Instrumental and Sensory Analysis of Volatile Organic Compounds in Porcine Adipose Tissue

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INSTRUMENTAL AND SENSORY ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN PORCINE ADIPOSE TISSUE

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DECLARATION

I hereby declare that all of the work contained within this thesis was carried out at Victoria University of Technology and the Victorian Institute of Animal Science, during my candidature as a PhD student. To the best of my knowledge no part of this thesis has been submitted in part or in full for any other degree or diploma at any other University. Moreover, I declare that no material contained within this thesis has been written or published by any other person, except where due reference has been made to individuals in the text.

Liliana Salvatore
January, 2003.

ABSTRACT

Boar taint has a major economic impact on the pig industry. A rapid method for the identification of malodour was also required by the pig industry. Electronic nose technology was thought to be a possible means for the detection of the complete odour profile of samples of boar fat. However, this was unsuccessful due to a humidity-related problem in the instrument. A more traditional method of GC-MS analysis compared with human sensory analysis was then conducted. The most widely held view is that the compounds skatole and androstenone are the main contributors to boar taint. However various studies have found that these compounds appear to differ in their contribution to boar taint and they have not always accounted for all malodour detected. A possible reason for this fact is that not all compounds have been accounted for when the analyses were made. The GC-MS analysis has confirmed that skatole and androstenone are significant contributors to boar taint, but further analysis was also conducted to determine the nature and extent of the effect of other compounds detected in the odour profile. This has shown that three other compounds contribute significantly to the odour of pork upon cooking. These have been identified tentatively from their mass spectra. Two of these, pentadecanal and 3-nonen-1-ol have been found to make a positive contribution to the odour profile, whilst the other 2-nonadecanone contributes in a negative manner. If only androstenone and skatole are taken into consideration when correlating chemical and sensory analysis, this accounts for only 37% of the variation. However when all five compounds (androstenone, skatole, pentadecanal, 2-nonadecanone and 3-nonen-1-ol) are used in the correlation, then one can account for 87% of the variation. The hitherto omission of these compounds may account for the variation in the comparison of chemical and sensory analysis of boar taint, seen in previous studies.

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ABBREVIATIONS

Androstenone = 5α-androst-16-en-3-one

ANOVA = analysis of variance

amu = atomic mass units

a_w = water activity

BAW = bulk acoustic wave

BHT = butylated hydroxy toluene

BHA = butylated hydroxyanisole

bp = boiling point

BSA = bovine serum albumin

CP = conducting organic polymer

ddH_2O = deionised and distilled water

e-NOSE = *e-NOSE* ™ *4000,* Neotronics Scientific Pty Ltd.

FSH = follicle stimulating hormone

GC = gas chromatography

GC-MS = gas chromatography mass spectrometry

h = hour

HPLC = high powered liquid chromatography

$HO^{\bullet}$ = hydroxyl radical

LH = luteinising hormone

LHRH = luteinising hormone releasing hormone

MDA = multiple discriminant analysis

3-methyl-indole = skatole

min = minute

mL = millilitre

mp = melting point

MHz = mega hertz

MOS = metal oxide sensors

MOSFET = metal oxide sensors field effect transistors

PCA = principal component analysis

ppm = parts per million

ppb = parts per billion

QMB = quartz micro balance

$R^{\bullet}$ = fatty acyl radical

RH = Fatty acid

$RO^{\bullet}$ = alkoxyl radical

$ROO^{\bullet}$ = peroxyl radical

RH = relative humidity

ROOH = hydroperoxide

RIA = Radio-immunoassay

SAW =surface acoustic wave

SED = standard error of deviation

sd = standard deviation

TBHQ = tertiary butyl hydroxy quinone

μm = micro metre

VOC = volatile organic compounds

CONVENTIONS

Data in tables that has a P-value that is significant at the 0.05% level is in bold to aid in the identification of significance in complex tables.

Descriptors of sensory measures such as *overall* are in italics to differentiate from a descriptor in the text being utilised as a normal adjective.

INSTRUMENTAL AND SENSORY ANALYSIS OF VOLATILE ORGANIC COMPOUNDS IN PORCINE ADIPOSE TISSUE

Chapter 1

OVERVIEW OF LITERATURE

1.0. INTRODUCTION

1.1. Boar taint and its commercial significance

The entire male pig has a commercial advantage over the castrate or female pig as a result of its ability to produce a leaner carcass with more efficient food conversion (Martin, 1969). These are two key factors in the profitability of pig enterprises. In addition there are also reduced costs associated with the more rapid growth of the entire male, reduced excretion of pollutants, reduced animal suffering, compared to castrated counterparts, as well as some improvement in aspects of meat quality, such as lower levels of fat (Bonneau, 1998). The exploitation of these advantages however, is hindered by the presence of off odours and flavours known as boar taint which results in a poorer perception of pork as a quality eating experience. This ultimately leads to a reduction in the consumption of pork, which is detrimental to the industry. Boar taint is rarely detected in the castrate or female pig (Hansson, 1980). It has been the subject of research for decades since the first report of Lerche (1936), with an aim to invent a method capable of removing or screening for all compounds contributing to taint. An inability to devise such a comprehensive method has hindered the rearing of entire male pigs on a large scale in many countries.

1.1.1. Compounds responsible for boar taint

Two major compounds are considered to be responsible for causing boar taint. Patterson (1968) reported 5α-androst-16-en-3-one (androstenone) as a major component of boar taint and showed that it had a urine-like odour. Walstra and Maarse (1970) found that 3-methyl-indole (skatole) was present at a higher concentration in boar fat and muscle, compared with barrows. The presence of this compound resulted in an abnormal faecal like odour and bitter taste (Vold, 1970). The minimum detectable concentration of the two major compounds ranges from 0.20-0.25 ppm for skatole (Vahlun, 1990; Berg *et al.*, 1993; Hansen Moeller and Godt, 1995) and from 0.5 to 1.0 ppm for androstenone (Bonneau, 1990). Additional

compounds that have been reported to contribute to taint include indole, (Annor-Frempong *et al.*, 1997c) and 5α-androst-16-en-3-ol, 5β-androst-16-en-3-ol and 3β-androst-16-en-3-one (Thompson *et al.*, 1972; Bonneau, 1982). Androstenone and related steroids are often referred to as the 16-androstene family of compounds. The authors Rius-Sole and Regeuiro have investigated the contribution of other compounds towards boar taint. Samples of fat that had low concentrations of skatole and androstenone but were still classified as tainted, were evaluated. The compound 4-phenyl-3-buten-2-one was found to possibly contribute to boar taint.

1.2. Boar taint and consumer acceptance

1.2.1. Correlation of 16-androstene compounds and skatole to sensory analysis

In a number of studies (Lundstrom *et al.*,1984, 1988; Mortensen and Sorensen, 1984; Walstra *et al.*, 1986; Andresen *et al.*, 1993; Bejerholm and BartonGade, 1993; Hansen-Moeller and Godt, 1995), skatole was reported to have a higher contribution to boar taint than androstenone. Mortensen and Sorensen (1984)) reported a correlation of 0.73 between skatole and odour. Lundstrom *et al.*, (1988) reported a significant correlation between both skatole (R = 0.65) and androstenone (R = 0.53) and boar odour. Although the correlation between skatole and boar taint scores was of the order of 0.7 it still explained only about 50% of the odour score. Other studies found the correlation co-efficient between skatole concentrations and boar odour sensory scores to range from 0.2 to 0.53 (Hansson *et al.*, 1980; Maarse *et al.*, 1972). Multiple regression analysis showed that again only 33% of the variation in boar odour intensity scores could be accounted for by the variation in skatole concentration (Hansson *et al.*, 1980)

Attempts to correlate sensory boar odour panel scores and quantitative measurements of androstenone have resulted in moderate correlation co-efficients (R = 0.3 to 0.8); Newell *et al.*, 1973; Malmfors and Andresen, 1975; Thompson and Pearson, 1977; Bonneau *et al.*, 1979; Bonneau and Desmoulin 1979; Forland *et al.*, 1980; Hansonn *et al.*, 1980; Bonneau, 1993; Bejerholm and Barton-Gade, 1993). These correlation co-efficients explain between 16-56% of the variation found among the sensory scores and the concentrations of androstenone (Thompson and Pearson, 1977; Bonneau, 1982). Bonneau *et al.*, (1992) demonstrated that the contribution of androstenone to boar taint was larger than that of skatole. The presence of skatole does not account for all the remaining unpleasant odour often associated with boar fat which is not accounted for by the presence of androstenone (Bonneau *et al.*, 1992). Squires *et al.*, (1992) could not find a significant correlation between

the levels of skatole in the fat of intact male pigs and the age and live weight of the animal, the authors suggested that androstenone was a better indicator than skatole.

Kaufmann *et al.*, (1976) concluded from consumer studies that androstenone concentrations of up to 0.1 ppm were acceptable in boar meat for human consumption. In another study consumers rated boar pork acceptable if it contained concentrations no greater than 0.5 ppm in fresh pork and 1.0 ppm in processed products. Fresh boar chops with androstenone levels above 1.0 ppm of fat also corresponded with a higher proportion of unpleasant judgements by the consumers (Desmoulin *et al.*, 1982).

The ratio of androstenone to androstenol in boar back fat was found to be 2.2:1 (Brennan et al., 1986). The androstenone content accounted for less than 40% of the variation in taint odour score. The concentration of androstenol is only 45% of the androstenone concentration in back fat, however it accounts for 7-16% of the variation in odour score, over and above that which was accounted for by androstenone (Brennan et al., 1986).

According to Berg *et al.*, (1993), androstenone and skatole have similar contributions to boar taint. Babol *et al.,* (2002) found that the 16-androstenes in salivary glands can be used to estimate boar taint however skatole measurement also needs to be included. Moreover, the simultaneous presence of androstenone and skatole odours has been known to further enhance the perception of taint (Lundstrom *et al.*, 1980; Walstra *et al.*, 1986; Bonneau *et al.*, 1992; Agerhem and Tornberg, 1994). Annor-Frempong *et al.*, (1997a) also reported a partial synergism between androstenone and skatole. Given the difficulty associated with subjective assessments made in sensory panels, the indications are that both compounds are important contributors to taint (Jeremiah *et al.*, 1999).

Factors such as temperature and method of cooking, percentage of fat, the use of spices along with the concentrations of androstenone and skatole have an effect on the level of taint (Bonneau *et al.*, 1992; McCauley *et al.,* 1997). Human perception of boar taint related odours is more difficult at room temperature than in heated products (Pearson *et al*, 1971; Desmoulin *et al.*, 1982). The cooking temperature appears to be a crucial element in the profile of taint. It was found that at a cooking temperature of 80°C, androstenone had a greater influence on pork loin abnormal flavour score than skatole, whereas at 68°C, the influence of skatole was higher (Agerhem and Tornberg, 1995).

High temperature cooking often releases odorous compounds and this is why often, upon cooking, odour is more correlated with boar taint than flavour (Matthews *et al.*, 2000). Some masking effects of boar taint can occur with curing and high temperature cooking during processing. Processing has been shown to improve acceptability of tainted meat (Bonneau *et al.*, 1980) and can possibly affect the respective contributions, of androstenone and skatole, to the unpleasant odours and flavours. A better consumer response can also be obtained if products are consumed at room temperature due to the minimal release of odourous compounds (Bonneau and Squires, 2000).

