Identification of androstenone sensitive subjects and their evaluation of different androstenone and skatole tainted meat products

Identifisering av androstenon sensitive subjekter og deres evaluering av ulike kjøtt produkter med androstenon og skatol

Philosophiae Doctor (PhD) Thesis

Kathrine Lunde

Dept. of Chemistry, Biotechnology and Food Science
Norwegian University of Life Sciences

Ås 2011

Thesis number 2011:12
ISSN 1503-1667
ISBN 978-82-575-0976-7

Acknowledgements

This thesis was written at Animalia and financially supported by the Research Council of Norway through project number 63164; “Boar meat-consumer aspects and exploitation of resources”.

First of all I would like to express my deep and sincere gratitude to all of my supervisors, Professor Bjørg Egelandsdal (principal supervisor, Norwegian University of Life Science), Adjunct Associate Professor Margrethe Hersleth (co-supervisor, Nofima Mat) and Ellen Skuterud (co-supervisor, Animalia). A special thank to Professor Bjørg Egelandsdal for her support, ideas, critical readings and great care.

My gratitude also goes to all participants in the project group: Bjørg Egelandsdal, Ellen Skuterud, Inghild A. Jørgenvåg, Anna Rehnberg, Audun Flåtten, Asgeir Nilsen and Elin Kubberød. Thank you for numerous ideas and fruitful discussions.

I would also like to thank all my co-authors and other co-workers in this project. In particular the practical assistance from the meat cutters at Animalia and Tom Chr. Johannesen and Karin Solgaard at Nofima Mat are highly appreciated.

A special thank also to Marit Rødbotten, Inger-Johanne Fjøsne and Aud Espedal at Nofima Mat for letting me use your extremely sensitive noses when testing out all the different androstenone solution and concentrations.

Last, but not least, my deepest gratitude goes to my family and friends for all your encouragement, understanding and care. My deepest love goes to my husband Ole Øystein and my children Mats, Iver and Even for making me realise what is most important in life.

Oslo, December 2010,

Kathrine Lunde

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Paper I – VI

Abstract

Castration of entire male pigs is widely used in Norway to prevent the unpleasant odour/flavour that may occur in meat from boars. Castration of entire male pigs is expected to be prohibited in Norway (and in Europe) in the future, and it is therefore important to gain more knowledge about the human perception of boar taint. Boar taint is mainly associated with the presence of two compounds, skatole and androstenone. Skatole is detected by 99% of the consumers and regarded as unpleasant, while the ability to perceive androstenone varies and is, at least partly, determined by the amino acid sequence of the human odour receptor OR7D4. The aim of the present thesis was to look at the possible challenges connected to a future production of entire males investigating the quality of the raw material and sensory perception of boar tainted meat.

The results presented in the thesis showed that approximately 39% of the Norwegian consumers were identified as androstenone sensitive based on results from a new method developed in this project. After relating the data from the consumer's androstenone sensitivity testing to their DNA, the result showed that the consumer's androstenone sensitivity corresponded with their DNA typing in all cases where the consumers were defined as sensitive. Since the developed sensitivity test gave no false positive genotypes, it can be recommended for setting androstenone thresholds in meat and for selecting assessors to sensory panels. This result was confirmed when sensory assessors from 4 European sensory panels evaluated meat with different levels of androstenone. All assessors were able to detect androstenone in pure form when recruited, but 26% of the assessors were defined as non sensitive by the method developed in this project

and did not react negatively on androstenone tainted meat as opposed to the sensitive assessors.

Results presented in this thesis also showed that skatole easily can be detected in low concentrations (0.15 ppm), both by sensory assessors and consumers. The Norwegian established practise with a sort out threshold value of 0.21 ppm may therefore provide negative reactions from the consumers. For androstenone, using a level of 3 ppm for sorting would be economically acceptable due to the low number of carcasses above 3 ppm (5.5%), but its odour may then be detected and not accepted by sensitive consumers during frying of the meat.

With the use of different production technologies (dry salted and fermented bacon) and addition of common and strong food flavour additives (liquid smoke) higher skatole levels were accepted by the consumers. In general, the skatole flavour seemed easier to mask than androstenone flavour.

Sammendrag

Kastrering av hanngris i Norge benyttes i stort omfang for å unngå den ubehagelige lukten/smaken som kan forekomme i kjøttet fra hanngriser. Kastrering av gris forventes å bli forbudt i Norge (og Europa) i fremtiden, og det er derfor viktig å skaffe mer kunnskap om forbrukernes oppfatning av rånelukt/smak. Rånelukt/smak assosieres hovedsakelig med komponentene skatol og androstenon. Skatol oppfattes som ubehagelig av 99 % av forbrukerne, mens evnen til å oppfatte androstenon variere og er, i det minste delvis, bestemt av luktreseptoren OR7D4. Målet med denne avhandlingen var å se på mulige utfordringer tilknyttet en fremtidig produksjon av hanngris ved å undersøke råstoffkvalitet og mulige anvendelsesområder. Hovedfokuset var produktkvalitet og den norske forbrukers oppfatning av rånelukt/smak.

Resultatene presentert i avhandlingen viser at ca 39 % av de norske forbrukerne ble definert som sensitive for androstenon etter å ha blitt testet med en ny metode for androstenonsensitivitet utviklet i prosjektet. Ved å relatere forbrukernes androstenonsensitivitet til forbrukernes DNA, fant man at forbrukernes androstenonsensitivitet var i samsvar med deres DNA profil i alle tilfeller hvor forbrukerne ble definert som sensitive. Siden den nye sensitivitetsmetoden ikke ga noen falske positive genotyper kan luktemetoden anbefales til å bestemme grenseverdier for androstenon i kjøtt, og til utvelgelse av dommere til sensoriske paneler. Dette resultatet ble bekreftet når sensoriske dommere fra 4 Europeiske sensoriske paneler bedømte prøver med ulikt innhold av androstenon. Alle dommerne hadde evnen til å kjenne androstenone i ren form når de ble rekruttert, men 26 % av dommerne ble definert som ikke sensitive på bakgrunn av metoden

utviklet i prosjektet. Disse reagerte heller ikke negativt på kjøtt med ulikt innhold av androstenon i motsetning til de sensitive dommerne.

Resultater presentert i avhandlingen viser også at skatol lett kan oppfattes i lave konsentrasjoner (0,15 mg/kg), både av sensoriske dommere og forbrukere. Den norske utsorteringsverdien på 0,21 mg/kg vil derfor kunne medføre negative forbrukerreaksjoner. Når det gjelder androstenon vil en utsorteringsverdi på 3 mg/kg være økonomisk akseptabelt på grunn av at få dyr (kun 5.5 %) i Norge har høyere androstenon innhold. Sensitive forbrukere vil likevel kunne reagere negativt (ikke akseptere) lukten fra 3 mg/kg med androstenone under steke prosessen.

Ved bruk av ulike prosesserings teknologier (tørrsaltet og fermentert bacon) og vanlige, sterke tilsetningsstoffer (flytende røykaroma) ble høyere nivåer av skatol akseptert av forbrukerne. Generelt så det ut til at skatol var lettere å maskere enn androstenon.

List of papers

I. Lunde, K., Skuterud, E., Nilsen, A. & Egelandsdal, B. (2009). A new method for differentiating the androstenone sensitivity among consumers. Food Quality and Preference 20, 304- 311.

II. Lunde, K., Skuterud, E., Egelandsdal, B., Font i Furnols, M., Nute, G., Bejerholm, C., Nilsen, A., Stenstrøm, Y.H. & Hersleth, M. (2010). The importance of the recruitment method for androstenone sensitivity with respect to the accurate sensory evaluation of androstenone tainted meat. Food Quality and Preference 21, 648-654

III. Lunde, K., Egelandsdal, B., Skuterud, E., Mainland, J.D., Lea, T., Hersleth, M. & Matsunami, H. (2010). Genetic variation of OR7D4 affects sensory perception of meat containing androstenone. Submitted to Plos Genetics.

IV. Lunde, K., Skuterud, E., Hersleth, M. & Egelandsdal, B. (2010). Norwegian consumer's acceptability of boar tainted meat with different levels of androstenone or skatole as related to their androstenone sensitivity. Meat science, 86 (3), 706-711.

V. Lunde, K., Egelandsdal, B., Choinski, J., Flåtten, A., & Kubberød, E. (2008). Marinating as a technology to shift sensory thresholds in ready-to-eat entire male pork meat. Meat Science, 80, 1264-1272

VI. Lunde, K., Skuterud, E., Lindahl, G., Hersleth, M. & Egelandsdal, B. (2010). Masking of boar taint in fermented, dry salted and brine injected bacons. Submitted to Journal of Food Science.

Introduction

The history and present practise in Europe regarding production of entire males depend on political choices made in each country. Thus some countries have extensively practised production of entire males e.g. England, while Norway has castrated all piglets (Fredriksen et al., 2009). Castration of male pigs is done to prevent an unpleasant odour/flavour that can occur in meat from boars. European countries are, however, aiming at a castration ban. It is therefore important to gain more knowledge about the Norwegian consumers' sensory perception of boar tainted meat, as this new situation may influence the demand for pork meat and will have large economical consequences for the industry.

Skatole and androstenone largely describe boar taint. Skatole is a faeces and manure smelling metabolite (Vold, 1970) of the amino acid tryptophane produced in the lower gut by intestinal bacterial flora. The ability to break down skatole changes during maturity of male pigs. Androstenone is a steroid structurally related to testosterone. The production of androstenone in the testis increases with maturity of the male pig. Androstenone is associated with a urine like flavour (Patterson, 1968). Both skatole and androstenone are highly fat-soluble compounds.

To be able to sort out carcasses that are unacceptable to consumers, more knowledge about the Norwegian consumer's sensory perception and acceptance of pork meat with different levels of skatole and androstenone is necessary. Knowledge of acceptance levels for skatole and androstenone

will make it possible for the pork meat industry to provide an estimate of the economic consequences of a change to entire male production. The thresholds used for sensory perception of the boar taint compounds androstenone and skatole today are usually 0.5 -1 ppm and 0.20- 0.25 ppm respectively (reviewed by Walstra et al, 1999). These levels are based on concentrations in fat and are determined by possible consumer reactions during consumption of pork. Using 0.20 ppm skatole as a threshold value for sorting out carcasses would mean that 7.7 % of all entire males produced in Norway must be sorted out. Using the most common sorting threshold for androstenone (1 ppm) 46.6 % of all entire males produced in Norway should be sorted out (Fredriksen, Hexeberg, Choinski, Ropstad & Nafstad, 2008). Sorting of carcasses will probably be based on both levels, so the percentages may be even higher. The income loss for the producers/industry will be substantial for each percentage of carcasses that need to be sorted out, and it is obvious that that there is a need to reduce the percentages of animals that needs to be sorted out.

Culture, experience and learning all impact food preferences, but genetic factors can also play a role in evaluating food. For example, genetic variation in the bitter receptor T2R38 affects sensitivity to Phenylthiocarbamide (PTC) (Kim, Jorgenson, Coon, Leppert & Risch, 2003) and correlates with food preferences (Dotson, Shaw, Mitchell, Munger & Steinle,2010). In addition to taste, odour is a major sensory component in flavour evaluation, yet how genetic variation in ORs affects food preferences remains unclear. Recent research has shown that detection of androstenone is, at least partly determined by the amino acid sequence of

the human odour receptor OR7D4 (Keller, Zhuang, Chi, Vosshall & Matsunami, 2007). Earlier studies have shown that consumers have different abilities to perceive androstenone (Wysocki & Beauchamp, 1984). Many consumers are insensitive to androstenone, but some consumers are highly sensitive and will react negatively upon exposure (Kline, Schwartz & Dikman, 2006). In a study on German and Spanish consumers Weiler, Fischer, Kemmer, Dobrowolski & Claus (1997) found that 31% of the German and 18% of the Spanish consumers were sensitive to androstenone. Large variation in androstenone sensitivity between countries makes it necessary to map each county separately since the fraction of androstenone sensitive consumers in a population is highly relevant as this figure could relate to the impact of specified androstenone levels on consumers' acceptance. Screening for androstenone sensitivity has earlier been performed by smelling pure crystals and rating the intensity on a seven point hedonic scale (Weiler et al., 2000), while others have used androstenone dissolved in mineral oil (de Kock, Heinze, Potgieter, Dijksterhuis & Minnaar, 2001) or androstenone in lard (Dijksterhuis et al., 2000) in a triangle test. In several studies the androstenone sensitivity has not been mapped at all. To what extent different presentation forms could affect the accuracy when allocating people as sensitive or non sensitive has not been discussed previously. There is a need for a standardized method that can be related to the consumer's apprehension of androstenone in meat products. Defining the consumer's androstenone sensitivity will be highly relevant before trying to define the acceptance threshold for androstenone in meat products. After knowing the consumer's androstenone sensitivity it will be

highly relevant to find methods to mask or reduce the perceived taint of androstenone in order to reduce economic losses.

Practically all consumers (99%) have the ability to perceive skatole (Weiler et al., 1997), and the compound can be detected in concentrations as low as 0.1 ppm (Bañón, Costa, Gil & Garrido, 2003; Font I Furnols, Guerrero, Serra, Rius & Oliver, 2000). Accordingly, the Norwegian established practise with a threshold value of 0.21 ppm skatole may be too high and negative reactions may occur from the consumer. Since the detection threshold (sensory assessors) for skatole appears to be as low as 0.1 ppm, it is important to find methods to mask or reduce the perception of boar taint avoiding negative reactions from consumers. At present, small quantities of tainted meat is used in different sausages; both dry-fermented and heat-processed. The meat processors are provided the skatole value of the back fat, and thereafter they can adjust their recipes using a large safety margin with respect to off-flavour. When castration is prohibited, the market situation will change dramatically, and it is therefore relevant to identify processing methods that can still provide high quality products to the consumer.

Objectives

The main objective of this thesis was to look at the possible challenges connected to a future production of entire males. The main focus was put on product quality and the Norwegian consumer's sensory perception of boar tainted meat.

To be able to solve the main objective of the study, the following three objectives were initially identified:

- Describe sensory quality of the raw material from entire males.
- Map the Norwegian consumers' sensitivity and acceptance of boar taint.
- Examine possible approaches for utilisation of the meat that needs to be sorted out to avoid negative consumer reactions.

Theory and approach

Instrumental measurements of skatole and androstenone

Various laboratory methods are in use in Europe for measuring skatole and androstenone. This includes different measurement principles and varying protocols for sample clean-up. This complicates the comparison between laboratories (Haugen, Lea & Lundby, 2010) in terms of; absolute thresholds values of consumers and sensory panels. Results from the recent ALSCADE inter laboratory comparison study showed a great need for a standardized and harmonized method for skatole and androstenone in male pig adipose tissue. This will be essential when comparing detection and acceptance threshold between countries. In addition, it is important to define if the skatole and androstenone values are reported *per* unit of fat or fat tissue, since fat tissue consists of approximately 78% fat. A reported fat tissue value will therefore be higher than values obtained in pure fat.

All skatole and androstenone values referred to in this thesis are values obtained in fat. Two different methods for analysing both skatole and androstenone were used during the experiments in this thesis because the analysing laboratory changed their analysing procedures during this project.

Table 1

Paper	Analytical method	
	Skatole	Androstenone
I	Automatic colorimetric assay	Elisa
II	HPLC	TR-FIA
III	HPLC	TR-FIA
IV	HPLC	TR-FIA
V	Automatic colorimetric assay	Elisa
VI	HPLC	TR-FIA

Determination of skatole using an automated colorimetric assay was performed after the method described by Hansen-Møller & Andersen (1994) and Mortensen & Sørensen (1984). Skatole was extracted from back fat in tris/acetone followed by addition of a colour agent. Absorbance was used for quantification of skatole. Skatole was also determined from extracted fat by HPLC (Agilent Technologies) using fluorescence detection according to a method developed by Gibis (1994). Automatic colorimetric assay measures skatole and indole together in homogenised fat, while using HPLC skatole and indole values are given separately and are analysed in melted fat.

The analysis of androstenone was based on the ELISA method of Claus, Herbert & Dehnhard (1997). Androstenone was determined using an extraction method followed by a commercial immunoassay (Ridel-del-Haen, Seelze, Germany).

Androstenone was also determined by a time-resolved fluorescent immunoassay as described by Tuomola, Harpio, Knuuttila, Mikola, & Løvgren (1997), modified by using antiserum produced and characterized by Andresen (1974). Androstenone was then analysed in melted fat using immunoassays in both methods.

GC-MS headspace analysis

This method has been used to support sensory and consumer analysis regarding if products really have different volatiles (paper V and VI). Identifying the specific components that the subjects respond to is, however, difficult due to the different human thresholds prevailing among volatile components.

Sensory evaluation

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyse, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone and Sidel, 1993).

Objective evaluation (trained sensory assessors)

The two main categories for methods in analytical evaluation of foods are difference testing and descriptive testing. Discrimination tests (difference test) answer whether any perceivable difference exist between to products,

the most well known methods being the triangle test and paired comparison tests (Lawless & Heymann, 1999; Meilgaard, Civille & Carr, 1999). Descriptive analysis is generally useful in any situation where a detailed specification of the sensory attributes of a single product or a comparison among several products is desired (Gillette, 1984). The descriptive sensory techniques allow for quantifying the perceived intensities of the sensory attributes of a product, and answers how products differ from each other. Sensory description of products obtained from descriptive profiling is frequently used to identify sensory properties that could be important for consumer acceptance. Descriptive profiling was used in all papers except paper IV. In paper III and VI descriptive analysis were used to select samples for further consumer testing.

The sensory panel used for descriptive profiling in this thesis was selected and trained according to guidelines in ISO 8586-1:1993 and the descriptive methodology used was in accordance with Generic Descriptive Analysis described by Lawless & Heymann. (1999). The samples were evaluated in a sensory laboratory designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory data.

Subjective evaluation (consumer testing)

In food research, it is obvious that the recognition threshold for a given flavour in a food would be useful to know. In the case of off flavours and taints, recognition may have a strong hedonic correlates in predicting consumers rejections (Lawless & Heyman, 1999).

Affective evaluation is a measure of consumers perception based on subjective responses with regard to preference and/or acceptance (Lawless et al., 1999; Meilgaard et al., 1999). Two main approaches in quantitative consumer testing exist, preference and acceptance measurements. In preference measurements the consumers has a choice, one product has to be chosen over one or more other products. However, it is important to consider that even though one product is chosen over another, the consumers might not like the product. Preference measurements were performed in one of the consumer studies in paper I.

Measuring the consumer's acceptance or hedonic liking, the consumers rate their responses to a product on a scale. The 7–point hedonic scale provides ratings of degree of liking of products, and provides measures of the size of difference between products. Hedonic ratings are applied in consumer testing in all papers except paper III and V.

Data analysis

The data analysis in this thesis is mainly performed by the statistical programs SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA), the open source software Panelcheck V 1.3.2 (http://www.panelcheck.com), Unscrambler (version 9.1, CAMO, Trondheim, Norway) and Minitab 14 (Minitab Inc., Pennsylvania, USA).

Various statistical methods have been used to analyse the data presented in this thesis.

Analysis of variance (ANOVA) is one of the most common statistical test performed on results from descriptive analysis and other test where more

than two products are compared using scale responses. It provides a sensitive tool for seeing whether treatment variables such as changes in levels of skatole and androstenone or different processing methods had an effect on the sensory properties of the product. Analysis of variance (Lea, Næs & Rødbotten, 1997) and Tukey's Multiple Comparison Test were used in all papers in the thesis to test for statistical significance between samples and sensitivity groups.

Principal component analysis (PCA) is a bilinear modelling method which gives an interpretable overview of the main information in multidimensional data tables. The information carried by the original variables is projected onto a smaller number of underlying "latent" variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on. By plotting the principal components one can view interrelationships between different variables, and detect and interpret sample patterns, groupings, similarities and differences. PCA was used on the sensory data in all papers to monitor the assessors in the principal components space according to their evaluations of the samples together with the average scores of the samples, showing product and attribute relationship.

In all papers, except paper V, the software Panelcheck was used to test both the performance of the entire sensory panel and also that of its individual members. Using this program it is easy to reveal which products or which

sensory properties the assessors may need further training or calibration in or simply disagree upon.

Main results of papers I - VI

This thesis focuses on evaluation of boar tainted meat using trained sensory assessors and consumers.

Paper I: The main objective of paper I was to develop a method to screen consumers for their androstenone sensitivity. Several different methods have been presented in the literature. To what extent different presentation forms could affect the accuracy when categorizing people as sensitive or insensitive had not been discussed in the scientific literature previously. The method developed in this study was a new procedure of testing consumers for their ability to perceive androstenone. The method involved intensity rating of androstenone crystals in water in a double 3 Alternative Forced Choice (AFC) test. In each of the 3 AFC tests two bottles with water and one bottle with androstenone were presented. The subjects rated intensity of the strongest odour on a Labelled Magnitude Scale after each test. The scale is anchored with “barely detectable” in the lower end and “strongest imaginable” in the higher end. The intensity scale is converted into numbers from 0 to 100, and the mean value of the two intensity ratings was used when defining the subjects as androstenone sensitive or insensitive. The results from the new method were validated for relevance by testing the consumers’ acceptance of boar tainted meat (odour and flavour). The results showed that the method separated well between sensitive and insensitive consumers. The sensitive group was defined as consumers that gave negative reactions to meat with higher levels of androstenone. The

insensitive group contained those consumers that gave no or positive reactions to androstenone tainted meat.

Knowledge of the fraction of androstenone sensitive consumers in a population is highly relevant as this figure are related to the acceptance of androstenone tainted meat and also provides a background for assessing economical consequences of sending entire male meat into the market. Results from the study showed that 39% of the Norwegian consumers were identified as androstenone sensitive.

Paper II: Recruitment of assessors for sensory profiling of boar tainted meat generally includes smelling of pure androstenone crystals. The aim of the research in paper II was to study the performance of sensory panellists concerning differentiation of meat samples with different levels of skatole and androstenone, with the main focus on androstenone. Our hypothesis was that being able to detect pure androstenone is not the same as giving a negative response, *i.e.* being sensitive to this component in meat. The assessors were tested with the sensitivity method developed in paper I, trying to show that this method would be suitable for recruiting assessors for evaluation of androstenone tainted meat.

The assessors (38) of 4 European sensory panels recruited according to ISO standards were reclassified in terms of their androstenone sensitivity. All 38 assessors were able to detect dry androstenone crystals during recruitment, but only 28 of the assessors were sensitive to androstenone when tested with the sensitivity method developed in paper I. The 28 androstenone sensitive assessors were able to detect androstenone odour in samples with androstenone $\geq$ 4.5 ppm and androstenone flavour in samples with

androstenone ≥ 3.7 ppm; all concentrations in fat. The 10 insensitive assessors could not detect androstenone even at 7.5 ppm despite the fact that all assessors detected dry androstenone crystals. These figures indicated that the method of recruiting assessors to a sensory panel was critical for the evaluation of androstenone tainted meat, and that the method developed in paper I was useful.

Paper III: All consumers (99%) have the ability to perceive skatole, but the ability to perceive androstenone varies among consumers. The ability to perceive androstenone is, at least partly, determined by the amino acid sequence of the human odour receptor OR7D4. The aim of the research in paper III was to relate OR7D4 genotype and androstenone sensitivity to the evaluation of meat samples with different levels of androstenone.
The result showed that subjects with at least one copy of the WM allele were classified as androstenone insensitive. Twelve of the sixteen subjects with the RT/RT genotype were classified as androstenone sensitive. The OR7D4 genotype explained 83% of the androstenone sensitivity, and confirmed the role of OR7D4 in olfactory sensitivity to androstenone. A portion of subjects can acquire sensitivity to androstenone after repeated exposure to androstenone. Although as a group there was no significant differences between intensity ratings before and after six weeks of daily exposure to androstenone, one RT/RT subjects who was initially classified as insensitive was reclassified as sensitive after the period of exposure. The consumer's evaluation of the samples showed that when consumers were divided by OR7D4 genotypes, there was a genotype effect on consumer's acceptance. RT/RT subjects disliked the flavour and odour more than the WM carriers.

The results from the sensory assessor's showed a significant interaction between androstenone concentrations and genotype for both odour evaluations, reflecting that subjects with the WM allele did not increase their intensity evaluations with androstenone content. Since the sensitivity test gives no false positive genotypes (all subject defined as sensitive had the RT/RT allele), it should be recommended for selecting assessors to panels and for setting androstenone thresholds in meat. The result of this paper suggests that the amount of sensitive consumers in Norway potentially need to be increased if all carriers of the RT/RT genotype can be sensitized.

Paper IV: In paper IV the Norwegian consumers' acceptability of pork meat with different levels of skatole and androstenone was studied. Knowledge of acceptance levels for skatole and androstenone will make it possible for the pork industry to provide an estimate of the economical consequences of a change to entire male production. The focus here was mainly on identification of consumers' androstenone thresholds using androstenone tainted meat. The consumers were segmented into sensitive and insensitive consumers prior to testing the meat using the method developed in paper I. The hypothesis was that a more correct estimation of the threshold value to androstenone would be achieved if consumers were classified with respect to androstenone sensitivity before they tested the tainted meat. Since insensitive consumers are expected to accept all levels of androstenone, the mean threshold of androstenone will be higher if results from insensitive consumers were included in the analysis. In addition, a correct percentage of consumers sensitive to androstenone and their acceptance threshold for

androstenone will give the pork industry better estimates of the economical consequences.

Androstenone insensitive consumers did not differentiate between reference (without androstenone) and androstenone tainted samples (≥ 7.5 ppm), meaning that the insensitive consumers accepted all levels of androstenone. Sensitive consumers gave a significant lower liking score for androstenone samples containing 3 ppm (and more) when evaluating these samples above the frying pan, but no significant difference was found between 3 ppm and reference samples when liking of the fried samples were evaluated. The same consumers differentiated samples with skatole flavour at 0.15 ppm. The Norwegian sort out threshold value today, 0.21 ppm skatole, may therefore lead to negative reactions from consumers. For androstenone, using a level of 3 ppm for sorting would be economically acceptable due to the low number of carcasses containing above 3 ppm (5.5%), but its odour may be detected (not accepted) by sensitive consumers during frying of the meat. Sorting thresholds used by the meat industry should be based on both skatole and androstenone values in combination since meat from entire males in most cases will contain both of these compounds. This suggests that samples containing either skatole above 0.1 ppm or androstenone above 2-3 ppm must be eliminated to avoid negative consumer reactions.

Paper V: Paper V investigated the effect of marinades on improving the eating quality in ready-to-eat boar meat, focusing on skatole using a trained sensory panel. The panel was not segmented in androstenone sensitive an insensitive assessors. The product used had fat content below 18.9%.

Liquid smoke and oregano extracts appeared to have the best potential for masking skatole off-flavour. Results from sensory analysis showed that marinated chops with skatole content of approximately 0.4 ppm were evaluated similar with respect to boar taint as samples made from castrates. Chops with skatole contents above 0.7 ppm remained unmasked despite the use of strongly flavoured marinades. Unmarinated chops served at 60°C were more tainted than those served at 15 °C, but scored lower for boar taint when reheated, although the concentrations of androstenone and skatole remained the same. The attribute manure related significantly to the skatole level of pork neck chops served to the sensory assessors. Common and strong food flavour additives like oregano extracts and liquid smoke affected the perception of boar taint. This study showed that meat samples with skatole levels up to 0.4 ppm could principally be used by the industry as raw material for pre-flavoured chops. Cold serving temperatures (15˚C) gave less perception of boar taint than serving at higher temperatures (approximately 60˚C). Reheating of pork neck chops tended to reduce the perception of boar taint. In general, it appears that volatile ingredients with low detection thresholds would be most successful in masking boar taint, and that it may be possible for the industry to use boar meat with higher skatole than 0.21 mg/kg using suitable processing.

Paper IV: Paper VI studied the possibility to reduce the perceived boar taint in bacon (fat % = 30-35) with the use of different production technologies (dry salting and brine injection) and fermentation. The main focus was on skatole. Bacon was analysed both by sensory descriptive analysis and

acceptance testing. Both sensory panel and consumers were pre-screened for androstenone sensitivity. The hypothesis was that with a suitable processing technology, bacon, despite the high fat fraction and high skatole (0.04 – 0.43) levels could be accepted among consumers. Results from the sensory profiling of bacon showed that smoke (brine injected samples) was effective in masking skatole, but did not have the same masking effect on androstenone. Dry salted bacon samples were given the highest mean values for both skatole odour and flavour, indicating that this process did not succeed in masking boar taint. The results also showed that the use of starter cultures lowered the perceived taint of skatole. In general, none of the technologies tested had a masking effect on androstenone. Comparing the results from the sensitive and non sensitive assessors it is obvious that the sensitive assessors' detected androstenone in all sample. Results from consumer testing showed that the dry salted bacon samples were given the highest liking scores by the Norwegian consumers when samples with higher levels of skatole were evaluated. These samples were not score significantly different from samples low in skatole; the reference sample included. These results indicated that the process of dry salting had a masking effect of skatole, and that it is possible for the industry to use meat with skatole up to 0.43 ppm (androstenone 1.61) without negative consumer reactions. This is in contrast to consumers that are able to detect skatole at 0.15 ppm in unprocessed meat samples with a lower fat %. No significant differences between dry salted samples and samples dry salted and fermented were found, indicating that the fermentation did not provide masking flavour beyond dry salting. The brine injected bacon samples, a common technology in the Norwegian industry today, were given the lowest

liking scores. This was probably due to the fact that these samples were too heavily smoked. Thus, smoke seemed to be effective in masking skatole, but the results may suggest that if liquid smoke is used to mask skatole there will be an upper concentration of liquid smoke aroma acceptable to the consumers. In general skatole seemed to be easier to mask than androstenone.

Conclusions

Results from this thesis have clearly shown the importance of testing sensory assessors and consumers for their ability to perceive androstenone before evaluating androstenone tainted meat. Using assessors or consumers not able to perceive androstenone will lead to incorrect estimates for acceptance of tainted meat since insensitive consumers accepted all levels of androstenone. The method developed in this thesis has shown to be useful in several studies. The OR7D4 genotype explained 83 % of the androstenone sensitivity, and the results showed that OR7D4 genotype and androstenone sensitivity correlated strongly with subject's evaluation of meat samples containing androstenone. Since the sensitivity test gives no false positive genotypes (all subjects defined as sensitive had the RT/RT allele), it should be recommended for selecting assessors to panels and for setting androstenone thresholds in meat. A total of 39% of the Norwegian consumers were defined as sensitive towards androstenone according to the method developed in this study. But the result suggests that the amount of sensitive consumers in Norway potentially need to be increased if all carriers of the RT/RT genotype can be sensitized.

The Norwegian consumers were able to detect skatole at 0.15 ppm in unprocessed samples, indicating at the sorting threshold of today, 0.21 ppm skatole, may lead to negative reactions from consumers. For androstenone, using a level of 3 ppm for sorting would be economically acceptable due to the low number of carcasses with androstenone levels above 3 ppm (5.5%), but using this threshold androstenone odour may be detected (not accepted) by sensitive consumers during frying of the meat.

Masking of skatole and androstenone was shown to be possible when adding marinade ingredients or using different production technologies. Smoke seemed to be effective in masking skatole, but if liquid smoke was used to mask skatole there seemed to be an upper concentration of smoke aroma acceptable to the consumers. In general, it appears that ingredients with low detection thresholds would be most successful in masking boar taint, and that it may be possible for the industry to use boar meat with higher skatole values than what is currently available in the Norwegian market today. Consumer testing of dry salted and fermented bacon showed that the processes of dry salting and fermentation also reduced the perceived taint of skatole. In general skatole seems to be easier to mask than androstenone.

Challenges and topics for further research

The study in paper I resulted in a new method to test consumers/assessors for their ability to perceive androstenone. This method was validated for relevance by evaluation of meat samples and also by defining the subjects DNA since the ability to perceive androstenone is, at least partly, determined by the amino acid sequence of the human odour receptor OR7D4 (Keller et al., 2007). The subject's androstenone sensitivity corresponded with their DNA typing in all cases where the subjects were defined as sensitive. This group gave also negative responses to androstenone tainted meat. Since the sensitivity test gave no false positive genotypes, it should be recommended for selecting assessors to panels and for setting androstenone thresholds in meat. A few of the subjects defined as insensitive had the genotype suggesting they had the ability to perceive androstenone, and one of these subjects was defined as sensitive after a 6 weeks sensitization experiment (daily exposure to androstenone). The results indicated that sensitization is possible when it comes to androstenone. Our data may suggest that 4 out of 16 could appear as false negative using the odour test developed in paper I. This is a high figure and then the percentage of consumers sensitive to androstenone in Norway may be 52% instead of 39%. In general there was a high correlation between the defined androstenone sensitivity, the DNA typing and the subject's evaluation of meat samples. However, further work is needed in order to understand sensitization of insensitive with the genotype expecting them

being able to perceive androstenone in order to achieve the highest accuracy regarding negative reactions to androstenone in meat in a specific country.

Entire male production in Norway will increase the percentage of animals that needs to be sorted to avoid negative consumer reactions. Knowledge of acceptance levels for skatole and androstenone will make it possible for the pork industry to provide an estimate of the economical consequences of a change to entire male production. Setting the acceptance thresholds for skatole and androstenone among Norwegian consumers was the aim of the study presented in paper V. The results in this paper showed that the consumers differentiated samples with skatole, with regard to flavour at 0.15 ppm. The Norwegian sort out threshold value of 0.21 ppm present sorting tresholds of skatole may therefore lead to negative reactions from consumers. For androstenone, the result showed that samples with 3 ppm androstenone were accepted by the androstenone sensitive consumers when they evaluated the fried samples (liking of both odour and flavour), but not accepted during frying of unflavoured meat samples. Using a level of 3 ppm for sorting would be economically acceptable due to the low number of carcasses above 3 ppm (5.5%) in Norway, but its odour may be detected (not accepted) by sensitive consumers during frying of the meat. If animals with androstenone levels above 2 or 3 ppm are to be eliminated in Norway this means that 17.3% or 5.5 %, respectively, of male carcasses will be rejected (Fredriksen et al. 2008). These figures actually suggest that a follow up study should be made with sensitive consumers using selected samples with androstenone contents between 2 and 3 ppm. This is highly relevant since the

economical consequences for the pork industry will be more critical if 17.3% in contrast to 5.5% of the entire males needs to be sorted out.

