat has been used, even though recent research has shown a higher concentration of skatole and indole in the belly fat (Wesoly, Stefanski, & Weiler, 2016). The suitability of the whole carcass for consumption is evaluated based on the neck sample. However, previous research has shown that different meat cuts from the same carcass can have different intensities of boar taint (Aaslyng, Broge, Brockhoff, & Christensen, 2015; Aaslyng, Broge, Brockhoff, & Christensen, 2016), but the distribution of the compounds and the resulting boar taint in a large sample of cuts are not known in detail. It is known that both skatole and androstenone are mainly fat-soluble, even though skatole is slightly more water-soluble than androstenone (Kock, Heinze, Potgieter, Dijksterhuis, & Minnaar, 2001). The relative importance of the two compounds analysed in the neck fat for boar taint might therefore depend on the fat content of the muscle. However, the fat content in the muscle might not just influence the content of the two compounds in the raw meat, but also influence the amount of the two compounds in the headspace of heated meat, since a low fat content might result in a higher concentration of the fat-soluble compounds in the headspace. The relation between the content of androstenone and skatole in the neck fat and the content and intensity of boar taint could therefore be muscle-specific. Several muscles have been included both in sensory studies and consumer studies, so it is known that boar taint is present in the loin (Dijksterhuis et al., 2000; Meier-Dinkel et al., 2013; Aaslyng et al., 2015; Aaslyng et al., 2016), the tenderloin (Byrne et al., 2008), bacon (Lesser, Baron, & Robb, 1977; Aaslyng et al., 2015) and the topside (Aaslyng et al., 2016) while the content of androstenone and skatole in these muscles has not yet been reported. So far, no reports have been published on the correlation between the concentration of skatole and androstenone in the

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neck fat and the sensory assessed boar taint and the concentration of the two compounds in the meat cuts.

The aim of this study was therefore to gain initial knowledge about: 1) the concentrations of skatole and androstenone in the neck fat versus different pork cuts from the same animal, 2) the concentrations of skatole and androstenone in the neck fat and the perceived sensory characteristics of the cooked meat, and 3) the concentrations of skatole and androstenone in the pork cuts and the perceived sensory characteristics of the cooked meat.

2. Materials and method

2.1. Screening and selection of pigs and meat

The carcasses used in this experiment were selected from among carcasses in another experiment described in Aaslyng et al. (2015). In short, meat from entire males and castrates was collected from a Danish commercial slaughterhouse between January and March 2013. The selection was focused on obtaining a variation in skatole and androstenone concentrations. On the day of slaughter, pigs were selected based on the slaughterhouse analysis of skatole equivalence (skatole and indole) (Mortensen & Sørensen, 1984) and a human nose test of the back fat (Meinert, Bejerholm, & Støier, 2011). On the day after slaughter, the pH was measured in the centre of the left loin (*m. longissimus thoracis et lumborum*) between the 4th and 5th lumbar vertebrae using a portable Knick pH-meter (Mettler Toledo, Glostrup, Denmark). If the pH was below 5.5 or above 5.8, the animal was discarded. The castrates were selected randomly at the slaughterhouse. Fourteen entire male pigs and one castrate were used in this study with concentrations of androstenone of up to 3.03 mg/kg and skatole of up to 0.70 mg/kg.

From each of the 15 pigs, neck fat and the following meat cuts were collected for analysis: neck (*m. longissimus thoracis*), loin (*m. longissimus thoracis et lumborum*), topside (*m. semimembranosus*), tenderloin (*psoas major*) and eye of round (*m. semitendinosus*). The meat cuts were chosen based on criteria covering the three main parts of the carcass with variations in fat content. The cuts with rind (e.g. loin) were de-rinded, and all the meat cuts were individually vacuum packed, aged for 4 days and subsequently frozen at −18 °C until analysis.

2.2. Analysis of neck fat

The human nose analysis was performed as described by Meinert et al. (2011). Approx. 5 g of back fat was cut into small pieces and transferred to a 100 mL conical flask, to which 75 mL of boiling water was added. The flask was covered with foil and equilibrated for 2 min before being assessed. Two assessors independently scored the odour on a categorical scale ranging from 0 (no boar taint odour) to 3 (very strong boar taint odour). The samples were assessed in a randomised order. Both assessors were sensitive to androstenone and skatole.

The chemical analysis of skatole and androstenone in the neck fat was performed at DMRI according to the HPLC-FD method described by Hansen-Møller (1994) and modified as described in Aaslyng et al. (2015).