1.2.2. Possible causes for inconsistencies between studies

The incidence of boar taint throughout the world is quite variable and the lack of consistency between results, obtained from various studies, may be due to a number of factors including:

(i) The differences in the characteristics of the meat samples, particularly their androstenone and skatole levels (Bonneau *et al.*, 2000a), resulting from differences in the breed, age and weight at slaughter, rearing conditions pre and post production, and the seasonal variation (Walstra *et al.*, 1999).

(ii) The different procedures and the difficulties associated with standardising procedures for the selection and training of the panel members (Dijksterhuis *et al.*, 2000).

(iii) The differences in the methodology and experimental design used for the sensory evaluation of meat odour and flavour, such as cooking procedures, different cuts and processing methods (Bonneau *et al.*, 2000a).

(iv) Variation in the sensitivity to androstenone is also partly under genetic and cultural influence (Wysocki and Beauchamp, 1984). A variable proportion of people are anosmic to androstenone (Gilbert and Wysocki, 1987) and for those who can detect it, the level of unpleasantness is also dependent on their degree of sensitivity (Weiler *et al.*, 2000).

(v) People vary in their sensitivity from time to time, as sensory thresholds are not static (Stevens and O'Connell, 1991).

(vi) The differences in the methodology for the analysis of compounds contributing to taint (Bonneau 1998).

(vii) Other compounds interfering with the odour profile (Babol *et al.*, 1996).

(viii) Cultural differences in meat consumption habits (Bonneau *et al.*, 2000a).

These factors will be discussed in greater depth in the following sections. An international study has recently been conducted, aimed at resolving the controversy around the respective

contributions of androstenone and skatole to boar taint (Bonneau *et al.*, 2000a). A group of entire males were selected from a large population of pigs for which the concentration of androstenone and skatole had been determined (Walstra *et al.*, 1999). Consumer surveys were conducted in seven European countries, with the meat samples from the selected animals (Matthews *et al.*, 2000). The results from the seven consumer surveys indicate that skatole makes a greater contribution than androstenone, towards the dissatisfaction of consumers with regard to the odour of entire male pork. However in relation to flavour, skatole and androstenone had a similar contribution, which was additive. The higher contribution of skatole is thought to be due to the relatively high proportion of consumers who are anosmic to androstenone which is approximately 15-30% of the population, depending on sex and geographical area (Griffiths and Patterson, 1970; Gilbert and Wysocki, 1987. No such anosmia however, exists for skatole (Weiler *et al.*, 1997).

1.2.3 Regional differences in the detection of taint

Many sensory studies have been conducted with regards to boar taint. Consumer studies, which employ untrained panellists, generally detect less difference between the meat of entire males and gilts or castrates. This is often in contrast to trained sensory panel studies that employ panellists who are screened for their sensitivity and undergo training in the detection of the taint compounds. Studies of British and Irish consumers have repeatedly shown that low levels of boar taint are detected. Kempster *et al.*, (1986) found no difference in consumer assessment of flavour or overall acceptability of leg, shoulder or loin joints, from entire males and gilts, in the UK. Both consumer and trained panels did not discriminate at all between the meat from entire males, gilts and castrates in several studies (Rhodes, 1972; Rhodes & Krylow, 1975; Wood *et al.*, 1986).

In an Irish study, where consumers were presented with bacon from entire males and gilts, no significant response for the flavour or aroma of the bacon was found, however, the trained sensory panellists detected a clear difference (Cowan and Joseph, 1981). Patterson *et al.*, (1990) reported that sensory panellists found differences in both odour and abnormal odour in cooked pork from male and female pigs but this was not reflected in consumer scores for fresh pork. Some reports have indicated very low incidence of boar taint even in heavy entire males (Cliplef *et al.*,1984; Judge *et al.*, 1990).

One reason in the past, for the generally low levels of boar taint in these countries and particularly the UK was probably the low market weight for pigs, that was approximately

65 kg. In countries where pigs are slaughtered at heavier weights, the response to entire male meat is less favourable. In many European countries, approximately 10-15% of entire males have been found to be tainted, as judged by trained panels (Malmfors & Hanson, 1974; Walstra, 1974; Malmfors & Nilsson, 1978; Arpa *et al.*,1988). The proportion of consumers judging the odour of boar meat to be unpleasant varied from 5-35%, compared to 3-10% in meat from gilts and castrates, as indicated in a review of nine consumer studies across six European countries (Malmfors and Lundstrom, 1983). The Dutch consumers were more responsive to odour, on cooking, than flavour, and more responsive to skatole than androstenone (Walstra *et al.*, 1986). A Spanish study however, indicated there was some adverse consumer reaction to pig meat from animals with high androstenone levels, particularly when cooked in the home (Diestre *et al.*, 1990). Similarly, Agerhem and Tornberg (1994) found a higher percentage of Swedish consumers who disliked the flavour or aroma of entire males compared with gilts.

In consumer trials, where entire males with live weights at about 80-100 kg were evaluated, it was confirmed that a small proportion of entire male carcasses had levels of boar taint unacceptable to some consumers (Mortensen *et al.*,1986). The rejection rate of carcasses in Denmark is 5-10% due to the high skatole concentrations (Kjeldsen, 1993). In the recent large European study an overall 22% of the samples from entire male pigs were disliked for their flavour and 34% were disliked for their odour. In contrast, 19% were disliked for flavour and 28% for odour for samples from gilts (Matthews *et al.*, 2000). This study however, was confounded with the problems associated with warmed over flavour as the samples were re-heated upon testing, possibly resulting in a smaller difference in the odour/flavour of meat from male and female pigs than would normally occur.

Weiler *et al.*, (2000) reported that 31% of the Spanish and 18% of German consumers were highly sensitive to androstenone with a significantly higher proportion of sensitive women. There was a tendency for consumers who were highly sensitive to androstenone, to score the high androstenone samples much lower than those with low concentrations, however this was not observed, for the mildly sensitive /insensitive consumers. This effect was further supported by the significant interaction between the concentration of androstenone and the sensitivity to androstenone found by the Spanish consumers ($P < 0.01$) for odour scores. This interaction observed for odour scores was not significant for flavour (Weiler *et al.*, 2000). In studies therefore that do not consider sensitivity to androstenone, the assumed acceptance of pork from entire males is unrealistically high for the sensitive consumer. This is particularly

important because the incidence of androstenone tainted carcasses is much more frequent compared to carcasses with high skatole levels (Bonneau *et al.*, 2000b).

These genetic and regional differences, partly explain the variability of consumer reactions to fresh and processed pork from entire males in different countries and various studies. The data illustrate the difficulty of getting a comprehensive estimate of the consumer reaction to pork from entire males, if sensitivity to androstenone is not considered. A single highly sensitive consumer per family can change the consumption habits of a whole family particularly if that person is responsible for food preparation.

1.2.4. Variation in sensitivity to androstenone

The ability to smell androstenone is genetically determined (Wysocki & Beachamp, 1984) and predominates in females. In some studies 35% of the population have been found to be extremely sensitive, another 15% detect a subtle odour although they are not offended by it and 40-50% of the population are not capable of detecting androstenone, even at a high concentration (Amoore *et al.*, 1977; Wysocki and Beachamp, 1984). In other reports 33% (Claus, 1983) and 25% (Elsely, 1968) of volunteers appeared to be anosmic to androstenone. In a study, with a reported 1.5 million participants, the proportion of consumers with complete anosmia for androstenone ranged between 24-26% for men and 16-18% for women in continental Europe, Asia and Australia. A substantially higher number of participants were found to be anosmic, in the US. There were 37.5% of males compared with 29.5% of females, found to be anosmic. This was also the case in the UK, with 30% of males compared with 20.9% of females, anosmic towards androstenone. The responses of the participants also varied widely from region to region within some countries (Gilbert and Wysocki, 1987).

Griffiths and Patterson (1970) demonstrated that when 100 panellists with equal numbers of male and females, were asked to analyse androstenone dried on the 5 cm^2 surface area of a watch glass, 7.6% of the women and 44% of the males could not detect it. In another study, 100 females and 100 males were tested for their ability to detect androstenone dried on the inside of a bottle. The results indicated that 46% of the males compared to only 24% of the females could not detect androstenone. Females also tended to rate the odour of androstenone and androstenol (Kloek, 1961) with higher intensity.

It is also possible to become more sensitive to boar taint after being exposed to it. The sensory threshold will decrease after repeated exposure to androstenone during training and testing (Wysocki *et al.*, 1989). This may explain why in studies by Matthew *et al.,* (2000) panellists who often cooked pork were more critical of it than those who never cooked it. Sensitivity to androstenone also appears to increase, for females, as they approach ovulation (Doty, 1981) and pregnant women perceive the odour as more intense compared to non-pregnant women (Gilbert and Wysocki, 1991). Some individuals, however, who can smell androstenone do not perceive it as an unpleasant odour (Labows and Wysocki, 1984). Gower *et al.*, (1985) found that 70% of the women, but only 15% of men rated the smell of androstenone as unpleasant. Skatole seems to be perceived by most people as an offensive off-odour. This would imply that screening for sensitivity to androstenone at least, would be an important factor when establishing a sensory panel, otherwise an underestimation of the level of taint affecting the sensitive consumers could occur, as indicated in the studies by Weiler *et al.*, (2000)

1.2.5. Sensory analysis methods

1.2.5.1. Types of sensory thresholds

There are several types of sensory thresholds that can be detected using a sensory panel. The difference or stimulus threshold requires one to taste/smell a solution in a concentration series and detect a different taste/smell to water or air, even though the basic taste or smell cannot be recognised. The recognition threshold requires one to continue tasting/smelling until a concentration is reached, at which the basic taste/smell is recognised correctly. The terminal threshold occurs above a certain concentration when the increasing concentration can no longer be differentiated (Saxby, 1996). The type of sensory test that should be used is also important. Some of these are described as (i) the paired difference test (ISO 5495:1983), (ii) the triangle test (ISO 4120 1983) and (iii) the duo-trio test (ISO 10399: 1991).

1.2.5.2. Factors of concern in difference tests

Difference tests are analytical methods and the panellist acts like an instrument that is to analyse whether or not there is a difference between two samples. Owing to the fact that the differences are small, these tests are very difficult and require precision and sensitivity, which can be acquired through training. The questions "Which is the odd sample" or "Which sample is stronger" are posed in such a way that no hedonic answer can be given. This is necessary to keep the method strictly analytical (Jellinek,1985; Stone and Sidel, 1993).

There are many factors to be considered when deciding which type of difference test should be used to determine the sensory thresholds of panellists. An ascending forced choice method takes into consideration the need for a precise and defined threshold level and accounts for some of the problems associated with sensory adaptation and observer fatigue. One should be aware, however, that many factors can affect the results of the threshold value when using an ascending forced choice method. Some of these factors are: (i) The number of alternatives i.e. the number of targets and blanks. (ii) The stopping rule or the number of correct steps in a row required to establish a threshold. (iii) The number of replicated correct trials required at any one step. (iv) The rule to determine at what concentration, the threshold value is assigned. For example, the individual threshold might be assigned at the lowest correct level or it could be assigned at the geometric mean between the lowest correct level and highest incorrect level. (v) The chosen step size in the increase of the concentration units, factors of 2 or 3 are common in taste and smell. (vi) The method of averaging or combining replicated ascending runs on the same individuals, as geometric means are common, but not universal (ISO 13301:2002; ISO 5496:1992).