Earlier studies dealing with odour and flavour characterization of processed products from entire males have shown that processing will lead to a higher acceptability of tainted meat (Walstra, 1974; Diestre, Oliver, Gispert, Arpa & Arnau, 1990; Bonneau, Le Denmat, Vaudelet, Veloso-Nunes, Mortensen & Mortensen, 1992b; Lunde, Egelandsdal, Choinski, Flåtten & Kubberød, 2008; Stolzenbach, Lindahl, Lundström, Chen & Byrne 2009). The higher acceptability can be explained by processing methods, addition of ingredients or as found by McCuley et al. (1997) that the higher acceptability could be explained more by the temperature of presentation rather than the processing itself. The androstenone content of Norwegian entire males (animals used for breeding) is not analysed in agreement with the practise of other countries. The main focus in several of the studies above has therefore been on skatole. In addition, trying to mask androstenone without defining the subject's androstenone sensitivity will lead to false results when insensitive subjects are included. The insensitive subjects will not react negatively to androstenone in meat at any level anyway. In general, the results presented in papers V and VI showed that masking of skatole seems to be easier than masking of androstenone. Further research is necessary trying to find ways of masking androstenone, when the androstenone sensitivity of the consumers is defined.

Smoke have shown to be effective in masking skatole in several studies, but the results presented in paper VI may suggest that if smoke is used to mask skatole there will be an upper concentration of smoke aroma acceptable to

the consumers. Finding the levels of smoke acceptable to consumers but still able to mask skatole will be relevant for the pork industry. Also further investigations into smoke composition could be relevant as it appears that some smoke components are not giving negative responses even if present at a high level.

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Abstracts of papers

Paper I

A new method for differentiating the androstenone sensitivity among consumers

A new method of testing consumers for their ability to perceive androstenone has been developed. The method used androstenone crystals in distilled water in foiled glass bottles. This presentation form made it easier to perceive androstenone while at the same time avoiding detectable smell from (mineral) oils.

The results from the new method were validated for relevance by testing the consumers' acceptance of boar tainted meat (odour and flavour). The results showed that the method separated sensitive and non sensitive consumers. The sensitive group was defined as consumers that gave negative reactions to meat with higher levels of androstenone. The non sensitive group contained anosmic consumers and those consumers that gave no or positive reactions to androstenone tainted meat.

Paper II

The importance of the recruitment method for androstenone sensitivity with respect to the accurate sensory evaluation of androstenone tainted meat

Four European sensory panels where all (38) assessors, when recruited, were able to detect dry androstenone crystals through smelling, were reclassified in terms of sensitivity using a recently developed sensitivity method based both on the assessor's ability to detect androstenone and the spontaneous descriptor used to describe the odour. The reclassification reduced the number of assumed androstenone sensitive assessors from 38 to 28. All 38 assessors evaluated 6 samples (at approx. 60°C) of minced meat low in skatole ($\leq$ 0.05 ppm) with androstenone contents from 3 to 9 ppm. The 28 androstenone sensitive assessors were able to detect androstenone odour in samples with androstenone $\geq$ 4.5 ppm and androstenone flavour in samples with androstenone $\geq$ 3.7 ppm; all concentrations in the fat. The 10 insensitive assessors could not detect androstenone even at 9 ppm despite the fact that all assessors detected dry androstenone crystals. The 10 insensitive assessors were present in 3 panels, the panels then having from 50 - 88.8% sensitive assessors. This showed that the method of recruiting assessors to a sensory panel was critical for the evaluation of androstenone tainted meat.

Paper III

Genetic variation of OR7D4 affects sensory perception of meat containing androstenone

Although odour perception impacts food preferences, the effect of genotypic variation of odorant receptors (ORs) on sensory perception of food is unclear. Human OR7D4 responds to androstenone, and genotypic variation in *OR7D4* predicts variation in the perception of androstenone. Since androstenone naturally occurs in meat derived from male pigs, we asked whether OR7D4 genotype correlates with either the ability to detect androstenone or the evaluation of pork tainted with varying levels of androstenone. Consistent with previous findings, subjects with two copies of the functional RT variant were more sensitive to androstenone than subjects carrying a non-functional WM variant. When pork containing varying levels of androstenone was cooked and tested, ssubject's with two copies of the RT variant as a group rated the androstenone-containing meat as less favourable than subjects carrying the WM variant. Our data suggest that the OR7D4 genotype predicts sensory perception of meat containing androstenone. This is the first demonstration that genetic variation in an odorant receptor alters food preferences.

Paper IV

Norwegian consumer's acceptability of boar tainted meat with different levels of androstenone or skatole as related to their androstenone sensitivity

The aim of work was to study Norwegian consumers' acceptance of pork meat with different levels of skatole and androstenone. One group of androstenone sensitive consumers (N=46) and one group of non sensitive consumers (N=55) participated in a home test and evaluated 11 samples with different skatole (range 0-0.35 ppm) and androstenone (range 0-9.0 ppm) levels. Liking of odour during frying and odour and flavour of the fried meat were evaluated. Results showed that the non sensitive consumers accepted all levels of androstenone in the samples. Sensitive consumers gave a significantly lower liking score for androstenone samples containing 3 ppm (and more) than the reference sample when evaluating these samples above the frying pan, but no significant difference were found between 3 ppm samples and reference samples when liking of fried meat was evaluated. This indicated that the sensitive consumers accepted 3 ppm in fried meat, but not if 3ppm was present in the sample during the frying process. The same consumer's differentiated skatole samples with regard to flavour at 0.15 ppm. The Norwegian established practise with a threshold value of 0.21 ppm skatole is higher than the value accepted by the consumers.

Paper V

Marinating as a technology to shift sensory thresholds in ready-to-eat entire male pork meat

This study investigated the effect of marinades on improving the eating quality in ready-to-eat boar meat. Neck chops with fat content below 18.9%, skatole ≤1.1 ppm (range 0.03 - 1.1) and androstenone ≤ 5.6 ppm (range 0.01 - 5.6) were used. In a screening experiment different marinades were tested for their ability to mask boar taint (defined as manure and urine odour and flavour). Liquid smoke and oregano extracts appeared to have the best potential for masking, and were studied in details. Results from the study indicated that marinated chops with skatole content of approximately 0.4 ppm appeared similar in boar taint to castrates. Chops with skatole contents above 0.7 ppm remained unmasked despite the use of strongly flavoured marinades. Unmarinated chops served at 60°C were more tainted than those served at 15 °C, but scored lower for boar taint when reheated, although the concentrations of androstenone and skatole remained the same. The fat content of the chops were not well correlated to the perception of boar taint. The attributes manure and urine were correlated to the level of skatole, but urine attribute was not a good indicator of the androstenone level.

Paper VI

Masking of boar taint in fermented, dry salted and brine injected bacons

The aim of the present work was to study the possibility to mask boar taint with the use of different production technologies: Dry salting, brine injection and dry salting plus fermentation. Bacon raw materials with different levels of skatole (range 0.04 – 0.43 ppm, fat values) and androstenone (range <1 – 3.21 ppm, fat values) were analysed by a trained sensory panel and a consumer panel.

Ten assessors evaluated the bacon samples and the results indicated that smoke was effective in masking skatole, but not androstenone. The process of dry salting did not succeed in masking boar taint, but used in combination with fermentation the perceived taint of skatole was reduced. The consumers (43) evaluated liking of odour during frying and odour and flavour of the already fried meat. Results from consumer testing showed that production of dry salted bacon made it possible for the meat industry to use boar meat with skatole levels up to 0.43 ppm in the fat (androstenone 1.61) without negative consumer reactions. Also dry salted and fermented bacon (starter cultures BFL-N16 and S-SX) was accepted by the consumers at a high skatole level of 0.35 ppm (androstenone 1.27 ppm).

Paper I – VI

Paper I

Food Quality and Preference 20 (2009) 304–311

Contents lists available at ScienceDirect

Food Quality and Preference

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

A new method for differentiating the androstenone sensitivity among consumers

K. Lunde [a,c,*], E. Skuterud [a], A. Nilsen [b], B. Egelandsdal [c]

[a] Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396, Økern, N-0513 Oslo, Norway
[b] Nofima Food, Osloveien 1, N-1430 Ås, Norway
[c] Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O. Box 5003, N-1432 Ås, Norway

ARTICLE INFO

Article history:
Received 20 April 2008
Received in revised form 7 November 2008
Accepted 4 February 2009
Available online 13 February 2009

Keywords:
Boar taint
Androstenone odour test
Consumer and sensory analysis

ABSTRACT

A new method of testing consumers for their ability to perceive androstenone has been developed. The method used androstenone crystals in distilled water in foiled glass bottles. This presentation form made it easier to perceive androstenone while at the same time avoiding detectable smell from (mineral) oils.

The results from the new method were validated for relevance by testing the consumers' acceptance of boar tainted meat (odour and flavour). The results showed that the method separated sensitive and non sensitive consumers. The sensitive group was defined as consumers that gave negative reactions to meat with higher levels of androstenone. The non sensitive group contained anosmic consumers and those consumers that gave no or positive reactions to androstenone tainted meat.

1. Introduction

Castration of male pigs is done to prevent an unpleasant odour/flavour that can occur in meat from boars. Castration of male pigs is expected to be prohibited in Norway in the future. Extensive work has shown that boar taint is mainly correlated with the presence of two compounds: skatole and androstenone (Patterson, 1968; Vold, 1970). Earlier studies showed that consumers have different abilities to perceive androstenone (Wysocki & Beauchamp, 1984). Many consumers are insensitive to androstenone, but some consumers are highly sensitive and will react negatively upon exposure (Kline, Schwartz, & Dikman, 2006). The consequences of letting boar meat enter the Norwegian market are yet unknown, because the Norwegian population has not been mapped or screened for androstenone sensitivity. Screening for androstenone sensitivity has earlier been performed by smelling pure crystals and by rating the intensity on a seven-point hedonic scale (Weiler et al., 2000), while others have used androstenone dissolved in mineral oil (De Koch, Heinze, Potgieter, Dijksterhuis, & Minnaar, 2001) or androstenone in lard (Dijksterhuis et al., 2000) in a triangle test. To what extent different presentation forms could affect the accuracy when allocating people as sensitive or non sensitive has not been discussed previously.

The fraction of androstenone sensitive consumers in a population is highly relevant as this figure could relate to the impact of specified androstenone levels on consumers' acceptance.

In this study, different carrier mediums for the androstenone compound were first screened on a sensory panel with people that were able to detect androstenone to find which presentation form gave the most intense odour and the most negative response. Then a questionnaire with a triangle approach combined with a label magnitude scale (LM scale) was developed. The method was thereafter used to test approximately 1200 consumers in the Norwegian market for their ability to perceive androstenone. The method developed here is also a relevant method to screen sensory assessors for their ability to perceive androstenone before evaluating androstenone tainted meat.

2. Materials and methods

The study consisted of four main parts: (i) testing different media as carriers of androstenone, (ii) developing a method for testing consumers for androstenone sensitivity, (iii) relating the outcome of the sensitivity test to the odour and flavour of boar meat samples using both a trained sensory panel and the consumer studies and (iv) screening the Norwegian population for androstenone sensitivity. The different parts in the study are shown in Fig. 1. The meat samples selected were necks and belly sides from entire male pigs with different combinations of skatole and androstenone.

2.1. Testing of different media as carriers of androstenone

Testing for androstenone sensitivity has earlier involved smelling pure crystals (Weiler et al., 2000), or androstenone crystals dissolved in mineral oil (de Koch et al., 2001). A trained laboratory sensory panel of nine assessors evaluated (rank order test) the

* Corresponding author. Address: Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396, Økern, N-0513 Oslo, Norway. Tel.: +47 22092317; fax: +47 22220016.
E-mail address: kathrine.lunde@animalia.no (K. Lunde).

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doi:10.1016/j.foodqual.2009.02.001

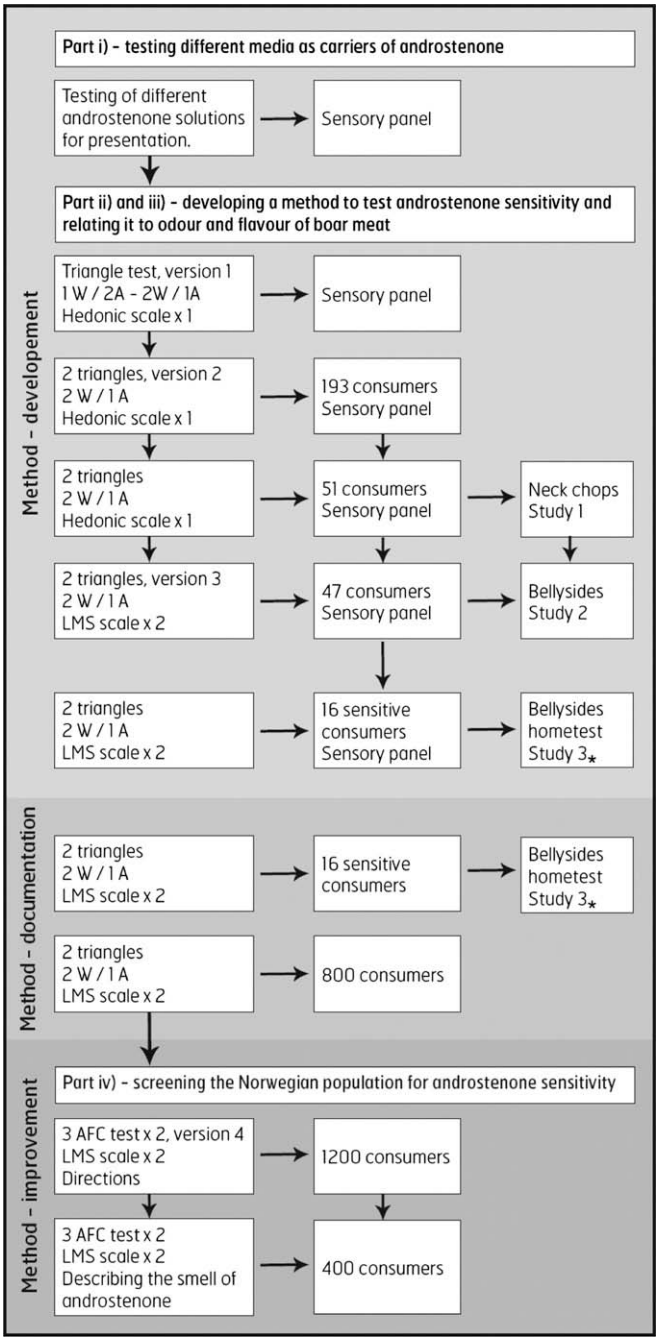

Fig. 1. The figure shows the different steps in the development of a method to test consumers for their ability to perceive androstenone. The four main parts in the study are also marked. *The belly side home test boxes are the same test evaluated twice but with different consumers. W = water and A = androstenone.

different systems for the presentation of androstenone from the lowest intensity to the highest intensity of odour. The assessors were all able to detect androstenone in pure form. The different systems of androstenone which were tested are presented below.

2.1.1. System 1

Pure androstenone crystals (5α-Androst-16-en-3-one) from Sigma–Aldrich, Co., Ltd., Poole. Crystals (0.0017 g) were tested in small glass bottles with a screw cap (Sigma–Aldrich, Z263133 Wheaton wide-mouth bottles with caps, 125 ml). The cap was only taken off when the bottle content was subjected to smelling.

2.1.2. System 2

Androstenone crystals dissolved in paraffin oil (Paraffinum liquidum, Unikem, Copenhagen). Androstenone crystals (0.0017 g) were dissolved in 10 ml paraffin oil in small glass bottles (Sigma–Aldrich, Z263133 Wheaton wide-mouth bottles with caps, 125 ml). The bottles were placed in a water bath with ultrasound (Bandelin Electronic, RK 106S, Germany) for 10 min.

2.1.3. System 3

Androstenone crystals in distilled water where the un-dissolved crystals were not removed. Androstenone crystals (0.0017 g) were dissolved in 10 ml distilled water in small glass bottles (Sigma–Aldrich, Z263133 Wheaton wide-mouth bottles with caps, 125 ml). The bottles were placed in a water bath with ultrasound (Bandelin Electronic, RK 106S, Germany) for 10 min. The un-dissolved crystals were not removed from the solution, and were floating on the saturated water solution.

2.1.4. System 4

Androstenone crystals in distilled water where the un-dissolved crystals were removed. Androstenone crystals (0.0017 g) were dissolved in 10 ml distilled water in small glass bottles (Sigma–Aldrich, Z263133 Wheaton wide-mouth bottles with caps, 125 ml). The bottles were placed in a water bath with ultrasound (Bandelin Electronic, RK 106S, Germany) for 10 min. Un-dissolved crystals were removed by filtration (Filter paper circles, Schleiche & Schüll GmbH, Germany).

2.1.5. System 5

Boar mate spray (Antec Boarmate, Antec International Limited, Suffolk, UK). Boar mate spray was sprayed on a filter paper (in a ventilating cupboard) until this paper was totally wet. The filter paper was dried so the carrier gases (isopropyl alcohol and liquefied petroleum gas (LPG)) from the spray evaporated. The dried filter paper was then put in a glass bottle (Sigma–Aldrich, Z263133 Wheaton wide-mouth bottles with caps, 125 ml).

All bottles were covered with an aluminium foil making it impossible to see their contents. The bottles were served to the sensory panel in a randomized order. The sensory assessors ranked the systems from low intensity to high intensity of odour. The assessors had 1 min break between each system, and were able to smell each system as many times as wanted.

The sensory assessors also evaluated the difference between pure water and oil. Twenty five millilitres of room tempered solutions were presented to the assessors in a triangle test made up with two bottles of water and one with oil. In addition, the odour and flavour of pure oil were evaluated. The evaluations were performed in laboratory equipped according to ISO 8589-1988. All the sensory studies have been performed in two replicates.

2.2. Procedure for testing consumers for androstenone sensitivity

Four different versions of the sensitivity test were evaluated to find the version that separated between the sensitive and non sensitive consumers. The different steps in the method development are shown in Fig. 1.

2.2.1. Version 1

The starting point was a triangle test (Lawless & Heymann, 1998) with three samples, two of them with the same content and one with a different content. The issue was to choose the sample with the content that was different from the other two. Two different triangles were tested for each consumer. One triangle with two bottles of water and one bottle with androstenone, and the other triangle with two bottles of androstenone and one with water. The triangle test was accompanied by a seven-point hedonic

scale in order to evaluate the intensity of the androstenone odour in the different bottles. Version 1 was tested on the sensory panel that consisted of nine persons all able to detect androstenone.

2.2.2. Version 2

A triangle with two bottles of water and one bottle with androstenone was chosen from version 1. To reduce the possibility of guessing correctly which bottle in the triangle was tainted, two consecutive triangles with a break (2 min) between were used. To be classified as sensitive to androstenone, the tainted bottle had to be correctly identified for both the triangles; thereby, the probability of guessing correctly was reduced from 1/3 to 1/9. After evaluating the last triangle a seven-point hedonic scale was still used to evaluate the intensity of the androstenone odour in the different bottles. In addition to the hedonic scale another scale was tested, the labelled magnitude scale (LMS). The LM scale is a quasi-logarithmic scale with label descriptors, which is equivalent to magnitude estimation (Green et al., 1996). The scale is anchored with "barely detectable" in the lower end, and "strongest imaginable" in the higher end. The intensity scale is converted into numbers from 0 to 100. The LM scales used to evaluate the intensity are shown outlined by boxes in Fig. 2. In contrast to standard rating scales where the responses are bound by labels as "very strong" or "extremely strong", the LM scale made it possible to relate the stimuli to the "strongest imaginable" stimuli experienced in everyday life. Instructions for using the scale were given according to Green, Shaffer, and Gilmore (1993) adjusted to our stimuli: "you will rate the intensity of the different solution in each triangle test by placing a mark on the labelled scale that best describes what you experience. You can use any part of the scale that seems appropriate for judging intensity. In making your judgements of intensity, you should rate the solution relative to the strength of sensations you have experienced in everyday life. Thus, "strongest imaginable" refers to the most intense sensation you have experienced smelling food and non-food items. This version was tested in two consumer trials (193 and 53 consumers) and by the sensory panel.

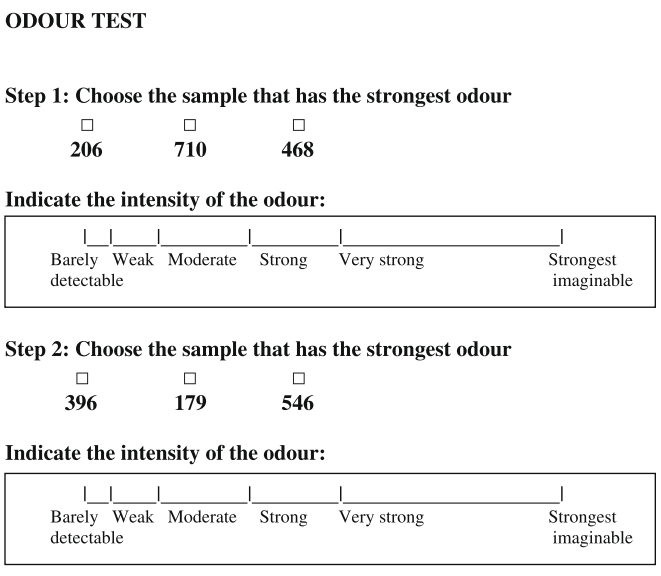

Fig. 2. The figure shows the questionnaire used in the final version of the sensitivity test that was used to screen consumers for androstenone sensitivity.

2.2.3. Version 3

Several consumers expressed that the bottle that was different in the first triangle had a stronger odour than the bottle that was different in the second triangle. It was then decided that the LM scale should be used after both triangles, and that the mean value of the intensity scores should be used in classifying consumers into sensitivity groups. Between the assessment of the two triangles the consumers had to take a break (approximately 2 min), using the time to fill in name, gender and age in the questionnaire.

The different versions of the method were tested in consumer trials during the method development. The presentation was changed as a result of experiences during the consumer studies. When the bottles with water were stored close to the androstenone bottle for a period, the water bottles also attained an androstenone odour. The androstenone odour from the water bottles was very much weaker than the androstenone odour from the bottle that actually contained androstenone. This version was tested in four consumer trials (47, 16, 16 and 800 consumers) and by the sensory panel.

2.2.4. Version 4

The guidelines to the consumers were changed from having to choose the bottle different from the two other bottles to choose the bottle with the strongest odour. Still two bottles with water and one bottle with androstenone were used. When the question was changed, the method changed from a triangle test to a 3-alternative forced test (3-AFC test) (Lawless & Heymann, 1998). As for the triangle test, the 3-AFC test was done twice by each consumer. The statistical calculations are the same as for the triangle test. In addition to changing the question, some new directions for use were made. The consumers were asked to smell each bottle only once. The water bottles were replaced with new bottles after every 20 consumers. The water used was Olden (Hansa Borg Breweries, Norway). All the bottles were covered with an aluminium foil to reduce perfume, lotion and similar from contaminating the glass bottles. The alumina foil on the androstenone bottles was changed when the two water bottles were replaced. In addition, to evaluate the intensity of androstenone in the two triangles the consumers were asked to describe the odour of the bottle identified as different. The last version was tested by 1200 consumers.

2.3. Relating the method to boar odour from meat

2.3.1. Skatole and androstenone

Skatole and androstenone values were measured in the back fat before processing. Skatole was determined using an automated colorimetric assay (Hansen-Møller & Andersen, 1994; Mortensen & Sørensen, 1984). The analysis of androstenone was based on the ELISA method of Claus, Herbert, and Dehnhard (1997). Androstenone was determined using an extraction method followed by a commercial immunoassay (Ridel-del-Haen, Seelze, Germany). The sensitivity of the Elisa method was 0.04 μg/g fat, and was more accurate than the method using high-performance liquid chromatography (HPLC) which has a sensitivity of 0.5–1 μg/g fat. In this study, when analyzing both these components, the analytical error (standard error) was approximately 0.1 ppm.

2.3.2. Sensory analysis done by a trained laboratory sensory panel

The sensory panel consisted of nine trained expert assessors with four to twenty years of experience in sensory profiling. The sensory laboratory was designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory

data (CSA, Compusense Five, Version 4.6, Canada). All assessors had the ability to detect androstenone in pure form.

The assessors evaluated the samples using a 9 cm unstructured continuous scale, where the left side of the scale corresponded to "low intensity" and the right side of the scale corresponded to "high intensity". The attributes employed for the evaluation were total intensity, boar (urine/manure), acid and rancid. The same attributes were used for both flavour and odour evaluation, and the sensory profile was the same in all the three studies (see below). The training of the assessors was done using meat from entire males with different levels of androstenone ($\leqslant$6.04 ppm) and skatole ($\leqslant$1.1 ppm) and a reference sample (castrate). The assessors were trained using the attributes (total intensity, boar, acid and rancid) in the profile.

During the method development, three different sensory evaluations on boar tainted meat (odour and flavour) were performed.

2.3.2.1. Study 1. Neck chops from five entire male pigs with low levels of skatole ($\leqslant$0.11 ppm) and different levels of androstenone were served the sensory panel. The androstenone levels evaluated are presented in Table 1. The samples (30 g), lean meat with adhering fat tissue, were fried in a warm frying pan for a few minutes until the samples were baked right through and then served warm (approximately 65 °C) on white plastic plates. Flavour and odour (all attributes) for all samples were evaluated in the booths on a 9 cm unstructured scale. The samples were served in a randomized order.

2.3.2.2. Study 2. Samples from the same animals in study 1 were evaluated again, this time as slices from the belly side. The fat content in the samples was approximately the same, but in study 1 the meat had adhering fat tissue while in study 2 the samples contained intramuscular fat. Sample 1 (lowest androstenone content) and sample 5 (highest androstenone content) from study 1 were used. Odour during frying and odour and flavour in the booths were evaluated for all the four attributes. The sensory assessors evaluated odour during frying by smelling directly above the frying pan. Sample 1 was evaluated first, and then the room was ventilated for 15 min before sample 2 was evaluated. New samples were made for the odour and flavour evaluation in the booths. These samples were fried as in study 1 and served warm (approximately 65 °C) on white plastic plates.

2.3.2.3. Study 3. Half a slice of belly sides from three entire male pigs with different levels of androstenone combined with low skatole values were evaluated by the sensory panel. The androstenone and skatole values are presented in Table 2. Odour during frying was evaluated by the sensory assessors smelling directly above the frying pan. The samples were evaluated in an increasing order of androstenone. The room was ventilated for 15 min between each sample. New samples were made for flavour and odour evaluation in the booths. The samples were fried as in studies 1 and 2 and served warm (approximately 65 °C) on white plastic plates, and were evaluated in a randomized order.

All the sensory studies (1–3) have been replicated.

Table 1
Study 1 and Study 2 (pan-fried) levels of androstenone and skatole in neck chops (1–5) from entire male pigs. Androstenone and skatole values were measured in backfat.

Chop	Androstenone (ppm)	Skatole (ppm)
1	0.37	0.03
2	0.82	0.09
3	1.60	0.11
4	3.81	0.03
5	6.04	0.05

Table 2
Study 3 levels of androstenone and skatole in the belly side slices from entire male pigs. Androstenone and skatole values were measured in backfat.

Slice	Androstenone (ppm)	Skatole (ppm)
1	0.16	0.07
2	1.26	0.07
3	3.00	0.07

2.3.3. Consumer studies

Samples from the same animals (and same muscle) as used in the sensory analysis (studies 1–3) were also evaluated in different consumer studies.

2.3.3.1. Study 1. Samples from the same animals (same muscle) as evaluated by the sensory assessors in study 1 (Table 1) were also evaluated by 51 consumers (33 women and 18 men). The consumers, 17 non sensitive, 17 medium sensitive and 17 highly sensitive, were recruited by the first version (version 1) of the sensitivity test. Liking of odour and flavour were evaluated on a seven-point hedonic scale from dislike very much (1) to like very much (7). The samples (30 g), lean meat with adhering fat tissue, were fried as for the sensory assessors and served warm (approximately 65 °C) in a randomized order. The consumers evaluated the samples in booths.

2.3.3.2. Study 2. The same consumers as in study 1 evaluated the two samples that were also evaluated by the sensory assessors (study 2). The consumers were tested with a new version 3 of the sensitivity test. The consumers were tested one by one, and there were no discussions between the consumers. The version divided the consumers into two groups (sensitive and non sensitive) instead of the former three groups. The low and high androstenone samples (samples 1 and 5, Table 1) were served as a paired comparative test (Lawless & Heymann, 1998), where the consumers were asked to choose the sample they liked the most, and describe what they did not like about the other sample. The samples (slices from the belly sides) were fried as earlier and evaluated warm in sensory booths. In addition to the paired comparative test, the consumers evaluated intensity and liking on a seven-point hedonic scale when smelling both samples during frying. The two samples were fried in two different rooms.

2.3.3.3. Study 3. Sixteen of the sensitive consumers from studies 1 and 2 evaluated the samples presented in Table 2 (slices from belly sides). This was done as a home test. The consumers got one slice from each belly side for the evaluation. Sample 2 or 3 (Table 2) was always served pair wise together with sample 1 (Table 2). The home test was done during minimum 3 days allowing the consumers to evaluate only one pair each day. The instructions were to evaluate sample 1 first, and the other samples afterwards. All samples were to be frozen until used. The consumers evaluated intensity and liking of odour during frying, and then liking of odour and flavour on the fried meat. The consumers used a seven-point hedonic scale with dislike very much on the left side and like very much on the right side. In addition detailed questions about the frying situation were asked, and the consumers were allowed to comment on each sample. The home test was repeated with 16 new consumers sensitive according to the test developed here. The different steps in the method development are shown in Fig. 1.

2.4. Screening the Norwegian consumers for androstenone sensitivity

Approximately, 1200 consumers all over Norway were tested for their ability to perceive androstenone using the test developed in this study (version 4). The consumers were tested at five shopping malls in different parts of Norway.

2.5. Statistical analysis of the results

Minitab release 14 (Minitab Inc., USA) and SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA) were used in significance testing (one way ANOVA), the mean sensory ratings provided by the sensory panel and the consumers. SPSS (SPSS Inc., Chicago) was used in cross tabulations of sensitivity, sex and age.

3. Results

3.1. Testing of different media as carriers of androstenone

The sensory assessors ranked the five different test solutions from the lowest intensity to the highest intensity of odour. Boar mate spray and androstenone crystals in water with the un-dissolved crystals removed were the solutions with the lowest intensities (not shown). The remaining solutions (pure crystals, androstenone crystal in oil and androstenone crystals in water) were evaluated again together with pure oil and water. The assessors ranging from the lowest intensity to the highest intensity were water, oil, oil with androstenone, pure crystals and water with androstenone. The results are presented in Table 3. There were no significant differences between the three methods involving visible crystals.

When evaluating the difference between pure water and oil in a triangle test (two with water and one with oil), a significant ($p \leqslant 0.05$) difference was found (not shown). The sensory assessors found the oil to differ significantly from water. The evaluation of odour and flavour of pure oil showed that the assessors used words such as vaseline, medicine, plastic and wax when describing the oil.

3.2. Procedure for testing consumers for androstenone sensitivity

The questionnaire used in the final version of the sensitivity test (version 4) is presented in Fig. 2. To be classified as sensitive, the mean value of the two intensity evaluations must be strong (35 when the LM scale is converted to numbers) or higher.

3.3. Relating the method to boar meat

3.3.1. Sensory analysis done by a trained laboratory sensory panel

No significant ($p > 0.05$) differences were found (boar attributes) by the sensory panel when the neck chops from different entire male pigs were evaluated (odour and flavour) in study 1. There was a tendency that samples with the skatole levels of 0.09 and 0.11 ppm explained more of the variance for the boar attributes (odour and flavour) than for the androstenone samples. In study 2, the low and the high androstenone samples from study 1 were evaluated again, this time as slices from belly sides (same animals). A significant difference ($p \leqslant 0.05$) was detected by the sensory panel for the boar attributes when smelling the samples above the frying pan. A significant ($p \leqslant 0.05$) difference was also found between these two samples when flavour and odour were evaluated in the booths. In study 3, the sensory assessors evaluated three samples with different levels of androstenone (Table 2). The results are presented in Fig. 3. The sample with the highest androstenone value ($A = 3$ ppm) was significantly different ($p \leqslant 0.05$) from the sample with the lowest androstenone value ($A = 0.16$) for all boar attributes. The sample with the highest level of androstenone was also significantly different ($p \leqslant 0.05$) from the androstenone sample with 1.26 ppm when the samples were evaluated in the booths (odour and flavour). No significant difference ($p > 0.05$) between these two samples ($A = 3.0$ and $A = 1.26$ ppm) was found when the assessors evaluated odour during frying. The sample with androstenone value of 1.26 ppm was significantly different ($p \leqslant 0.05$) from the lowest androstenone sample ($A = 0.16$ ppm) when evaluating odour during frying, but not when odour and flavour were evaluated in the booths.

Table 3
Sensory assessors' evaluation (rank order test) of the different systems with androstenone from the lowest (1) to the highest (5) androstenone intensity. The mean values of the assessors are shown.

Water	1.00c
Oil	2.22bc
Oil with androstenone	3.56ab
Androstenone crystals	3.78ab
Water with androstenone	4.44a

Different letters within the same column indicate significant differences ($p \leqslant 0.05$).

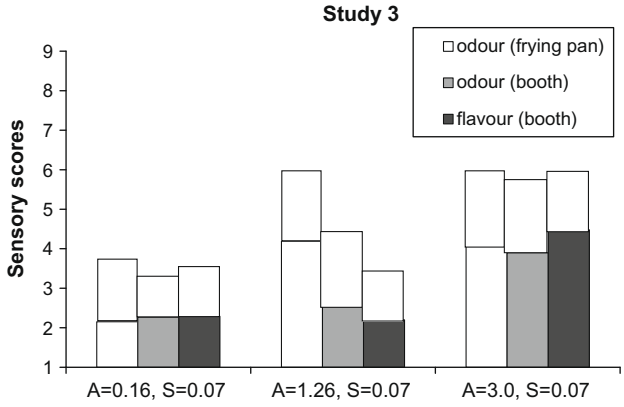

Fig. 3. Sensory assessors evaluation (boar attributes) of the three samples with different levels of androstenone (Table 2). Odour during frying and odour and flavour in the booths were evaluated. Mean values and standard deviations are shown.