2.3. Sample preparation of meat for chemical analysis

Meat (50–100 g) was trimmed and blended in a bowl chopper. The comminuted meat was transferred to a petri dish and spread out in a thin, even layer, frozen overnight in a freezer and lyophilised for approx. Three days. The weight loss was measured by weighing the sample before and after drying. Dried meat (1.00 g) was transferred to a centrifugal glass tube, and an internal standard mixture (3 mL, 0.033 mg/L and 0.33 mg/L of 2-methylindole and androstanone, respectively) was added and the glass capped. The suspension was shaken overnight at 45 °C followed by homogenisation using a Polytron PT 3100D. The homogenate was sonicated for 3 h, centrifuged at room temperature at 5000 rpm and analysed by HPLC-FD analysis (Hansen-Møller, 1994). The concentration obtained from the HPLC analysis was expressed as μg/g dried meat. The results were then calculated as μg/g meat based on the weight loss during freeze-drying and the initial sample mass.

2.4. Analysis of meat cuts

The HPLC-FD method was also used for the analysis of the meat cuts. However, a new method involving lyophilisation of the meat cuts was developed for the sample preparation.

2.4.1. Chemicals

The reference standards indole, skatole (3-methylindole) and androstenone (5α-androst-16-ene-3-one) and the internal standard 2-methylindole were obtained from Sigma Aldrich (St. Louis, MO, USA). Androstanone (5α-androstan-3-one) was obtained from Steraloids Inc. (Newport, RI, USA). Reagents were obtained from VWR International (Merck, Darmstadt, Germany) and were of analytical grade or HPLC grade. Dansylhydrazine was obtained from Sigma Aldrich (St. Louis, MO, USA). Demineralised water was treated in a Milli-Q Plus water purification system from Millipore (Bedford, MA, USA).

2.4.2. High-performance liquid chromatography

2.4.2.1. HPLC system 1. The HPLC systems were Hitachi systems from Merck. Each system consisted of an AS-4000 autosampler, an L-6200 gradient pump, an F-1080 fluorescence detector and an L-5025 column oven. The column was a Hypersil ODS (3 μm, 60 × 4.6 mm I.D. Hewlett Packard) operated at 40 °C. A diluted phosphate buffer was made (3.13:1:45.88) 0.2 M potassium phosphate buffer/acetic acid/water. The mobile phase buffers used were: (A) (99:1 THF/water)/diluted phosphate buffer (1:2.23), (B) acetonitrile/(99:1 THF/water)/diluted phosphate buffer (1:1.43:1.77) and (C) (99:1) THF/water (V/V). The following gradient profile was used: 0–7.5 min 69% A; 0% B; 31% C; 1.2 mL/min; 7.5–17.0 min, 41% A; 24% B; 34% C; 1.4 mL/min; 17.5–19.1 min, 10% A; 0% B; 90% C; 1.4 mL/min 19.1–19.8, 69% A; 0% B; 31% C; 1.2 mL/min 19.8 min 69% A; 0% B; 31%C; 1.2 mL/min.

2.4.2.2. HPLC system 2. The HPLC systems were an Agilent 1100 system from Agilent Technologies and an AS-4000 autosampler from Merck-Hitachi. The Agilent system consisted of a G1379A degasser, a G1312A binary pump, a G1321A FLD fluorescence detector and a G1316A column oven. The column was a Poroshell 120 SB-C18 (2.7 μm, 75 × 4.6 mm I.D. Agilent Technologies) operated at 55 °C. The mobile phase buffers used were: (A) 95:5:0.1 water/methanol/trifluoroacetic acid and (B) 95:5:0.1 methanol/water/trifluoroacetic acid. The following gradient profile was used: 0–3.0 min 38% A; 62% B; 1.4 mL/min; 3.0–7.5 min 50% A; 50% B; 1.4 mL/min; 7.5–12.0 min, 5% A; 95% B; C; 1.6 mL/min; 12.0–19.1 min, 5% A; 95% B; 1.6 mL/min 19.1–19.8, 38% A; 62% B; 1.4 mL/min.

The fluorescence detector was used with excitation at 285 nm and emission at 340 nm for the first seven minutes (indole and skatole) and then excitation at 346 nm and emission at 521 nm (dansylhydrazones of androstenone and androstanone).

2.4.3. Limits of detection and quantification

The limits of detection (LOD) were determined in standard solutions with a signal-to-noise ratio of 3:1. The limits of quantification (LOQ) were determined as ten times the detection limit (Hansen-Møller, 1994). The LODs for indole and skatole were <3 ng/lyophilised meat and for androstenone <20 ng/g lyophilised meat, while the LOQs for indole and skatole were 30 ng/g lyophilised meat and for androstenone 200 ng/g lyophilised meat.

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