At each point a different choice can be made, however, it is important to decide which method is most appropriate and then report the proceedings accurately. The number of methods that have been used to screen panellists for their sensitivity to androstenone and skatole can be observed in the studies by Judge *et al.*, (1990), De Vries and Walstra, (1993), Laing (1996), McCauley, *et al.*, (1997) and many others. These papers indicate a large variation in the screening methodology for the selection of candidates in a boar taint sensory panel. Due to these differences and the variable sensitivity of the individual, it is therefore not surprising that the results obtained from different boar taint sensory panels, are not always consistent.

1.3. Physiological influences on taint compounds

1.3.1. Effect of seasonal variation

Artificial light regimes, mimicking spring and autumn photoperiods, produced in boars, lower androstenone and skatole concentrations, with the lengthening of days (Andersson *et al.*, 1995; Andersson *et al.*, 1997b). This was coincident with the development of the sexual maturation characteristics. Claus (1979) also noted higher concentrations for androstenone during autumn, but this was in older boars. Keller *et al.*, (1997) also found that androstenone

levels were affected by season in boars used for artificial insemination. The androstenone concentration decreased with increasing day length and increased as day length was reduced. Higher concentrations of androstenone were found in winter compared to early summer or autumn, but the differences were found to be insignificant (Falkenberg and Blowdow, 1981). Neupert *et al.*, (1995) also using different light regimes, could detect only weak effects on androstenone as well as on skatole.

In another study, there were no differences between the light regime groups for skatole concentrations for animals slaughtered in the cooler months (Andersson *et al.*, 1997b). However, seasonal variation appears to have an effect on skatole (Andersson *et al.*, 1995; Walstra *et al.*, 1999). Hansen *et al.*, (1993) and Lundstrom and Malmfors (1993) ascribed the higher skatole concentrations to the higher temperatures over the warmer periods. These groups explained the higher concentrations of skatole in animals by the higher concentrations of skatole in the air and the higher skatole load from faeces, favoured by high temperatures during the summer. Hansen (1998) reported that, in the week before slaughter, skatole concentrations may increase or decrease if the animals were heavily fouled or clean, respectively.

In summary, most findings for androstenone had a meaningful physiological background, corresponding to wild boars and older domesticated boars. Under commercial conditions the differences for androstenone between the summer and winter seasons are small to insignificant. For skatole however, a more definite seasonal effect was demonstrated, the concentrations of skatole were, on average, lower when slaughtered in winter than in summer and this was thought to be attributed to the lower temperatures (Walstra *et al.*, 1999).

1.3.2. Effect of weight

Studies have shown a highly variable relationship between odour and either weight or age (Claus, 1975a; Andresen, 1976; Malmfors *et al.*, 1978; Claus and Hoffman, 1980; Bonneau, 1982). Falkenberg and Blowdow (1981) and Neupert *et al.*, (1995) found no correlation between body weight and androstenone levels. No relationship was found between concentrations of androstenone in fat and quick or slow growth rate (Andresen, 1976; Bonneau, 1982). Generally though, the slaughter weight among other things is known to influence the androstenone content in boars (Andresen,1976; Bonneau and Desmoulin,1980; Bonneau *et al.*, 1992). Studies to determine whether it was possible to reduce the incidence of boar odour by decreasing weight at slaughter have been conducted (Le Denmat *et al.*,

1993). In boars slaughtered at approximately 105 kg, androstenone was the main contributor to boar taint. At the lighter weight of approximately 90 kg, skatole made a larger contribution than androstenone. In general, the eating quality of boars tended to be more closely related with skatole than with androstenone concentration, irrespective of the slaughter weight. However, it was also noted that the two compounds did not account for all off odours (Le Denmat *et al.*, 1993).

Contrary to this, Berg *et al.*, (1993) found a significant relationship between the androstenone content and the slaughter weight for male pigs ($P = 0.002$; $R^2 = 0.396$). He concluded it was important to keep the slaughter weights low to reduce the risk for androstenone off-odour. The number of pigs exhibiting high concentrations of androstenone tended to increase as pigs became older and heavier (Claus, 1975a; Ruszczyc *et al.*, 1981). A sharp increase in both plasma and fat concentrations of androstenone occurs when the pigs are approximately 200 days old (Claus, 1975a; Claus and Hoffmann, 1980). Fast-growing boars slaughtered at live weights of 100 to 120 kg also had higher concentrations of androstenone (Asp and Hansson,1972; Malmfors and Hansson, 1974; Bonneau and Desmoulin, 1979)

Androstenone levels differed markedly from each other when comparing the extreme weight groups (Malmfors *et al.*, 1978; von Seth *et al.*, 1995). Androstenone concentrations were higher at the higher weights (Hoppenbrock, 1993). It was reported that as the weight of entire males increased from 85 to 95 kg the number of entire males with androstenone concentrations above the 0.5 and 1.0 ppm thresholds also increased (Walstra and Garssen, 1995). This increase probably reflected sexual maturity. The sorting of entire samples into 33.3 percentiles in relation to their weight showed increasing androstenone levels with increasing weight (Salvatore *et al.*, 1995). Moss *et al.*, (1997) also concluded from their study that there was some increase in skatole concentrations at the higher weight. Squires and Lou (1995) found higher skatole levels with increasing weight in the Hampshire breed, but not in other breeds studied. Hennessy *et al.*, (1995) found no increase in skatole with increasing weight.

Studies by Walstra and Garssen, (1995) indicated that although the correlation coefficients between the carcass weight and the boar taint compounds were low, the relationship was significant due to the wide range of slaughter weights and the high number of animals involved. Androstenone and skatole concentrations generally increased with heavier carcass weight and generally decreased with lean meat percentage. Therefore, although carcass weight can significantly contribute to the prediction of the androstenone and skatole

concentrations, the percentage of the variance accounted for is quite low. This is possibly because the relationship of growth to the onset of maturity is a fairly rapid one, although quite variable among individuals resulting in a weak correlation. It would thus seem to be of little practical value to have a limit on weight, as long as the normal weight ranges are involved. A method of controlling boar taint would be to slaughter intact male pigs at a lighter slaughter weight. However, this does not allow producers to take full advantage of the growth efficiency of boars in the 80 to 120 kg live weight range and may only be partially effective.

1.3.3. Effect of different breeds

Breed exerted a major influence on meat quality, cooking and palatability traits (Jeremiah *et al.*, 1999). The most palatable meat was produced by Hampshires and the least desirable meat produced by Yorkshires. In more recent years the carcass weights have tended to increase and the breed mix has included the dark skinned Duroc genotypes. Both of these factors have tended to increase the level of taint in these animals (Squires, 1999).

Xue *et al.,* (1996) conducted a study with 228 intact Duroc, Hampshire, Landrace and Yorkshire males. The proportion of tainted carcasses determined by the sensory panel was 5% for androstenone and 11.4% for skatole with a combined total of 15% tainted from either source. Sensory analysis of taint showed a significantly lower proportion of tainted carcasses of the Hampshire breed compared to other breeds. There was a significantly higher proportion of Duroc pigs having levels above the threshold for 16-androstene steroid in salivary gland and fat, than in the other breeds. Landrace pigs had the lowest average concentration of steroid and skatole in the fat tissue. Duroc boars also had significantly higher androstenone levels compared to the Large White x Landrace pigs in studies by Salvatore *et al.*, 1995. Squires *et al.*, (1992) in Canada found 59% of the Duroc breed of pigs to have androstenone concentrations above the acceptable sensory threshold. The proportion of animals with high androstenone levels was much lower (6-9%) for the other breeds such as Yorkshire and Landrace. Similarly, Bonneau *et al.*, (1979) measured higher levels of androstenone in the Pietrain breed compared to the Belgian x Landrace pigs. Concentrations of androstenone in fat were also found to be higher in Landrace compared to Large White or Yorkshire boars (Rhodes and Patterson, 1971; Malmfors *et al.*, 1978).

1.4. Androstene compounds and taint

1.4.1. Levels of 16-androstene compounds in tissue and plasma

Androstenone acts as a male pheromone in pigs and is closely linked to testicular function. The concentration of the 16-androstene steroids in pigs is much lower in blood compared to fatty tissues. The concentration of both androstenone, and androstenol are in the order of 11 ppb of plasma in mature boars (Bonneau, 1982). In fat however, the concentration of androstenol is 10 fold greater and the concentration of androstenone is 100 fold greater, compared to that found in plasma. The salivary gland has the highest steroid levels, with androstenone levels at 5-10 ppm of tissue and androstenol levels at 30-80 ppm of tissue in mature boars (Squires, 1990). The ratio of androstenone to androstenol in boar back fat was found to be 2.2:1 (Brennan *et al.,* 1986). The concentration of androstenol and androstenone in the back fat of *ad libitum* boars increased sharply during the 100-130 kg growth period. There was a one-unit increase in odour intensity scores and a 2-3 fold increase in fat steroid levels, in boars that were fed *ad libitum* during this growth period. The relationship between androstenone content and odour intensity of fat is linear up to a maximum value of 3.42 ppm of androstenone in fat (Brennan *et al.*, 1986).

1.4.2. Excretion and secretion of androstenone

Androstenone being lipophilic is stored in the adipose tissue. However, androstenone levels in fat drop sharply after castration (Claus, 1976) with its half-life being in the range of a few days to several weeks (Bonneau *et al.*, 1982a). The androstenone concentration in adipose tissue can therefore be reduced to acceptable levels by inhibiting 16-androstene production several weeks before the day of slaughter. A major source of 16-androstenes is synthesized in the testes of pigs with a lesser contribution from the adrenals (Gower, 1972, 1984). Androstenone in the testes is released into the spermatic vein, and then into the systemic circulation where it is taken up by the salivary gland and adipose tissue. This is why peripheral plasma levels of androstenone are about six times lower than those in the spermatic vein (Hurden *et al.*, 1984; Groth and Claus, 1977). Androstenone is concentrated in the salivary glands by a specific binding protein known as pheromaxein (Booth, 1984) where it is then partly reduced to 5α-androst-16-en-3-ol and, to a lesser extent, to 5β-androst-16-en-3-ol (Claus, 1979). Indeed, high levels of androstenone and androstenol are measurable in the salivary glands where the steroids are stored, concentrated and subsequently released into the saliva (Patterson, 1968). During sexual activity, profuse salivation occurs allowing the odour of

androstenone and androstenol to be transmitted to the sow from the boar (Reed *et al.*, 1974, Perry *et al.*, 1980).

1.4.3. Social interactions affecting androstenone concentration

The social environment can also affect the concentration of taint compounds in the pig. Andresen (1975a) and Claus and Alsing (1976) noted that when boars were socially integrated with gilts, the plasma concentrations of androstenone decreased sharply. Concentrations of androstenone in fat were reported to be higher in boars reared only with boars (Lightfoot, 1979; Bonneau and Desmoulin, 1980). This confirmed an earlier trial by Walker (1978); however, Walker was unable to repeat his findings in a second trial. Patterson, (1982) also found that boars raised with gilts tended to have lower concentrations of androstenone in their adipose tissue than those raised only with boars.