3.3.2. Consumer studies

No significant ($p > 0.05$) differences between the samples were detected when the consumers evaluated the different neck chops (study 1) from the entire males presented in Table 1. No differences regarding how androstenone sensitive, medium sensitive and non sensitive consumers, defined by version 2 of the test, evaluated the samples for the perception of boar taint were shown. When the consumers evaluated the low and the high androstenone samples in a paired comparative test (study 2), there were no significant ($p > 0.05$) differences in how the consumers, both androstenone sensitive and non sensitive (grouped according to version 3), evaluated the odour and flavour of the served meat. These two samples were also evaluated during frying. The sensitive group detected a significant ($p \leqslant 0.05$) difference between the low and high samples when they evaluated liking of odour during frying. For the non sensitive group, there were no differences in how they evaluated these two samples during frying. The difference in how sensitive and non sensitive consumers evaluated the two samples during frying is presented in Fig. 4.

The sensitive consumers (version 3) from studies 1 and 2 also evaluated slices of belly sides from the entire male pigs described in Table 2. Samples 2 ($A = 1.26$, $S = 0.07$) and 3 ($A = 3.0$, $S = 0.07$) were significantly ($p \leqslant 0.05$) different from the reference sample for liking odour during frying, but not when odour and flavour were evaluated in served samples.

3.4. Screening the Norwegian consumers for androstenone sensitivity

Of the 1200 Norwegian consumers tested, approximately 39% were found to be sensitive for androstenone (version 4). The re-

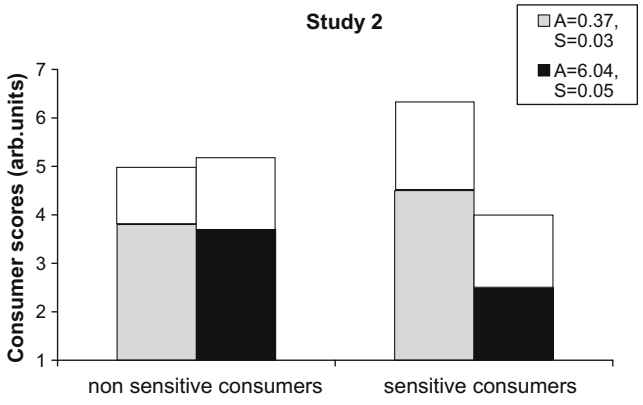

Fig. 4. Liking (1 = dislike very much, 7 = like very much) of odour when sensitive (21) and non sensitive (25) consumers evaluates the low ($A = 0.37$) and high ($A = 6.04$) androstenone samples in study 2. The sensitive consumers detected a significant ($p \leqslant 0.05$) difference between the two samples (low and high androstenone). Mean values and standard deviations are shown.

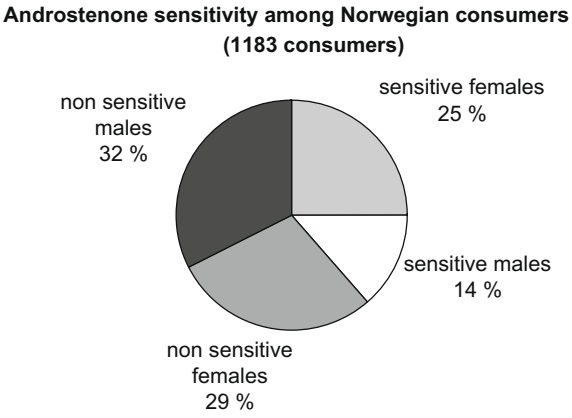

Fig. 5. The figure shows the androstenone sensitivity among Norwegian consumers. Of the 1183 consumers that were tested 39% were sensitive towards androstenone.

sults are shown in Fig. 5. When sensitivity to androstenone was grouped according to sex, 46.4% of the females and 26.3% of the men were found to be sensitive to this compound.

3.5. The androstenone odour

The 400 consumers that were screened for androstenone sensitivity were asked to describe the smell of the androstenone odour. Some of the words used by the sensitive and non sensitive consumers to describe androstenone are presented in Fig. 6. In the sensitive group, consumers gave negative reactions to meat with higher levels of androstenone. In the non sensitive group, consumers gave no or positive reactions to androstenone tainted meat.

4. Discussion

4.1. Testing of different media as carriers of androstenone

Based on the results from the sensory ranking of the different test bottles, androstenone dissolved in water was chosen for testing consumers for androstenone sensitivity. This presentation form made it easier to perceive androstenone while at the same time avoiding detectable smell from (mineral) oils. The water solubility of androstenone is 0.00023 g/l at 25 °C (Amoore & Buttery, 1978),

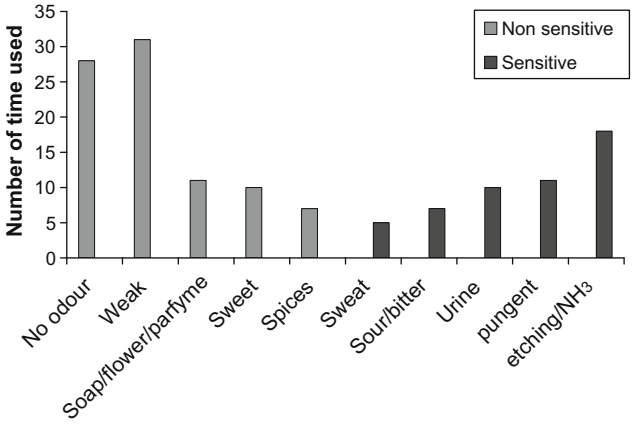

Fig. 6. The words used by sensitive and non sensitive consumers to describe the androstenone odour.

and the water solution with androstenone crystals was always saturated with androstenone because only a small amount of the androstenone crystals was dissolved.

Androstenone has a higher solubility in oil, but the sensory assessors sensed the odour of the oil. The sensory panel evaluated oil and water in a triangle test to confirm this result, and found a significant difference between oil and pure water. Words such as vaseline, medicine, plastic and wax were used to describe the oil despite this being a mineral oil that is supposed to be odourless and stable. Because of this it was decided that androstenone dissolved in water was to be used as a carrier when testing consumers for androstenone sensitivity. No statistical difference was found between the bottles containing the same amount of dry androstenone, either dry or floating on distilled water. However, some introductory experiments suggested a tendency for crystals on water to provide the strongest odour.

4.2. Procedure for testing consumers for androstenone sensitivity

The starting point was a triangle test using three bottles, two of them with the same content and one with a different content. Two different types of triangles were tested. Using two bottles of androstenone was not well received for highly sensitive persons. After smelling one of the bottles with androstenone, the highly sensitive persons lost their ability to perceive androstenone for a short while. Because of this a triangle with two bottles of water and one bottle of androstenone was used. Whether or not expressing no differences among the samples should be an option was discussed. If the consumers could have this option the possibility of guessing right might be reduced. Another way of reducing the chance of guessing right is using two test sessions after each other (version 2). To be classified as sensitive both triangles must be correctly identified. Two different scales were tested: the seven-point hedonic scale as used in the earlier testing and the labelled magnitude scale (LMS). After testing consumer with the two scales and relating this to how the consumers evaluated meat from entire male pigs (2.3.3 consumer studies), the LM scale was chosen. The background for this was that this scale appeared to separate the consumers better in two groups (a sensitive and a non sensitive group) than did the hedonic scale. Several consumers commented that they thought that the different bottles in the first triangle had a stronger smell than the different bottles in the second triangle, even though they paused a few minutes between these two triangles. Therefore, the LM scale was used after both triangles, and the mean value of the two intensity ratings was used when dividing the consumers into different groups (version 3). When bottles with

water were stored close to androstenone bottles during testing they started to smell of androstenone as well, but the smell was much weaker. The sensitive consumers had no problems differentiating between the androstenone bottle and the water bottles that started to smell of androstenone. The water bottles that started to smell of androstenone was still a problem. The time needed to contaminate the water bottles depended on the number of consumers tested during a period, i.e. the number of times the bottles were opened. The optimal triangle would be with new water bottles for each consumer, but this was not possible in practice when screening a large number of consumers. The method was changed from a triangle test to a 3-alternative forced test (3-AFC), where the question asked was to select the bottle with the strongest odour (version 4). To be sure that the disturbing factor with the smell of androstenone on the water bottles was minimized, some new directions for use were made (see Section 2.2 method development, version 4).

4.3. Relating the method to boar meat

4.3.1. Sensory analysis done by a trained laboratory sensory panel

The sensory evaluations of meat from entire male pigs were performed in order to relate the sensitivity test to scores given when evaluating the tainted meat samples. The odour and flavour evaluation of the five samples with different levels of androstenone (Table 1) showed that the sensory assessors had problems differentiating the samples for the boar attributes even though the high levels of androstenone were present. The two samples with skatole 0.09 and 0.11 ppm (Table 1) obtained the highest mean values for both boar odour and flavour. The level of skatole needed for clear cut identification by trained sensory panels is suggested to be 0.1 ppm (Bañón, Costa, Gil, & Garrido, 2003; Font I Furnols, Guerrero, Serra, Rius, & Oliver, 2000). Despite the high androstenone values and that all of the sensory assessors had the ability to detect androstenone, skatole values around the identification threshold explained most of the variance between these samples. Even though the samples in study 1 had higher androstenone values than in study 3 (where significant differences were found), the sensory assessors did not find any significant differences between these samples. This seems to be due to the higher skatole values in study 1, and that skatole is easier perceived and will be a disturbing factor when both components are in the same sample. This study aims at developing a method to perceive androstenone and relating it to meat boar meat and with this information the rest of the androstenone samples were chosen with as little skatole as possible. This is in some agreement with the results reported by Dijksterhuis et al. (2000) that the perception and recognition of androstenone smell seemed to be more difficult than that of skatole.

In an earlier study (Agerhem & Tornberg, 1995), androstenone had a larger influence at higher temperatures. Therefore, assessing odour during frying was included. The sensory assessors evaluated two of the samples again; the low (0.37 ppm) and the high (6.04 ppm) androstenone samples (different muscle), both these samples having skatole values below 0.05 ppm. A significant difference between these two samples for odour during frying, and odour and flavour evaluated in the booths was found. When the sensory assessors evaluated the androstenone samples in study three, they found a significant difference between the highest (A = 3.0 ppm) and the lowest (A = 0.16) androstenone samples for both odour during frying and odour and flavour evaluated in the booths. The highest androstenone sample was also significantly different from the sample with the androstenone value of 1.26 ppm when the assessors evaluated odour and flavour in the booths, but not when odour during frying was evaluated. The sample (A = 1.26 ppm) was significantly different from the lowest sample (A = 0.16 ppm) when odour during frying was evaluated, but not when evaluating odour and flavour in the booths. This shows that the assessors find it easier to detect the androstenone odour during frying (higher temperatures), and that the androstenone value needs to be higher (A = 3.0 ppm) to be detected in samples evaluated in the booths.

4.3.2. Consumer studies

The consumers evaluated five neck chops with different levels of androstenone combined with low levels of skatole (Table 1). Despite some high levels of androstenone (6.04 ppm, back fat value), there were no significant differences between the samples; sensitive, medium sensitive and non sensitive consumers gave the same scores for samples with androstenone values of 6.0 ppm and 0.37 ppm. Based on this, the consumers were asked to evaluate two of the samples again, the samples with the lowest (0.37 ppm) and highest (6.04 ppm) androstenone values. The samples were evaluated in a paired comparative test so the consumers could evaluate the two samples at the same time, and really compare them. The evaluation of odour and flavour in the booths showed the same insignificant result as in the first evaluation, between sensitive and non sensitive consumers. But in this study the consumers also evaluated intensity and liking of odour during frying. The consumers classified as sensitive for androstenone by the sensitivity test developed found a significant difference between the low and the high androstenone samples. The consumers classified as non sensitive did not differentiate the odour of the two products. The results from this study showed that the sensitivity test developed in this study is related to how sensitive consumers responded to meat with androstenone (>0.37 ppm) during frying, but not when evaluating odour and flavour on the served meat. To verify the results the sensitive consumers evaluated samples with different androstenone levels combined with low skatole levels (Table 2) in a home test. The skatole levels were below the identification threshold (0.1 ppm) for trained sensory panels (Bañón et al., 2003; Font I Furnols et al., 2000). In this study, there was also a significant difference in liking of odour during frying between the androstenone samples and the reference sample, but not when evaluating odour and flavour on served meat. The home test was repeated with new consumers to verify the results. Androstenone odour was more pronounced during frying, but not in the served meat (androstenone $\leqslant$ 3 ppm). These results were not in agreement with de Koch et al. (2001). They found that individuals that are sensitive to the odour of androstenone would become more aware of its presence when the product has cooled down and eaten (androstenone $\leqslant$ 3.4 ppm). Matthews et al. (2000) found skatole and androstenone to explain similar proportions of the variation in the flavour score, and that skatole explained more of the variation in the odour score. These consumers did not evaluate the samples during frying, and were not tested for their ability to perceive androstenone in pure form. Consumers insensitive to androstenone will not be influenced by androstenone in meat samples. However, Agerhem & Tornberg (1995) found that androstenone had a larger influence at higher temperatures. This is in agreement with what was found in this study that higher temperatures (frying) will make the appearance of androstenone more obvious than when serving the samples warm (65 °C).

4.4. Screening the Norwegian consumes for androstenone sensitivity

Of the approximately 1200 Norwegian consumers tested in this study, 46.4% of the females and 26.3% of the men had the ability to perceive androstenone. In the earlier studies, it has been estimated that approximately 50% of adults cannot perceive an odour when presented with androstenone. Of the 50% that can detect it, 15% detect a sandalwood like odour and are not offended by it, and the

remaining 35% are highly sensitive to it and are likely to find it offensive, saying it smelled like urine or sweat (Wysocki & Beauchamp, 1984). It is also reported that when exposing non sensitive subjects (not anosmic) systematically to androstenone their ability to perceive androstenone is induced (Wysocki, Dorries, & Beauchamp, 1989). This indicates that the non sensitive consumers that will not react or use positive words when smelling androstenone can have an induced ablility to percieve androstenone if they are systematically exposed, and that the sensitve group might be lagrer. Weiler et al. (2000) reported that 18% of the German and 31% of the Spanisch participants were highly sensitive to androstenone, with a higher proportion of women. The results found by Weiler et al. (2000) are somewhat lower than those found in this study.

4.5. The androstenone odour

The words used to describe the androstenone odour were different between the groups defined as sensitive and non sensitive consumers using our test. The sensitive consumers mainly used negative loaded words such as ammonia, urine, chemical and sweat when describing the smell of androstenone. In the other end of the scale, the non sensitive (and anosmic) consumers mainly used neutral and positive loaded words such as nothing, weak, flower and soap when they described the androstenone odour. The words used in describing the androstenone odour by the consumers confirmed the dividing of consumers into sensitive and non sensitive groups with the sensitivity test developed in this study. In the region between the two distinct groups of consumers, the use of positive and negative words was more mixed. This is the region on the LM scale (strong) where the limit for dividing into the different groups is. The consumers' willingness to put their mark just below or just above the word strong on the LM scale will probably vary, and some consumers will accordingly then be allocated to the wrong sensitivity group. The number of consumers in this area is relatively small (approximately 5%), and will only to a small extent affect the results when a larger group of consumers is tested. However, the fraction of sensitive consumers in a population is highly relevant when calculating the economical consequences if castration is banned, and then even small error will have some economical consequences.

5. Conclusion

The method developed in this study is a new way of testing consumers for their ability to perceive androstenone. The sensitivity test separated the consumers into largely two groups: sensitive consumers describing androstenone odour with negative words and non sensitive/anosmic consumers that will not react or use positive words when smelling androstenone. The sensitive consumers will react on meat from entire male pigs with different levels of androstenone during frying. The sensory assessors will also react on meat with high androstenone values (A = 3.0 ppm) when evaluating the samples in the booths (odour and flavour).

Acknowledgements

The Norwegian Research Council is thanked for their grant to this activity through Project Number 63164; "Boar meat-consumer aspects and exploitation of resources". Marit Rødbotten and Aud Espedal at Nofima Food are thanked for their assistance during the pre testing of the different androstenone systems. Elin Kubberød at Nofima Food and Anna C. Rehnberg at Animalia are thanked for their contribution and assistance throughout the whole project. Bente Fredriksen at Animalia is thanked for useful comments for the preparation of this paper.

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Paper II

Food Quality and Preference 21 (2010) 648–654

Contents lists available at ScienceDirect

Food Quality and Preference

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

The importance of the recruitment method for androstenone sensitivity with respect to accurate sensory evaluation of androstenone tainted meat

K. Lunde [a,b,*], E. Skuterud [a,b], B. Egelandsdal [b], M. Font i Furnols [c], G.R. Nute [d], C. Bejerholm [e], A. Nilsen [f], Y.H. Stenstrøm [b], M. Hersleth [f,b]

[a] Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396, Økern, N-0513 Oslo, Norway
[b] Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O. Box 5003, N-1432 Ås, Norway
[c] IRTA, Finca Camps i Armet, E-17121 Monells, Girona, Spain
[d] Division of Farm Animal Science, University of Bristol, Langford BS40 5DU, United Kingdom
[e] Danish Meat Association, Maglegaardsvej 2, 400 Roskilde, Denmark
[f] Nofima Mat AS, Osloveien 1, N-1430 Ås, Norway

ARTICLE INFO

Article history:
Received 12 August 2009
Received in revised form 7 April 2010
Accepted 7 April 2010
Available online 10 April 2010

Keywords:
Androstenone sensitivity
Boar taint
Inter collaborative test
Sensory analysis

ABSTRACT

Four European sensory panels where all (38) assessors, when recruited, were able to detect dry androstenone crystals through smelling, were reclassified in terms of sensitivity using a recently developed sensitivity method based both on the assessor's ability to detect androstenone and the spontaneous descriptor used to describe the odour. The reclassification reduced the number of assumed androstenone sensitive assessors from 38 to 28. All 38 assessors evaluated 6 samples (at approx. 60 °C) of minced meat low in skatole (⩽0.05 ppm) with androstenone contents from 3 to 9 ppm. The 28 androstenone sensitive assessors were able to detect androstenone odour in samples with androstenone ⩾4.5 ppm and androstenone flavour in samples with androstenone ⩾3.7 ppm; all concentrations in the fat. The 10 insensitive assessors could not detect androstenone even at 9 ppm despite the fact that all assessors detected dry androstenone crystals. The 10 insensitive assessors were present in 3 panels, the panels then having from 50% to 88.8% sensitive assessors. This showed that the method of recruiting assessors to a sensory panel was critical for the evaluation of androstenone tainted meat.

1. Introduction

Castration of entire male pigs is widely used in Norway and many other countries (Fredriksen et al., 2009). This is done to prevent the unpleasant odour/flavour that may occur in meat from boars. Castration of entire male pigs is expected to be prohibited in Norway (and in Europe) in the future, and it is therefore important to gain more knowledge about the human perception of boar taint. Boar taint is mainly associated with the presence of two compounds, skatole and androstenone. Skatole is a metabolite of the amino acid tryptophan produced in the lower gut by intestinal bacterial flora. Skatole is associated with a faecal like odour (Vold, 1970). Androstenone is a steroid closely related to testosterone. Production of androstenone in the testis increases with the maturity of the male pig. Androstenone is associated with an urine like flavour (Patterson, 1968). Skatole is perceived by 99% of the consumers and regarded as unpleasant (Weiler, Fischer, Kemmer, Dobrowolski, & Claus, 1997), while the ability to perceive androstenone varies among consumers (Wysocki & Beauchamp, 1984). Many consumers are insensitive to androstenone, but some consumers are highly sensitive and will react negatively upon exposure (Kline, Schwartz, & Dikman, 2006; Lunde, Skuterud, Nilsen, & Egelandsdal, 2009; Weiler et al., 2000). Recent research has shown that the human odorant receptor, OR7D4, is involved in the ability to perceive androstenone. Depending on OR7D4 genotype, human subjects differ in sensitivity (Keller, Zhuang, Chi, Vosshall, & Matsunami, 2007).

Previous studies have shown that trained sensory panellists may find it difficult to differentiate between androstenone and skatole. Dijksterhuis et al. (2000) found, in a study with seven trained sensory panels from different European countries that ratings of the attribute urine correlated positively with androstenone in most panels; however, skatole also had a positive correlation with the attribute urine. The same was shown for the manure attribute. Manure had a positive correlation with skatole, but manure also had a positive correlation with androstenone in some panels. Accordingly, these results demonstrated the confusion between androstenone and skatole odour in heated meat. All samples in

* Corresponding author at: Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396, Økern, N-0513 Oslo, Norway. Tel.: +47 22092317; fax: +47 22220016.
E-mail address: kathrine.lunde@animalia.no (K. Lunde).

0950-3293/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodqual.2010.04.002

that study contained combinations of both skatole and androstenone, with the low skatole group containing ⩽0.10 ppm skatole. Results have shown that sensory assessors are able to detect skatole as low as 0.10 ppm (Bañón, Costa, Gil, & Garrido, 2003; Font I Furnols, Guerrero, Serra, Rius, & Oliver, 2000; Lunde et al., 2009). Using samples containing both compounds may therefore contribute to confusion between these compounds. Therefore, the samples analyzed in this study contain only skatole or androstenone. This is possible with addition of synthetic skatole and androstenone to meat from castrates.

Another factor that may influence the panel's performance is the number of attributes used for profiling. Dijksterhuis et al. (2000) used eight attributes to describe the boar tainted samples in his study. Using several attributes when describing each of the components may make the differentiation between the components more difficult. In the same study, urine was used as an attribute for androstenone. Results from a study by Lunde et al. (2008) showed that the attribute urine was not an appropriate indicator of the androstenone level. These results were confirmed by another study performed by trained sensory assessors in Norway. The results showed that only 1 of 12 androstenone sensitive assessors related the androstenone odour with the word urine (unpublished results). The other 11 assessors described androstenone with words that also were used to describe the skatole odour (naphthalene, boar). In addition, the androstenone odour has been described by 400 Norwegian consumers during a screening of the Norwegian population for their androstenone sensitivity (Lunde et al., 2009). Urine was only used, as a spontaneous first choice, to describe androstenone by few (11) consumers. These results confirmed that urine is not the most relevant attribute to use when evaluating androstenone. The profile in this study was therefore kept as simple as possible, and the attributes used for sample evaluation were only androstenone and skatole.

In general, the perception and recognition of androstenone seemed to be more difficult than that of skatole (Dijksterhuis et al., 2000). This may be related to the assessor's ability to perceive androstenone. Recruitment of assessors for sensory profiling of boar tainted meat today often includes smelling pure androstenone crystals. In the recent study by Lunde et al. (2009) a new way of testing consumers/assessors for androstenone sensitivity was developed. The method involved intensity rating of androstenone crystals in water in a double 3 alternative forced choice (AFC) test. In each of the 3 AFC tests two bottles with water and one bottle with androstenone were presented. The subjects rated intensity of the strongest odour on a labelled magnitude scale after each test. The mean value of the two intensity ratings was used when defining the subjects as androstenone sensitive or non sensitive. The test revealed that an assessor able to detect pure androstenone was not necessary related to the same person's negative experience with boar tainted meat. Using assessors only able to detect pure androstenone, but not sensitive (as in giving a negative response) to this component in meat, can be an explanation as to why the perception and recognition of androstenone have seemed to be more difficult than that of skatole. The aim of the present research was to study the performance of sensory panellists in four different sensory panels concerning differentiation of meat samples with different levels of skatole and androstenone. However, the main focus was on the androstenone content, where our hypothesis was that being able to detect pure androstenone are not the same as giving a negative response, i.e. being sensitive to this component in meat. A person could also give a neutral or even a positive response. The hypothesis tested was that the androstenone sensitivity among consumers in meat is what matters, and that the method described by Lunde et al. (2009) would be suitable for recruiting assessors for their sensitivity towards androstenone in meat samples.

2. Materials and methods

2.1. Meat samples with different levels of skatole and androstenone

The sensory analysis in this study was performed on eleven samples of meat (minced meat) with different levels of skatole and androstenone. Synthetic skatole (3-methylindole) and androstenone (5α-androst-16-en-3-one) from Sigma–Aldrich, Co. Ltd., Poole were added to fat tissue from castrates (skatol ⩽0.05 ppm) and mixed with meat from *Semimembranous* muscle according to the experimental design. The skatole and androstenone levels (fat values) in the design are shown in Table 1. The reason why the skatole levels in the samples was ⩽0.05 ppm and not zero, was due to the fact that the average skatole level among castrates in Norway is 0.07 ppm.

2.1.1. Sample preparation

Preparation of samples was done at Nofima Mat (Norway) and distributed to the participating countries. The samples were frozen until analysed. Fat from different castrates with skatole ⩽0.05 ppm were mixed in a bowl chopper (Vacuum chopper Kilia 30L VAOU 2000s, Fritz Reimers GmbH, Kiel, Germany). Synthetic skatole and androstenone were dissolved in 10 ml ethanol, and then added in the chopped fat mixtures. Two fat mixtures with androstenone (4 and 10 ppm) and two fat mixtures with skatole (0.45 and 1 ppm) were prepared. Three replicates were taken from each of the four fat batches to confirm the amount of skatole and androstenone added. Eleven different batches were then made by mixing fat with meat (*Semimembranous* muscle) to obtain the skatole and androstenone values in the design. To each batch 1% water and 1% salt were added. Samples (50 g) with thickness of approximately 2 mm and diameter of approximately 15 cm were made by hand, then vacuum-packed and kept frozen (−20 °C) until analyzed.

2.2. Measurements of skatole and androstenone

Skatole and androstenone values were measured in the minced fat tissue before processing. Determination of skatole and indole in extracted fat were carried out by HPLC (Agilent Technologies) using fluorescence detection according to a method developed by Gibis (1994). Androstenone content in the fat was measured by a time-resolved fluorescent immunoassay as described by Tuomola, Harpio, Knuuttila, Mikola, and Løvgren (1997), modified by using antiserum produced and characterized by Andresen (1974).

Synthetic skatole and androstenone were compared to the corresponding biological compounds using NMR spectra. NMR spectra were recorded in $CDCl_3$ using the solvent as the reference set at 7.24 for the ^{1}H NMR and 77.23 for the ^{13}C NMR values.

Table 1
The skatol and androstenone levels of the boar tainted samples evaluated by the four different sensory panels across Europe.

Sample	Androstenone (ppm)	Skatole (ppm)
A3.0	3.0	⩽0.05
A3.7	3.7	⩽0.05
A4.5	4.5	⩽0.05
A5.2	5.2	⩽0.05
A6.0	6.0	⩽0.05
A9.0	9.0	⩽0.05
S0.15	0	0.15
S0.25	0	0.25
S0.30	0	0.30
S0.35	0	0.35
Reference	0	⩽0.05

The skatole and androstenone values were measured in fat. All samples had 20% fat content.

2.3. Sensory analysis

2.3.1. Sensory panels: Recruitment criteria, experience and testing locations

The four sensory panels participating in this study are described in Table 2.

Panel 673 was a sensory laboratory panel consisting of eleven trained assessors with 4–20 years of general experience in sensory profiling. The panel had several years of experience with evaluation of boar tainted meat, especially during the last 5 years. All assessors in panel 673 had been tested with androstenone crystals in pure form and were able to detect this compound.

Panel 437 consisted of 8 assessors, all able to detect androstenone in pure form. This panel was only used for boar taint evaluations, and training of the panel was specific for boar taint and followed the procedure described by Font i Furnols (2000). Four of the assessors had the first boar taint training and evaluation 10 years ago and the other four about 4 years ago.

Panel 234 consisted of nine assessors with 1–19 years of general experience in sensory profiling. The sensory panel was recruited according to the methods outlined in ISO 8586-1, 1993. Screening for boar taint included exposure to pure samples of androstenone and skatole, all assessors were able to detect androstenone according to this method. Only two assessors had participated in projects with boar tainted meat 15 years ago. The other assessors had no experience analysing boar tainted meat samples.

Panel 359 was a 10 member sensory panel recruited according to the methods outlined in ISO 8586-1, 1993 but given additional training in the sensory assessment of boar taint. Screening for boar taint included exposure to pure samples of androstenone and skatole. All panel members were able to detect androstenone in pure form. General experience for individual panel members ranged from 2 to 18 years.

All panels evaluated the samples in sensory laboratories designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory data.

2.3.2. Testing assessors for androstenone sensitivity

All assessors participating in this study were tested for their ability to detect androstenone crystals in pure form. In addition, all the assessors were tested for their ability to perceive androstenone by the method described by Lunde et al. (2009). Using this method, earlier results have shown that there is a difference between being able to detect pure androstenone and being sensitive to this component in meat. The method divided the subjects in two groups; sensitive and non sensitive subjects. The sensitive group was defined as subjects that gave negative reactions to meat with higher levels of androstenone. The non sensitive group contained subjects that gave no or positive/neutral reactions to androstenone tainted meat. When referring to the assessor's androstenone *sensitivity* in this article the sensitivity was tested by the method described by Lunde et al. (2009). Assessors tested for their ability to perceive androstenone by other methods, were described as assessors able to *detect* androstenone.

Table 2
The four sensory panels (across Europe) participating in this study.

Panel	Assessors	Sensitive assessors	Experience as assessors (years)	Experience assessing boar taint (years)
673	11	7	4–20	≈5, frequently
437	8	8	None	4–10, infrequently
234	9	8	1–19	None
359	10	5	2–18	Experienced

Androstenone sensitivity was defined by the method described by Lunde et al. (2009). All assessors were, however, able to detect androstenone in pure form.

2.3.3. Sensory profile

Differentiating between the boar attributes skatole and androstenone have proved to be difficult (Dijksterhuis et al., 2000). Earlier results by panel 673 have shown that by using relatively few attributes the assessors were able to distinguish between skatole and androstenone to a higher degree, therefore the number of attributes in this profile was kept as low as possible. Accordingly the profile used consisted of the attributes skatole (intensity of skatole), androstenone (intensity of androstenone) and rancid (intensity of all rancid odours (grass, hay, paint, stearine). Rancid was included as an attribute in the profile since rancidity is one of the more common off-flavours in pork meat.

2.3.4. Training of assessors

A common training procedure for the four panels was developed. Three meat samples were used; a reference sample (no skatole or androstenone added), a sample with high skatole content (0.35 ppm) and a sample with high androstenone content (9 ppm). The skatole and androstenone levels in the training samples corresponded to the highest skatole and androstenone levels of samples in the experiment. The assessors evaluated the samples using a 9 cm unstructured continuous scale, where the left side of the scale corresponded to "low intensity" (1) and the right side of the scale corresponded to "high intensity" (9). The assessors were trained in the odour and flavour description of the following attributes: skatole, androstenone and rancid. Training included perception of the attributes during frying (only odour) and evaluation in the booth (odour and flavour). The scales used in the training session are shown in Fig. 1.

2.3.5. Sensory analysis of boar tainted samples

The sensory assessors evaluated both odour above a frying pan (sniffing above the pan) and odour and flavour in the booths. Evaluated attributes were intensity of skatole, androstenone and rancidity. The same attributes were used for both odour and flavour evaluation.

The heat treatment of the samples was performed by the panel leaders after a standardized method. The frozen samples were fried in neutral oil in a pre-heated pan with lid. The samples for evaluation in the frying pan were divided in 5 parts (approx. 10 g) and fried with a lid for 1 min before the lid was taken off and the assessors sniffed one by one (while still frying the samples). Five or six samples were evaluated in each frying session, with a short break (2 min) between each of the samples. During this break the room

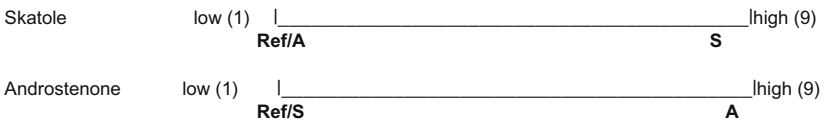

Fig. 1. The figure shows how the training samples were to be evaluated by the sensory assessors. The high skatole sample (S) was placed on the right end of the scale (high intensity) while the reference sample (ref) and high androstenone sample (A) was placed on the left side (low intensity). This was the same for the androstenone evaluation (androstenone sample on the right and reference and skatole on the left side of the scale).

was ventilated. The frying pan was cleaned with washing-up liquid and rinsed thoroughly between each sample.

Samples evaluated in the booth (odour and flavour) were fried in a warm pan with lid for approximately 1 min on each side; until well done. The assessors got approximately 25 g of each sample; the samples were divided before frying. The samples were served by a temperature of 60 °C in boxes (suitable for sensory analysis) with a lid. The assessors evaluated the odour after taking the lid off, and then the flavour. The assessors rinsed their mouths with water and/or some neutral crackers between the samples. Each of the sessions in the booth consisted of five or six samples, with a short break (2 min) between.

The samples were served replicated in a randomized order in each session. All sensory panels evaluated the samples in the same order to obtain more comparable results as the number of individuals in each panel was different. The samples were served the sensory assessors in 8 sessions with a minimum 10 min break between each session. Odour during frying and odour and flavour assessments was run in different sessions. The analysis was carried out separately for each panel.

2.4. Statistical analysis

Three of the sensory panels used a scale from 0 to 9 while the fourth panel used a scale from 1 to 9. All the results were therefore converted into a nine point scale (1–9) by use of the following equation:

$$\text{New value} = \frac{(B-A)*x \pm Ab - Ba}{b-a} \quad (1)$$

where x is the old value, $a = 0$, $b = 9$, $A = 1$ and $B = 9$.