The effects of environmental rearing conditions on the development of the young boar were also studied by Andresen (1976). He observed that one animal in each of the three groups of young boars raised in a single-sex environment developed much higher levels of androstenone compared with the other animals in the group, thus suggesting that social hierarchy may affect the 16-androstene steroid formation in boars. However, Narendran *et al.*, (1980) disputed this theory, finding that the dominant and subordinate boars all had similar plasma concentrations of androstenone.

Giersing *et al.*, (2000) however, found that the social rank appears to influence the plasma concentration of androstenone, testosterone and skatole and the weight of bulbo-urethral glands. High androstenone concentrations in the dominant male did not suppress the androstenone concentration in the other males but rather appeared to enhance it possibly due to the stimulating effect of androstenone and possibly the mating activity.

1.4.4. Genetics and its effect on androstenone

Several researchers (Rhodes and Patterson, 1971; Malmfors *et al.*, 1978; Willeke *et al.*, 1987) have found that the level of androstenone expressed in an animal is a heritable trait. In a review by Willeke (1993) the heritability of androstenone ranged from 0.3 – 0.9 with the concentration of androstenone being successfully reduced in genetic selection programmes. The research groups, however, found that selection also resulted in decreased concentrations of testosterone, suggesting the likelihood of adverse consequences on growth performance

and carcass composition; indeed androgens and oestrogens have an anabolic influence on muscle growth (Grigsby *et al.*, 1976; Fowler 1976).

Some phenotypic characteristics such as fatty acid levels have a low but significant correlation with androstenone (Malmfors *et al.*, 1978; Bonneau *et al.*, 1979; Bonneau and Desmoulin, 1980). Conversely, in other phenotypic (Malmfors *et al.*, 1978) and genetic (Jonsson and Andresen, 1979) studies, no relationship was found between androstenone levels in fat and growing performance or carcass composition criteria. Andresen and Bakke (1975) and Andresen (1976) also observed no difference in androstenone levels between two lines of boars selected for quick or slow muscular growth.

Squires, (1996) found that studies conducted on the synthesis of androstenone in pig testes suggests the involvement of the cytochrome P450 enzyme system, specifically cytochrome P450c17, to be responsible for the synthesis of androgens. The synthesis of androstenone was thought to also require the presence of another protein known as cytochrome b5 (Squires, 1996). It has subsequently been found that the increased levels of the low molecular weight immunoreactive cytochrome b5 protein and not cytochrome P450c17 is related to the increased production of testicular 16-androstene steroid and therefore its accumulation in fat (Davis and Squires, 1999). Such results suggest that selecting for reduced levels of the cytochrome b5 protein in the testes may also result in lower levels of 16-androstene steroid in fat ultimately reducing the level of taint in boars.

The presence of a major gene responsible for the levels of androstenone has been demonstrated by studies conducted by Fouilloux *et al.*, (1997). Bidanel *et al.*, (1997) found a genetic linkage between the levels of androstenone in fat and a marker in chromosome 7. The current hypothesis is that the major gene is situated on chromosome 7 and this is associated with the levels of the low molecular weight isoform of cytochrome b5 controlling the synthesis of 16-androstene steroids in male pigs.

1.5. Skatole and boar taint

1.5.1. Formation of skatole

Skatole, the other chemical considered to be a major contributor to boar odour, has a strong faecal-like smell and produces a bitter taste (Vold, 1970, Walstra and Maarse, 1970). Skatole is formed from the biochemical breakdown of tryptophan by intestinal micro-organisms and is absorbed by the hindgut of the pig (Lundstrom *et al.*, 1988). The *lactobacillus sp.* are

thought to be responsible for the production of skatole (Yokoyama & Carlson, 1979). A sufficient population of *lactobacillus* in the gut of the pig along with an appropriate diet will promote the degradation of tryptophan into skatole. The *lactobacillus sp.* can also produce indole (Hansson *et al.*, 1980). The mechanisms underlying the uptake of skatole into fatty tissue are not well defined and therefore its physiological role is not fully understood. Skatole is found not only in boars but significant levels can also be found in some gilts (Hansson *et al.*, 1980). This may therefore be the cause of the so-called "gilt-taint".

1.5.2. Excretion of skatole

The half-life for skatole which is approximately 10 h, is quite short (Friis, 1993). This would suggest that any treatment used to reduce the levels of skatole in the carcass, need only be administered in the last few days before slaughter. The skatole concentrations in fat will also decrease after castration because the presence of skatole depends, in turn, on the presence of the testes. Skatole levels are generally very low in fat from gilts and castrates (Pedersen *et al.*, 1986).

1.5.3. Factors affecting skatole concentrations in boars

1.5.3.1. Genetics and skatole concentration

Wide variations in skatole levels among different herds have been reported (Kjeldsen, 1993). Skatole levels are generally higher in the back fat of boars than in castrates or gilts (Hansson *et al.*, 1980, Bejerholm and Barton-Gade, 1993). There are several theories as to why entire male pigs have significantly higher or lower concentrations of skatole in body tissues resulting in odour problems. Skatole is rapidly absorbed in the gut and metabolised in the liver (Agergaard and Laue, 1993). A recessive gene which is thought to be expressed in the liver under certain environmental conditions could be responsible for the reduced degradation of skatole in these animals (Lundstrom *et al.*, 1994). It can also be hypothesized that this major gene is associated with the cytochromes which are known to affect the levels of skatole in boars (Babol *et al.*,1997).

A study regarding the involvement of the cytochrome P450 in the liver and its effect on the metabolism of skatole, Squires (1996), has shown that males with high levels of skatole in fat, had lower levels of a particular isoform of P450 known as cytochrome P4502E. Cytochrome P4502E was identified by the reaction with a specific antibody using the

technique of Western blotting. Genetic markers to identify pigs with high levels of the cytochrome P4502E in livers are being developed (Squires, 1996). It was found that low levels of cytochrome P4502E1 have been thought to result in high levels of skatole in boars (Squires and Lundstrom, 1997). Skatole is thought to be subsequently metabolised by the cytochrome P4502E1 to produce 6-hydroxy skatole and other similar skatole metabolites (Babol *et al.*, 1998).

1.5.3.2. The effect of hormones and diet

The presence of male hormones appears to influence the presence of skatole (Babol *et al.*, 1999) and this theory is further supported by the fact that in the absence of testosterone in the boar, the skatole levels also diminish. Babol *et al.*, (1999) have indicated that the relationship between the level of androstenone and skatole in the adipose tissue is a result of the link between the synthesis of androstenone in the testes rather than the metabolism of androstenone and skatole in the liver. Doran *et al.*, (2002) however, have found that skatole induces the expression of the CYP2E1 protein and androstenone antagonises the effect of skatole on CYP2E1 expression. The results of their studies suggest that defective expression CYP2E1 in some pigs could be due to excessive concentrations of androstenone which prevent CYP2E1 induction by skatole. As a result skatole metabolism is reduced and skatole is accumulated in adipose tissue.

The presence of testosterone in male pigs in combination with high levels of oestrogens, promotes high levels of IGF-1 in the intestine. It was found that gut cell mitosis is stimulated by the growth factor IGF-1 (Raab *et al.*, 1997). In addition, the mitotic rate is elevated when high amounts of purines are available in the diet, allowing for a more rapid DNA and RNA synthesis. The provision of high-energy diets is also thought to be linked to the level of cell debris that is shed (Claus *et al.*, 1994). This high energy combined with high purines in the diet lead to a remarkable increase of gut cell mitosis that is accompanied by an increase in cell death. A dramatic rise in the concentration of skatole in the blood plasma occurs as a result of the increased levels of apoptopic cells providing a good substrate for skatole formation (Claus and Raab, 1999).

Feedstuffs, however, with a high fibre content (Agergaard *et al.*, 1995) and more generally those containing a high level of non-digestible carbohydrate which can be degraded in the hind gut (Claus *et al.*, 1994) elicit a reduction in skatole production and storage. A number of feedstuffs containing high amounts of fermentable carbohydrate can also be incorporated

into the diets, resulting in decreased concentrations of skatole (Jensen *et al.*, 1995, 1997; Andersson *et al.*, 1997). The addition of inulin reduces the production of skatole (Claus *et al.*, 1994). Inulin, a complex carbohydrate, is particularly effective because it cannot be metabolised by mammalian enzymes and so it passes through to the hind gut without digestion. The bacteria are therefore able to ferment the inulin whilst cell growth reduces the amount of tryptophan available for skatole production. This theory is reinforced by the fact that the addition of tryptophan to the feed does not increase skatole production as the tryptophan is rapidly absorbed by the pig (Claus *et al.*, 1994). Short-term feeding, i.e. 5 days, with diets containing inulin can produce a decrease in adipose skatole between 50% and 78%, compared to pre-treatment levels (Claus *et al.*, 1994). Similar results have been obtained with the inclusion of sugar beet pulp containing pectins whose digestibility is similar to that of inulin (Kjeldsen, 1993). To further reduce the levels of skatole in carcasses it is recommended to restrict feed for 48 h and withhold feed for 12 h prior to slaughter (Kjeldsen, 1993). Increasing the water consumption and the addition of antibiotics such as Avotan™ and Virginiamycin™ that reduce the growth of *lactobacillus* also lowers skatole levels (Kjelsden, 1993). The addition of zinc bacitracin (Hansen *et al.*, 1997) or zeolite (Baltic *et al.*, (1997) to the diet is also effective in reducing skatole levels.

1.5.3.3. The effect of environmental factors

Changes in the environment of the hind gut, particularly pH, can alter the microbial population and therefore change the indole-to-skatole ratio. *In vitro* studies conducted by Jensen and Jensen (1993) have shown that the relative production of skatole to indole was increased at pH 5 but was dramatically reduced at pH 8 in comparison to pH 6.5. Another environmental factor that affects the skatole concentrations in pig adipose tissue is the stocking density of pigs. Skatole can be absorbed from the faeces via the lungs or skin (Hansen *et al.*, 1993). High stocking rates, particularly in warm weather, will increase skatole production and accumulation in the animal.

1.6. The control of boar taint

1.6.1. Castration

Castration of male pigs has been traditionally used as a means of preventing the development of undesirable off-odours and off-flavours resulting from boar taint (Walstra, 1974). Other reasons for castration have been: (i) the easier control of the animal behaviour and (ii) the higher propensity of castrated animals to deposit more fat, which has been a desirable quality

until quite recently (Bonneau and Squires, 2000). However, there are significant economic advantages to be gained by producers if intact male pigs are utilised for meat production (Walstra and Kroeske, 1968). Intact male animals grow more rapidly, utilise feed more efficiently and produce carcasses with higher retail yields (Martin, 1969). In fact, the feed efficiency of castrates and muscle content is reduced by 5-10%, with the carcass fat content increasing up to 26% compared to intact boars (Walstra and Kroeske, 1968; Campbell and Taverner, 1988; Dunshea *et al.*, 1993). Many fattening trials over the last 30 years clearly demonstrate that boars are superior to castrates and gilts in their fattening traits and lean meat percentage (Campbell and Taverner, 1988; Judge *et al.*, 1990). Castration also involves the possible risk of infection (Brooks *et al.*, 1986). There is increasing pressure from the animal welfare perspective to abandon castration altogether (Bonneau and Squires, 2000). There is also an environmental issue in favour of rearing entire male pigs because they produce less nitrogenous waste excretion (Bonneau, 1998).

1.6.2. Immunocastration

1.6.2.1. Immunisation against androstenone

Alternative methods of castration that would alleviate stress and other undesirable side effects associated with a surgical procedure are of interest to those concerned with animal welfare. Several researchers have investigated the possibility of actively immunising young boars against androstenone in order to reduce its concentration in serum and fat. Claus (1975b) conjugated this steroid to bovine serum albumin (BSA), and vaccinated young boars. High titres of specific antibody were produced in the pigs; however, the concentrations of androstenone in adipose tissue were not significantly different between immunised and non-immunised control boars. Shenoy *et al.*, (1982) conjugated androstenone to bovine thyro-globulin and administered it subcutaneously in a water-in-oil emulsion. This procedure produced both highly specific antibody titres and a 67% decrease in the concentration of androstenone in the adipose tissue of immunised boars slaughtered at 135 kg. However, the organoleptic evaluation indicated that the meat samples from the immunised boars appeared to be tainted whilst the meat samples from the gilts appeared to taste normal.

Williamson and Patterson (1982) achieved a 69% decrease in androstenone concentrations in the adipose tissue of boars immunised against androstenone conjugated to bovine serum albumin. There were no detrimental side-effects on growth, testicular development and spermatogenesis, nor were there any abnormal features during histological examination of testicular, bulbo-urethral, prostate or kidney tissues. However, no results of organoleptic

evaluation of either meat or samples of fat from the immunised boars were reported in this study (Williamson and Patterson, 1982). In other studies, despite high antibody titres to androstenone in the immunised pigs, there was little or no effect on boar taint (Williamson *et al.,* 1985). One of the problems with vaccination against specific androgens is that it continues to allow the testes to synthesise many other steroids and also skatole may still be present to contribute to taint.

1.6.2.2. Immunisation against LHRH

The production of 16-androstene steroids can also be reduced by interfering with the function of gonadotropins and this can be accomplished by generating antibodies against luteinising hormone releasing hormone (LHRH) otherwise known as gonadotropin releasing hormone. Immunisation of boars against hypothalamic LHRH, reduces the production of pituitary peptide hormones that largely drive and control testicular development and function (Xue *et al.*, 1994; Meloen *et al.*, 1992; Falvo *et al.*, 1986; Hagen *et al.*, 1988). LHRH is released into the hypophygeal portal blood vessel and stimulates the secretion of pituitary luteinising hormone (LH) and follicle stimulating hormone (FSH). The interruption of LHRH by the active production of antibodies against this hormone inhibits the action of LHRH on the pituitary gland. There is a reduction in both LH and FSH concentrations in the blood when the activity of LHRH is blocked. This will ultimately suppress testes function and the production of hormones, like testosterone and androstenone, and thus remove the undesirable boar taint before the pigs are slaughtered (Falvo *et al.*, 1986). Skatole levels are dependent on the presence of testosterone and other like hormones, therefore in the absence of these hormones, it is expected that the concentration of skatole in adipose tissue will decline. In fact, the levels of skatole were found to be reduced in boars vaccinated with LHRH, compared to non-vaccinated boars in a study by Dunshea *et al.*, 2001.

Caraty and Bonneau (1986) vaccinated boars with LHRH antibody coupled to BSA, at approximately 14 weeks of age and demonstrated that androstenone levels were reduced to low levels similar to those observed in surgically castrated males. Bonneau *et al.*, (1994) demonstrated that anti-LHRH immunisation can be very effective in reducing the levels of boar taint while having limited consequence on the performance of the animals. It was found that the immunocastrates and entires both had a higher feed conversion efficiency of about 10-12% and a 3-4% higher lean meat yield compared to castrates. The concentration of androstenone was also reduced from 0.66 ppm of fat in entires to 0.21 ppm of fat in immunocastrates. Several other authors also found that vaccination against LHRH was

highly effective in stopping testicular steroidogenesis also resulting in the reduction of the concentrations of androstenone and skatole in fat. This occurred without causing any adverse effects on growth rate or back fat thickness (Hennessy *et al.*, 1994, 1995, 1997; Dunshea *et al.*, 2001; Metz *et al.*, 2002). The approach carried out by Hennessy *et al.*, (1997) and Dunshea *et al.*, (2001) has been so effective that it has formed the basis for a commerical vaccine for the suppression of boar taint.

1.7. Analytical procedures for detecting compounds contributing to taint

A large number of methods have been utilised in the analysis of taint. Methods involving GC, GC-MS, HPLC, Radio-immunoassay and ELISA have been established over the decades and they will be discussed in the next section. Many of these analytical methods are not suitable for rapid on-line analysis for the detection of all compounds contributing to boar taint. Such a method is still required by the industry however, it has not yet been discovered.

An alternative method to reduce the intrinsic level of taint in pork is to identify the tainted carcasses by some form of analytical screening. In this case the frequency of the number of tainted animals that pass through the slaughter line is important. If this number is high, then this approach is uneconomical. If the proportion is low then the screening system needs to be accurate. The "hot iron vial test" (Jarmoluk, 1970) was initially used to screen for taint at the slaughter line, however this method is subjective and can lead to inconsistent results. Several analytical methods have been developed, which ultimately are more reliable and consistent, in an attempt to screen for the compounds relating to taint. This has resulted in the development of a number of immunological, colorimetric and chromatographic methods which measure the levels of skatole and androstenone in carcasses.

1.7.1. Chromatographic methods for quantitation of 16-androstene steroids and skatole

1.7.1.1. Quantitation of androstenone by GC

Gas chromatography (GC) was one of the earliest methods for the detection of the 16-androstene steroids. However, most early methods of quantitating these steroids involved tedious, steam or distillation extraction techniques and labor-intensive thin layer chromatography for purification of samples prior to GC and mass spectrometric analysis (Patterson, 1968; Claus, 1970; Thompson *et al.*, 1972; Fuchs, 1972; Kaufmann *et al.*, 1976). Other methods (Claus and Hoffmann, 1971) not only involved these tedious techniques, but

also incorporated the use of expensive radio-isotopes as internal standards in the gas chromatographic analyses.

One of the better early GC methods for the quantitative determination of the androstene steroids employed a deuterium isotope as an internal standard and utilised selected ion monitoring gas chromatography-mass spectrometry (Thompson and Pearson, 1977). This method is capable of quantitating each of the 16-androstene steroids and also possesses comparable sensitivity (pg levels) to radio-immunoassay (RIA) methods. However, the technique requires laborious sample preparation and sophisticated gas chromatography-mass spectrometry (GC-MS) equipment. In GC analysis the detection of androstenone and skatole has been made with a range of detectors. Examples are the flame ionisation detector (Porter *et al.*, 1989), the electron capture detector (de Brabander and Verbeke, 1986), the thermionic specific detector (Peleran and Bories, 1985) and the mass spectrometer (Garcia-Regueiro and Diaz, 1989; Kwan *et al.*, 1992). Careful extraction of the samples is required for chromatographic analysis, to ensure good resolution. A method using supercritical fluid extraction coupled to GC-MS in the selected ion monitoring mode for the detection of androstenone has also been established. The detection limit for this assay is 0.05 ppm of fat using optimised conditions (Magard *et al.*, 1995). Another method incorporating the analysis of androstenone and skatole by supercritical fluid extraction and GC-MS was also established (Zabolotsky *et al.*, 1995; Tuomola *et al.*, 1998). Supercritical fluid extraction could possibly provide, a rapid extraction method for the detection of compounds associated with taint.

Whilst the concentration of androstenone in blood and adipose tissue has been studied extensively using gas chromatography, the contribution of other 16-androstene steroids has not been studied so thoroughly. Gas chromatographic measurement of 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol is very tedious and the concentrations usually found in boar fat barely exceed the lowest range of sensitivity of the method (Bonneau, 1982) making it difficult to conduct routine measurements of these compounds.

1.7.1.2. The quantitation of androstenone by headspace analysis techniques

Headspace analysis is an effective method for the detection of compounds contained in vapours. There are several different types of headspace analysis such as the Tenax™ trap, purge-and-trap and the static or dynamic headspace methods. Viallon *et al.*, (1992) utilised the purge-and-trap method in the analysis of "boar taint" and correctly classified 78% of the samples as tainted/non-tainted according to the concentration of androstenone in the fat. It is

possible to distinguish contents lower or higher than 0.5 ppm in adipose tissue (Viallon *et al.*, 1992). Along with androstenone, 55 other molecules were also identified by GC-MS and the compounds belong to the chemical families of aldehydes, ketones, alkanes, alkenes, alcohols, aromatic compounds, chloro compounds, terpenes, lactones, furans and pyrazines. Lipid oxidation is the likely biochemical origin of some of these compounds.

1.7.1.3. Quantitation of androstenone by pyrolysis mass spectrometry

This examines the headspace profile of a sample by the coupling of pyrolysis to mass spectrometry, which then acquires 200-300 mass fragments and therefore provides many descriptors of the substance for analysis. The stepwise discriminant analysis of the results from the pyrolysis mass spectrometry study indicated that 98% of the duplicates were successfully analysed (Berdague *et al.*, 1996). When the pyrolysis mass spectrometry data were analysed by neural network discriminant analysis, all samples were correctly classified and 100% of the samples were also correctly classified in the cross validation. Further work is required for these systems to be optimised for on-line analysis (Berdague *et al.*, 1996).

1.7.1.4. Quantitation of skatole by GC

Maarse *et al.*, (1972) developed a GC method for the analysis of skatole in adipose tissue. The extraction yield of this component is quite low and cannot be measured accurately hence the method is semi-quantitative. Another method for fat skatole measurement was developed by Peleran and Bories (1985). This method involves several elaborate extraction procedures to remove skatole from fat after which the skatole is analysed by GC-MS with a selected ion monitoring system.

1.7.1.5. Quantitation of skatole and androstenone by GC-MS headspace analysis

Dynamic headspace assessment of androstenone, skatole and indole by GC-MS detection was conducted by Garcia-Regueiro *et al.*, (1995). The skatole and indole concentrations in the vapour phase were approximately 20-30% of the concentration found in the fat. The concentration of androstenone in the vapour phase, however was only 5% of that found in the fat, although the concentration of androstenone in the fat was several fold higher than skatole. It appears as though skatole can be more easily detected because of its higher volatility, despite its lower concentration in samples of fat. This may indicate that the conditions of the odour evaluation may influence the relative partitioning of boar taint compounds between the solid and vapour phases (Garcia-Regueiro *et al.*, 1995).