The open source software Panelcheck V 1.3.2 (http://www.panelcheck.com) and principal component analysis (PCA) were used to compare the sensory evaluations performed by the different sensory panels, and to monitor the assessors in the principal components space according to their evaluations of the samples together with the average scores of the samples. Correlations between odour and flavour attributes were found using 2D scatter plots in Unscrambler (version 9.1, CAMO, Trondheim, Norway). Analysis of Variance (ANOVA), two-way model with assessor effects was performed on the descriptive data in order to identify attributes that differentiated between samples ($p < 0.05$). ANOVA was done by use of SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Measurements of skatole and androstenone

Synthetic skatole and androstenone were compared to biological compounds with NMR and were found to be 99.9% pure. The skatole and androstenone values referred to in this text were values measured in fat (not fatty tissue), and the levels are presented in Table 1.

3.2. Androstenone sensitivity

All assessors participating in the sensory analysis in this study were able to detect androstenone in pure form. Since earlier results have shown that being able to detect androstenone in pure form not necessary are related to androstenone sensitivity in meat products (Lunde et al., 2009), the assessors were also tested for their ability to perceive androstenone by the method developed by Lunde et al. (2009). Results from this sensitivity testing are shown in Table 2. The assessors were divided in two groups, sensitive (28) and non sensitive (10) after the method by Lunde et al. (2009), expecting the sensitive group to react negatively on meat with higher levels of androstenone. Ten assessors were defined as non sensitive expecting them not being able to perceive androstenone in tainted meat samples although these assessors were all able to detect androstenone in pure form. Using the method presented by Lunde et al. (2009) the assessors were also asked to describe the odour. The words used to describe the androstenone odour by the assessors are presented in Table 3. The result showed that the sensitive and non sensitive assessors used different words when describing androstenone. The sensitive assessors used negative words while the non sensitive assessors used more neutral (or positive) words.

3.3. Sensory analysis of boar tainted samples

The results from panel 234, 437 and 359 showed that the assessors in these panels found it more difficult to differentiate between the samples when sniffing above the frying pan than when evaluating odour and flavour of the fried samples. This is in contrast to panel 673, especially when it came to the evaluation of androstenone odour. This can partly be explained by the fact that panel 673 had used this procedure on several evaluations, and that sniffing above the frying pan may have been more difficult to standardize. Therefore, only the results from the evaluation of the samples in booths are presented. Four of the samples evaluated by the sensory panels contained different levels of skatole. The results from the skatole evaluation is only commented on briefly because these results only confirm earlier results found in other studies and do not contribute to new findings.

Looking at the difference between sensitive and non sensitive assessors in each panel was not interesting due to the low number of insensitive assessors in most panels. The differences between the sensitive and non sensitive assessors are therefore presented at the end, grouping assessors independent of their panels. The results presented from the androstenone evaluations in each of the panels are the results from the androstenone sensitive assessors (sensitivity defined by Lunde et al. (2009)).

3.3.1. Sensory analysis; panel 673

Results from the analysis of variance are presented in Table 4. Panel 673 consisted of 11 assessors able to detect androstenone, 7 of them sensitive to androstenone by the method described by Lunde et al. (2009). The assessors were experienced with evaluation of meat from entire male pigs. Results from evaluation of the androstenone samples showed that the androstenone samples were ranked almost according to their androstenone content. The androstenone sensitive assessors found a significant difference ($p \leqslant 0.05$) between the reference sample and the androstenone samples containing 4.5 ppm or higher when evaluating the androstenone odour. The correlation between androstenone odour

Table 3
The words used to describe the androstenone odour by the sensory assessors in the four different panels.

Panel	Sensitive assessors	Non sensitive assessors
673	Naphthalene, androstenone (3), urine, boar (2)	Sea, moist, chemical, pricking
437	Urine (5), androstenone (4), sweat (3), manure	
234	Piggery, urine (2), stale (2), chemical	Perfume
359	Urine, piggy, male hormone (2), mothballs (2)	Perfume, musty, light ammonia

The letters in parenthesises after the words used to describe androstenone indicates how many assessors that used that word. Some assessors have used more than 1 word to describe androstenone.

Table 4
Evaluation of androstenone samples by four different sensory laboratory panels across Europe.

Panels	637 (7)		437 (8)		234 (8)		359 (5)	
Samples (ppm)	Androstenone odour	Androstenone flavour	Androstenone odour	Androstenone flavour	Androstenone odour	Androstenone flavour	Androstenone odour	Androstenone flavour
Reference	1.45 a	1.33 a	2.03 a	1.76 a	1.52 a	2.31 a	1.77 a	1.78 a
A3.0	3.31 ab	3.85 ab	2.91 a	2.98 ab	1.81 a	2.24 a	2.04 a	2.02 ab
A3.7	2.84 ab	3.71 ab	2.86 a	4.46 b	2.07 a	3.31 a	2.23 a	2.19 ab
A4.5	5.69 b	5.62 b	2.84 a	3.83 b	2.42 a	3.21 a	2.50 a	2.27 ab
A5.2	3.90 b	4.97 b	2.32 a	4.02 b	2.99 a	3.14 a	2.64 a	2.49 ab
A6.0	4.20 b	4.33 b	2.41 a	3.64 b	2.91 a	3.33 a	2.40 a	3.18 b
A9.0	6.06 b	6.22 b	3.04 a	4.69 b	3.08 a	3.79 a	2.89 a	3.50 b

The mean values of the sensitive assessors are presented. The assessors evaluated the samples using a 9 cm unstructured continuous scale, where 1 corresponded to "low intensity" and 9 corresponded to "high intensity" of the attribute. Different letters within the same column indicate significant differences ($p \leqslant 0.05$). The androstenone values of the samples are given in ppm (mg/kg). Letters in parenthesis after the panel number are the number of sensitive assessors in each panel.

and flavour was found to be relatively high (0.86), and the results from evaluation of androstenone flavour gave corresponding results. The reference sample did not differ significantly from the lowest androstenone samples (3 and 3.7 ppm), but the androstenone samples had higher mean values than the reference sample. The higher mean values might indicate that some of the assessors also detected androstenone in the samples with the lowest androstenone content.

3.3.2. Sensory analysis; panel 437

Panel 437 consisted of 8 sensitive assessors (defined by the method described by Lunde et al. (2009)). The assessors were recruited only to analyse boar taint, and were experienced in the assessment of boar tainted meat. Results from evaluation of the androstenone odour showed no significant differences between any of the samples (Table 4). The mean values ranged from 2.03 (ref) to 3.04 (9 ppm androstenone), and this is relatively low values considering the high androstenone levels. Significant differences were found for androstenone flavour and consequently, the correlation between androstenone odour and flavour was low (0.55). The reference sample scored significantly lower than the other androstenone samples except for the sample with the lowest androstenone value (3 ppm). The results showed that androstenone flavour was easier to detect than the androstenone odour.

3.3.3. Sensory analysis; panel 234

Panel 234 consisted of 9 trained sensory assessors all able to detect androstenone, 8 of them sensitive to androstenone by the method described by Lunde et al. (2009). The assessors had no experience with evaluation of meat from entire male pigs. The results from the analysis of variance are presented in Table 4. The results showed that there were no significant differences between any of the samples when evaluating the androstenone odour or flavour. Looking at the mean values of the androstenone samples the sensitive assessors' ranking of samples correlated positively with the androstenone content ($R^2 = 0.81$ for odour; $R^2 = 0.74$ for flavour). The ranking of samples according to the androstenone content indicate that the assessors detected androstenone in the samples with the higher androstenone contents. The mean values for androstenone flavour ranged from 2.31 (ref) to 3.79 (9 ppm). The mean values for androstenone flavour were higher than the mean values for androstenone odour; this indicated that also this panel found it easier to detect the androstenone flavour compared to the androstenone odour. The results suggested that the sensitive assessors needed more experience/exposure to boar tainted meat to be able to differentiate better between the samples, and that their experience as assessors in general did not contribute to accurate sensory analysis of the tainted meat.

3.3.4. Sensory analysis; panel 359

Panel 359 consisted of 10 assessors able to detect pure androstenone, only 5 of them sensitive by the method described by Lunde et al. (2009). The assessors in this panel were experienced with sensory analysis of boar tainted meat. Results from the evaluation of the androstenone samples by the sensitive assessors (5) are presented in Table 4. No significant differences between the samples

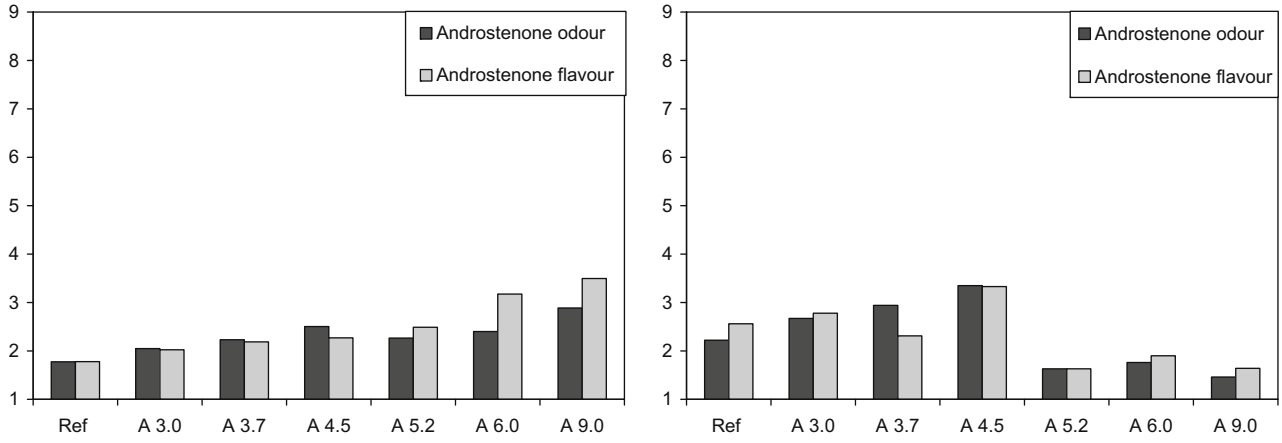

Fig. 2. The figure shows the ranking of the androstenone samples according to the androstenone content by panel 359. The figure to the left shows the ranking of the samples by the sensitive assessors (5), while the figure to the right shows the ranking of the samples by the non sensitive assessors (5).

Table 5
Evaluation of the androstenone samples by the sensitive and non sensitive assessors (independent of panels).

Samples (ppm)	Sensitive assessors (28)		Non sensitive assessors (10)	
	Androstenone odour	Androstenone flavour	Androstenone odour	Androstenone flavour
Reference	1.56 a	1.81 a	2.18 a	3.07 a
A 3.0	2.54 ab	2.82 ab	3.08 a	3.24 a
A 3.7	2.52 ab	3.54 bc	3.14 a	3.11 a
A 4.5	3.37 bc	3.82 bc	3.87 a	3.53 a
A 5.2	2.89 bc	3.74 bc	2.64 a	2.60 a
A 6.0	3.00 bc	3.64 bc	2.74 a	3.03 a
A 9.0	3.78 c	4.60 c	2.79 a	3.07 a

Sensitivity was tested by the method described by Lunde et al. (2009). All assessors were able to detect androstenon in pure form when recruited for sensory analysis. The mean values of the assessors are presented. The assessors evaluated the samples using a 9 cm unstructured continuous scale, where 1 corresponded to "low intensity" and 9 corresponded to "high intensity" of the attribute. Different letters within the same column indicate significant differences ($p \leqslant 0.05$). The androstenone values of the samples are given in ppm (mg/kg).

were found when evaluating androstenone odour. Finding significant difference using only 5 assessors can be difficult due to the low number of assessors. However, the ranking of the samples corresponded with the androstenone content in the samples. The mean values of the androstenone samples ranged from 1.77 (ref) to 2.89 (9 ppm). This was a low score considering the amount of androstenone added, but was in agreement with panel 437 and 234. The assessors found a significant difference ($p \leqslant 0.05$) between the reference sample and the samples with androstenone contents of 5.2 and 9 ppm when evaluating androstenone flavour, the correlation between androstenone odour and flavour was 0.84. The ranking of the samples (androstenone flavour) corresponded with the amount of androstenone added. Since this panel had equal numbers of sensitive and non sensitive assessors, comparison of the sensitive and non sensitive assessor's evaluation of the same samples was possible. The non sensitive assessors (all able to detect androstenone) could not rank the androstenone samples according to the androstenone content as opposed to the sensitive assessors. The results are presented in Fig. 2. The results from this panel showed the importance of using assessors sensitive to androstenone, and not assessors just able to detect androstenone in pure form.

3.4. Sensitivity testing of assessors to be used in evaluation of boar tainted meat

The assessors were also grouped according to their androstenone sensitivity independent of their allocation to a sensory panel. The results from the evaluation of the androstenone samples are presented in Table 5. The sensitive assessors (28) found a significant difference between the reference sample and the samples containing 4.5 ppm or higher when evaluating androstenone odour. Upon evaluating androstenone flavour of the same samples a significant difference between the reference sample and the samples containing 3.7 ppm or higher was found. The non sensitive assessors ($n = 10$) did not find any significant differences between any of the androstenone samples evaluated; this was the same for both the odour and flavour attributes.

3.5. Evaluation of the skatole samples

The results from the evaluation of the skatole samples from the 38 assessor in the 4 panels (independent of their allocation to a sensory panel) demonstrated the ability to detect skatol at 0.15 ppm (not shown).

4. Discussion

4.1. Meat samples

Skatole can be detected in low concentrations, 0.1 ppm (Bañón et al., 2003; Font I Furnols et al., 2000; Lunde et al., 2009). Studying androstenone without the influence of skatole can therefore be difficult with the use of biological material. Previous results from panel 673 (Table 2) have shown that low levels of skatole influenced the evaluation of the androstenone samples, and therefore assessors' perception of androstenone was studied for samples strongly reduced in skatole.

Another challenge with the use of biological material is presenting the assessors the same meat as the amount of meat from each muscle is only enough for few assessors. Using several animals in groups defined by their skatole and androstenone levels, as done in the study by Dijksterhuis et al. (2000), may contribute to more noise in the results because small variations in skatole and androstenone levels can influence the assessor's evaluations. In this study, addition of synthetic skatole and androstenone to meat from castrates made it possible to study androstenone without the influence of skatole.

4.2. Sensitivity testing of assessors to be used in evaluation of boar tainted meat

Screening for the ability to detect androstenone is sometimes included when assessors are recruited for sensory analysis. The screening is performed in different ways, often with pure androstenone crystals (Weiler et al., 2000). Sensory assessors can be able to detect androstenone in pure form, but this is not necessarily related to their ability to detect androstenone in meat products. The results presented in Table 2 showed that the androstenone sensitivity did not correlate with the assessor's ability to detect androstenone in pure form. All assessors (38) participating in this study were able to detect androstenone in pure form, but ten of them were not sensitive to androstenone by the method described by Lunde et al. (2009). Using panel 359 as an example, all the sensory assessors in this panel were able to detect androstenone in pure form. However, used in a panel to evaluate androstenone tainted meat samples, they were not able to rank the samples according to measured level of androstenone. In this experiment, the assessors in panel 359 were also screened for androstenone sensitivity by the method described by Lunde et al. (2009). Using this method, only five of ten assessors that were able to detect pure androstenone were sensitive to the component in meat. Evaluation of the androstenone samples by the sensitive assessors showed that the assessors ranked the androstenone samples according to the androstenone content. The low number of sensitive assessors used in the analysis can explain why their detection threshold was high. Evaluation of the androstenone samples by the non sensitive assessors (but able to detect androstenone) showed that there was no longer a system of the ranking of samples according to the androstenone content.

Grouping the assessors according to their androstenone sensitivity independent of their allocation to a sensory panel, the results showed that the method used to test the assessors for their ability to perceive androstenone was important (Table 5). All the assessors participating in this study were able to detect androstenon in pure form. The ten assessors defined as non sensitive by the method described by Lunde et al. (2009) indicated no significant differences between any of the samples despite high androstenone values (9 ppm). On the other hand, the androstenone sensitive assessors (28) found significant differences between the reference sample and samples with androstenone content above 3.7 ppm.

The results showed that including the non sensitive assessors (but able to detect androstenone) reduced the accuracy of the sensory profile of the androstenone tainted meat. It is therefore necessary to carefully select the method of recruiting assessors for evaluation of androstenone tainted meat. Assessors with the ability to detect pure androstenone crystals are not the same as sensitive assessors that will react negatively on meat with higher levels of androstenone.

In a study by Weiler et al. (2000) consumers were checked for their androstenone sensitivity. The results showed that a significantly higher proportion of the sensitive consumers were women. In this study the difference between male and female assessors are not relevant as long as the assessors are sensitive to androstenone, the sex of assessors does not influence the analysis.

4.3. Evaluation of the androstenone samples

Some of the panels (sensitive assessors) have given the high androstenone samples relatively low intensity scores. The reasons for different use of scale like for panel 673 and 239 cannot be fully explained. Issues around drift in perceived intensity and up regulation and down regulation of androstenone receptors with time are not presently understood.

Androstenone flavour was easier detected than the androstenone odour in most panels. This can be due to the fact that the samples were fried in a pan, and then put in boxes with lids. Some of the androstenone odour could have volatilized during the cooking. Font i Furnols et al. (2009) found higher scores for androstenone odour than for androstenone flavour when the samples were cooked individually in a closed container.

4.4. Evaluation of the skatole samples

In general the evaluation of androstenone seems to be more difficult than the evaluation of skatole (Dijksterhuis et al., 2000). This was confirmed in our investigations. The result from the 38 assessors in the 4 panels (independent of their allocation to a sensory panel) demonstrated the ability to detect skatol at 0.15 ppm. This level is in agreement with literature (Bañón et al., 2003; Font I Furnols et al., 2000; Lunde et al., 2009).

5. Conclusion

The assessors (38) of 4 European sensory panels recruited according to ISO standards were reclassified in terms of their androstenone sensitivity. All 38 assessors were able to detect dry androstenone crystals, but only 28 of the assessors were sensitive to androstenone when tested with the sensitivity method developed by Lunde et al. (2009). The result showed a significant difference between the sensitive (28) and non sensitive (10) group in their evaluation of the androstenone samples, and the method developed by Lunde et al. (2009) for screening assessors with regard to androstenone sensitivity was shown to be useful. Long working experience as assessors in general did not seem to be important when evaluating androstenone tainted meat, but the method used to screen the assessors for their androstenone sensitivity was highly important.

The results from the skatole samples evaluated in this study confirmed the results from earlier published data, that skatole easily can be detected at low concentrations (0.15 ppm).

Acknowledgements

The Norwegian Research Council is thanked for its grant to this activity through Project Number 63164; "Boar meat-consumer aspects and exploitation of resources". Olaf Bjørkeli at Nortura Rushøgda is thanked for his sampling of suitable animals for the experiment. The meat cutters at Animalia and Tom Chr. Johannesen and Karin Solgaard at Nofima Mat are thanked for their assistance in the sample preparation. Ellen Dahl at Norwegian School of Veterinary Sciences is thanked for her cooperation with the skatole and androstenone analysis.

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Paper III

Genetic variation of OR7D4 affects sensory perception of meat containing androstenone

Lunde, K. [1,2], Egelandsdal, B. [2], Skuterud, E.[1,2], Mainland, J.D. [4], Lea, T. [2], Hersleth, M.[2,3] & Matsunami, H. [4]

[1]Norwegian Meat Research Centre, Lørenveien 38, P.O.Box 396 Økern, N-0513 Oslo, Norway

[2]Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O.box 5036, N-1432 Ås, Norway

[3]Nofima Mat, Osloveien 1, N-1430 Ås, Norway

[4]Department of Molecular Genetics and Microbiology, Duke University Medical Centre, Research Drive, Durham, North Carolina 27710, USA.

*** Corresponding author. Tel.: +4722092317; fax: +4722220016.**
***E-mail address*: kathrine.lunde@animalia.no**

Abstract

Although odour perception impacts food preferences, the effect of genotypic variation of odorant receptors (ORs) on the sensory perception of food is unclear. Human OR7D4 responds to androstenone, and genotypic variation in *OR7D4* predicts variation in the perception of androstenone. Since androstenone is naturally present in meat derived from male pigs, we asked whether OR7D4 genotype correlates with either the ability to detect androstenone or the evaluation of pork tainted with varying levels of androstenone. Consistent with previous findings, subjects with two copies of the functional RT variant were more sensitive to androstenone than subjects carrying a non-functional WM variant. When pork containing varying levels of androstenone was cooked and tested by sniffing and tasting, subjects with two copies of the RT variant rated the androstenone-containing meat as less favourable than subjects carrying the WM variant. Our data suggest that the OR7D4 genotype predicts sensory perception of meat containing androstenone. This is the first demonstration that genetic variation in an odorant receptor alters food preferences.

Keywords: boar taint, androstenone sensitivity, androstenone sensitization, OR7D4.

1. Introduction

Culture, experience and learning all impact food preferences, but genetic factors can also play a role in evaluating food. For example, genetic variation in the bitter receptor T2R38 affects sensitivity to phenylthiocarbamide (PTC) [1] and correlates with food preferences [2]. In addition to taste, odour is a major sensory component in flavour evaluation, yet how genetic variation in ORs affects food preferences remains unclear. Androstenone, a steroid structurally related to testosterone, is a known pheromone in boars [3]. Androstenone, in combination with skatole, makes up the primary component of boar taint, an unpleasant odour and flavour found in pork derived from male pigs [4]. Skatole is a metabolite [5] of the amino acid tryptophan produced in the lower gut by the intestinal bacterial flora and has a faecal odour. Almost all consumers (99%) have the ability to perceive skatole [6] , and the compound can be detected in concentrations as low as 0.1 ppm [7,8,9]. Androstenone is found in pork from male pigs in the range of 0-6.4 ppm. Although castration reduces the amount of androstenone in pork, the European Union recently proposed to ban castration due to animal welfare concerns [10]. This has reinvigorated the study of consumer perception of pork containing androstenone.

Unlike skatole, androstenone is perceived differently among people, with descriptions ranging from urine and sweat to vanilla and sweet [11,12]. Different studies have shown that while some subjects are insensitive to androstenone, others are highly sensitive and will react negatively upon exposure [13] . Androstenone in meat has been associated with flavours described as urine-like, etching, pungent and sour [4,14].

A recent survey showed that 39% of Norwegian consumers were identified as androstenone-sensitive, with negative reactions to meat containing higher levels of androstenone [9]. The fraction of androstenone-sensitive consumers in a population is highly relevant, as this figure could relate to the impact of specified androstenone levels on consumers' acceptance, providing a background for assessing economical consequences of sending meat from uncastrated males into the market.

The ability to perceive androstenone correlates strongly with genetic variation of the human odour receptor OR7D4 [15]. A cell-based screen using an expression library of human ORs identified OR7D4 as a major androstenone receptor. We refer to the most common allele of this receptor, or the reference sequence, as OR7D4 RT, and to the other allele, which is a common variant of OR7D4 contains two non-synonymous single-nucleotide-polymorphisms (SNPs) in complete linkage disequilibrium, resulting in two amino acid substitutions (R88W, T133M) as OR7D4 WM.. In cell-based assays, OR7D4 RT responds to androstenone while OR7D4 WM shows diminished responses. In a previous study, subjects with OR7D4 RT/WM and WM/WM genotypes were less sensitive to androstenone and found the odour less unpleasant than the subjects with the RT/RT genotype [15]. However, it is not known whether OR7D4 affects flavour perception of food containing androstenone such as pork.

Repeated exposure to androstenone induces increased sensitivity to androstenone, but only in about half of the exposed subjects [16,17,18,19,20]. Understanding how sensitivity to androstenone changes with respect to OR7D4 genotype may help us understand the mechanisms

underlying the perception of and sensitization to androstenone, as well as estimate consumer acceptance of meat with boar taint.

The aim of the present study is to relate both OR7D4 genotype and sensitivity to androstenone to the evaluation of meat samples with different levels of androstenone. We confirmed that OR7D4 genotype predicts sensitivity to androstenone and furthermore, influences the evaluation of androstenone-tainted meat samples.

Materials and methods

Recruitment of subjects

Subjects for this study were recruited following sensitivity testing in Norway [9]. All subjects gave consent to participate, and were financially compensated for their time and efforts. The participants were informed about the project and procedures according to instructions from The National Committees for Research Ethics in Norway. The participants were able to drop out at any time during the study without consequence. A total of 23 subjects were recruited: 13 consumers and 10 professional sensory assessors.

Sampling of blood, isolation of DNA and DNA typing

Approvals to collect, export and analyse the DNA of recruited subjects were given by the Regional Committees for Medical Research Ethics in Norway, the Norwegian Directorate for Health and the Norwegian Social Science Data Services. Trained health care personnel collected the blood samples and DNA was isolated at the Norwegian University of Life Science using

the method described by Keller et al. [15]. For sequencing, human genomic DNA was amplified with HotStar Taq (Qiagen) with primers upstream (5'AAGTGATGACAAGCTGAGCTGC-3') and downstream (5'CCACAACATTTGCCTTAGGGGTA-3') of the OR7D4 open reading frame. The PCR products were then Sephadex G50-purified (GE HealthCare) and sequenced with a 3100 or 3730 Genetic Analyzer (ABI Biosystems).

Androstenone sensitivity among participating subjects

The subjects participating in this study were selected among subjects who were previously tested for their ability to perceive androstenone in a large screening of androstenone sensitivity done in Norway in 2008 [13]. The method involved the intensity rating of androstenone crystals in water in a double 3-Alternative Forced Choice (AFC) test. In each of the 3 AFC tests, two bottles with water and one bottle with androstenone were presented and the subject chose the sample with the strongest odour. Moreover, the subjects rated the intensity of the strongest odour on a Labelled Magnitude Scale. This scale was anchored with "barely detectable" at the lower end and "strongest imaginable" at the higher end. The qualitative intensity scale was converted to a quantitative one from 0 to 100, and the mean value of the two intensity ratings was used to define the subjects' relative androstenone-sensitivity. Twelve sensitive and eleven non-sensitive subjects were selected for further testing.

Androstenone sensitization with time

All subjects participating in this study were exposed to androstenone daily for six weeks after the initial testing. The sensitization experiment was performed after the evaluation of meat samples (see below) in all cases except one, because this consumer forgot the samples in the freezer. The androstenone solution used in the sensitization experiment was the same as the solution used in the sensitivity test (0.0017 g androstenone crystals added to 10 ml water). This amount ensures that the water was saturated with androstenone for an extended period. The subjects were told to store the bottle at room temperature and to sniff the bottle after taking the cap off once daily.

Evaluation of meat samples

The subjects evaluated meat samples with different levels of androstenone. In this study, seven samples of minced meat with different levels of androstenone were evaluated. Fat from different castrates with skatole levels at ≤ 0.05 ppm (skatole is naturally present among castrates in Norway at an average level of 0.07 ppm, but samples that had ≤ 0.05 ppm skatole) were mixed with synthetic androstenone (5α-androst-16-en-3-one) from Sigma–Aldrich, Co Ltd (Poole) dissolved in 10 ml ethanol.

The fat tissue was mixed with meat from *Semimembranous* muscle according to the experimental design shown in Table 1. Sample preparation was done at Nofima Mat in Norway, and is described in detail by Lunde et al. [14]. 1% water and 1% salt were added to each batch. Samples (50 g) with a thickness of approximately 2 mm and a diameter of approximately 15 cm were made by hand, then vacuum-packed and kept frozen (- 20˚C). The samples were

similar to a product already produced in the Norwegian market. The subjects were requested to keep the samples frozen until they were fried.

Instrumental measurements of skatole and androstenone

Skatole and androstenone values were measured in the fat mixtures before processing. Skatole was determined in extracted fat by HPLC (Agilent Technologies) using fluorescence detection according to a method developed by Gibis [21]. The androstenone content was determined by a time-resolved fluorescent immunoassay as described by Tuomola et.al. [22], modified using antiserum produced and characterized by Andresen [23].

Synthetic skatole and androstenone were compared to the biological compounds using NMR spectra. NMR spectra were recorded in $CDCl_3$ using the solvent as the reference set at 7.24 for the ^{1}H NMR and 77.23 for the ^{13}C NMR values.

Consumer testing

The samples (minced meat) with different levels of androstenone (Table 1) were fried in a preheated frying pan and evaluated by 13 consumers in a home test during a period of several days. If more than one sample was evaluated during a day, the consumers were instructed to have at least a one-hour break while ventilating the room before evaluating the next sample. Between each sample, the consumers were told to clean the frying pan with soap and rinse thoroughly. Liking of odour during frying, liking of odour of the fried meat, and liking of flavour during eating were evaluated on a seven point scale with “dislike very much” rated as a “1” and “like very much”

rated as a “7”. In addition, the consumers were allowed to comment on each sample.

The samples were evaluated in the order as they appeared in the questionnaire, which was randomized for each subject. The samples were evaluated before the sensitization experiment in all cases except one who became sensitive after training. The subjects were classified as sensitive or insensitive by the method described by Lunde et al. [9].

Sensory analysis by assessors

The sensory analysis was performed by the sensory panel at Nofima Mat in Norway. The panel consisted of 10 trained (7 sensitive) assessors with 4 to 20 years of general experience in sensory profiling. The panel has had several years of experience evaluating boar tainted meat, especially during the last 5 years. The samples were evaluated in a sensory laboratory designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory data.

Sensory profile

The profile used was the same as the profile used in the study with four sensory panels across Europe [14]. The profile consisted of the attributes skatole (intensity of skatole), androstenone (intensity of androstenone) and rancid (intensity of all rancid odours--grass, hay, paint, stearine). Rancid was included as an attribute in the profile since rancidity is one of the more common off-flavours in pork meat.

Training of assessors

The sensory assessors were experienced in the evaluation of boar-tainted meat, and were recently trained on boar-tainted meat samples. The training of assessors was therefore done using three samples: one reference sample (no androstenone or skatole added), one sample with high androstenone content (7.5 ppm) and one sample with high skatole content (9.0 ppm). The androstenone level in the training samples corresponded to the highest androstenone level of samples in the experiment. The samples were evaluated on a 9 point unstructured continuous scale, where a "1" corresponded to "low intensity" and a "9" corresponded to "high intensity". The assessors were trained using the attributes in the profile. Training included perception of the attributes during frying (only odour) and evaluation in the booth (odour and flavour).

Sensory analysis of boar tainted samples

The assessors evaluated the odour of the sample above the frying pan both during and following frying. They then evaluated the flavour of the finished sample by consuming the meat. The assessors evaluated the rancidity of the meat as well as the intensity of skatole and androstenone. The same attributes were used for both odour and flavour evaluation.

The frozen samples were fried in neutral oil in a pre-heated pan with lid. The samples evaluated in the frying pan (odour) were divided in 5 parts (approx. 10 g each) before frying. The samples were fried in a pan covered with a lid for 1 minute before the lid was taken off and the assessors then sniffed the samples one by one while still frying them. The frying pan was cleaned with soap and rinsed thoroughly between each sample.

Samples evaluated in the booth (odour and flavour) were fried in a warm pan with a lid on top for approximately 1 minute on each side until the meat was well-done. The assessors divided the samples into approximately 25 g portions before frying. The samples were served at a temperature of 60 ˚C in boxes suitable for taste analysis with a lid. The assessors evaluated odour after taking the lid off, and then flavour while eating. The assessors rinsed their mouths with water and/or some neutral crackers between the samples. The samples were served in a randomized order. Odour assessments during frying and odour and flavour assessments after frying were run in different sessions, with a break (30 minutes) between sessions.

Statistical analysis

Statistical analyses were performed in JMP 9 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

OR7D4 genotype predicts androstenone sensitivity

The subjects' ability to detect androstenone were tested and correlated with their OR7D4 genotype. When subjects were divided into sensitive and insensitive cohorts according to Lunde et al. [9], we found that all subjects with at least one copy of the WM allele were classified as androstenone-insensitive. Twelve of the sixteen subjects with the RT/RT genotype were classified as androstenone-sensitive. The OR7D4 genotype explained 83% of the androstenone sensitivity classification (Fisher's exact test, $p < 0.0013$) and 40% of the variation in intensity ratings. These data are consistent with

the previously published findings [15] and confirm the role of OR7D4 in olfactory sensitivity to androstenone (Figure 1).

A portion of subjects in prior studies were shown to be sensitized to androstenone after repeated exposure to androstenone [16,17,18,19]. The subjects' sensitivity to androstenone was therefore compared before and after daily exposure to androstenone over a period of six weeks. Although as a group there was no significant difference between intensity ratings before and after sensitization (Wilcoxon, $p = 0.72$), one RT/RT subject who was initially classified as androstenone-insensitive was reclassified as sensitive after the sensitization period. As a result, we concluded that the OR7D4 genotype best explained the intensity of androstenone after sensitization rather than the intensity of androstenone at the initial screening (Figure 2). In the future, more subjects need to be tested to determine whether RT/RT subjects are more likely to be sensitized to androstenone.

OR7D4 genotype predicts acceptance of meat containing androstenone

The next question was whether OR7D4 genotype correlated with the perception of meat samples tainted with androstenone. Synthetic androstenone was added in the samples evaluated in this study so that androstenone sensitivity could be studied without the influence of skatole, while synthetic skatole was used as a control. This is important given that small amounts of skatole can influence the analysis and that skatole can be detected at levels as low as 0.1 ppm [7,8,9] . In addition, the variation in the

samples presented to the subjects was minimized as all samples contained the same amount of fat, skatole and androstenone (Table 1).

The quality of synthetic skatole and androstenone was also measured. The samples were compared to biological compounds using NMR and were found to be 99.9% pure. The skatole and androstenone values referred to in this text were values measured in fat (not fatty tissue), and the levels are presented in Table 1. The levels of androstenone were within the naturally-occurring range.

Consumer testing

To test the effect of OR7D4 genotype on meat preference containing androstenone, we first tested naïve consumer subjects for their odour and flavour perception of the samples. Consumers as a group tended to dislike meat flavour containing more androstenone; an ordinal logistic regression showed that consumer evaluations predicted the androstenone content of the samples when rating the flavour ($p < 0.014$), but not the odour (during frying $p = 0.20$; finished $p = 0.29$).