1.7.1.6. Quantitation of androstenone and skatole by HPLC

HPLC analysis of androstenone involves its conjugation to a chromophore for fluorescent detection since androstenone does not have a strong fluorescence nor does it absorb strongly in the ultraviolet region. Skatole however has a strong intrinsic fluorescence. The HPLC analysis of skatole utilises reverse phase columns eluted with either isocratic or gradient solvent systems along with either ultraviolet or fluorescence detection (Garcia-Regueiro and Diaz, 1989; Hansen-Moeller, 1992). Rapid methods for the extraction of skatole from samples of fat for HPLC analysis have been developed (Dehnhard *et al.*, 1990; Hansen-Moeller, 1992;). An HPLC method for the combined analysis of androstenone, skatole and indole has also been developed (Hansen-Moeller, 1994). Hansen Moeller and Andresen (1994) showed good correlation, i.e. an R value of 0.975, with this HPLC method and the rapid on-line colorimetric method established by Mortensen and Sorensen, (1984). A very sensitive HPLC method was also developed for the analysis of skatole and indole in porcine tissue, i.e. in fat and salivary glands, as well as serum samples (Tuomola *et al.*, 1996; Garcia-Regeuiro and Rius, 1998). Close correlations were found between the concentrations of fat and serum skatole and it was proposed that routine measurement of skatole in serum could be utilised for the detection of taint

1.7.2. Immunological methods

1.7.2.1. Quantitation of androstenone by Radio-Immunoassay

The development of the RIAs for plasma (Andresen, 1974) and fat androstenone (Claus, 1974; Andresen, 1975b) enables semi-routine measurements to be performed. The RIA technique is more sensitive and faster than GC techniques but is restricted to laboratories specialised to handle radioactive isotopes. A simplified but less accurate, RIA (Andresen, 1979) may be more suitable for large-scale measurement, namely for systematic checking of boar carcasses. No method is currently available for measuring 5β-androstenone. Bicknell and Gower (1976) developed an RIA method for the detection of 5α-androst-16-en-3α-ol in plasma. The contribution of these steroids however, has not been investigated thoroughly and therefore their exact contribution to boar taint is unknown.

1.7.2.2. Quantitation of androstenone and skatole by ELISA methods

An enzyme immunotest method was developed for commercial purposes (Walstra and Moermann, 1981). The microtitre enzyme immunoassay is a rapid and practical routine method for the determination of androstenone in the adipose tissue of pigs (Claus *et al.*, 1988). The method measures pg quantities of the 16–androstene steroid with an enzyme conjugate, rather than employing radioisotope-labelled analogs. The coefficient of variation of this assay, is only slightly higher than that of the RIA and is sufficient for all practical purposes. Due to the improved procedure for sample preparation, it is also less time consuming than the RIA methods described earlier. Thus one person can measure about 64 biological samples per day. The preparation of samples containing volatile substances needs extreme care and experience to obtain reliable results and the use of an external quality control system is recommended (Claus *et al.*, 1988; Abouzied *et al.*, 1990). This rapid ELISA test for androstenone was compared to HPLC analysis of androstenone which resulted in a high correlation coefficient of 0.979 (Claus *et al.*, 1997). Further advances have been made with the mobile automated enzyme immunoassay determining the concentrations of androstenone in the adipose tissue of pig carcasses. This method will analyse 24 samples in 75 min (Axmann and Ebrecht, 1994).

A direct competitive ELISA for quantitation of skatole in porcine serum has been developed (Singh *et al.*, 1988). The test utilises skatole antibodies immobilised on microtitration wells and skatole enzyme conjugate as a tracer. The test is rapid and measures skatole levels in boars, although there is some cross reactivity with tryptophan. The test utilises simple preparation and can be automated to screen for thousands of animals in high-speed slaughter lines. This test along with an androstenone ELISA could provide a strategy for the comprehensive screening of taint. The production of large quantities of monoclonal antibodies required for commercial purposes however, could be problematic.

1.7.2.3. Quantitation of androstenone by Time-Resolved Fluorimetry

A competitive time-resolved fluoro-immunoassay based on a dissociation-enhanced lanthanide (DELFIA) system has been established to determine the concentration of androstenone in sera or in a sample extracted from fat (Tuomola *et al*, 1997). Fluorescent labels provide for a method that is sensitive and by using time-resolved fluorescence, the

method also has limited background interference. This DELFIA system utilizes a non-fluorescent chelate that is employed to bind europium to the analyte, i.e androstenone. After the bio-affinity reaction is completed, the europium ions are dissociated from the chelate by an enhancement solution that forms new fluorescent complexes. The fluorescent label is excited and the measurement of light occurs after a fixed time when most of the background interference has disappeared. The sensitivity range for sera, using this method, is between 1-90 ppb and the intra and inter coefficients of variation are 5.4 and 8.1%, respectively. The sensitivity range for fat is between 50-3000 ppb and the intra and inter coefficients of variation are 6.0 and 8.9%, respectively. A significant correlation was found between the concentration of androstenone in fat and sera ($P < 0.0001$) indicating this method could be used for routine screening of taint (Tuomola *et al.*, 1997).

1.7.3. Colorimetric assays

1.7.3.1. Quantitation of androstenone by colorimetric assay

A rapid colorimetric method for the analysis of the 16-androstene steroids has been developed and involves a sequence of complicated purification procedures (Squires, 1990; Squires *et al.*, 1991; Squires *et al.*, 1993). The method requires the extraction of the 16-androstenes from the samples of fat or salivary glands with methanol and then concentrated using a C18 column. Cholesterol is removed from the fat extracts by a digitonin column placed before the C18 column. All of the 16-androstenes produce a colour for this test based on a reaction between resorcaldehyde and sulfuric acid in glacial acetic acid (Brooksbank and Haselwood., 1961). The results are expressed as equivalents of androstenone for samples derived from fat and equivalents of 5α-androst-16-en-3α-ol for samples from salivary glands. The coefficient of variation in this method using salivary glands is less than 4%. Good correlations ($R = 0.8$) have been found between off-aroma and off-flavour sensory scores versus the total 16-androstene steroid levels in salivary glands, by a trained sensory panel (Squires *et al.*, 1991).

1.7.3.2. Quantitation of skatole by colorimetric assay

A rapid colorimetric assay for the detection of skatole has also been developed (Mortensen and Sorensen, 1984). The method is based on the analysis of skatole and is adapted to an automated system in which 150 samples per hour can be analysed. Danish slaughter houses have been equipped to carry out rapid skatole analysis (Sandersen, 1993). The method of analysis is not very specific; compounds with similar chemical characteristics may interfere

with the analysis and therefore the results are presented as skatole equivalents (SE units). Hansen-Moeller and Andresen (1994) reported that this "skatole equivalents" method has a high correlation (R = 0.975) with skatole using the HPLC procedure by Hansen-Moeller, (1994). The rapid measurements for skatole were carried out using this automated colorimetric method in the large European study conducted by Walstra *et al.*, (1999).

1.8. Electronic Noses

The "electronic noses" are based on a technology that incorporates an array of sensors each of which has partial specificity and thus produces an odour fingerprint that can be identified by a pattern recognition system (Strike *et al.*, 1999). The series of chemical sensors respond to the volatile chemicals within a headspace over a sample. They often respond to the same chemicals that are responsible for the sensation of smell in humans but importantly they can also respond to other chemicals that are odourless to humans (Gardner, 1996).

A current issue with boar taint is finding a rapid effective mechanism capable of sorting tainted pig carcasses for quality control purposes. Several studies found that the direct use of the analytical concentrations of androstenone and skatole are an inadequate basis for sorting due to the presence of other compounds (Babol *et al.*, 1996; Xue *et al.*, 1996a; Annor-Frempong *et al.*, 1998; Jeremiah *et al.*, 1999a). Different combinations of these compounds could be unique in their characteristics, implying that the responses need to be measured and used as a basis for an effective classification system. It is therefore important not only to identify the compounds that contribute to taint, but rather develop a system capable of measuring the complete odour profile of samples. A well-defined odour profile without taint should be compared with the odour profiles of a sample being analysed and any significant deviation from the non-tainted profile would result in the rejection of the relevant carcass (Haugen and Kvaal, 1998).

1.8.1. Different sensor types

Gas sensors are based on: (i) physical or chemical adsorption and desorption, (ii) optical absorption or (iii) chemical reactions that can take place on the surface and/or in the bulk of the sensor material. A series of different detection principles can be used in chemical gas sensors which are: heat generation, conductivity, electrical polarisation, electrochemical activity, optical properties, dielectric properties and magnetic properties. The most commonly used sensor technologies that have shown to be successful in food analysis can be

divided into two major groups, the hot and cold sensors. The most frequently used sensors in commercial instruments are the metal oxide semiconductors (hot sensors) and the organic polymers (cold sensors).

1.8.1.1. Hot sensors

The "hot sensors" consist of metal oxide semiconductors (MOS) and metal oxide semi-conducting field effect transistors (MOSFET) which operate at elevated temperatures. The MOSFET sensor consists of a doped semiconductor and an insulator (oxide) covered by a catalytic metal (Lundstrom *et al.*, 1990). Variation in the gaseous odour is indicated by the output signal, based on a change of potential in the sensor due to electrical polarisation, when molecules react on the catalytic surface. The MOSFET sensors operate at temperatures between 100-200°C. The MOS sensors (Gardner *et al.*, 1991) rely upon the reaction between adsorbed oxygen on the oxide surface with incoming molecules. In this case the output signal is derived from a change in conductivity of the oxide caused by a reaction with the volatile compounds. These sensors operate at a higher temperature of 200-500°C. In both sensor types, however, reducing or oxidising compounds interact with the sensor surface. Their selectivity and sensitivity characteristics are dependent on the temperature and choice of metal (Haugen and Kvaal, 1998).

1.8.1.2. Cold sensors

Cold sensors operate at ambient temperature. They consist of the conducting organic polymers (CP), oscillating sensors, optical sensors or electrochemical cells. Conducting organic polymers are made of polymers such as polypyrrole, polyaniline and polythiophene (Bartlett *et al.*, 1989) and under the right conditions, are electrically conductive. These polymers are deposited between two electrodes and used as gas sensors. A change in the conductivity of the polymer results from exposing the sensor to a volatile component. This change may be monitored by passing a current between the two electrodes and observing the change in the voltage across the conductive polymer (Gardner, 1996). By altering the functional groups or the structure of the polymer and using different doping ions, the selectivity and the sensitivity of the polymers can be altered.

Conducting polymer electronic noses have a sample vessel that is manufactured from an odour-free material and is of sufficient size to hold several grams of sample. The instrument produces a seal between the head and vessel and is capable of purging the sample with a

reference gas. When exposed to a vapour containing a chemical to which the sensor is responsive, each sensor exhibits a characteristic response, dependent upon the chemical interactions between the sample and the sensor. The data collected from the sensor array for a particular sample can be interpreted as a pattern of responses, or "fingerprint" representative of that sample (Gardner 1996).

Oscillating sensors are based on the principle, that firstly, there is adsorption of molecules onto a sensing layer. Frequency waves are then generated down the surface of a quartz or silica substrate onto which a thin coating has been applied. Upon exposure to an analyte the gas molecules adsorb onto the sensing layer and there is a change in the wave frequency. This is due to the increase in mass and sometimes a change in the viscosity of the sensing layer (Gardener, 1996). Examples of these are the surface acoustic wave (SAW) and the quartz micro balance (QMB) also called bulk acoustic wave (BAW) sensors. QMB sensors (Nakamoto *et al.*, 1995) consist of a piezoelectric quartz oscillator coated with a sensing membrane, whereas the SAW (Liron *et al.*, 1997) sensors consist of two pairs of finger structure electrodes fabricated onto a piezoelectric substrate with a sensing layer between them. The selectivity and sensitivity of the sensor is determined by the composition of the coated sensing layer and the frequency of operation. SAW sensors operate at much higher frequencies such as 50-1000 MHz compared to the QMB sensors that operate at 5-30 MHz (Haugen and Kvaal, 1998).

Another type of sensor that is used in electronic noses is the optical gas sensor. In these sensors, a light source excites the gas that ultimately results in a signal from optical properties either from absorbance, reflectance, fluorescence or chemiluminescent reactions. One other type of sensor is the electrochemical cell consisting of several electrodes and an electrolyte. The gas molecules are either oxidised or reduced at the working electrode, whilst the opposite reaction takes place at the counter electrode. The reaction between the analyte generates a voltage between the electrodes which is measured as the output signal.

More recently the SAW and QMB sensors have been implemented in commercial instruments. Commercial hybrid electronic noses are also being produced, by combining different kinds of sensor technologies in the same instrument, which can be adapted for many different applications. The response and recovery times for any of the sensor types will be dependent on the particular compound and its concentration. However, given that the various sensor types operate under different conditions of temperature and utilise different carrier gases etc, it remains to be seen how useful these hybrid instruments will be in the years ahead

(Haugen and Kvaal, 1998). In general, the hot sensors are more robust and less sensitive to moisture with their sensitivity ranging from 100 ppm to 0.1 ppm (Gardner and Bartlett, 1994). The conducting organic polymer sensors and SAW sensors are more sensitive, with an ability to detect volatiles down to ppb levels (Gardner, 1996; Shaffer *et al.*, 1998) however conducting polymers are also sensitive to moisture (Schaller *et al.,* 2000).

1.8.2. Critical factors in odour measurement using electronic nose

Flavour of foods is made up of two components; the volatile and the non-volatile components. The non-volatile compounds react with the biological sensors on the tongue contributing to the taste sensation. The volatile compounds which interact with sensors in the nose are responsible for odour and are by far the most important in defining the flavour of a product (Manual on sensory testing method, 1986). Other factors that can affect odour, such as enzyme and chemical oxidation, can lead to rancid like changes which are present in high lipid containing products due to the formation of aldehydes and ketones. All of these compounds are typically present in low concentrations in the vapour phase, and the human can detect these at the parts per billion level or even lower. It is therefore necessary that any sensor used in an electronic nose must respond to the different functional groups and be able to detect these at similar concentrations to the human (Pendick, 1997).

The analysis of odour vapours is also dependent on other factors. The moisture content and the temperature of the sample is important, as well as the temperature at the time of measurement. The shape of the free space and the speed of air and flow pattern in the measurement room may cause some variation. The possible contamination of the headspace with minuscule solids and the drift caused by ageing effects of the sensor must be monitored. Finally, the method of processing and interpretation of the data requires careful consideration (van Dijk 1995).

1.8.3. Analysis of androstenone and skatole using electronic nose

Annor-Frempong *et al.,* (1998) evaluated the ability of a conducting polymer electronic nose (*e-NOSE*) to discriminate between different concentrations of skatole, androstenone and also mixtures of skatole and androstenone. They investigated the responses of the *e-NOSE* to odour intensity levels and compared these results with those determined by a trained sensory panel. Samples were prepared with various concentrations of skatole and androstenone in odourless, partially hydrogenated vegetable oil. The odour intensity of these samples was determined using a trained, ten-member sensory panel. The same samples were evaluated by

the *e-NOSE* for 60 s in triplicate at room temperature. Both the odour intensity levels and the responses from the 12 sensors of the *e-NOSE* were analysed using principal component analysis (PCA) and multiple linear discriminant analysis (MDA).

The results showed that the *e-NOSE* could discriminate between increasing levels of skatole and increasing levels of androstenone as did the sensory panel. However, the ability of the *e-NOSE* to differentiate between androstenone and skatole combinations was not as discriminatory as the sensory panel (Annor-Frempong *et al.*, 1998). This study however showed good correlation between *e-NOSE* sensor responses and the sensory panel studies with regard to taint, implying the possibility for its use in identifying tainted carcasses.

An electronic nose with five commercially available metal oxide semiconductor sensors was used to analyse adipose tissue grouped into two androstenone concentrations which were <0.7 and >1.7 ppm. The statistical processing of the data indicated that 84.2% of the samples were classified in their respective groups (Bourronet *et al.*, 1995).

1.9. Other compounds contributing to odour profile of pig fat

The sorting of carcasses according to fat androstenone and skatole concentrations does not always account for all the odours in tainted meat when compared with the sensory attributes of meat, from castrates and females pigs. This implies that other malodorous compounds are likely to contribute to taint (Babol *et al.*, 1996; Xue *et al.*, 1996a; Jeremiah *et al.*, 1999a). A possible source of other odorous compounds is the lipid oxidation of fatty acids during the cooking process.

Food oils and fats contain mixtures of saturated and unsaturated fatty acids esterified to glycerol. There are three main groups of fatty acids: saturated, mono-unsaturated and poly-unsaturated fatty acids (PUFAs). A large proportion of the fatty acids present in oils and food lipids are palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids (Saxby, 1996). Oxidation of saturated fatty acids is extremely slow at low temperatures. However, at elevated temperatures, for example in the frying operation, oxidation of saturated fatty acids can take place. It is generally the unsaturated fatty acids that are more susceptible to oxidation than the saturated fats. The double bonds present in unsaturated fatty acids cause them to be more chemically reactive than the saturated fatty acids. The greater the extent of unsaturation, the greater the reactivity (Saxby, 1996). The position and geometry of the double bonds also affect the chemical reactivity. The *cis*

isomers of acids oxidise more readily than the corresponding *trans* isomers, and conjugated double bonds are more reactive than non-conjugated ones (Saxby,1996). In general, the fatty acid composition affects the odour profile, and the fatty acid composition of the lipids is also related to the components of the diet (Timon *et al.*, 2001).

In foodstuffs, deterioration of unsaturated lipids can occur both by enzymic and non-enzymic (oxidative) mechanisms. Oxidative deterioration of food lipids generally occurs between oxygen and unsaturated fatty acids, which may or may not be a part of the oil or the phospholipid. This process of oxidation leads to the development of various unpleasant odours and flavours, generally called "rancid" in oils and lipid containing foods, and thus renders them unpalatable. In some foods, such as, butter, cheese, cucumber and cooked chicken, a limited degree of lipid oxidation under certain conditions, is desirable in order to produce typical and characteristic flavours of pleasant fried food aromas.

1.9.1. The lipid oxidation process

The removal of a hydrogen atom from a methylene carbon in a fatty acid (RH) is the first step in the lipid oxidation process. The RH represents the unsaturated fatty acid with H attached to the allylic carbon. This process becomes easier as the number of double bonds in the fatty acid increases, which is why the polyunsaturated fatty acids are particularly susceptible to oxidation (Halliwell and Chirico, 1993). During the process of oxidation $O_2^{\bullet-}$, H_2O_2 and $HO^{\bullet}$ are produced at a low rate. The hydroxyl radical ($HO^{\bullet}$) is the most potent oxygen radical encountered in biological systems. The first step in lipid oxidation can be catalysed by a hydroxyl radical ($HO^{\bullet}$) or by certain iron-oxygen complexes (e.g. ferryl or perferryl radicals)

$$RH + HO^{\bullet} \rightarrow R^{\bullet} + H_2O \qquad 4.1$$

The fatty acyl radical ($R^{\bullet}$) reacts rapidly with O_2 to form a peroxyl radical ($ROO^{\bullet}$):

$$R^{\bullet} + O_2^{\bullet} \rightarrow ROO^{\bullet} \qquad 4.2$$

The $ROO^{\bullet}$ is more highly oxidised than the fatty acyl radical or the fatty acid itself and will therefore preferentially oxidize other unsaturated fatty acids and propagate the chain reaction (Morrissey *et al.*, 1998)

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet} \qquad 4.3$$

The primary products of lipid oxidation, called hydroperoxides (ROOH), are tasteless and odourless. It is the decomposition of hydroperoxides and secondary volatile oxidation products that give rise to off flavours in oils, fats and lipid containing foods. Lipid hydroperoxides formed in the propagation reaction are both products of oxidation and substrates for further reaction with Fe^{2+} and Cu^{2+} to yield other $ROO^{\bullet}$ and alkoxyl radicals ($RO^{\bullet}$) (Morrissey *et al.*, 1994).

The highly reactive $ROO^{\bullet}$ and $RO^{\bullet}$ can initiate further reactions, much like those in equation 4.3 and the following:

$$RO^{\bullet} + RH \rightarrow ROH + R^{\bullet} \qquad 4.4$$

The $RO^{\bullet}$ can also undergo β-scission and subsequently degrade to an alkyl radical ($R'CH^{\bullet}{}_2$) and a range of aldehydes (R"CHO) depending on the particular hydroperoxide initially present (Morrissey *et al.*, 1994):

$$RO^{\bullet} \rightarrow R'CH_2^{\bullet} + R''CHO \qquad 4.5$$

The alkyl radical ($R'CH_2^{\bullet}$) can initiate further chain reactions resulting in the formation of ethane and pentane, whilst aldehydes, such as hexanal, malondialdehyde and 4-hydroxynonenal, can react readily with ε-amino groups of proteins to yield Maillard-type complexes (Morrissey *et al.*, 1998). It is these compounds that will ultimately affect the odour/flavour of foods.

There are many factors that affect the rate of oxidation of fats or food lipids such as the degree of unsaturated lipid, transition metals, biological catalysts and heat (Saxby, 1996). Factors that may slow down the rate of oxidation are anti-oxidants, synergists and storage under an inert gas (Saxby, 1996). Different foods may contain various non-lipid components that can also affect the rate of lipid oxidation. The effects of these factors on lipid oxidation are discussed below.

1.9.2. Factors affecting the rate of lipid oxidation

The oxidation rate increases, as the temperature increases (Saxby, 1996). However, in multi-component oil and lipid containing foods, the effect of temperature is not simple. This is because the oxidation rate of the various reactions that comprise the chain reaction do not vary equally as the temperature is changed (Saxby, 1996). There may be a critical temperature above which the rate of one reaction becomes faster than that of a second reaction, both being responsible for oil deterioration (Labuza and Kamman, 1983). Therefore, the rate limiting reaction at one temperature may not be the limiting reaction at a different temperature. For example, linoleic acid at 25°C forms mainly the *trans-trans* hydroperoxide, where at 0°C mainly the *cis-trans* hydroperoxide is formed (Curda and Poulsen, 1978).

The length and type of storage are major components affecting lipid oxidation (Zanardi *et al.*, 2000). The concentration of oxygen also affects the rate of oxidation. The rate of oxidation is approximately proportional to the oxygen pressure when the oxygen pressure is low and conversely, it is independent of the oxygen pressure when the oxygen supply is unlimited (Saxby, 1996). The surface area also influences the effect of oxygen pressure on the oxidation rate. The rate of oxidation increases in proportion to the fat surface exposed to oxygen or air (Saxby, 1996). Any reduction in the oxygen pressure becomes less effective, if the ratio of available surface-to-fat volume increases.