When the subjects were divided by OR7D4 genotypes, there was a genotype effect on consumer preference. RT/RT subjects disliked the flavour and odour of the finished samples more than the WM carriers, but not the odour during frying (flavour, $p < 0.001$; finished, $p < 0.002$; during frying $p=0.23$) (Figure 3).

Four of the subjects classified as insensitive to androstenone had the RT/RT genotype. One of these subjects was classified as sensitive after six weeks of daily exposure to androstenone. This subject gave low liking scores for

androstenone after the sensitization experiment, consistent with the observation that this subject had been sensitized. Consumers have not experienced androstenone-containing meat since there has not been meat production from intact males for years. The data raise the possibility that more consumers will show low liking evaluations to male meat when exposed to androstenone more frequently as a result of a castration ban.

Assessor testing

Trained assessors are widely used in evaluating meat samples. To test OR7D4 genotype effects on meat evaluation containing androstenone, we trained and tested assessors with meat samples containing androstenone (see Materials and Methods for details). An ordinal logistic regression showed that the assessors' androstenone intensity evaluations predicted the androstenone content of the samples when rating the flavour and odour of the finished sample, but not the odour while frying (flavour, $p < 0.0043$; finished, $p < 0.05$; during frying $p = 0.14$) (Figure 4). When we divided the subjects by OR7D4 genotype, there was a significant interaction between androstenone concentration and genotype for both odour evaluations (during frying, $p < 0.01$; finished $p < 0.006$), reflecting the observation that subjects with the WM allele did not increase their intensity evaluations with androstenone content. However, assessors with the WM allele gave flavour ratings that varied with the androstenone content of the samples and there was no interaction effect ($p = 0.83$). This may be due to higher androstenone concentrations in meat containing 7.5ppm androstenone. Though future research is necessary to confirm, this finding raises the possibility that

people with the WM allele can be trained to evaluate androstenone flavour, but not odour, in meat samples.

Our data raise the possibility that the detection of androstenone flavour in the mouth was more sensitive than the detection of the androstenone odour by sniffing; this is consistent with the results from the evaluation and previously published results [14], but the cause is unclear. Androstenone may be vaporized more efficiently in the mouth when evaluating flavour. Alternatively, other volatiles might mask androstenone odour when smelling. Another possibility is that humans might be more sensitive to androstenone when sensing retronasally. These possibilities are not mutually exclusive and future study is necessary to address these issues.

Conclusion

The results showed that OR7D4 genotype correlated strongly with androstenone sensitivity as well as the subject's perception of meat samples containing androstenone. Our study suggests that functional variation in an OR alters food preferences. Further work is needed to understand how an individual's unique OR repertoire contributes to overall flavour evaluation and preference of meat and other foods.

Acknowledgement

We thank the Norwegian Research Council for its grant to this activity through project number 63164; "Boar meat-consumer aspects and exploitation of resources". We thank Olaf Bjørkeli at Nortura Rushøgda for his sampling of suitable animals for the experiment. We thank the meat

cutters at Animalia and Tom Chr. Johannesen and Karin Solgaard at Nofima Mat for their assistance in the sample preparation. We thank Ellen Dahl at the Norwegian School of Veterinary Sciences for her cooperation with the skatole and androstenone analysis. We thank Y. Dong and Y.R. Li for reading and editing. JM is supported by a NIH-NRSA fellowship and HM is supported by the NIH.

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Table 1
The androstenone levels of the boar tainted samples evaluated in this study.

Sample	Androstenone (ppm)	Skatole(ppm)
Reference	0	≤0.05
A3	3	≤0.05
A3.7	3.7	≤0.05
A4.5	4.5	≤0.05
A5.2	5.2	≤0.05
A6	6	≤0.05
A7.5	7.5	≤0.05

The androstenone values were measured in fat. All samples had 20 % fat content.

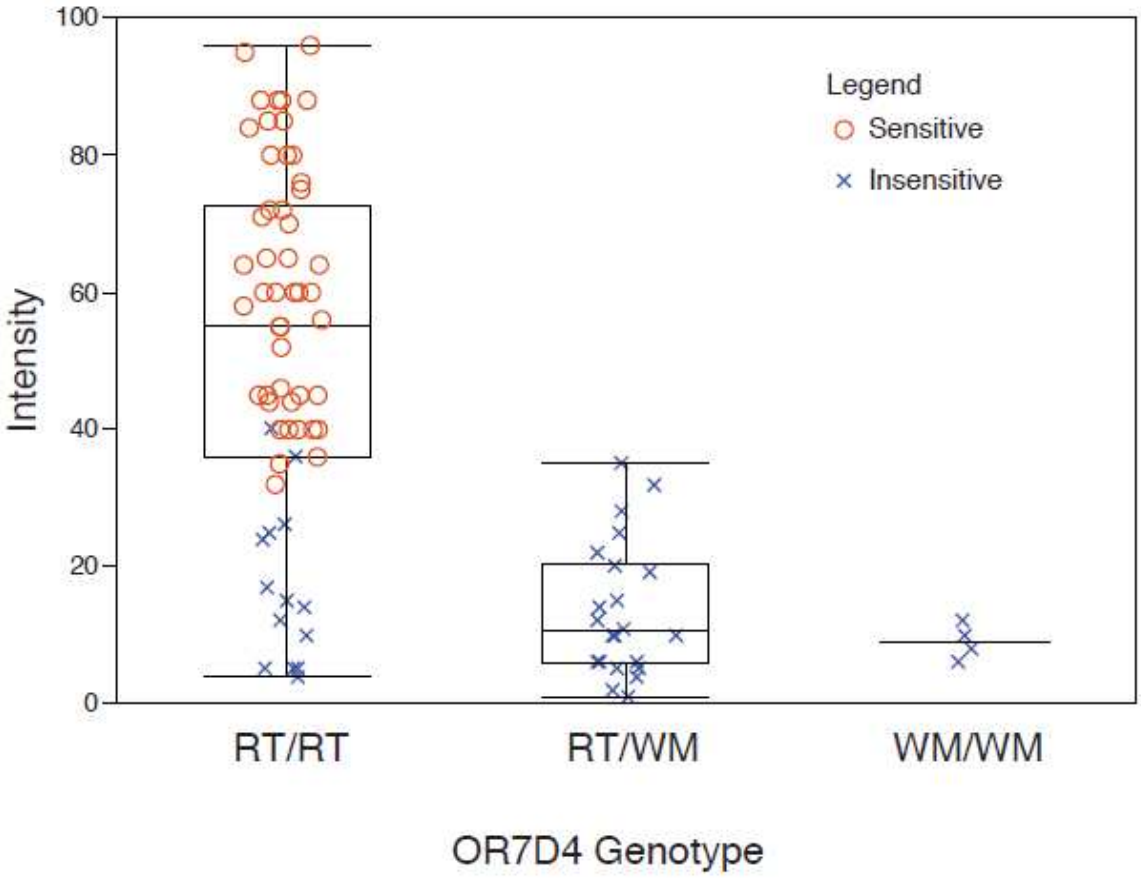

Figure 1. Genotypic variation in OR7D4 accounts for 40% of the variance in androstenone intensity. Subjects identified as sensitive to androstenone by the 2-trial 3AFC test are represented by circles, and subjects identified as insensitive are represented by Xs. Each subject rated the intensity of androstenone four times.

Note that none of the subjects classified as sensitive carry the WM allele.

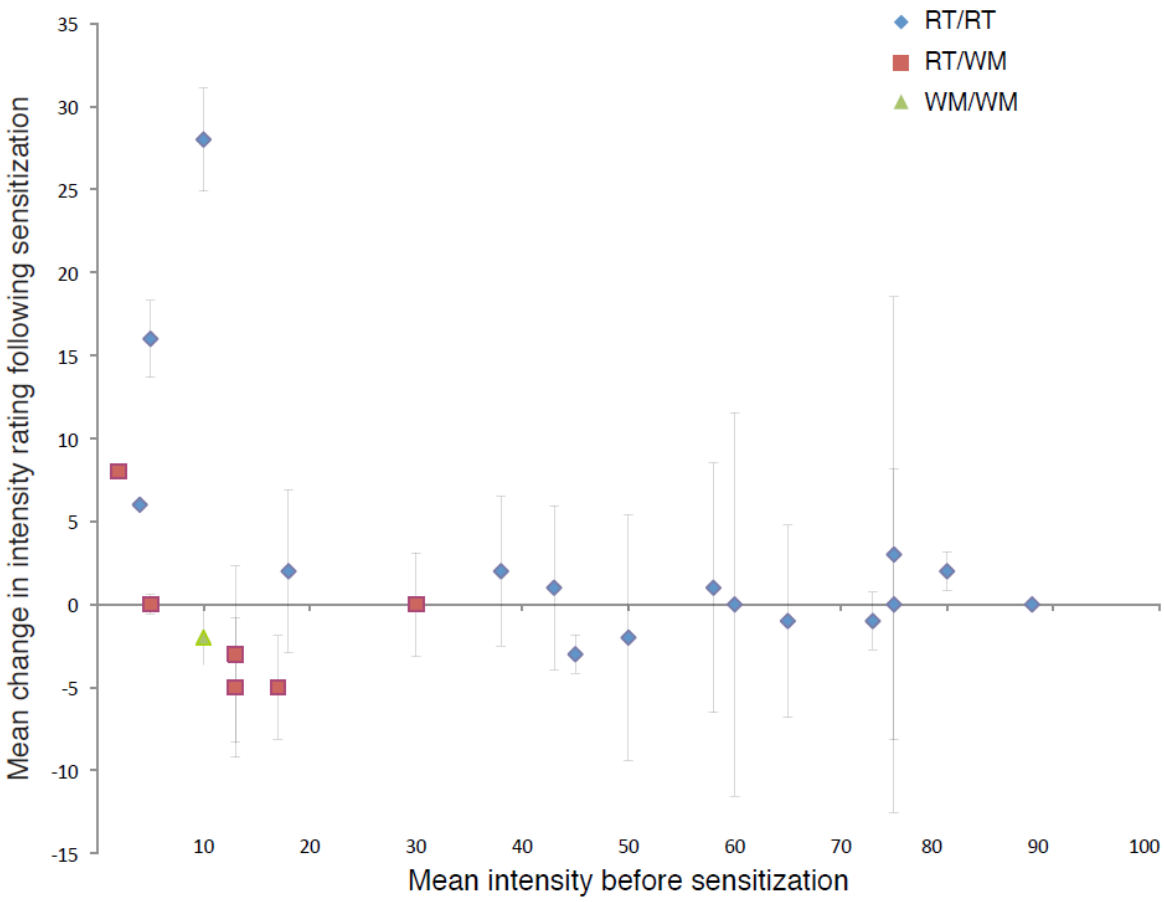

Figure 2. Change in intensity ratings following sensitization. The y-axis represents the mean of all possible pairings of ratings before sensitization with ratings after sensitization. Error bars represent standard error.

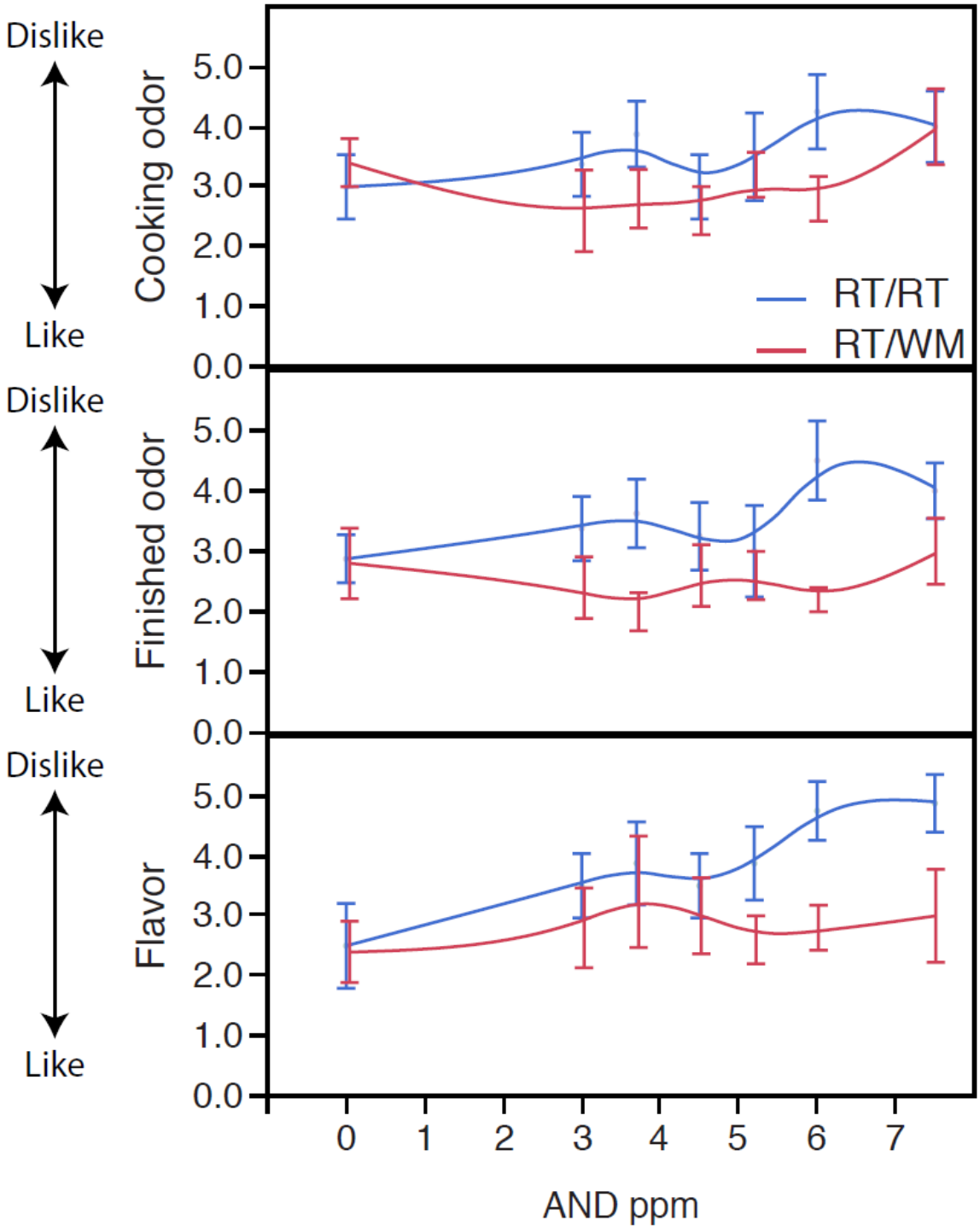

Figure 3. Consumer evaluation of meat samples. Error bars represent standard error and lines represent a smoothing spline.

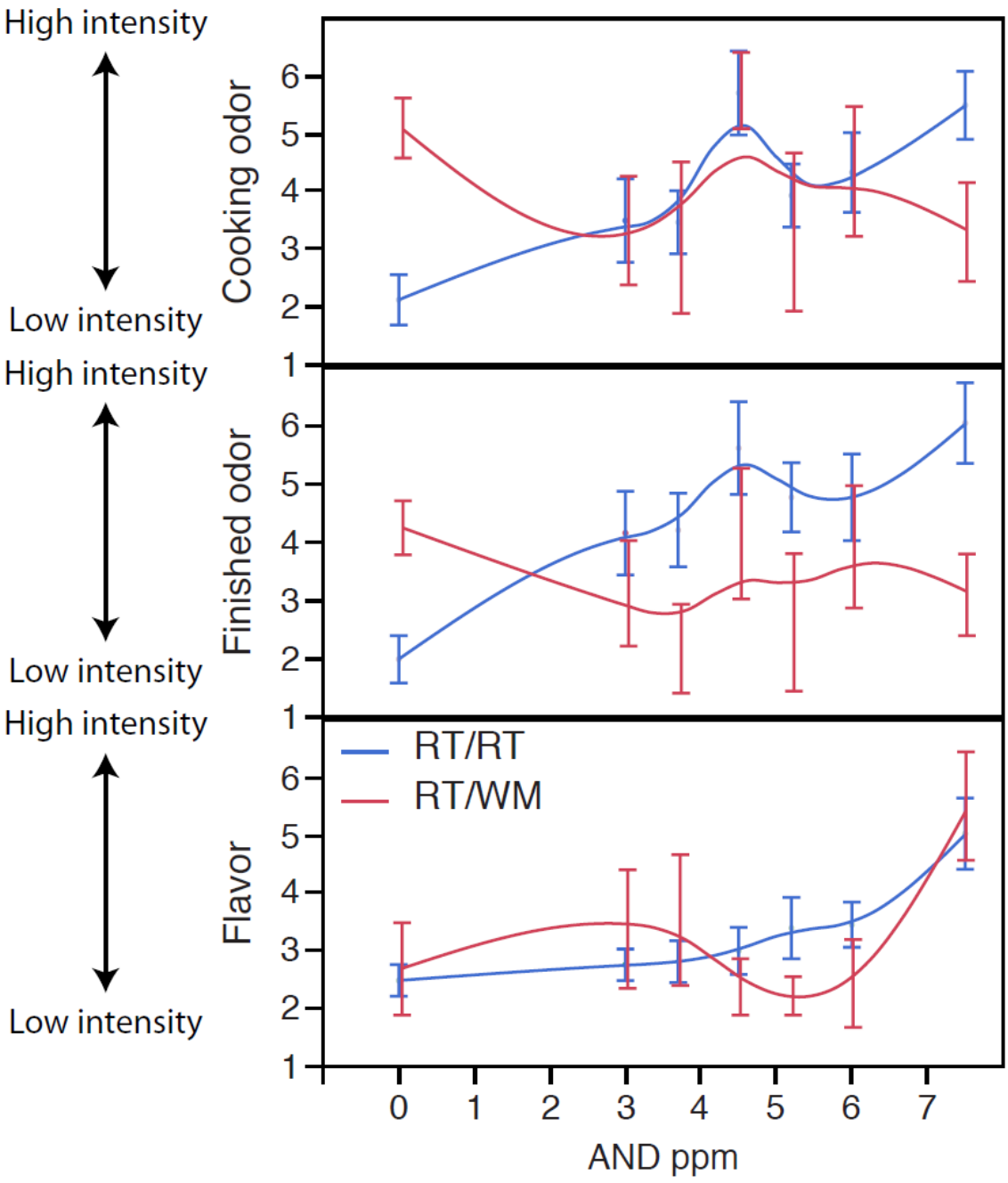

Figure 4. Sensory assessor evaluation of meat samples. Error bars represent standard error and lines represent a smoothing spline.

Paper IV

Meat Science 86 (2010) 706–711

Contents lists available at ScienceDirect

Meat Science

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

Norwegian consumers' acceptability of boar tainted meat with different levels of androstenone or skatole as related to their androstenone sensitivity

K. Lunde [a,b,*], E. Skuterud [a,b], M. Hersleth [c], B. Egelandsdal [b]

[a] Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396 Økern, 0513 Oslo, Norway
[b] Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O. Box 5003, 1432 Ås, Norway
[c] Nofima Mat, Osloveien 1, 1430 Ås, Norway

ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 2 June 2010
Accepted 8 June 2010

Keywords:
Boar taint
Androstenone sensitivity
Consumer accept

ABSTRACT

The aim of work was to study Norwegian consumers' acceptance of pork meat with different levels of skatole and androstenone. One group of androstenone sensitive consumers (N = 46) and one group of non sensitive consumers (N = 55) participated in a home test and evaluated 11 samples with different skatole (range 0–0.35 ppm) and androstenone (range 0–9.0 ppm) levels. Liking of odour during frying and odour and flavour of the fried meat were evaluated. Results showed that the non sensitive consumers accepted all levels of androstenone in the samples. Sensitive consumers gave a significantly lower liking score for androstenone samples containing 3 ppm (and more) than the reference sample when evaluating these samples above the frying pan, but no significant difference were found between 3 ppm samples and reference samples when liking of fried meat was evaluated. This indicated that the sensitive consumers accepted 3 ppm in fried meat, but not if 3 ppm was present in the sample during the frying process. The same consumer's differentiated skatole samples with regard to flavour at 0.15 ppm. The Norwegian established practise with a threshold value of 0.21 ppm skatole is higher than the value accepted by the consumers.

1. Introduction

The history and present practise in Europe regarding production of entire males depends on political choices made in each country. Thus some countries have extensively practised production of entire males e.g. England, while Norway has castrated all piglets. Castration of piglets is expected to become prohibited in Norway in the future. It is therefore important to gain more knowledge about the Norwegian consumers' perception of boar tainted meat, as this new situation may influence the demand for pork meat and will have economic consequences for the industry. Skatole and androstenone largely describe boar taint. Skatole is a faeces and manure smelling metabolite (Vold, 1970) of the amino acid tryptophane produced in the lower gut by intestinal bacterial flora. Androstenone is a steroid structurally related to testosterone. Androstenone was earlier associated with a urine like flavour (Patterson, 1968), but later the flavour has been described as more diverse (Annor-Frempong, Nute, Whittington & Wood, 1997; Lunde, Skuterud, Nilsen & Egelandsdal, 2009). Both skatole and androstenone are fat-soluble compounds. Skatole is perceived by 99% of consumers and regarded as unpleasant (Weiler, Fischer, Kemmer, Dobrowolski & Claus, 1997), while the ability to perceive androstenone varies among consumers (Lunde et al., 2009, Wysocki & Beauchamp, 1984). Recent research has shown that detection of androstenone is, at least partly, determined by the amino acid sequence of the human odour receptor OR7D4 (Keller, Zhuang, Chi, Vosshall & Matsunami, 2007). Thus, many consumers are highly sensitive to androstenone and will react negatively upon exposure (Kline, Schwartz & Dikman, 2006). In a recent consumer study by Lunde et al. (2009) a total of 39% of Norwegian consumers were identified as androstenone sensitive. These consumers rated meat samples with a high androstenone level significantly lower than samples with a low androstenone level when they evaluated liking of odour during frying. The consumers not sensitive to androstenone are expected to accept all levels of androstenone in androstenone tainted samples. To be able to sort out carcasses that are unacceptable to consumers, more knowledge about consumer's acceptance of pork meat with different levels of skatole and androstenone is necessary.

Matthews et al. (2000) did a consumers study in seven European countries and showed that skatole contributed more than androstenone to the dissatisfaction of consumers. In this study the consumers were not classified with regard to their ability to perceive androstenone. Such a classification may have a large impact on sensitivity and acceptance (Lunde et al., 2009; Weiler et al., 2000). Results have shown that sensory assessors are able to detect skatole at levels as low as 0.10 ppm (Bañón, Costa, Gil & Garrido, 2003; Font I Furnols, Guerrero, Serra, Rius & Oliver, 2000; Lunde et al., 2009). Results from the sensory panel at Nofima Mat (Norway) indicated that skatole levels lower than 0.1 ppm (0.07 ppm) contributed more to boar taint than did higher (>3 ppm) androstenone levels in the same samples. Since the detection threshold of skatole is

* Corresponding author. Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396 Økern, 0513 Oslo, Norway. Tel.: +47 22092317; fax: +47 22220016.
E-mail address: kathrine.lunde@animalia.no (K. Lunde).

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doi:10.1016/j.meatsci.2010.06.009

this low, using samples containing both compounds may induce confusion regarding identification of skatole and androstenone. Providing entire male meat samples with low skatole levels (<0.05 ppm) in combination with different levels of androstenone were difficult since the average skatole level is 0.8 ppm among Norwegian boars (Fredriksen, Hexeberg, Choinski, Ropstad & Nafstad, 2008). To be sure that the androstenone levels of the samples were evaluated with minimal influence of skatole, addition of synthetic skatole and androstenone to meat from castrates (analyzed for skatole) were chosen. The reason being that it would be relevant to know the acceptability related to only androstenone in meat since this will give input to a debate regarding the need to sort for androstenone in meat.

In addition to sensory detection of boar taint during consumption of pork, sensitive consumers are likely to detect boar taint during frying and preparation of the meal. The intensity of odour during frying is described as more intense than the odour of the fried, served product (Lunde et al., 2009). Accordingly, negative experiences with boar tainted meat will influence consumers repeat purchasing decisions (Bryhni et al., 2002, 2003).

The aim of the present research was to study Norwegian consumers' acceptance of pork meat with different levels of skatole and androstenone. Knowledge of acceptance levels for skatole and androstenone will make it possible for the pork industry to provide an estimate of the economical consequences of a change to entire male production. The focus here is mainly on identification of consumers' androstenone thresholds using androstenone tainted meat. The consumers were segmented into sensitive and non sensitive consumers prior to testing the meat using a recently developed method (Lunde et al., 2009). The method identifies sensitivity to androstenone using intensity responses to water saturated with androstenone in combination with a negative response to the aroma. This sensitivity test appears to give a good segmentation into sensitive and non sensitive persons as judged from a recent investigation (Lunde et al., unpublished). The hypothesis was that a more correct estimation of the threshold value to androstenone would be achieved if consumers were segmented with respect to androstenone sensitivity before they tested the tainted meat. Since non sensitive consumers should accept all levels of androstenone, the acceptance threshold of androstenone will be higher if non sensitive consumers were included with sensitive consumers during a consumer test. In addition, the percentage of consumers sensitive to androstenone and their acceptance threshold for androstenone will give the pork industry better estimates of the economical consequences than if non sensitive consumers are included.

2. Materials and methods

2.1. Preparation of meat samples

Studying the sensory perception of androstenone without the influence of skatole might be difficult with the use of biological materials because skatole can be detected at levels as low as 0.1 ppm (Bañón et al., 2003; Font I Furnols et al., 2000; Lunde et al., 2009), and possibly even lower levels. Another challenge with the use of biological material occurs when high fat percentages are required (20% or more), as providing representative samples each muscle is only sufficient for serving a few consumers. Using several animals in groups defined by their skatole and androstenone contents, as done in the study by Matthews et al. (2000), may contribute to uncontrolled noise in the results as small variations in skatole and androstenone levels between animals may influence the consumer's evaluations. Therefore, in the present study, synthetic skatole and androstenone were added to meat from castrates instead of using meat from boars. In addition, when using synthetic samples all the parameters (fat content, androstenone and skatole content) are better controlled than when using biological samples. No limitations in relevance of the results obtained are foreseen by using synthetic skatole and androstenone in the meat, provided the compounds are of high purity and evenly distributed in the batches.

Synthetic skatole (3-methylindole) and androstenone (5α-androst-16-en-3-one) from Sigma-Aldrich, Co Ltd, Poole were added to fat tissue from castrates (skatole ≤0.05 ppm) and mixed with meat from *Semimembranous* muscle according to the experimental design. The reason why the skatole levels in the samples were ≤\0.05 ppm and not zero was due to the fact that even castrates in Norway contain some skatole.

Preparation of samples was done at Nofima Mat in Norway. Fat from different castrates with skatole ≤0.05 ppm were mixed in a bowl chopper (Vacuum chopper Kilia 30L VAOU 2000s, Fritz Reimers GmbH, Kiel, Germany). Synthetic skatole and androstenone were dissolved in 10 ml ethanol, and then added to the chopped fat mixtures. Two fat mixtures with androstenone (4 and 10 ppm) and two fat mixtures with skatole (0.45 and 1 ppm) were prepared. Three replicates were taken from each of the four fat batches to confirm the amount and homogeneity of the added skatole and androstenone. Eleven different batches were then made by mixing fat (20%) with meat (*Semimembranous* muscle) to obtain skatole and androstenone concentrations according to the design (Table 1). To each batch 1% (w/w) water and 1% (w/w) salt were added. Samples (50 g) with thickness approximately 2 mm and diameter approximately 15 cm were made by hand, then vacuum-packed and kept frozen (−20 °C) until distributed to the consumers. The samples were similar to a product already established in the Norwegian market, however, without the addition of spices. The consumers were requested to keep the samples frozen until fried.

2.2. Instrumental measurements of skatole and androstenone

Skatole and androstenone values were measured in the fat mixtures before processing. Skatole was determined in extracted fat by HPLC (Agilent Technologies) using fluorescence detection according to a method developed by Gibis (1994). The androstenone content was determined by a time-resolved fluorescent immunoassay as described by Tuomola, Harpio, Knuuttila, Mikola & Løvgren (1997), modified using antiserum produced and characterized by Andresen (1974).

Synthetic skatole and androstenone were compared to the biological compounds using NMR. NMR spectra were recorded in $CDCl_3$ using the solvent as reference; set at 7.24 for the ^1H NMR and 77.23 for the ^{13}C NMR values.

2.3. Pre-screening for skatole and androstenone thresholds with a sensory laboratory panel

A pre-screening for skatole and androstenone thresholds by a sensory laboratory panel was performed on twenty-four samples containing different levels of skatole and androstenone to be able to a choose a selection for further consumer testing. Seven sensitive assessors

Table 1
The skatol and androstenone levels of the boar tainted samples evaluated by the Norwegian consumers (101).

Sample	Androstenone (ppm)	Skatole (ppm)
Reference	≤0.05	≤0.05
A3.0	3.0	≤0.05
A3.7	3.7	≤0.05
A4.5	4.5	≤0.05
A5.2	5.2	≤0.05
A6.0	6.0	≤0.05
A9.0	9.0	≤0.05
S0.15	≤0.05	0.15
S0.25	≤0.05	0.25
S0.30	≤0.05	0.30
S0.35	≤0.05	0.35

The skatole and androstenone values were measured in fat. All samples had 20% fat content.

evaluated the samples containing androstenone, while all 9 assessors evaluated the skatole samples. The difference is due to the fact that androstenone insensitive assessors were allowed to score skatole tainted samples but not androstenone tainted samples in accordance with Lunde et al. (2010). The sensory panel was used routinely for assessing boar tainted meat samples. The training of assessors was therefore done using only three samples; a reference sample (no androstenone or skatole added), a sample with high skatole content (0.35 ppm) and a sample with high androstenone content (9.0 ppm). The skatole and androstenone levels in the training samples corresponded to the highest skatole and androstenone levels of samples in the experiment. The samples were evaluated in a sensory laboratory designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory data. The sensory assessors evaluated both odour above a frying pan (sniffing above the pan) and odour and flavour in fried samples. Attributes used were skatole (intensity of skatole), androstenone (intensity of androstenone) and rancid (intensity of all rancid odours (grass, hay, paint, and stearine)). Rancid was included as an attribute in the profile since rancidity is one of the more common off-flavours in pork meat and thus can mask or reinforce boar taint.

The same attributes were used for both odour and flavour evaluation. The assessors evaluated the samples using a 9 cm unstructured continuous scale, where the left side of the scale corresponded to “low intensity” (1) and the right side of the scale corresponded to “high intensity” (9).

Samples (24) evaluated in the pre-screening experiment are presented in Table 2, and were evaluated the same way as the training samples using the same attributes. The samples were served replicated in a randomized order in each session. Odour during frying and odour and flavour assessments in booths was run in different sessions.

2.4. Consumer testing

2.4.1. Testing of androstenone sensitivity among consumers

Consumers' androstenone sensitivity has proved to be an important factor in the acceptability of pork (Lunde et al., 2009; Weiler et al., 2000). Evaluating androstenone samples without knowledge about the consumer's androstenone sensitivity will result in a higher average acceptance than when only sensitive consumers are included. Earlier results showed that non sensitive consumers will accept all levels of androstenone in the samples. In this study the consumers were chosen on their androstenone sensitivity in order to be able to compare the two group's (sensitive/non sensitive) perception of the androstenone samples. The acceptance of androstenone was based on the sensitive consumer's evaluation of the androstenone samples. The consumers participating in this study (N = 101) were tested for their ability to perceive androstenone in a screening of the Norwegian population using the method described by Lunde et al. (2009), and later some of these consumers were used even though the number of people in each group was not identical. The number of consumers in each group should have been 55/55, but some consumers never returned the results. The number of consumers in each group was of comparable size (46 sensitive and 55 non sensitive). The sensitive group is defined as consumers giving negative reactions to meat with higher levels of androstenone (above 3 ppm, fat value). The non sensitive group is defined as consumers giving no or positive reactions to androstenone tainted meat.

Table 2
The samples evaluated in a pre-screening by a trained sensory laboratory panel.

Sample	Androstenone (ppm)	Skatole (ppm)
Reference	0.0	≤0.05
1	2.0	≤0.05
2	3.0	≤0.05
3	3.7	≤0.05
4	4.5	≤0.05
5	5.2	≤0.05
6	6.0	≤0.05
7	7.0	≤0.05
8	8.0	≤0.05
9	9.0	≤0.05
10	10.0	≤0.05
11	0.0	0.10
12	0.0	0.15
13	0.0	0.25
14	0.0	0.30
15	0.0	0.35
16	2.0	0.10
17	4.5	0.10
18	9.0	0.10
19	2.0	0.20
20	4.5	0.20
21	9.0	0.20
22	2.0	0.35
23	4.5	0.35
24	9.0	0.35

The skatole and androstenone values were measured in fat. All samples had 20% fat content.

2.4.2. Consumers evaluation of meat samples

The samples (minced meat) with different levels of skatole and androstenone (Table 1) were evaluated by the consumers in a home test, during several days. If more than one sample was evaluated each day, the consumers were instructed to have at least a one hour break while ventilating the room before evaluating the next sample. The frozen samples were fried in a preheated frying pan in a manner typical for this type of product. The consumers were instructed to clean the frying pan with washing-up liquid and rinse the pan thoroughly between each sample. Both liking of odour during frying and liking of odour and flavour of the fried meat were evaluated. The consumers evaluated the samples on a seven point hedonic scale with dislike very much (1) on the left side and like very much (7) on the right side. In addition, the consumers were allowed to comment on each sample. The samples were evaluated in the order they appeared in the questionnaire. The samples were thus evaluated in a randomized order.

2.5. Statistical analysis

The open source software Panelcheck V 1.3.2 (http://www.panelcheck.com) and Principal Component Analysis (PCA) were used to compare the sensitive and non sensitive consumers. Correlations between odour and flavour attributes were found using 2D scatter plots in Unscrambler (version 9.1, CAMO, Trondheim, Norway). Analysis of Variance (ANOVA) was performed on the consumer data to identify differences between samples (one model for skatole and one for androstenone) and sensitivity groups ($p<0.05$) with Tukey's Studentized Range (HSD) test. ANOVA analysis was performed in SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA). A pair wise T-test was performed to find significant differences between the reference sample and the sample containing 3 ppm androstenone when an increasing number of consumers was randomly selected in permutations (1000 for each level of consumers). The T-test was performed using R (open source software: http://www.r-project.org/).

3. Results and discussion

3.1. Instrumental measurements of skatole and androstenone

Synthetic skatole and androstenone were compared to their biological counterparts by NMR and were found to be 99.9% pure. The skatole and androstenone values referred to are presented in Table 1 (refers to values in fat). Three replicates were taken from each of the batches with added skatole and androstenone to confirm the amount of skatole and androstenone added. The results (not shown) indicated that the added compounds were evenly distributed in the fat batches.

3.2. Pre-screening for skatole and androstenone thresholds with a sensory laboratory panel

The results from the pre-screening study (not shown) were used for choosing samples for further consumer testing. The results from the evaluation of the androstenone samples showed that the assessors were able to detect this component in samples containing 6 ppm (odour) and 5 ppm (flavour). Evaluation of androstenone odour above the frying pan showed that the assessors found no significant differences between any of the samples, but the mean values were higher for samples containing androstenone with 3 ppm or above than for samples containing lower levels of androstenone (1 and 2 ppm). It was assumed that the trained assessors would detect androstenone with higher precision than the consumers; therefore samples with androstenone contents below 3 ppm were not chosen for the consumer study.

The results from the evaluation of the skatole samples showed that the assessors were able to detect skatole at 0.1 ppm. This is in agreement with earlier results (Bañón et al., 2003; Font I Furnols et al., 2000; Lunde et al., 2009). Samples with skatole contents of 0.15 ppm and above were therefore chosen for the consumer study.

Samples containing both skatole and androstenone were also evaluated by the sensory panel. The results showed that the assessors found it extremely difficult to differentiate between skatole and androstenone when both components where present in the same sample. Also in a study by Lunde, Egelandsdal, Choinski, Flåtten & Kubberød (2008) results from evaluation of samples containing both compounds showed that differentiating between the attributes when both compounds were present in the same samples was difficult, and the interaction effect between the compounds was not significant. Therefore, these samples were not evaluated by the consumers.

None of the samples were found to be rancid by the sensory assessors.

3.3. Consumer testing

3.3.1. Androstenone sensitivity

The ability to perceive androstenone is determined, at least partly, by the human odour receptor OR7D4 (Keller et al., 2007). Approximately 60% of Norwegian consumers are insensitive to androstenone, but about. 40% of consumers are highly sensitive and will react negatively to meat containing higher levels of androstenone (Lunde et al., 2009). The evaluation of the androstenone samples was therefore performed with two groups, sensitive and. The number of consumers was of comparable size (46/55). The results are presented in Table 3 and show a significant difference ($p \leq 0.05$) in liking of frying odour between the reference sample and the other samples for sensitive consumers. For odour and flavour, significant difference were found between the reference sample and the samples containing 3.7 ppm androstenone or higher.

No significant differences were found between the reference samples and any of the androstenone samples for non sensitive consumers, neither for liking of odour above the frying pan, or for liking of odour and flavour of the fried meat (Table 3).

This study focuses on consumers acceptance of androstenone samples containing 3.0 ppm or higher. This level was chosen based on the results by the trained sensory panel (presented above). The sensory assessors were not able to detect androstenone at this level (3 ppm), neither in the booths nor above the pan during frying. The results of the consumer study indicated that samples with lower levels of androstenone (2 ppm or possibly lower) should have been included in order to obtain more detailed information about levels of acceptance for androstenone during frying.

The samples evaluated by the consumers in this study were also evaluated by four sensory panels in Europe (Lunde et al., 2010). The results from three of the panels showed that the assessors found it more difficult to differentiate between the samples when sniffing above the frying pan than when evaluating odour and flavour of the fried samples. This is in contrast to the fourth panel and the consumers in this study. However, the only sensory panel in Lunde et al. (2010) able to differentiate between the androstenone samples during frying was still unable to detect androstenone at 3 ppm, indicating that sensing a meat sample with 3 ppm androstenone during frying is not always possible for a sensory panel.

Table 3
Evaluation (mean liking) of the androstenone samples by the Norwegian consumers (101).

	Sensitive consumers (46)			Non sensitive consumers (55)		
Sample	Odour (frying)	Odour	Flavour	Odour (frying)	Odour	Flavour
Reference	4.17a	4.78a	5.11a	4.27	4.75	4.91
A 3.0	3.17b	4.30ab	4.35ab	4.10	4.58	4.86
A 3.7	2.87b	3.98b	3.91b	3.75	4.27	4.47
A 4.5	3.37b	4.04b	4.00b	4.10	4.31	4.56
A 5.2	3.17b	3.83b	3.70b	3.80	4.27	4.27
A 6.0	2.80b	3.67b	3.54b	3.97	4.27	4.38
A 9.0	2.78b	3.72b	3.56b	4.07	4.30	4.16

The consumer's evaluated liking on a 7 point hedonic scale. Different letters within the same column indicate significant differences ($p \leq 0.05$). The androstenone values of the samples are given in ppm.

Finding significant differences is easier when more subjects are included in the analysis. To see how the consumer's ability to detect differences between the samples was affected by the number of consumers participating in the analysis, permutations with random selection of the sensitive consumers (different number of consumers in each permutation) were performed. The results are presented in Fig. 1 and show that when selecting seven random sensitive consumers (1000 permutations) a significant difference between the reference sample and the sample containing 3 ppm androstenone could only be found in 19.6% of cases. Using 14 sensitive consumers a significant difference between the two samples was found in 41.3% of cases. Using 42 sensitive consumers a significant difference between the samples was found in 100% of cases.

Since liking is regarded as a response to androstenone for these samples that are low (≤ 0.05 ppm) in skatole and otherwise identical, the results suggest that the consumers gave good precision. This suggests that they worked at their own speed and found the task relatively easy, possibly due to the fact that they were segmented for their ability to perceive androstenone.

The sensory panel, despite the training that normally provides high precision, could not identify the sample with 3 ppm androstenone. Lunde et al. (2010) reported that training was not very important with respect to perceiving androstenone, in addition, the calculations done

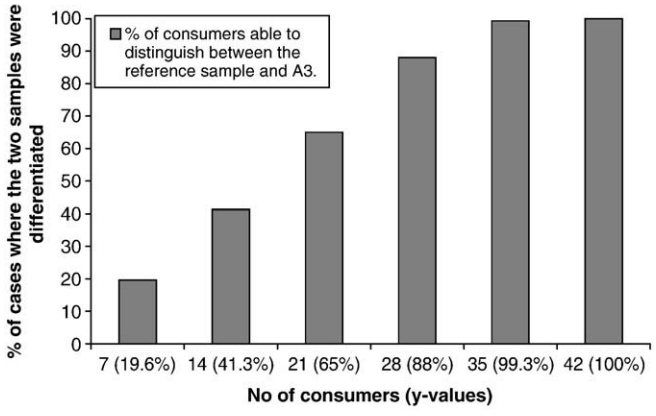

Fig. 1. The figure shows the sensitive consumers ability to differentiate between the reference sample and the sample containing 3 ppm androstenone ($p \leq 0.05$) using an increasing number of consumers (odour above frying pan). For each level of consumers 1000 permutations were made.

on the consumer data (Fig. 1) suggest that the probability of obtaining false negative results is reasonably high for meat samples with 3 ppm androstenone even for trained panels.

Thus, the results reported here and elsewhere (Lunde et al., 2010); suggests it is difficult to detect 3 ppm in meat samples and that the detection threshold of androstenone is probably between 3 and 2 ppm.

3.3.2. Evaluation of the skatole samples

Skatole is perceived and regarded as unpleasant by practically all consumers (Weiler, et al., 1997). The consumers were therefore not split into 2 groups when analyzing the samples containing skatole. Table 4 shows no significant differences in liking ($p>0.05$) of all five samples when odour above the frying pan was evaluated. Evaluation of liking of odour on the fried meat revealed a significant higher liking score for the reference sample ($p\leq0.05$) than for the skatole samples containing 0.25 ppm or higher. Liking of flavour showed that the reference sample was significantly different ($p\leq0.05$) from all samples containing skatole, indicating that consumers can detect skatole flavour at levels as low as 0.15 ppm, similar to trained assessors. Accordingly the Norwegian established threshold value of 0.21 ppm skatole may be too high.

In general, the differences in mean liking between the reference sample and the samples containing skatole were relatively small both for odour and flavour evaluations. That boar taint (skatole and androstenone attributes) was more pronounced during evaluation of flavour compared to odour agrees with results of four sensory panels when evaluating the same samples in an intercollaborative test (Lunde et al., 2010). The fact that skatole can be detected at lower concentrations than 0.21 ppm agree with results in other studies, (0.07 ppm–0.15 ppm) (Bañón et al., 2003, Font I Furnols et al., 2000, Lunde et al, 2009). Different methods are used when analyzing for skatole, but all show that skatole can be detected at low concentrations.

3.3.3. Economic consequences

Entire male production in Norway will increase the percentage of animals that need to be sorted to avoid negative consumer reactions. The threshold used for sorting out tainted carcasses on the slaughter line in Norway today is 0.21 ppm for skatole. This threshold may be too high as many assessors (and consumers) can detect skatole at 0.10 ppm. Using 0.20 ppm skatole as a threshold value for sorting carcasses would mean that 7.7% of all entire males produced in Norway must be rejected (Fredriksen et al., 2008), and still some negative consumer reactions would be expected. Changing the threshold value for sorting to 0.1 ppm would lead to 21.9% of the carcasses being eliminated, but negative consumer reactions would probably be avoided.

The androstenone content of Norwegian entire males (animals used for breeding) is not analyzed in agreement with the practise of other countries. If animals with androstenone levels above 2 or 3 ppm are to be eliminated in Norway this means that 17.3% and 5.5%, respectively, of male carcasses will be rejected (Fredriksen et al. 2008). These figures actually suggest that a follow up study should be made with sensitive consumers using selected samples with androstenone contents between 2 and 3 ppm.

Table 4
Evaluation (mean liking) of the skatole samples by the Norwegian consumers (N = 101).

Sample	Odour (frying pan)	Odour	Flavour
Reference	4.23	4.76a	4.97a
S 0.15	3.95	4.37ab	4.39b
S 0.25	3.92	4.23b	4.29b
S 0.30	4.00	4.09b	3.72c
S 0.35	4.02	4.30b	4.23bc

The consumer's evaluated liking on a 7 point hedonic scale. Different letters within the same column indicate significant differences ($p\leq0.05$). The skatole values of the samples are given in ppm.

Sorting thresholds used by the meat industry must be based on both skatole and androstenone values since meat from entire males in most cases will contain both compounds. Calculations performed by Fredriksen et al. (2008) showed that when sorting out percentages were based on both skatole and androstenone values (high correlation between skatole and androstenone levels in boars), the sorting out percentages will be higher than the percentages presented above.

4. Conclusion

Androstenone insensitive consumers did not differentiate between reference (without androstenone) and androstenone tainted samples, meaning that the non sensitive consumers accepted all levels of androstenone. Sensitive consumers gave a significant lower liking score for androstenone samples containing 3 ppm (and more) when evaluating these samples above the frying pan, but no significant difference was found between 3 ppm and reference samples when likings of the fried samples were evaluated. This indicated that samples with 3 ppm androstenone were accepted by the sensitive consumers when they evaluated the fried samples, but not accepted during frying.

The same consumers differentiated samples with skatole, with regard to flavour at 0.15 ppm. The Norwegian sort out threshold value of 0.21 ppm skatole may therefore lead to negative reactions from consumers. For androstenone, using a level of 3 ppm for sorting would be economically acceptable due to the low number of carcasses containing above 3 ppm, but its odour may be detected (not accepted) by sensitive consumers during frying of the meat. Sorting thresholds used by the meat industry must be based on both skatole and androstenone values in combination since meat from entire males in most cases will contain both of these compounds. This suggests that samples containing either skatole above 0.1 ppm or androstenone above 2–3 ppm must be eliminated to avoid negative consumer reactions. We are sceptical of reliable identification of interaction effects between androstenone and skatole, and would not recommend lowering individual thresholds for androstenone and skatole to compensate for an uncertain interaction effect.

Acknowledgements

The Norwegian Research Council is thanked for its grant to this activity through project number 63164; “Boar meat-consumer aspects and exploitation of resources”. Olaf Bjørkeli at Nortura Rushøgda is thanked for his sampling of suitable animals for the experiment. The meat cutters at Animalia and Tom Chr. Johannessen and Karin Solgaard at Nofima Mat are thanked for their assistance in the sample preparation. Ellen Dahl at Norwegian School of Veterinary Science is thanked for her cooperation with the skatole and androstenone analysis. Solve Sæbø is thanked for his assistance with the permutation calculations.

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Paper V

Meat Science 80 (2008) 1264–1272

Contents lists available at ScienceDirect

Meat Science

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

Marinating as a technology to shift sensory thresholds in ready-to-eat entire male pork meat

K. Lunde [a,b,*], B. Egelandsdal [b], J. Choinski [c], M. Mielnik [d], A. Flåtten [a], E. Kubberød [d]

[a] Norwegian Meat Research Centre, Lørenveien 38, P.O. Box 396, Økern, N-0513 Oslo, Norway
[b] Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O. box 5036, N-1432 Ås, Norway
[c] Nortura Rudshøgda, P.O. Box 70, N-2366 Rudshøgda, Norway
[d] Matforsk, Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway

ARTICLE INFO

Article history:
Received 15 July 2007
Received in revised form 13 May 2008
Accepted 28 May 2008

Keywords:
Androstenone
Skatole
Boar taint
Marinating
Sensory thresholds

ABSTRACT

This study investigated the effect of marinades in improving the eating quality in ready-to-eat boar meat. Neck chops with fat content below 18.9%, skatole ⩽1.1 ppm (range 0.03–1.1) and androstenone ⩽5.6 ppm (range 0.01–5.6) were used. In a screening experiment different marinades were tested for their ability to mask boar taint (defined as manure and urine odour and flavour). Liquid smoke and oregano extracts appeared to have the best potential for masking, and were studied in detail. Results from the study indicated that marinated chops with skatole content of approximately 0.4 ppm appeared similar to castrates in boar taint. Chops with skatole contents above 0.7 ppm remained unmasked despite the use of strongly flavoured marinades. Unmarinated chops served at 60 °C were more tainted than those served at 15 °C, but scored lower for boar taint when reheated, although the concentrations of androstenone and skatole remained the same. The fat content of the chops was not well correlated to the perception of boar taint. The attributes manure and urine were correlated with the level of skatole, but urine attribute was not a good indicator of the androstenone level.

1. Introduction

Castration of entire male pigs is widely used in Norway and in many other countries. Castration is done to prevent an unpleasant odour and flavour (boar taint) in meat from entire males. Boar taint is mainly associated with two components, androstenone and skatole. Androstenone is a steroid closely related to testosterone. The production of androstenone in the testis increases with maturity of the male pig. Androstenone is associated with a urine-like flavour (Patterson, 1968). Skatole is formed in the gut by microbial degradation of the amino acid, tryptophan. The ability to break down skatole changes during maturity of male pigs. Skatole is associated with manure-like flavour (Vold, 1970).

In 2001 the Norwegian authorities decided that castration of male pigs should be forbidden in Norway from 2009. Due to problems with tainted meat in the market place there is a need for updated knowledge about processing opportunities for this raw material. Studies have shown that the defect (boar taint) is better tolerated in processed products (Bañón, Costa, Gil, & Garrido, 2003; Bonneau et al., 1992; Diestre, Oliver, Gispert, Arpa, & Arnau, 1990) than in retail cuts. Even though minced meat products constitute a large product share (approx. 50%), there is also a large market segment based on retail cuts. The retail cut segment also contains more highly priced products. At present, small quantities of tainted meat are used in different sausages: both dry-fermented and heat-processed. The meat processors may obtain the skatole value of the back fat, and thereby adjust their recipes using a large safety margin with respect to off-flavour. When castration is prohibited, the market situation will change dramatically, and it would be relevant to identify processing methods that could still provide high quality products to the consumer.

Not all consumers have the ability to sense androstenone. In a study on German and Spanish consumers Weiler, Fischer, Kemmer, Dobrowolski, and Claus (1997) found that only 31% of the German and 18% of the Spanish consumers were sensitive to androstenone. In contrast to androstenone, skatole is perceived by 99% of the consumers and is regarded as unpleasant (Weiler et al., 1997). The contribution of skatole and androstenone to boar taint has been investigated in a number of different studies. Dijksterhuis et al. (2000) confirmed what was found by Frempong, Nute, Whittington, and Wood (1997), that both compounds are important in boar taint perception, although with a stronger negative reaction towards skatole (Cameron et al., 2000). Bonneau et al. (1992) also found that cooked ham sensory odour scores were more related to skatole than to androstenone content. Mortensen, Bjerholm,

* Corresponding author. Address: Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O. box 5036, N-1432 Ås, Norway. Tel.: +47 22092317; fax: +47 22220016.
E-mail address: kathrine.lunde@animalia.no (K. Lunde).

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doi:10.1016/j.meatsci.2008.05.035

and Pedersen (1986) found that when boars with low skatole content were used in meat production there were only a few adverse comments on meat quality compared with barrows. The level of skatole needed for clear cut identification by trained sensory panels is suggested to be 0.1 ppm (Bañón et al., 2003; Font I Furnols, Guerrero, Serra, Rius, & Oliver, 2000). On the other hand, a threshold of 0.25 ppm (backfat value) was identified by The Danish Slaughter Industries as an economically feasible threshold for sorting of entire male carcasses (Andersen, 2005). There is no similar experience in Norway, but at present any entire male pig with a skatole value below 0.20 ppm is regarded as untainted. A big effort is now being made to obtain an estimate of the prevailing Norwegian skatole level distribution in pigs.

The interest in time efficient alternatives for traditional meals is growing since the spare time of consumers is decreasing. The consumption of ready-to-eat meals is rapidly growing in Europe. In this respect, the concept of enhancing meat has been very successful in the USA (Miller, 1998). Marinating is a variant of enhancement. Marinating adds taste and aroma to the meat, and has the potential to mask the off-flavour due to boar taint. Previously very few studies have been devoted to identifying thresholds of boar taint within the framework of modern meat processing. McCauley et al. (1997) found that sweet and sour marinades did not totally mask the boar taint (oven cooked pork), but the intense odour and flavour of the marinades confused the assessors. They found significant differences ($p \leqslant 0.05$) between the low taint group (skatole: 0.06 ± 0.045 ppm, androstenone: 0.25 ± 0.28 ppm) and the high taint group (skatole: 0.17 ± 0.06 ppm, androstenone: 1.1 ± 0.6 ppm) for both boar odour and flavour. There was no significant difference between the reference group (female pig carcasses, skatole: 0.04 ± 0.01 ppm) and the low taint group. Even though they found a significant difference between the low and high taint groups for the marinated samples, all the marinated samples had lower average intensities for boar odour and flavour than the same samples served unmarinated. This indicated that marinating had a masking effect.

2. Materials and methods

The aim of the present study was to investigate the effect of marinades on neck chops with much higher concentrations of androstenone and skatole than levels investigated by McCauley et al. (1997). A screening study with the purpose of selecting candidate ingredients for the marinades was carried out (Exp. 1). The ingredients that seemed to have a potential in masking boar taint were combined and tested in a new experiment (Exp. 2). In this experiment oregano and liquid smoke seemed to be effective in sensory masking of androstenone and skatole. Oregano and liquid smoke were not used in the same marinade in experiment 2, and another experiment (Exp. 3) was then performed to obtain the right concentrations of oregano and liquid smoke in the same marinade. Finally, an experiment (Exp. 4) was conducted to demonstrate how the assessors' scores for boar attributes would change with increasing amount of smoke.

2.1. Meat samples

Neck chops were selected based on androstenone and skatole levels measured in pure back fat. Breed was regarded as irrelevant in the study. Meat samples were collected for the different experiments (Exps. 1–4):

Experiment 1: Screening of candidate ingredients for marinades using two necks with skatole levels around 0.5 ppm combined with different androstenone values (A = 1.5 and A = 2.4 ppm).

Experiment 2: An experiment with marinated products from 12 entire males and 4 castrates (skatole levels: 0.03–0.68 ppm; androstenone levels: 0.09–4.0 ppm). The pH values of the meats were 5.64–6.22, and the fat levels of the chops were 5.0–18.9% (mean value: 10.0%; standard deviation: 4.3%). The correlation between androstenone and skatole was 0.12 ($p = 0.19$).

Experiment 3: A second experiment with marinated products from 12 entire males, two castrates and one sow (skatole levels: 0.1–1.1 ppm; androstenone levels: 0.01–5.6 ppm). The pH values of the meats were 5.65–6.44, and the fat levels of the chops were 2.5–15.9% (mean: 8.24%; standard deviation: 3.5%). The correlation between androstenone and skatole was 0.16 ($p = 0.56$).

Experiment 4: A detailed study on smoke flavour using one neck with skatole level at 0.64 ppm and androstenone level at 1.00 ppm.

The necks were vacuum-packed and kept frozen (−40 °C) until used, and then thawed and processed. Some of the necks were frozen for a long period (maximum 6 months) because of problems finding animals with right levels of skatole and androstenone.

2.2. Methods

2.2.1. pH and fat content

pH was measured with a Beckman φ31 pH meter (electrode INLAB427, Switzerland). The fat content was measured on homogenised meat using NMR (Nuclear Magnetic Resonance) as described by Wold, Lundby, and Egelandsdal (1999).

2.2.2. Skatole and androstenone

Skatole was determined using an automated colorimetric assay (Hansen-Møller & Andersen, 1994; Mortensen & Sørensen, 1984). Skatole was extracted from back fat in tris/acetone followed by addition of a colour agent. Absorption was used for quantification of skatole. The analysis of androstenone was based on the ELISA method of Claus, Herbert, and Dehnhard (1997). Androstenone was determined using an extraction method followed by a commercial immunoassay (Ridel-del-Haen, Seelze, Germany). In experiment 3, skatole and androstenone values were measured in fat before heat treatment and after storage and reheating. In the other experiments (Exps. 1, 2 and 4) skatole and androstenone were only measured in fat before heat treatment. The analytical error in this study when analysing both of these components was approximately 0.1 ppm.

2.2.3. Rancidity

Due to the long storage period in the freezer, the samples were examined for rancidity using both sensory and chemical analysis. A decision was then made about which samples to use for experiment 3. The unmarinated samples were measured for rancidity with a standard extraction method for 2-thiobarbituric acid reactive substances (TBARS) (Sørensen & Jørgensen, 1996), and then quantified using a standard curve for TEP (1.1.3.3-tetraethoxypropane). The marinated samples were also analysed for TBARS, but the extraction was replaced with distillation (Tarladgis, Watts, Younathan, & Dugan, 1960) because of the nitrite content of the marinades. When employed on the same nitrite-free systems, the deviation between the two methods was small for products with low TBARS values.

2.3. Processing and sampling

Experiment 1: Screening procedure: 20 grams of meat (approximate cubes) was soaked in marinade (meat:marinade ratio,

1: 4), and left for equilibration overnight before heating in small plastic bags to 72 °C. These small samples were evaluated by a screening panel (see Section 2.4).

Experiment 2: Half a pork neck (both sides were used, *i.e.* four marinades multiplied by ½ neck multiplied by 12 animals) was injected twice with marinade (at 4 °C) in a multineedle injection machine (Sukner SG, Bremgarten, Switzerland) and placed in a room at 4 °C. A 20% pump was used for this purpose. The necks were rapidly packed in "cook-shrink" bags (Cook-tite 82 from BM Food Tech, Bernis, Swansea, England), quickly shrunk (at 90 °C) and then left overnight in a room at 4 °C. The next morning each sample was cooked to an internal temperature of 72 °C in a cooking/smoking cabinet (Doleschal Unitronic SC2000, Steyr, Austria). After 10 days of storage at −2 °C, the packed samples were transferred to the sensory laboratory for reheating to an internal temperature of 72 °C, and were then evaluated by the sensory panel. The necks from the castrates were marinated in the same way as the necks from the entire males.

Experiment 3: The necks (both left and right side for each animal) were cut into seven parts. For each animal seven different treatments were tested. Pure pork backfat was included in the study to see the difference between pure pork backfat and neck chops with different fat content (between 2.5% and 15.9% fat). For each animal the effect of marinades, serving temperature and reheating were tested. The treatments are presented in Table 1. The multineedle injection machine used in study 2 was not used in study 3 because of the sample size (small samples). The marinating was then done by tumbling (Hollstein & Fuhrmann, GmbH. KG, A-1200 Wien). Twenty gram marinade was added directly into the cook-shrink bag (Cook-tite 82 from BM Food Tech, Bernis, Swansea, England) with 100 g meat, and tumbled for 20 min at max speed in a room at 4 °C. The samples were left for 3 days (4 °C) before being sliced and vacuum-packed in portion sizes (approx. 30 g). These samples were then transferred to the sensory laboratory for heating and evaluation. The samples (30 g) were heated to an internal temperature of 72 °C, and kept in warm metal boxes on a hot-plate (65 °C) on the table in front of each assessor until the analysis (a few minutes later). The samples (30 g) that were to be heated twice to investigate the effect of reheating were first heated in the cooking/smoking cabinet (Unitronic SC2000, Doleschal, Austria), stored for 7 days and then transferred to the sensory laboratory for reheating and evaluation. The necks from the castrates and the sow were treated the same way as the necks from the entire males.

Experiment 4: One neck was cut into five parts to test the differences in smoke flavour. The different combinations are presented in Table 2. The marinating was done by tumbling as in experiment 3. The samples were left for 3 days (4 °C) before heating to an internal temperature of 72 °C in a cooking cabinet (Electrolux combined steamer, CS 7 Gourmet, Stockholm, Sweden). The samples that were treated with real smoke (samples 4 and 5) were first dried and then smoked. The smoking was done with beech chips at 25 °C and 60% humidity for 30 and 60 min, respectively, in a cooking/smoking cabinet (Doleschal Unitronic SC2000, Steyr, Austria). The samples were then transferred to the sensory laboratory for reheating and evaluation. The reheating and evaluating procedure was done in the same way as for the samples in experiment 3.

Table 1
The different treatments that were tested in experiment 3

Sample	Heating	Serving temperature	Marinade
1	Once	Warm[a]	Unmarinated
2	Once	Cold[b]	Unmarinated
3	Twice	Warm	Unmarinated
4	Twice	Warm	High oregano–high liquid smoke
5	Twice	Warm	Low oregano–low liquid smoke
6	Twice	Warm	Low oregano–high liquid smoke
7	Twice	Warm	High oregano–low liquid smoke
8	Twice	Warm	Pure pork backfat

[a] Warm was approximately 60 °C.
[b] Cold was approximately 15 °C.

Table 2
The different treatments tested in the detailed study on smoke flavour (Exp. 4)

Sample	Treatment
1	Unmarinated
2	Marinated in brine mixture[a]
3	Marinated in high oregano high smoke (from Exp. 3)
4	Marinated in high oregano high smoke (from Exp. 3) + 30 min of real smoke in a smoking cabinet
5	Marinated in high oregano high smoke (from Exp. 3) + 60 min of real smoke in a smoking cabinet

[a] Wela 63/398997 fra SFK Foods A/S, Copenhagen, Denmark: contained phosphates E450/451, dextrose, fructose and ascorbate.

2.4. Marinade ingredients

The ingredients for the marinades were chosen in several steps. The screening of ingredients was done by a five-person panel. One person was formally trained as a chef, and the other four persons were professionals from the Meat Research Institute. They all had the ability to perceive skatole and androstenone in pure form. These five people selected a limited number of ingredients that gave marinades with a genuine flavour for experiment 2, where pre-cooked and reheated products were served to a trained sensory panel. Four marinades were used in experiment 2. The composition of the four marinades is presented in Table 3. Based on the results of experiment 2, a third experiment (Exp. 3) was carried out with even fewer ingredients. The marinades used in experiment 3 are shown in Table 4. It was impossible to obtain the same liquid smoke aroma as used in experiment 2, therefore a new

Table 3
The composition of the four marinades used in experiment 2 (in grams per kg marinade)

Ingredients	Marinade 1	Marinade 2	Marinade 3	Marinade 4
Salt (NaCl)	40.0	59.4	52.8	56.4
Sodium nitrite	0.24	0.36	0.32	0.34
Ascorbate	1.2	1.8	1.6	1.3
Phosphate (E451, E450) (as P_2O_5)	14.3	18.8	16.9	17.9
Dextrose	12.8	16.9	17.5	16.1
Fructose	3.7	3.7	3.3	n.a.
Soya sauce (Kikkoman Corp.)	250	n.a.	n.a.	n.a.
Liquid smoke (Wright's, USA)	3.7	9.9	n.a.	n.a.
Oregano, oleoresin (Kalsec, USA)	n.a.	n.a.	0.5	n.a.
Garlic powder (E.H.Woree, Germany)	n.a.	n.a.	4.4	3.5
Paprika, extract (Chr.Hansen, Spania)	n.a	1.2	n.a	n.a
Tabasco (McIlhenny Co., USA)	n.a.	n.a.	n.a.	35.2
Bacon flavour (Perfecta Limited, UK)	n.a	n.a.	23.4	23.4
Lemon pepper (SFK Foods A/S, Denmark)	2.5	n.a.	n.a.	n.a.
Tomato pure (Sopps, Norway)	n.a.	n.a.	88.0	n.a.
Onion extract (Nopal, Norway)	n.a.	n.a.	2.2	n.a.

n.a. = not applied.

Table 4
The marinades used for marinating boar-tainted neck chops in experiment 3

Ingredients	High oregano–high smoke (g)	Low oregano–low smoke (g)	Low oregano–high smoke (g)	High oregano–low smoke (g)
Smoke EZ-C3 (Red Arrow, USA)	100	54	100	54
Aro-smoke P-50 (Red Arrow, USA)	0.2	0.108	0.2	0.108
Oregano oleoresin (Kalsec, USA)	1.6	0.4	0.4	1.6
Brine mixture[a]	120	120	120	120
Nitrite salt (0.6% nitrite)	120	120	120	120
Water	1760	1760	1760	1760

[a] Wela 63/398997 fra SFK Foods A/S, Copenhagen, Denmark.

(blended) liquid smoke, selected among different commercial types, were used in experiments 3 and 4 (Table 4).

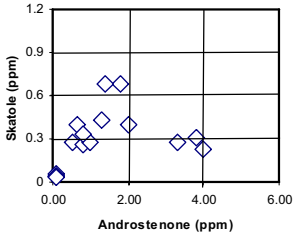
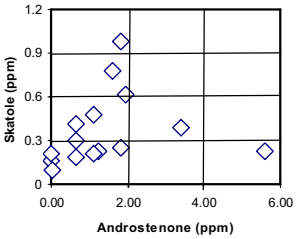
The high and low levels of oregano and liquid smoke (Exp. 3) were determined by the screening panel. Samples with different levels of oregano and liquid smoke were prepared in the same way as the samples in experiment 3 (Section 2.3, Exp. 3). The samples were evaluated warm (approx. 60 °C) using an unstructured scale from low to high intensity of the marinade attributes (oregano and liquid smoke). The low and high values were set based on the criteria that the lower limit should have a low intensive flavour of the marinade ingredients and the upper limit should have distinct, but not overwhelming, flavour of the marinade ingredients.

Table 5 shows the screening for useful ingredients. On the left, 26 ingredients are listed. These were blended to provide 17 marinades (Løvlund, 2002). The 26 ingredients were selected based on recommendations from commercial providers of ingredients and the trained chef. All the marinade ingredients were in the sensory profile. That means that when a marinade contained oregano, oregano odour and flavour were evaluated. The marinade ingredients used in the experiments were chosen on the basis of how the marinade attributes related to skatole and androstenone. Four marinades were then selected as having a potential for reducing the perception of boar taint (Exp. 2). Ingredients common to all four marinades (Table 5) were chosen to improve water-binding, and to reduce the possibility of warmed-over-flavour. Only two of the marinade ingredients in experiment 2, oregano and liquid smoke were identified as potent ingredients to mask androstenone and skatole, respectively, and these were tested in different combinations in experiment 3.

2.5. Sensory analysis

The screening of marinade ingredients (Exp. 1) was done as an open discussion session with samples, but where the screening

Table 5
The identification of ingredients suitable for marinating entire male pork meat

Exp. 1 (screening)	Exp. 2	Exp. 3
Salt, phosphate, nitrate, soy sauce, meat broth Black pepper, lemon pepper, cayenne pepper, red curry paste, tabasco (mild), mustard, Fructose, xylose, honey Garlic powder, onion extract, oregano extract, paprika extract, tomato-purée, lemon juice, lime juice Cognac aroma, fermented milk, beer, bacon-aroma, liquid smoke	1. Liquid smoke, paprika extract 2. Soy sauce, liquid smoke, lemon pepper 3. Garlic and onion powder, tomato-purée, oregano extract 4. Tabasco(mild), garlic powder, bacon aroma All systems (1-4) contained: NaCl, phosphate (E450/451), ascorbate, nitrite, dextrose/ fructose	Liquid smoke & oregano extract combinations Added to all combinations: NaCl, phosphate (E450/451), ascorbate, nitrite, dextrose/ fructose See Table 2
Skatole 0.5 ppm Androstenone: not measured Fat: Not measured	 Fat: 5.0 -18.9%	 Fat: 2.5-15.9%

The exact recipes for marinades 1–3 were given by Egelandsdal et al. (2004). The composition of marinade 4 is given above.

panel wrote their personal scores using a scale from 1 to 5. One corresponded with too little and 5 corresponded with too much of the flavour components.

The sensory panel consisted of 10 trained expert assessors screened for sensory abilities (basic tastes, colour vision, odour detection and tactile sensibility) as well as ability to communicate sensory descriptors of products as recommended in ISO (1993). The sensory laboratory was designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory data (CSA, Compusense Five, Version 3.80, Canada, 1999). The sensory study in experiment 2 was done during 3 days with two sessions each day. Experiment 3 was evaluated during 4 days with two sessions each day, and experiment 4 was carried out during one day (two sessions). All the samples were served in completely randomised order, and odour and flavour were evaluated in the same sessions. The samples were served both warm (60 °C) and cold (15 °C) in experiment 3. In experiment 2, only a sub selection of the samples was served cold (15 °C), and in experiment 4 all samples were served warm (60 °C). In experiment 2 the bags containing ½ pork necks were opened in the sensory laboratory and sliced into smaller samples so each assessor got half a slice with a thickness of 1 cm. These slices were reheated individually in a new packing material before being served to the panel. In experiments 3 and 4 the samples were of portion sizes (30 g) and were vacuumed-packed in small bags one by one. The assessor had to open each sample and could then perceive the odour directly from the meat in the bag.

The attribute profile used was the one defined by Dijksterhuis et al. (2000), modified to include characteristic flavours describing the ingredients of the marinades. In experiments 3 and 4 the profile was simplified (removing the following attributes: abnormal, pig, sweet, metal and sweat) because the assessors did not use the scale for these attributes (very low standard deviations). The training of the assessors was done using boar meat and a reference sample (castrate/sow). The assessors were trained using all attributes in the profile. The assessors were trained before experiment 2 (3 days) and were trained again before experiment 3 (1 day). Before experiment 3 the assessors were trained only on the attributes oregano and liquid smoke. In addition to the training in these experiments they had some pervious training on boar-tainted meat.

The assessors used intensity scores from 1 to 9; where 9 corresponded to the highest intensity score. The assessors were all sensitive to skatole and androstenone in pure form (Lunde et al., in preparation).

2.6. Statistical analysis

The sensory responses were analysed using proc GLM (general linear model) in SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA). The model had sample, animal and sensory assessor as main effect. The interaction terms were sample * animal, sample * assessor and assessor * animal. The models for oregano, smoke, urine and manure (odour and flavour) were all significant ($p < 0.0001$). The effect of sample, animal and assessor on the above eight attributes also had $p < 0.001$ except for the effect of animal on oregano odour ($p = 0.01$). In general, the interaction term assessor * sample was the largest interaction term and significantly ($p < 0.001$) explained variance for oregano, smoked, urine and manure * assessor (18.4%, 14.3%, 11.1% and 6.8%, respectively). Statistical significance for the effect of treatment and the effect of animal were found using Tukey's test in SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA). For each meat sample, the average score of the assessors was estimated and presented. Correlation between odour and taste and correlation between different treatments and attributes were found using 2D scatter plots in Unscrambler.

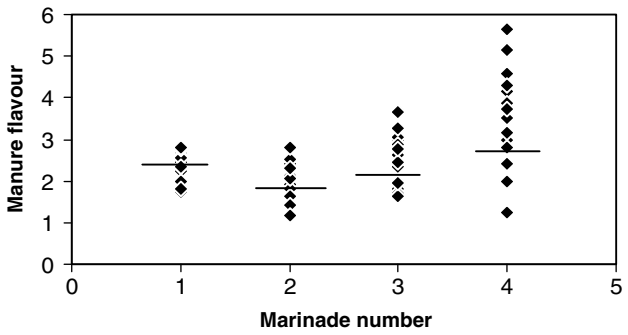


Fig. 1. The intensity score for manure flavour for marinades 1–4 (Exp. 2 (Table 4)) is given. The mean intensity of the four castrates is indicated as: ––.

Relations between different treatments and sensory attributes scores were studied using linear regression in Unscrambler, version 9.1 (CAMO, Trondheim, Norway). Also stepwise linear regression in Minitab 14 (Minitab Inc., Pennsylvania, USA) was used to test for the importance of fat content in regression models.

3. Results and discussion

3.1. Choice of ingredients

Table 5 shows how the screening for useful ingredients in the marinades progressed. In experiment 2, only five of the attributes had a standard deviation for sensory score above 1.0. These five attributes were urine, manure, garlic, oregano and smoke. A high standard deviation of an attribute indicated that the assessors found it easy to distinguish between the low and high level of that attribute, and also that the assessors spanned the intensity scale to a larger degree. In the same experiment (Exp. 2) odour and flavour were highly correlated ($R^2 = 0.80–0.99$) for the attributes urine, manure, smoke and oregano, and were treated as one attribute. Therefore only the flavour attributes were chosen for presentation here.

Fig. 1 displays how the marinated meat from the entire males was assessed relative to the castrates (Exp. 2). Marinade 4 showed the lowest reduction in perception of boar taint (manure). Marinade 3 had an intermediate perception score of manure, while marinades 1 and 2 gave the lowest perception score of manure. For marinade 1 there was no significant correlation between the manure attribute and skatole level ($p = 0.44$), as found for the three other marinades. The attribute urine correlated with both skatole and androstenone levels for marinades 2, 3 and 4, indicating that the attribute was not a unique descriptor of androstenone.

As liquid smoke was used in both marinades 1 and 2, and was negatively correlated to the manure flavour ($p = 0.004$), it was assumed that liquid smoke was an important factor for the reduction of manure flavour. The oregano flavour used in marinade 3 yielded the highest standard deviation of all the ingredients (sensory score; 2.7 ± 2.7) and had a very distinct flavour. Oregano was uncorrelated to skatole and androstenone levels as well as to manure. Unexpectedly, oregano was positively related to urine ($p = 0.028$). However, this relationship was not significantly reproduced in experiment 3.

In experiment 3 it was reconfirmed that the assessors easily perceived the four attributes smoke, oregano, urine and manure, and that the assessors spanned the entire intensity scale. The odour and flavour attributes for urine, manure and oregano were highly correlated ($R^2 = 0.92–0.95$) as in experiment 2. However, there was no correlation between flavour and odour for the smoke attribute, even though this correlation was found to be the highest one in experiment 2. The reason for this disagreement seemed to

be the change in preparation routine from experiment 2 to experiment 3.

3.2. Content of skatole and androstenone

Table 5 shows that higher values for both androstenone and skatole content were used in experiment 3 than in experiment 2. Animals that scored high for both androstenone and skatole concentrations could not be acquired for any of the two experiments.

3.3. The sensory attributes urine and manure, and their relation to androstenone and skatole

In experiment 3 the attribute manure had a positive correlation to skatole for all the different treatments. Skatole was also significantly ($p \leqslant 0.05$) related to both manure and urine for all treatments (Table 6).

From Table 6 it is obvious that there was a substantial inconsistency in how the attribute urine related to androstenone. Apparently, urine related more consistently to skatole, which was also observed in experiment 2. As the correlation between androstenone and skatole in experiment 3 was 0.16, it seemed unlikely that the assessors could determine androstenone through its correlation to skatole. Thus there are only two possibilities: the assessors were not trained well enough to differentiate between the attributes urine and manure, or they found it difficult to relate the urine flavour to androstenone. Even though they all have demonstrated the ability to sense the chemically pure component it might not be related to real life situations. At present it is unclear why urine was not a good descriptor of the androstenone level in the samples. In experiment 3 the sample containing the highest level of skatole (1.1 ppm) combined with a low androstenone level gave the highest mean value of urine flavour, but it was not significantly different ($p > 0.05$) from the sample with the highest level of androstenone (5.59 ppm) combined with a much lower (0.23 ppm) skatole level. Meat with high levels of androstenone combined with lower levels of skatole (but above the detection threshold 0.1 ppm) thus gave as intense urine flavour as meat with high levels of skatole. According to Dijksterhuis et al. (2000) the scoring of boar taint attributes is complex and can be confusing even for a trained panel and the perception of androstenone seems to be more difficult than that of skatole. Font I Furnols et al. (2000) related the urine attribute, both flavour and odour, to the samples which were high in androstenone and low in skatole (0.1 ppm).

3.4. Effect of garlic, oregano and smoke on the sensation of boar taint (manure and urine)

Garlic has a distinct flavour that is easy to recognize. However, when the criterion was a tasteful marinade, garlic did not reveal a sufficient masking effect on boar taint. The content of allicin and other flavour components and flavour precursors in garlic varies among different products (Yu, Wu, & Ho, 1994). Therefore some garlic products could still be relevant additives to tainted meat, despite the results reported in Fig. 1.

Oregano also has a distinct flavour that is easily detected. Only one oregano extract was tested in this study. The flavour of oregano will depend on several different compounds, and could be discriminated by their contents of *p*-cymene, γ-terpinene, *cis*- and *trans*-sabinene hydrate, borneol, terpinen-4-ol, α-terpineol, thymol and carvacrol (Figueredo, Chalchat, & Pasquier, 2006). The extract used in this study contained mainly different types of alkenes; the dominant volatiles being the terpenes (alkenes): α-pinene and β-pinene. Their flavour was described as pine/turpentine (GC–MS headspace analysis, not shown). When marinated in oregano (high and low concentration), meat from castrates could be ranked as having a stronger manure flavour (mean values) than samples having 0.3 ppm skatole. This may be occasional, but it could be a good indicator of the assessors not being able to differentiate well between castrates and lower skatole samples when the samples were marinated in oregano.

During experiments 2 and 3 it became apparent that the smoke flavour was easily identified. When the preparation routine was changed (from experiment 2 to experiment 3 to accommodate the need for more and therefore smaller samples), the assessors assessed samples in the packing materials used in the smoke-heat cabinet. No smoke flavour was intentionally added in the cabinet, nor was the cabinet used to generate smoke. Even though such cabinets were cleaned after use, smoke was still present and easily detected by the assessors who also gave a rather high score for the odour of smoke from the packing material. The assessors gave all such samples higher intensities for the odour of smoke, and this may have interfered with the correlation between flavour and odour of smoke that otherwise is present. Subsequently, a fourth experiment was conducted to demonstrate how the assessors will change their scores for manure with increasing amount of smoke (Table 7) on a tainted (skatole: 0.64 ppm) neck chop. The assessors evaluated samples that were not smoked, and samples with alternating treatment with smoke (Exp. 4). The assessors scored high on smoke flavour as soon as the liquid smoke was added, but did not differentiate the samples thereafter. Obviously the perception of smoke appeared nonlinear (Table 7). The fourth experiment had again a high correlation between smoke flavour and odour ($R^2 = 0.97$). Smoke, in this case volatiles remaining in the cabinet as remnants from a smoke generator that used beech chips, therefore contains flavour components with very low thresholds. This can easily be detected on items stored in a pilot plant where smoking occasionally is performed.

Smoke flavour and odour were negatively correlated to manure flavour and odour which means that more smoke made skatole less pronounced. It should be noted that scores around 1.7–1.9 for

Table 6
The relations between the sensory descriptors manure and urine flavour and the level of the boar taint components skatole and androstenone in the meat (Exp. 3) given as *p*-values (n.s means $p > 0.05$)

Taste	Um-1	Um-2	O–S	o–s	o–S	O–s
Manure						
Skatole	0.010	0.005	0.000	0.000	0.000	0.000
Androstenone	n.s	n.s	n.s	n.s	n.s	n.s
Urine						
Skatole	0.006	0.008	0.000	0.000	0.000	0.000
Androstenone	n.s	n.s	n.s	n.s	0.023	0.009

The data relate to samples served warm.
Um-1: unmarinated heated once; Um-2: unmarinated heated twice; O: high oregano; o: low oregano; S: high smoke; s: low smoke.

Table 7
Effect of different concentrations of smoke, both liquid and smoke generated from beech chips

Treatment	Smoke taste	Smoke smell	Manure taste	Manure smell
Unmarinated	1.01^{b}	1.00^{b}	4.03^{a}	4.23^{a}
In brine mixture[A]	3.04^{b}	2.17^{b}	5.17^{a}	5.74^{a}
High oregano–high smoke	4.47^{ab}	4.37^{a}	1.70^{b}	1.59^{b}
High oregano–high smoke + 30 min with real smoke	5.39^{a}	5.21^{a}	1.91^{b}	1.66^{b}
High oregano–high smoke + 60 min with real smoke	5.45^{a}	5.30^{a}	1.86^{b}	1.71^{b}

The mean values of assessors are shown (Exp. 4).
[A] Wela 63/398997 fra SFK Foods A/S, Copenhagen, Denmark. Different letters within the same column indicate significant differences ($p \leqslant 0.05$).

manure flavour and odour is a very low score for a sample having 0.64 ppm skatole (see also Table 8).

The smoke flavours used in experiments 2 and 3 were somewhat different. The smoke flavour used in experiment 2 was high in phenols, while the smoke flavour used in experiment 3 was high in volatile aldehydes. With respect to the two different smoke aromas, it is not possible to arrive at any conclusion as they were not applied to the same meat samples. However, when oregano levels were high, low smoked samples with skatole $\leqslant$0.41 ppm were close to the highest ranked castrate with respect to mean intensity of manure. With high levels of smoke, both samples with skatole 0.41 and 0.48 ppm scored lower than the castrate with the highest ranking for manure (Exp. 3). In experiment 2, all entire males with added smoke flavour (marinade 1) scored lower than the castrate with the highest score for manure.

These results suggest that it is possible to reduce the perception of boar taint in meat samples having higher skatole values than 0.20 ppm using strongly flavoured marinades such as those commercially available. For consumer acceptance it would be highly relevant to test marinated neck chops up to 0.4 ppm in skatole content.

3.5. Effect of serving temperature and reheating

Table 8 shows the mean scores of the assessors for the different treatments studied in experiment 3. The highest mean values for manure were given for (unmarinated) backfat, but were not significantly ($p > 0.05$) different from the scores for unmarinated meat. For manure, pure backfat was significantly ($p \leqslant 0.05$) different from the marinated meat samples. This was also the case for urine taste that had the highest intensity in pure backfat, and was significantly ($p \leqslant 0.05$) different from all the marinated samples.

The assessors seemed to detect the flavour and odour of manure in the reference samples, although to a lesser extent than in the boar samples. This is probably due to the mean skatole values (0.15 ppm) for the reference samples. This value might represent the skatole level in the Norwegian market today. As for the reference samples, the marinated samples had lower mean values than pure backfat and unmarinated meat (heated once and served warm) for the attributes urine and manure.

In food serving the temperature at the time of consumption is important. Boar taint is more easily revealed when served warm (Williams, Pearson, & Webb, 1963). The manure flavour dropped (from 4.76 to 3.61) when the meat was served cold compared to the unmarinated samples heated once and then served warm. The perception of manure of cold, unmarinated products was similar to the perception of manure for meat samples heated twice and evaluated warm, and not significantly ($p > 0.05$) different from the marinated samples. The tendency of less manure flavour in cold compared to warm samples found in this study agrees with several previous investigations (De Koch, Heinze, Potgieter, Dijksterhuis, & Minnaar, 2001; Desmoulin, Bonneau, Frouin, & Bidard, 1982; Pearson, Ngoddy, Price, & Larzelere, 1971; Williams et al., 1963), showing that boar taint is more intense in warm than cold products. The smoke odour on the plastic bags interfered in the relationship between odour and flavour for the smoke attribute. In addition, it is also possible that the smoke odour on the plastic bag lowered the mean values for manure when the samples were served cold and reheated. If that was the case, the mean values for the urine attribute should also be lower for cold and reheated samples, but only the reheated samples are affected in this study. Although androstenone and skatole are not highly volatile compounds (García-Regueiro, Rius, & Díaz, 1995), a proportion of these substances are evaporated during the cooking process and it is easily detected when cooked meat is assessed in a heated state (Font I Furnols, Gispert, Diestre, & Oliver, 2003). There was no significant ($p > 0.05$) drop in urine flavour when the unmarinated meat was served cold instead of warm.

A reduction in mean values for the manure attribute was observed when the unmarinated meat was served reheated. The sensory scores for rancidity and warmed-over-flavour varied more (as indicated by standard deviations) in experiment 3 compared to experiment 2 (0.4 versus 0.1, respectively). However, this phenomenon was not elucidated in experiment 2. This was due to the fact that the reference samples in experiment 2 were marinated; castrate meat samples containing the same antioxidants as the meat from entire males.

All samples from experiment 3 were measured for rancidity, both chemically and by sensory descriptive analysis. Several attempts have been made to determine the threshold in sensory perception of oxidation in relation to the value of TBARS measured chemically. Tarladgis et al. (1960) suggested that oxidation was perceived at TBARS values in the range of 0.5–1.0 mg/kg in pork. TBARS values for the unmarinated samples before heat treatment ranged from 0.10 to 0.40 mg/kg, while the unmarinated samples heated twice had TBARS values ranging from 0.31 to 0.80 mg/kg. All of the marinated samples remained unchanged or had reduced TBARS (compared with unmarinated samples) when heated/reheated, as expected, since several antioxidants were present in the marinades. The results are presented in Table 9. The sensory panel in this study was highly trained with respect to rancidity and would be expected to detect WOF/rancidity in a descriptive

Table 8
Effect of the treatments tested in experiment 3 (all animals)

Treatment	Smoke	Oregano	Urine	Manure
Unmarinated 1h	1.00^{c}	1.03^{b}	4.55^{ab}	4.76^{a}
Unmarinated 1h + 1c	1.99^{bc}	1.15^{b}	3.91^{abc}	3.61^{b}
Unmarinated 2h	2.62^{b}	1.19^{b}	3.52^{bc}	3.54^{bc}
Pure fat	1.02^{c}	1.05^{b}	4.89^{a}	5.10^{a}
OREGANO–SMOKE 2h	4.54^{a}	4.01^{a}	3.33^{bc}	2.93^{bc}
Oregano–smoke 2h	4.67^{a}	1.96^{b}	3.25^{bc}	2.78^{bc}
Oregano–SMOKE 2h	4.49^{a}	1.58^{b}	3.33^{bc}	3.18^{bc}
OREGANO–smoke 2h	4.21^{a}	3.15^{a}	2.83^{c}	2.48^{c}

The mean values of assessors for the attributes smoke, oregano, urine and manure are shown.
1h: heated once, served warm; 1h + 1c: heated once, served cold; 2 h: heated twice, served warm. OREGANO: high concentration; oregano: low concentration; SMOKE: high concentration; smoke: low concentration. All the marinated samples were served warm. Different letters within the same column indicate significant differences ($p \leqslant 0.05$).

Table 9
TBARS values for the different samples in experiment 3

Animal	Unmarinated (untreated)	Unmarinated (heated twice)
1	0.16	0.56
2	0.33	0.79
3	0.21	0.50
4	0.10	0.50
5	0.12	0.61
6	0.35	0.39
7	0.12	0.43
8	0.22	0.53
9	0.16	0.31
10	0.23	0.42
11	0.40	0.43
12	0.18	0.42
13	0.13	0.84
14	0.16	0.36
15	0.11	0.35

The TBA values are given in mg/kg.
The TBARS values for the marinated samples heated twice were unchanged/reduced when compared with the unmarinated untreated samples.

test. Sensory scores for rancid flavour and odour were higher for unmarinated samples heated twice, but did not differ significantly from the other samples. It appears that the presence of warmed-over-flavour reduced the perception of urine and manure. But it is a possibility that the odour of smoke on the plastic bags affected the mean values of the assessors for the samples served cold and reheated. Skatole and androstenone values were recorded in pork back fat both before and after processing (Table 10). No significant difference ($p > 0.05$) in boar taint components caused by heat treatment was found. Accordingly, that phenomenon cannot explain the apparent reduction in manure and urine in reheated entire male pig meat samples. The average difference between skatole and androstenone before and after heat treatment was of the same magnitude as the analytical error for skatole and androstenone found in this study. In contrast Bonneau, Desmoulin, and Frouin (1980) observed a reduction of androstenone in cooked hams and sausages, and reported a reduction after cooking of 46% and 23%, respectively. However, little is known about how these compounds are degraded during the processing (Babol & Squires, 1995).

3.6. Effect of animal

It seems from Table 6 that all marinated samples still relate strongly to skatole (Exp. 3), and all entire male samples were recognized for their boar taint. However, for the marinated samples, only the sample containing the most skatole (1.1 ppm) was significantly different from the other samples. The rest of the samples were not significantly different from the castrates and the sow. This shows that the marinades had a masking effect on skatole. The results obtained in experiment 3 are apparently in conflict with the results of experiment 2. The discrepancy between experiment 2 and experiment 3 can partly be explained by the fact that there were two samples with more extreme values for skatole (i.e. 0.78 and 1.1 ppm in Exp. 3) compared to experiment 2 (Table 5). Despite the substantial amounts of strongly flavoured volatiles in the product, these two samples have caused the sensory scores for manure flavour to differ significantly. This shows that masking of boar taint in samples with skatole content ranging from 0.7 to 0.8 will cause problems, even with highly aromatic ingredients.

3.7. Effect of fat content in the chops

No significant change (Exps. 2 and 3) in explaining the variation in manure or urine could be obtained by including the fat content of the chops in linear regressions models. This means that for fat levels between 2.5% and 18.9%, no significant ($p > 0.05$) variation in urine and manure flavour and odour could be related to the level of fat. Nevertheless, there existed significant differences in these attributes between very high-fat products (pork backfat >70% fat) and a mean fat level of 8.24% (Exp. 3).

Table 10
Changes (Δ) in skatole and androstenone content in back fat due to heat treatment

Skatole values before heat treatment (ppm)	Δ skatole (ppm) (before – after heat treatment)	Androstenone values before heat treatment (ppm)	Δ androstenone (ppm) before – after heat treatment)
0.41	0.10	0.65	−0.03
0.30	0.17	0.64	−0.07
0.78	0.09	1.62	0.16
0.53	−0.23	1.94	−0.23
0.25	−0.04	1.84	−0.40
0.48	0.07	1.11	0.00
0.23	−0.04	1.24	0.03
0.21	0.02	1.12	−0.03
0.39	0.07	3.40	0.15
0.23	−0.06	5.59	−0.19
0.30	0.06	0.64	−0.06
1,10	0.44	1.82	0.22
0.16	−0.06	0.01	−0.02
0.21	0.05	0.01	0.00
0.10	−0.11	0.03	0.01
	0.11 (average)		0.11 (average)

4. Conclusion

The attribute manure related significantly to the skatole level of pork neck chops served to the sensory assessors. The attribute urine also related significantly to the level of skatole, but did not serve as a robust indicator of androstenone level. Common and strong food flavour additives like oregano extracts and liquid smoke affected the perception of boar taint. This study shows that meat samples with skatole levels up to 0.4 ppm can be used by the industry as raw material for pre-flavoured chops. Cold serving temperatures (15 °C) gave less perception of boar taint than serving at higher temperatures (approximately 60 °C). There was also a tendency in the results that reheating of pork neck chops reduced the perception of boar taint. The sensory panel did not detect any relationship between manure or urine and the fat level in neck chops (fat varied between 2.5% and 18.9%). In general, it appears that volatile ingredients with low detection thresholds would be most successful in masking boar taint, and that it may be possible for the industry to use boar meat with higher skatole values than currently available in the Norwegian market today. In addition, it seems that both cold serving and reheating of products from entire male pigs reduced the perception of boar taint.

Acknowledgements

The Master of Science students Elise Løvlund and Mia Høver are thanked for their hard work and creativeness during the screening experiments 1 and 2. Karin Solgaard and Tom Chr. Johannesen at Matforsk are thanked for their assistance throughout the whole project. Nortura, represented by Ragnhild Solheim, is thanked for its generosity when granting permission to publish the composition of the four recipes used in experiment 2 and for their support through the project "Marinated products from pork" granted by the Norwegian Research Council. The staff at Nortura Rudshøgda are thanked for their sampling of suitable animals for all experiments. Finally, The Norwegian Research Council is thanked for its second grant to this activity through Project No. 63164: "Boar meat – consumer aspects and exploitation of resources". The national representative of Red Arrow is also thanked for his cooperation during the searching for liquid smokes.

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Paper VI

Masking of boar taint in fermented, dry salted and brine injected bacons.

Lunde, Kathrine [1,2 *], Skuterud, Ellen[1,2], Lindahl, Gunilla [3,4], Hersleth, Margrethe [2,5] & Egelandsdal, Bjørg [2]

[1]Norwegian Meat Research Centre, Lørenveien 38, P.O.Box 396 Økern, N-0513 Oslo, Norway

[2]Institute of Chemistry, Biotechnology and Food Science, University of Life Science, P.O.box 5036, N-1432 Ås, Norway

[3]Department of Food Science, Faculty of Life Science, University of Copenhagen, Rolighedsvej 30. 1958 Fredriksberg C, Denmark

[4]Department of Food Science, Swedish University of Agricultural Sciences, P.O.Box 7051, 750 07 Uppsala, Sweden

[5]Nofima Mat, Osloveien 1, N-1430 Ås, Norway

Abstract

The aim of the present work was to study the possibility to mask boar taint with the use of different production technologies: Dry salting, brine injection and dry salting plus fermentation. Bacon raw materials with different levels of skatole (range 0.04 – 0.43 ppm, fat values) and androstenone (range <1 – 3.21 ppm, fat values) were analysed by a trained sensory panel and a consumer panel.

Ten trained assessors evaluated the bacon samples and the results indicated that smoke was effective in masking skatole, but not androstenone. The process of dry salting did not succeed in masking boar taint, but used in combination with fermentation the perceived taint of skatole was reduced. The consumers (43) evaluated liking of odour during frying and odour and

flavour of the already fried meat. Results from consumer testing showed that production of dry salted bacon made it possible for the meat industry to use boar meat with skatole levels up to 0.43 ppm in the fat (androstenone 1.61) without negative consumer reactions. Also dry salted and fermented bacon (starter cultures BFL-N16 and S-SX) was accepted by the consumers at a high skatole level of 0.35 ppm (androstenone 1.27 ppm).

Keywords: Boar taint, sensory analysis, consumer testing, masking, fermentation, bacon

*** Corresponding author. Tel.: +4722092317; fax: +4722220016.**

***E-mail address*: kathrine.lunde@animalia.no**

1. Introduction

Production of entire males depends on political choices made in each country. European countries are, however, aiming at a castration ban. Boar taint is mainly described by the compounds, skatole and androstenone. Skatole is a faeces and manure smelling metabolite (Vold, 1970) of the amino acid tryptophan produced in the lower gut by intestinal bacterial flora. Most consumers (99%) have the ability to perceive skatole (Weiler, Fischer, Kemmer, Dobrowolski & Claus, 1997), and the compound can be detected in concentrations as low as 0.1 ppm (Bañón, Costa, Gil & Garrido, 2003; Font I Furnols, Guerrero, Serra, Rius & Oliver, 2000; Lunde, Skuterud, Nilsen & Egelandsdal, 2009). Androstenone is a steroid structurally related to testosterone. Androstenone was earlier associated with a urine like flavour (Patterson, 1968), but later the flavour has been described as more diverse (Annor-Frempong, Nute, Whittington & Wood, 1997; Lunde et al., 2009). In contrast to skatole, the ability to perceive androstenone varies among consumers. Recent studies have has shown that detection of androstenone is, at least partly, determined by the amino acid sequence of the human odour receptor OR7D4 (Keller, Zhuang, Chi, Vosshall & Matsunami, 2007).

Earlier studies dealing with odour and flavour characterization of processed products from entire males have shown that processing will lead to a higher acceptability of tainted meat (Walstra, 1974; Diestre, Oliver, Gispert, Arpa & Arnau, 1990; Bonneau, Le Denmat, Vaudelet, Veloso-Nunes, Mortensen & Mortensen, 1992; Lunde, Egelandsdal, Choinski, Flåtten & Kubberød, 2008; Stolzenbach, Lindahl, Lundström, Chen & Byrne 2009). The higher acceptability can be explained by processing methods, addition of

ingredients or, as found by McCuley et al. (1997), the temperature of the sample presentation rather than the processing itself. Walstra (1974) showed that smoked sausages produced with up to 25% strongly tainted meat were accepted when consumed cold and sausages with respectively 6% and 12 % tainted meat were accepted if consumed warm. In a study by Lunde et al. (2008) it was shown that addition of liquid smoke to tainted meat affected the perception of skatole odour and flavour. Stolzenbach et al. (2009) also showed that the addition of liquid smoke masked the odour perception of boar taint in fermented sausages (skatole < 0.1 – 0.89 ppm and androstenone 0.0 – 7.4 ppm) while aroma produced by different starter cultures was insufficient to completely mask the perception of boar taint.

Both skatole and androstenone are highly fat-soluble, and it has been suggested that the fat level in products is important for consumer's negative reaction to boar tainted meat. Most meat products have a fat fraction below 30%. One exception is bacon with a fat fraction up to 30-35%, although leaner types exist. In addition, both skatole and androstenone are volatile compounds which easily will be detected during frying (Lunde et al., 2009). Bacon is produced using a variety of recipes and processes (Andersen, 2004) and therefore this product is interesting to study when working with masking of boar taint.

The aim of the present research was to investigate the sensory acceptability of bacon produced from entire males using different processing technologies (salting, smoking and starter cultures). The bacon was evaluated by sensory descriptive analysis and consumer testing. This investigation differs from previous investigation for two reasons: 1) both sensory panel and consumers

were pre-screened for androstenone sensitivity and 2) the upper androstenone (3.21 ppm) and skatol (0.43 ppm) levels were, based on previous investigations (Lunde et al., 2008, Lunde et al., 2009, Lunde et al., 2010, Lunde, Skuterud, Egelandsdal & Hersleth, 2010), selected as possible relevant upper limits for a processed products in this fat category. Thus the hypothesis was to prove that with a suitable processing technology, bacon, despite its high fat fraction and high skatole and androstenone levels could be accepted among consumers.

2. Materials and methods

2.1. Production methods

2.1.1. Bacon production technologies

Three different production technologies were used: fermentation/dry salting, salting and brine injection. When starter cultures were added to the belly sides, they were added with the salt mixture. The amount of salt added gave a final salt concentration of 3.5% in the dried product. The salt mixture consisted of two parts of vacuum salt (Basic Chemicals Division, Netherland) and 1 part of nitrite salt (sodium nitrite content, Basic Chemicals Division, Netherland). The acetate mixture used consisted of 1 part dextrose (BASF AG, Denmark) and 0.355 part sodium ascorbate. The amount of these mixtures used was 30 g of the salt mixture and 0.335 g of the ascorbate mixture per kg meat.

All the salt was added at the same time, some of the salt was rubbed into the rind while the rest covered as much of the meat as possible. The salted belly sides (fermented/dry salted and dry salted only) was then vacuumed and kept at 4˚C for eleven days. The samples with added starter cultures were then

kept at 23˚C for twenty-four hours to stimulate fermentation; the other samples remained at 4˚C. The samples (except the brine injected samples) were smoked. The smoking was done using beech chips at 35˚C and 60 % humidity in a cooking/smoking cabinet (Doleschal Unitronic SC2000, Steyr, Austria). Smoking was carried out after the following procedure: 10 min pre heating, 15 min drying, 30 min smoking and 5 min ventilation. The meat was kept at 4˚C for twenty-four hours before vacuumed and frozen. The injected brine used consisted of 1600 g of NaCl (vacuum salt), 800 g nitrite salt, 200 g dextrose and 50 g ascorbate plus water providing a total of 10 kg brine. Liquid smoke was added to the brine injected (12.5 % increment) samples by the following procedure; 1 min of dipping in liquid smoke followed by 1.5 min of dripping. The liquid smoke (Enviro 24PA, Red Arrow) used was the same smoke as the commercial producer of the reference sample used in the consumer testing. The meat was vacuumed and kept at 4˚C for twenty-four hours before frozen.

2.1.2 Screening of starter cultures for bacon production

Starter cultures for the main experiments were selected in two steps. Step one (S1) used aerobic fermentation of bacon sides with and without smoking, while step two was performed as anaerobic fermentation without smoking. Step one used five different starter cultures (Table 1) tested on pieces of belly sides (from a castrate), half the samples being smoked after fermentation. The production (S1) started with adding 70% of the salt mixture to the meat together with the ascorbate mixture and the starter culture. After four days at 4˚C the remaining salt (30%) was added, and the fermentation continued for another seven days. Otherwise the process was as

carried out above. In step two some changes were made. All the salt was added at the same time and the meat was vacuumed and kept at 4˚C for twelve days. None of the samples in step two were smoked because the motivation was to evaluate the aromas from the starter cultures without the influence from the smoke aroma.

A sensory test (employees at the University of Life Science) on fried slices was arranged after both steps to choose the two starter cultures with the most pleasant/aromatic flavour. Odour and flavour on fried bacon was evaluated on a seven point hedonic scale with dislike very much (1) on the left side and like very much (7) on the right side. Based on these two screening tests and bacterial counts for compatibility of the starter cultures (results not shown), BFL-N16 (*Lactobacillus sakei* and *Staphylococcus carnosus* and S-SX (*Staphylococcus xylosus*) were selected as cultures for the main experiment.

2.2 Design of the main bacon production experiment

Belly sides from seven boars (two belly sides from each boar) with different combination of skatole and androstenone were used for the main experiment. The belly sides from each of the seven boars were divided in two, providing four pieces of belly sides from each boar. Accordingly, it was possible to compare the different production technologies using the same biological material.

The different production technologies, fermentations and levels of skatole and androstenone in the samples produced are presented in Table 2. The samples were named with a combination of a letter and a number. The letters A to G indicated the skatole and androstenone levels of the samples while the numbers indicated the production technologies (1-4). This means that all

samples numbered 1 were treated with the starter culture BFL-N16 (*Lactobacillus sakei* and *Staphylococcus carnosus*, dry salted process), all samples numbered 2 were treated with the starter culture S-SX (*Staphylococcus xylosus*, dry salted process), all samples numbered 3 were dry salted and all samples numbered 4 were brine injected. When the samples were evaluated by the sensory panel and the consumers, the samples were given a three numbered code.

2.3 Instrumental, chemical and microbiological measurements

2.3.1 Instrumental measurements of skatole and androstenone

Skatole and androstenone values were measured in the fat mixtures before processing. Skatole was determined in extracted fat by HPLC (Agilent Technologies, Santa Clara, CA, USA) using fluorescence detection according to a method developed by Gibis (1994). The androstenone content was determined by a time-resolved fluorescent immunoassay as described by Tuomola, Harpio, Knuuttila, Mikola, & Løvgren (1997), modified by using antiserum produced and characterized by Andresen (1974).

2.3.2 Microbiological measurements

Samples for microbiological measurements were taken before processing, after salting and after fermentation. The total number of bacteria was measured using melted PCA (from Merck, aerobic incubation at 30 ˚C for 3 days). The growth of the starter cultures were measured with the use of LBS agar (from Merck) for Lactobacillus (anaerobic incubation at 30 ˚C for 4

days) and Baird-Parker with egg yolk tellurite enrichment for Staphylococcus (from Oxoid, aerobic incubation at 37 ˚C for 2 days).

2.3.3 Measurement of volatile compounds

Volatile compounds were measured by Dynamic Headspace – Gas chromatography – Mass spectrometry (HSGCMS). The headspace volatile compounds of bacon were isolated by a dynamic headspace analyzer Teledyne Tekmar HT3 (Teledyne Tekmar, Ohio, USA) coupled to an Agilent gas chromatograph 6890N (Agilent Technologies, Santa Clara, CA, USA). The gas chromatograph was equipped with a 30 m x 0.25 mm. DB-WAXETR fused silica capillary column (film thickness 0.5 µm, J&W Scientific, USA), and the injector inlet temperature was 250 ˚C. The carrier gas was helium at a flow rate of 1.0 ml/min. The oven temperature program was: 30 ˚C for 10 min, heating rate: 1.0 ˚C min^{-1} up to 40 ˚C, heating rate: 3.0 ˚C min^{-1} up to 70 ˚C, heating rate: 6.5 ˚C min^{-1} up to 230 ˚C and 5 min at 230˚C. The GC column was connected to the ion source (temperature 230 ˚C) of an Agilent 5975 (Agilent Technologies Santa Clara, CA, USA) quadrupole mass spectrometer (interface line 250 ˚C). The mass spectrometer was operating in the scan mode within a mass range of m/z 30 – 550 at 1 scans s^{-1}. Ionization was done by electronic impact at 70 eV, calibration was done by autotuning. Compounds were first tentatively identified by computer-matching of mass spectral with those in the NIST 05 Mass Spectral Library (Agilent Technologies, Santa Clara, CA, USA). The compounds 2-butanone, 2-furfural (both from Fluka) and 2H-furanone (Alfa Aesar) were purchased in pure form and used for validation and calibration of their contents in bacon for this project. All other compounds used here (except ethanone,

1-(2-furanyl) that were tentatively identified) been identified from pure components in other projects using the above set-up

2.4 Sensory analysis

The sensory analysis (Quality Descriptive Analysis) was performed by the sensory panel at Nofima Mat in Norway. The panel consisted of ten trained assessors with 4 to 20 years of general experience in sensory profiling. The panel had several years of experience with evaluation of boar tainted meat, especially during the last 5 years. The samples were evaluated in a sensory laboratory designed according to guidelines in ISO (1988) with separate booths and electronic registration of sensory data. The assessors were sorted according to their androstenone sensitivity based on the sensitivity testing with the method described by Lunde et al. (2009) when evaluating the samples containing androstenone. Of the ten assessors used in the evaluation, six of them were sensitive to androstenone. Only the results from these six assessors (sensitive) were used in the evaluation of the androstenone samples. Evaluation of the skatole samples were performed without sensitivity grouping since all assessors were able to perceive skatole (tested when recruited to the panel).

2.4.1 Sensory profile

Differentiating between the boar taint attributes skatole and androstenone has proved to be difficult (Dijksterhuis et al., 2000). Earlier results by the sensory panel at Nofima Mat have shown that by using relatively few attributes the assessors were able to distinguish between skatole and androstenone to a higher degree, therefore the number of attributes in this

profile was kept as low as possible. Accordingly, the profile used consisted of the attributes acid (intensity of a sour/sweet fruit acid odour), skatole (intensity of skatole), androstenone (intensity of androstenone), smoke (intensity of smoke) and rancid (intensity of all rancid odours; grass, hay, paint, stearine). Rancid was included as an attribute in the profile since rancidity is one of the more common off-flavours in pork meat.

2.4.2 Sensory analysis of fermented bacon

The trained sensory assessors evaluated odour and flavour of the attributes defined in the profile using a 9 cm unstructured continuous scale, where the left side of the scale corresponded to “low intensity” (1) and the right side of the scale corresponded to “high intensity” (9). The 28 samples evaluated are presented in Table 2. The frozen samples were fried in margarine (Melange, Mills DA, Oslo) in a pre-heated pan with lid for approximately 1 minute on each side; until well done. The frying pan was cleaned with washing-up liquid and rinsed thoroughly between each sample.

The assessors got half a slice of bacon (thickness 2-3 mm). The samples were served at a temperature of 60 ˚C in boxes (suitable for sensory analysis) with a lid. The assessors evaluated odour after taking the lid off, and then flavour. The assessors rinsed their mouths with water and/or some neutral crackers between the samples. The samples were served replicated in a randomized order.

2.5 Consumer testing

The consumers participating in this study (43) were selected among consumers that previously were tested for their ability to perceive

androstenone by the method described by Lunde et al. (2009) in a large screening of androstenone sensitivity in the Norwegian population. The method divided the consumers in two groups, sensitive and non sensitive consumers. The sensitive group (20 consumers) was defined as consumers that gave negative reactions to meat with different levels of androstenone. The non sensitive group (23 consumers) consisted of consumers that gave no or positive reactions to androstenone tainted meat. Since practically all consumers (99%) have the ability to perceive skatole (Weiler et al., 1997) the consumers were not tested for their ability to perceive skatole before analyzing the samples.

Nine of the twenty-eight samples (Table 2) evaluated by the sensory assessors were chosen for consumers testing (Table 3). The samples were selected to represent all production technologies, and both low and high values of skatole and androstenone. In addition a reference sample (brine injected, assumingly from a castrate) produced by a commercial producer (Nortura SA, Norway) was included. The samples with different levels of skatole and androstenone (Table 3) were analysed by 43 consumers in a home test during a period of several days. If more than one sample was evaluated during a day, the consumers were instructed to have at least one hour break while ventilating the room before evaluating the next sample. The samples were fried in a preheated frying-pan. Between each sample, the consumers were told to clean the frying-pan with washing-up liquid and rinse thoroughly. Liking of odour during frying, and liking of odour and flavour on the fried meat were evaluated on a seven point hedonic scale with dislike very much (1) on the left side and like very much (7) on the right side. In addition, the consumers were allowed to comment on each sample.

The consumers were asked to evaluate the samples in the order which appeared in the questionnaire (randomized).

2.7 Statistical analysis

Principal component analysis was made using Unscrambler (version 9.1, CAMO, Trondheim, Norway). Weighting according to 1/st-dev was used since the components appeared with rather different amounts in headspace. Full crossvalidation was used.

The open source software Panelcheck V 1.3.2 (www.panelcheck.com) was used both on consumer and descriptive sensory data to compare the subject's evaluation of the bacon samples, and to identify differences between the samples. Analysis of Variance (ANOVA) was performed on both the consumer and descriptive sensory data in order to identify differences between samples and sensitivity groups ($p < 0.05$).

ANOVA analysis was performed in SAS Release 8.2 (SAS Institute Inc., Cary, NC, USA) and in Minitab 15 (Minitab Inc, Pennsylvania, USA).

3. Results and Discussion

3.1 Screening of starter cultures for bacon production

The pre-screening of starter cultures focused on selecting starter cultures that provided the most aromatic flavour to salted bacon. The amount of starter culture used was doubled compared to general instructions because of the small production scale. This was also done to make sure that the starter cultures would rapidly overcome the house/endogenous flora. Three of the five starter cultures tested in step one (aerobe fermentation with and without smoking) was chosen for further testing. The cultures chosen were S-SX,

SM-75 and BLF-N16 as these cultures provided the most aromatic odours. After the anaerobe fermentation (without smoking) in step two, the same cultures were chosen. The cultures finally chosen for the main experiment were S-SX and BLF-N16 since these two cultures were given the highest liking scores in screening tests.

3.*2 Instrumental, chemical and microbiological measurements*

3.2.1 Microbiological measurements

Microbiological measurements were made in order to verify that the added starter cultures dominated the samples after fermentation. The results from the microbiological measurements of bacon are presented in Table 4. The starter cultures used for fermentation dominated the counts after the fermentation process. The two starter cultures was significantly different with the starter culture BFL-N16 (1) giving the highest bacterial number of *Lactobacillus spp* after fermentation, but no difference in *Staphylococcus spp*. The sample that was only dry salted contained significantly less counts. However, the counts were nevertheless increased for the *Staphylococcus spp* (Table 4). This may explain why this technology scored similarly to fermented bacons for aspects like volatiles and sensory attributes (see below).

3.2.2 Volatile compounds

Bacons smoked with beech chips in the smoking cabinet contained significantly more 2-furfural ($p < 0.001$) and 3-furaldehyde ($p = 0.023$) among the volatiles than the bacons produced with liquid smoke (Figure 1). These are typical degradation product from cellulose. Acetone and 2-butanone

(both $p < 0.001$) were typical for the dry salted bacons, and samples made with Technology 1 (fermented) clustered most closely to these volatiles (Figure 1). 2(5H)–furanone and methylacetate ($p < 0.01$) characterized the bacons produced with liquid smoke. 2(5H)–furanone is typical in some hard wood smoke extracts that also contain larger amounts of acetic acid (http://www.leffingwell.com/smoke1.htm). Acetic acid only tended ($p=0.18$) to be more typical for the commercial liquid smoke. The mean content of 2-furfural (Technology 1-3) was 6 ppm, for Technology 4 the mean content of 2H-furanone in the bacon was 8 ppm. The brine injected samples scored at average approx. 3 times stronger for sensory volatile smoke attributes (not shown) than did the commercial sample used for consumer testing. The dry salted and fermented samples (Technology 1 and 2) were grouped regarding smoke volatiles (Figure 1), and were significantly different ($p<0.05$, ANOVA, data not shown) from the brine injected samples.

3.3 Sensory analysis of bacon

The sensory panel evaluated twenty-eight samples produced by different technologies and with different levels of skatole and androstenone (Table 2).

3.3.1 Evaluation of the brine injected (and dipped in liquid smoke) samples.

The results from the evaluation of the skatole attributes for the brine injected samples (Technology 4) are presented in Table 5. All samples were given relatively low scores for both skatole odour and flavour (results for 10 assessors). The results from the ANOVA showed that there were no significant differences between any of the samples even though the skatole level of the samples ranged form 0.04 to 0.43 ppm. The highest mean value

given for any sample was 2.52; this value was given to the sample with the lowest skatole level.

Results from the evaluation of the androstenone attributes for the samples with high levels of androstenone (group C) are presented in Table 6. The assessors were grouped according to androstenone sensitivity. Brine injection was not favourable with respect to giving low intensities for androstenone flavour and odour, but was not significantly differentiated from any technology.

Figure 2 shows that the brine injected samples had high scores for smoke flavour and odour in the plot. ANOVA on the sensory data showed that all brine injected samples (except C4, androstenone sample) were given significantly ($p \leq 0.05$) higher scores for these two attributes (smoke) than the rest of the samples. Sample C4 was indicated as low in characteristic volatiles in Figure 1. Liquid smoke was effective in masking skatole since high scores were given for the sensory smoke attributes in combination with the low scores given for the skatole attributes (see E4 and G4 in Figure 2 and Table 5). Samples E4 and G4 were the samples with the highest scores for the 2(5H) furanone; the indicator molecule for the liquid smoke used here. This agrees well with Lunde et al. (2008) and Stolzenbach et al.(2009); smoke can be effective in masking skatole. On the other hand, addition of liquid smoke did not seem to have a strong masking effect on androstenone. The brine injected androstenone sample (C4) was given high scores for androstenone odour and flavour.

3.3.2 Evaluation of the dry salted samples

The results from the evaluation of the skatole attributes for the dry salted samples (Technology 3) are presented in Table 5. The results showed that dry salting in general was the technology where the samples with the highest skatole values were given the highest mean values for both skatole odour and flavour. Samples containing skatole ≥ 0.31 ppm (except sample with skatole 0.39 ppm) were given significantly higher scores for both skatole odour and flavour that the samples containing 0.04 ppm skatole. In general, the mean values given by the assessors both for skatole odour and flavour were relatively low considering the high levels of skatole in the samples, but still the results indicated that the assessors detected skatole in the samples with the higher levels of skatole.

The results from the evaluation of the androstenone attributes (Table 6) showed that the dry salted samples together with the BFL-N16 fermented samples were given lower numerical mean values for androstenone odour and flavour than the brine injected and the S-SX fermented samples. The low number of sensitive assessors (6) used can probably explain why this differences was not significant even though the differences in mean values were large. Still, the mean value of the samples (dry salted) were 4.84 (odour) and 4.86 (flavour), indicating that the assessors detected androstenone in these samples. None of the technologies tested succeeded in masking androstenone since these values were significantly higher than the scores obtained for the non sensitive assessors.

3.3.3 Evaluation of the fermented samples

The results from the evaluation of the skatole attributes for the fermented and dry salted (Technology 1 and 2) samples are presented in Table 5. Using the starter culture BFL-N16 showed that the assessors in general gave low scores for both skatole odour and flavour. The sample with the highest skatole level (0.43 ppm) did not score significantly different from the samples with the lowest skatole levels (0.04 ppm) when skatole odour and flavour were evaluated. The mean value was somewhat higher (2.52), but still relatively low considering the amount of skatole in the sample. The samples containing 0.35 and 0.39 ppm skatole were given significantly higher scores than the low skatole samples (0.04 ppm), but still the mean values were relatively low (3.37 and 3.22 respectively). Why these two samples were given higher scores for skatole odour and flavour than the sample with the highest skatole level (also higher androstenone level) is difficult to explain. The same results were found for both odour and flavour. The result presented above indicated that fermentation with the use of BFL-N16 combined with dry salting gives a possibility for the meat industry to use higher skatole levels than the threshold value used in Norway today (0.21 ppm).

For the starter culture S-SX low scores were in general given for both skatole odour and flavour by the assessors. The sample with the highest skatole level (0.43 ppm) did not score significantly different from the samples with the lowest skatole levels (0.04 ppm) when skatole odour or flavour was evaluated. But as for fermentation with BFL-N16 the sample with 0.35 ppm skatole (androstenone 1.27) was given significantly higher score than the low skatole samples. The reason why the lower skatole sample (0.35 ppm) was given higher scores for skatole odour than the high skatole sample (0.43

ppm) cannot be explained by the results. The same was found for the sample with a skatole value of 0.23 (androstenone 3.21 ppm). The results from evaluation of skatole flavour showed the same as when skatole odour was evaluated. In general low values were given for skatole odour and flavour for all the fermented samples indicating that also fermentation with the use of S-SX combined with dry salting gives a possibility for the meat industry to use higher skatole levels than the threshold value used in Norway today (0.21 ppm).

The results from the evaluation of the androstenone attributes (Table 6) showed that the samples fermented with BFL-N16 (and dry salted) were given similar scores for both androstenone odour (4.98) and flavour (4.57) as the samples only dry salted. This indicated that fermentation with BFL-N16 did not have good enough masking properties, but it gave the lowest mean androstenone flavour among the techniques and its androstenone flavour was significantly lower than that obtained by the other starter culture. The use of S-SX in fermented bacon did not succeed in masking androstenone either, the samples fermented with this starter culture were actually given higher scores for androstenone than the other fermented and dry salted samples.

3.4 Consumer testing

Nine samples produced by different technologies using two types of starter cultures and with different levels of skatole and androstenone were evaluated by the consumers (Table 3).

The results from the consumer's evaluation of the different samples are presented in Table 7. The results showed that the consumers' tended to give

lower liking scores for odour during frying than for odour and flavor evaluations given post-frying. The reference sample (commercially produced bacon) was not given significantly higher liking scores than any of the other samples during frying. The dry salted samples G3 despite its high content of skatole and relatively high content of androstenone were liked well during frying. Post-frying, the reference sample was still not scored significantly higher than the other samples. Brine-injected samples scored low (flavor) and in particular the flavor of E4 was disliked. This sample is also indicated as an extreme sample in Figure 1. Looking at the consumer's evaluation (and comments, not shown) of this sample combined with the sensory evaluation where the brine injected samples were described as low in skatole but high in smoke, the results indicated that the consumers reacted negatively to the type of smoke used in the sample, and possibly also the amount of specific components in the smoke. Thus, albeit smoke seemed effective in masking skatole (Table 5), the results may suggest that at least when certain liquid smokes are used to mask skatole, as done for E4, there would be an upper limit of smoke aroma acceptable to the consumer.

It is worth noting that fermentation Technology 2 (BFL-N1) was liked due to the high mean scores obtained, but since the technology cannot mask androstenone, it is not liked as much for C2 samples.

Evaluation of the samples high in androstenone (C2 and C4) was as for the assessors performed with two groups, sensitive and non sensitive consumers. The androstenone samples (C2 and C4) were in general given lower liking scores by the androstenone sensitive consumer than by the non sensitive

consumers, indicating that the consumers detected androstenone in these samples. This seemed to be the case also when the samples were evaluated by the sensory assessors since the androstenone sensitive assessors gave relatively high scores for androstenone attributes for sample C2 and C4 (Table 6) compared to non sensitive assessors. The mean liking scores were higher when liking of flavour was evaluated compared to liking of odour, indicating that androstenone will be accepted in higher concentrations when flavour is evaluated compared to odour. This is in agreement with earlier results (Lunde et al. 2009, Lunde et al., 2010).

The fermented bacon samples were all dry salted. To be able to see the difference between the fermentation and the dry salting a direct comparison of the samples E1, E2 and E3 was possible (Table 7). E1 and E2 were added starter cultures while E3 only was dry salted (all with skatole 0.35 ppm). No significant differences between the samples were found during frying and odour post-frying. However, the dry salting process gave significantly higher flavour scores than brine injection. Fermentation in addition to dry salting did not contribute more than only dry salting in masking of boar taint. The dry salted and fermented bacons samples were not given significantly different liking scores from the reference sample, indicating that skatole levels up to 0.35 ppm were accepted by the consumers in dry salted and fermented bacon samples. The technique that we have used for dry salting also allowed for increases in bacterial counts and in staphyloccocus species (Table 4). This fact may reduce the difference between added starter culture and a competitive background flora.

In a recently study by Lunde et al. (2010) on the Norwegian consumer acceptance of boar tainted meat the result showed that the consumers were able to detect skatole in meat samples as low as 0.15 ppm (20% fat). Comparing that result to what was shown in this study where consumers not were able to detect skatole at 0.43 ppm in dry salted bacon or skatole at 0.35 ppm in fermented bacon it was obvious that the processing of bacon was efficient in masking boar taint (skatole) at those levels.

4. Conclusion

Results from the sensory profiling of bacon showed that smoke (brine injected samples) was effective in masking skatole, but did not have the same masking effect on androstenone. The dry salted bacon samples were given the highest mean values for both skatole odour and flavour and the sensory panel detected skatole at 0.31 ppm indicating that this process did not succeed in masking boar taint. The results also showed that the use of starter cultures lowered the perceived taint of skatole. In general, none of the technologies tested had a masking effect on androstenone. Comparing the results from the sensitive and non sensitive assessors it is obvious that the sensitive assessors' detected androstenone in all samples (3.21 ppm). Results from consumer testing showed that flavour was accepted to a higher degree than odour for all samples. The dry salted bacon samples were given the highest liking scores by the Norwegian consumers when samples with higher levels of skatole (0.35 and 0.43 ppm) were evaluated. These samples were not score significantly different from samples low in skatole; the reference sample included. These results indicated that the process of dry salting had a masking effect of skatole, and that it is possible for the industry

to use meat with skatole up to 0.43 ppm (androstenone 1.61) without negative consumer reactions. This is in contrast to consumers that are able to detect skatole at 0.15 ppm in unprocessed meat samples with a lower fat percent. No significant differences between dry salted samples and samples dry salted and fermented were found, indicating that the fermentation did not provide masking flavour beyond dry salting. The brine injected bacon samples, a common technology in the Norwegian industry today, were given the lowest liking scores. This was probably due to the fact that these samples were too heavily smoked, and not because of the skatole content of the samples. Thus, smoke seemed to be effective in masking skatole, but the results may suggest that if liquid smoke is used to mask skatole there will be an upper concentration of liquid smoke aroma acceptable to the consumers.

Acknowledgement

The Norwegian Research Council is thanked for its grant to this activity through project number 63164; “Boar meat-consumer aspects and exploitation of resources”. Ellen Dahl at Norwegian School of Veterinary Science is thanked for her cooperation with the skatole and androstenone analysis**.** Kari Olsen, Olga Shapovalova Lothe and Kirsti Hagenes at University of Life Science are thanked for their assistance with sample preparation and analysis. Tom Chr. Johannesen at Nofima Mat assisted us well with the bacon production. Tina Hornbæk and Karsten Tjener at Chr. Hansen are thanked for their cooperation during the selection and screening of starter cultures, and Ellen Freberg at Nortura is thanked for providing the liquid smoke.

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Table 1
The different starter cultures for bacon production tested in the pre-screening experiments.

Culture	
S-SX	containing *Staphylococcus xylosus*
SM-75	containing *Staphylococcus carnosus* and *Staphylococcus equorum*
CS 299	containing *Staphylococcus carnosus*
BFL-C08	containing *Staphylococcus carnosus* and *Debaromyces hansenii*
BLF-N16	containing *Lactobacillus sakei* and *Staphylococcus carnosus* subsp. *carnosus*

All of the starter cultures tested was produced by Chr. Hansen A/S.

Table 2.
Seven pork side samples with different contents of androstenone and skatol produced with 3 different bacon technologies; brine injection, dry salting and dry salting including fermentation (2 different starter cultures). All 28 productions were evaluated by a sensory panel.

		Samples			
Skatole (ppm)	Androstenone (ppm)	BFL-N16	S-SX	Dry salted	Brine injected
0.04	< 1	A1	A2	A3	A4
0.05	< 1	B1	B2	B3	B4
0.23	3.21	C1	C2	C3	C4
0.31	1.37	D1	D2	D3	D4
0.35	1.27	E1	E2	E3	E4
0.39	1.15	F1	F2	F3	F4
0.43	1.61	G1	G2	G3	G4

The samples added different starter cultures were produced by dry salting. The letters indicate the skatole and androstenone levels of the samples while the numbers indicate the production technologies.

Table 3.
The nine bacon samples evaluated by the Norwegian consumers (43).

		Samples			
Skatole (ppm)	Androstenone (ppm)	BFL-N16	S-SX	Dry salted	Brine injected
0.04	< 1	A1		A3	
0.23	3.21		C2		C4
0.35	1.27	E1	E2	E3	E4
0.43	1.61			G3	

In addition to the nine samples a reference sample produced by a commercial producer was included. The samples added different starter cultures were produced by dry salting. The letters indicate the skatole and androstenone levels of the samples while the numbers indicate the production technologies.

Table 4
Microbiological measurements LOG10 TVC (means) + upper st.dev) taken from the bacon samples before processing and after fermentation.

		Fermented samples		
	Initial,unfermented *	BFL-N16(1)	S-SX(2)	Dry salted(3)
PCA	4.4 ± 0.3^{a}	9.6 ± 0.1^{b}	8.2 ± 0.2^{c}	5.3 ± 0.4^{a}
LBS	2.5 ± 0.3^{a}	9.2 ± 0.2^{b}	7.3 ± 0.1^{c}	1.7 ± 0.3^{a}
BP	3.6 ± 0.4^{a}	9.7 ± 0.3^{b}	8.5 ± 0.2^{b}	5.8 ± 0.4^{c}

* The brine injected samples and unfermented samples had the same counts since these were measured before applying smoke/smoke flavour. Different letters in superscript in each row indicate significantly different ($p<0.05$) TVC (Tukey's test); the superscript can only be compared in rows. PCA =total number of bacteria, LBS =Lactobacillus and BP=Staphylococcus.

Table 5
Sensory evaluation of the skatole attributes for samples produce by the different technologies by (fermentation, dry salting and brine injection).

			BFL-N16		**S-SX**		**Dry salted**		**Brine injected**	
Group	Skatole (ppm)	Androstenone (ppm)	Skatole odour	Skatole flavour	Skatole odour	Skatole flavour	Skatole odour	Skatole flavour	Skatole odour	Skatole flavour
A	0.04	< 1	1.12a	1.21a	1.92a	1.67a	1.22 a	1.51 a	1.72	2.08
B	0.05	< 1	1.67ab	1.53ab	1.49a	1.97a	1.14 a	1.18 a	1.92	2.52
C	0.23	3.21	2.16ab	2.30abc	4.57b	3.89b	2.62 abcd	2.07 abc	1.88	1.75
D	0.31	1.37	1.82ab	1.99abc	1.56a	1.58a	3.48 bcd	3.66 bc	1.63	1.95
E	0.35	1.27	3.37b	3.17bc	4.81b	4.41b	3.95 cd	3.61 bc	1.46	2.13
F	0.39	1.15	3.22b	3.70c	2.06a	2.03a	2.01 abc	2.31 ab	2.02	1.90
G	0.43	1.61	2.52ab	2.95abc	1.81a	2.06a	4.56 d	4.58 c	1.52	1.95

The mean values of the assessors are presented. The assessors evaluated the samples using a 9 cm unstructured continuous scale, where 1 corresponded to “low intensity” and 9 corresponded to “high intensity” of the attribute. Different letters within the same column indicate significant differences ($p \leq 0.05$). The skatole and androstenone values of the samples are given in ppm (mg/kg).

Table 6
Sensory evaluation (sensitive assessors) of the androstenone attributes for samples produced by the different technologies (fermentation/drysalting, dry salting and brine injection).

Sample	Skatole	Androstenone	Technology	Androstenone odour		Androstenon flavour	
C1	0.23	3.21	BFL-N16	4.98	(2.72)	4.57 a	(2.80)
C2	0.23	3.21	S-SX	5.94	(2.21)	6.34 b	(2.80)
C3	0.23	3.21	Dry salted	4.84	(1.46)	4.86 ab	(1.83)
C4	0.23	3.21	Brine injected	6.07	(3.42)	5.69 ab	(4.30)

The mean values of the sensitive assessors (6) are presented. The mean values of the non sensitive assessors are also shown in the parenthesis. The assessors evaluated the samples using a 9 cm unstructured continuous scale, where 1 corresponded to “low intensity” and 9 corresponded to “high intensity” of the attribute. Different letters within the same column indicate significant differences ($p \leq 0.05$). The skatole and androstenone values of the samples are given in ppm (mg/kg).

Table 7
Liking of fermented bacon by Norwegian consumers.

Sample	Skatole	Androstenone		Technology	Odour (frying)	Odour	Flavour
Ref				4.23 abc	4.56 abc	4.91 ab	
A1	0.04	< 1	S_SX	4.86 a	4.98 a	5.33 a	
A3	0.04	< 1	Dry salted	4.44 ab	4.84 ab	4.74 ab	
C2	0.23	3.21	BFL-N16	3.50 c (4.53)	3.88 c (4.58)	4.63 ab	(5.05)
C4	0.23	3.21	Brine injected	3.33 c (3.95)	3.79 c (4.47)	4.13 bc	(4.11)
E1	0.35	1.27	S-SX	3.58 c	3.93 c	4.56 ab	
E2	0.35	1.27	BFL-N16	3.63 c	4.00 c	4.60 ab	
E3	0.35	1.27	Dry salted	3.93 bc	4.12 bc	4.40 b	
E4	0.35	1.27	Brine injected	3.63 c	3.88 c	3.37 c	
G3	0.43	1.61	Dry salted	4.19 abc	4.56 abc	4.58 ab	

The skatole and androstenone values are given in ppm. The mean values of the consumers (43) are presented. The mean values of the androstenone samples (C2 and C4) are the mean values of the androstenone sensitive consumers only(24) with the mean values of the non sensitive consumes given in parenthesis. The consumer's evaluated liking on a 7 point hedonic scale. Different letters within the same column indicate significant differences ($p \leq 0.05$).

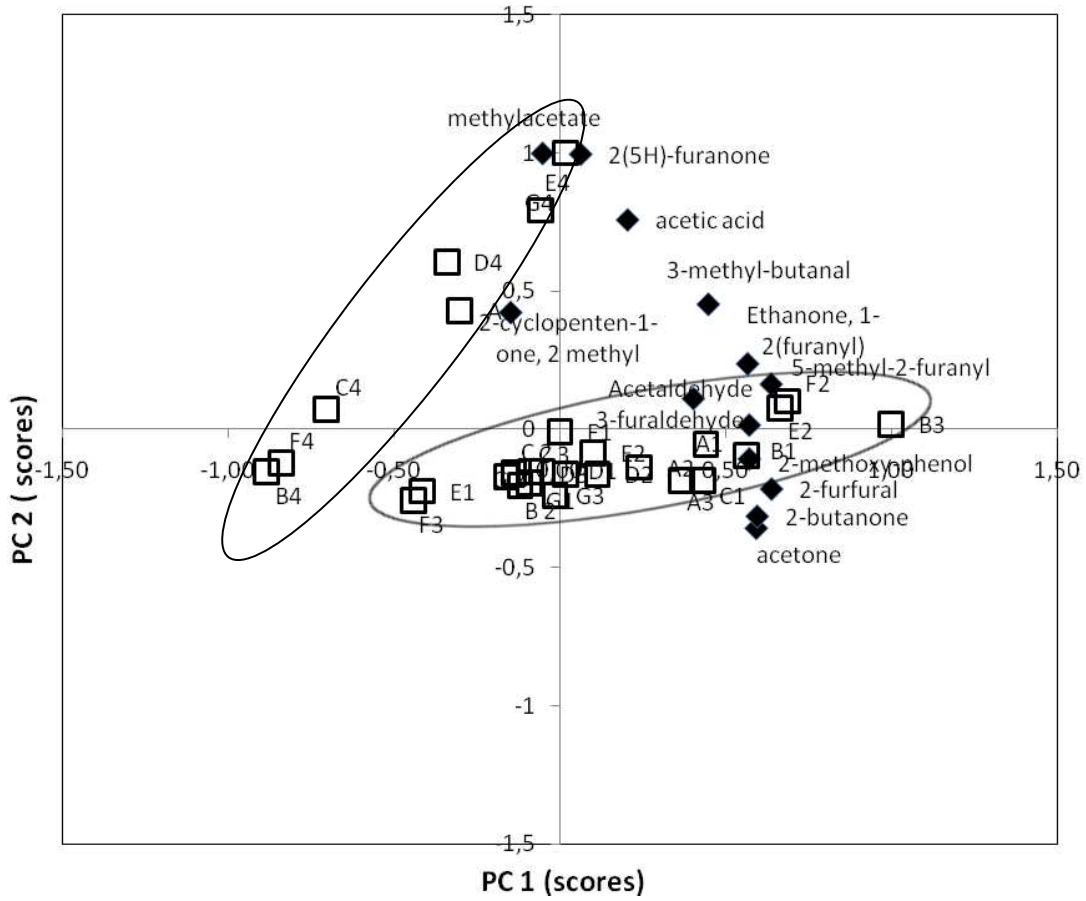

Figure 1. The figure shows a biplot (PC 1 versus PC 2) of the more dominant volatiles from the different samples smoked with beech chips (technology 1-3, ellipsoide) and the samples dipped in liquid smoke (technology 4; ellipsoide).

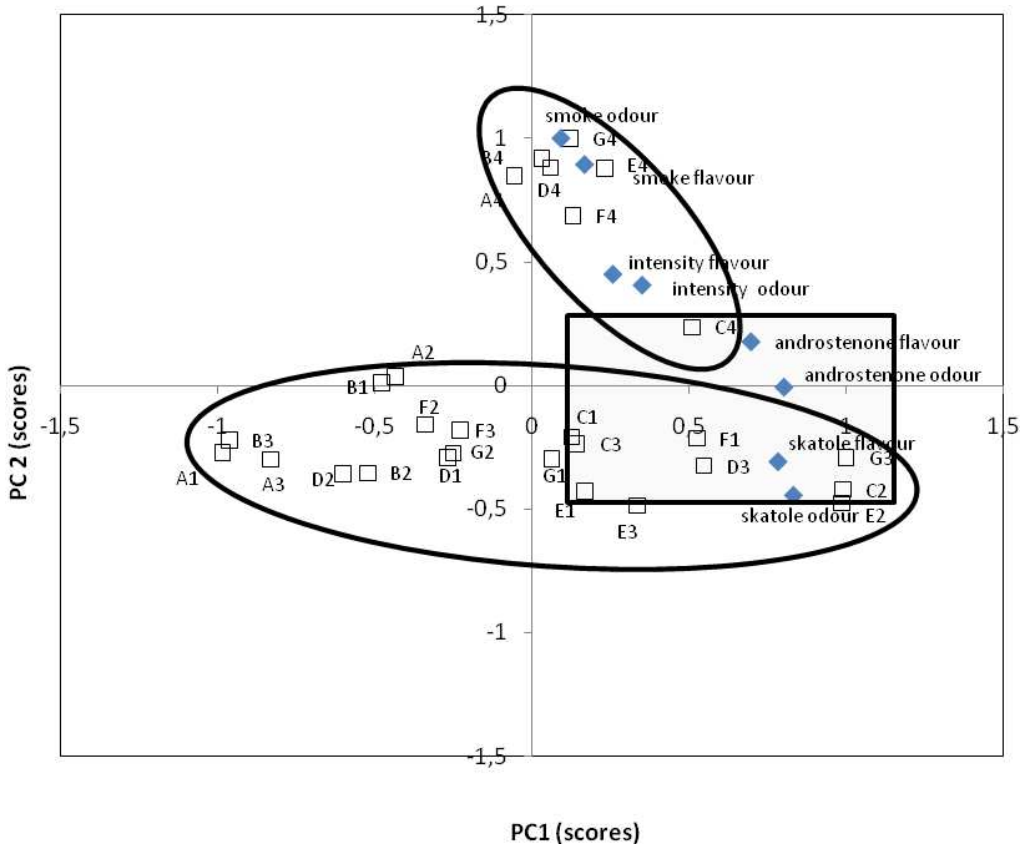

Figure 2. The figure shows the biplot (PC 1 versus PC 2) of some sensory attributes for bacon produced with different technologies. Technologies 1-3 and Technology 4 are surrounded by ellipsoids . The rectangle indicates the score of samples with androstenone at 3.21 ppm (C1-C4).