The rate of lipid oxidation in foods can also depend strongly on the moisture content of foods (Karel, 1980). The parameter water activity (a_w) which is equal to p/p_o where p represents the partial pressure of water in food and p_o represents the vapour pressure of water, governs the distribution of water between different food components (Saxby, 1996). Oxidation proceeds rapidly in dried food where the moisture content is low. Lipid oxidation is retarded when foods have a water activity of 0.3. The presence of moisture is thought to decrease the catalytic activity of trace metals, by quenching free radicals, and by promoting non-enzymic browning, which produces compounds with anti-oxidant activities (Saxby, 1996). All these factors aid in the reduction of lipid oxidation activity. At higher water activities (a_w), the rate of oxidation is accelerated probably due to the fact that the catalysts present in foodstuffs are mobilised to a greater extent (Saxby, 1996).

Trace metals catalyse the oxidation of fatty acids in some foodstuffs. In most foods, trace metals occur naturally in either free or bound forms (Saxby, 1996). Copper, iron, manganese and nickel are all examples of transition metals, possessing two or more valency states with a suitable oxidation-reduction potential to catalyse lipid oxidation. At very low concentrations, for example 0.01 ppm for copper, if present in the active state, can accelerate the rate of oxidation to give rise to off-flavours in soyabean oil, rapeseed or highly unsaturated fish oils (Saxby, 1996). In addition, heavy metals, such as lead or cadmium, further catalyse the decomposition of hydroperoxides formed during lipid oxidation resulting in the development of off-flavors and odours. Heavy metals can also be involved in further oxidation of the secondary products (Pokorny, 1987).

Factors that slow down the rate of oxidation in oils and food lipids are termed anti-oxidants which also protect oil-soluble vitamins, carotenoids and other nutritive ingredients. Undesirable changes such as discoloration in meat products that are caused by oxidation are also delayed (Gregory, 1984; Miles *et al.*, 1986). Phenolic anti-oxidants, whether naturally occurring such as tocopherol or flavonoids, or whether they are part of a group of synthetic compounds such as butylate hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroxyquinone (TBHQ) or alkyl gallates terminate and inhibit the free radical chain reactions of oxidation (Kocchar, 1988; Scott, 1993). This is achieved by quenching free radicals and by acting as hydrogen donors. There are also chelators such as citric acid, phosphoric acid, EDTA, amino acids etc., that also promote the action of phenolic anti-oxidants. The reaction mechanisms of anti-oxidants are described:

$$AH + RO^{\bullet} \rightarrow ROH + A^{\bullet} \qquad (4.1)$$

$$AH + ROO^{\bullet} \rightarrow ROOH + A^{\bullet} \qquad (4.2)$$

The $RO^{\bullet}$, $A^{\bullet}$, $ROO^{\bullet}$ represent free radicals. The anti-oxidant (AH) reacts with alkoxyl ($RO^{\bullet}$) or peroxyl radical ($ROO^{\bullet}$) preventing these radicals from further reacting with fatty acids. The RH and ROOH represent the unsaturated fatty acid and esters with H attached to the allylic carbon. The inhibitory reaction (4.2) is the most important and primarily influences the overall inhibition rate constant.

$$RH + A^{\bullet} \rightarrow AH + R^{\bullet} \qquad (4.3)$$

$$AH + RO^{\bullet} \rightarrow [ROO^{\bullet}\ AH]\ \text{Complex} \qquad (4.4)$$

The stable resonance hybrid of the anti-oxidant free radical $A^{\bullet}$ products of the termination reaction, and the products of the termination reactions (4.5) to (4.8), are incapable of initiation or propagation of the chain reactions and therefore prevent further oxidation. The products of the termination reaction are AA, RA, ROOA (Saxby, 1996).

$[ROO^{\bullet}AH]$	$\rightarrow$	Non radical products	(4.5)
$A^{\bullet} + A^{\bullet}$	$\rightarrow$	AA	(4.6)
$A^{\bullet} + R^{\bullet}$	$\rightarrow$	RA	(4.7)
$A^{\bullet} + ROO^{\bullet}$	$\rightarrow$	ROOA	(4.8)

1.9.3. Products of lipid oxidation

1.9.3.1. Aldehydes and ketones

In the absence of anti-oxidants and under vigorous conditions, lipid oxidation will proceed to produce by-products such as ketones, short chain fatty acids, alcohols and hydrocarbons and aldehydes. The aliphatic aldehydes are important volatile breakdown products because: (i) they can be major contributors to odours and flavours in food products, (ii) they often contribute to unpleasant odours, and (iii) at extremely low levels, many aldehydes contribute to the desirable flavours of many foods (Saxby, 1996). The creamy flavour of butter is derived from trace amounts (1 ppb) of 4-cis-heptenal which has a threshold level of 0.04 ppb (McGill *et al.*, 1977). Octa-decadienoic acid has been reported to be the precursor of this aroma compound (Badings, 1984). The 2,4-decadienals have also been reported to have oily/fatty, deep frying odours (Saxby, 1996). The relationships between the molecular structure of aldehydes and flavour intensity is rather complex and does not follow any particular pattern. The odours of *cis*-3-hexenal and *cis*-4-heptenal, for example, are much more intense than the corresponding *trans*-3- hexenal and *trans*-4-heptenal. In the case of 6-nonenal the situation is reversed with the *cis* isomer having a less intense flavour compared to the *trans* isomer (Keppler *et al.*, 1977).

Aldehydes such as hexenal, pentanal, heptenal and octanal which arise from unsaturated fatty acid oxidation are abundant in the subcutaneous fat of hams (Grosch, 1987). Owing to its abundance in foodstuffs, linoleic acid and its glycerides are among the most important precursors to many aldehyde compounds (Grosch, 1987). The formation of two major aldehydes, hexanal and 2,4-decadienal can be explained by the thermal decomposition of

linoleate 1-OOH and 9-OOH hydroperoxides, respectively. Other aldehydes such as the epoxyaldehyde, have an extremely low odour threshold and have been shown to contribute to the warmed over flavour of stored beef (Konopka and Grosch, 1991). The aldehyde malonaldehyde, an almost odourless and flavourless compound, is often used as an indicator of the level of lipid oxidation that has occurred in foodstuffs.

Another major group of compounds arising from the oxidation of unsaturated fatty acid is the aliphatic ketones, which can also contribute to the desirable and undesirable flavours of oils and food products. The methyl ketones are produced from their precursors by hydrolysis and decarboxylation. The reaction is accelerated by heat. Langler and Day (1964) showed that methyl ketones, at concentrations far below their flavor threshold values, produce unpleasant off-flavours. Methyl ketones are also responsible for the development of off flavours in dairy products, vegetable oils and animal fats. Mookherjee *et al.*, (1965) suggested that the oxidation of aldehydes resulted in the formation of C3-C9 methyl ketones in stale crisps. The compounds 3-octanone and 5-undecanone have been found in cooked chicken (Nonaka *et al.*, 1967). Propanone is the most prevalent ketone in the subcutaneous fat of cured ham (Larick *et al.*, 1992) whilst the ketones 2-heptanone, 2-octanone, 2-nonanone and 2-decanone have been detected in the interior of the ham (Timon *et al.*, 2001). Methyl ketones are also formed in mould-ripened cheeses to produce desirable flavours, particularly when there are high concentrations of 2-heptanone and 3-nonanone (Badings, 1984; Kinsella and Hwang, 1976). These compounds along with short-chain fatty acids and lactones, are also important flavour constituents of butter aroma however, any imbalance among these substances can cause flavour defects.

The sensory thresholds of the methyl ketones can vary with carbon chain length and are significantly higher than those of their isomeric aldehydes. The odour thresholds of vinyl ketones are in the range of the most potent aldehydes. The flavours of vinyl ketones are usually more unpleasant than the saturated ketones. The metallic flavour detected in oxidised butter and safflower oil is caused by the presence of octen-1-one (Stark and Forss, 1962). Many reaction mechanisms have been suggested for the formation of this vinyl ketone from poly-unsaturated fatty acids (Hammond and Hill, 1964). The decomposition of linoleic hydroperoxide has been thought to give rise to 1-penten-3-one and the sharp, fishy odour in oxidised fats (Stark *et al*, 1967).

1.9.3.2. Other compounds

Aliphatic alcohols can also make contributions to the off-flavours produced by the oxidative deterioration of food lipid. Their sensory thresholds are significantly higher than those of the corresponding aldehydes and therefore their contribution to off-odour is small (Saxby, 1996). Many of the saturated and unsaturated alcohols however contribute to the desirable attributes of fruits and vegetables (Forss, 1972) and some are commercially important flavour and fragrant compounds. The flavour becomes stronger as the chain length increases, as for example trans-2-hexen-1-ol has a sweet wine odour whilst the higher C8-C12 alken-1-ols have fatty odours (Forss, 1972). Alcohols such as 1-heptanol, 1-octanol, 1-octen-3-ol have been detected in the interior of dry cured hams (Timon *et al.*, 2001). Pentanol has been reported to be derived from hexanal which arises from the decomposition of 13-OOH linoleate hydroperoxide. The reduction of off-odour and/or flavour can occur, even if a small amount of potent, off-flavour aldehyde, above the detection level, can be transformed into the alcohol, given that alcohols have a significantly higher flavour threshold compared to their corresponding aldehydes. The quenching of off-flavours arising from aldehydes by the addition of alcohol dehydrogenase and NADH to certain foods can be effective (Eriksson *et al.*, 1977).

In many oils, however, the presence of as little as 0.1% of free fatty acids (FFAs) can reduce the period of time that the oil has a natural resistance to oxidation (Hartman, 1975). This could be due to the FFAs altering the effectiveness of the naturally occurring phenolic anti-oxidants, thereby accelerating the decomposition of the hydroperoxides. The presence of relatively high amounts of FFAs in oils can increase the uptake of catalytic trace metals, such as Fe and Cu, from storage equipment, further enhancing the rate of oil oxidation (Saxby, 1996).

Short-chain aliphatic acids C1-C12 also play significant roles in the desirable aromas as well as in the off-flavours of many foodstuffs. Owing to the higher thresholds of fatty acids, they may not always be present in sufficient quantity to contribute to the off-flavours and odours arising from oxidative deterioration of unsaturated lipids (Saxby, 1996). They are however, produced during the oxidation of higher fatty acids and their glyceride esters. Horvat *et al.*, (1969) identified 12 fatty acids including C1 and C5-C9 from the oxidation of methyl linoleate at room temperature. Other types of acids such as keto, hydroxy and dicarboxylic acids have been isolated from heated frying oils (Kawada *et al.*, 1967). Keto and hydroxy acids are important precursors of desirable flavour/odour compounds. Unsaturated acids

such as 2- and 3- alkenoic acids are also formed when oils and fats are heated in the presence of oxygen (Kawada *et al.*, 1967). Some unsaturated lactones, for example gamma-lactone of 4-hydroxy-2-nonenoic acid, also contribute to the pleasant flavour of deep-fried chicken.

Saturated hydrocarbons are also known to possess high sensory threshold values and weak flavours. The C5-C10 alk-1-enes, alkynes and some alkadienals have been observed to have moderately potent flavours (Evans *et al*, 1971). Some hydrocarbons have been detected in the fat of dry cured hams and are associated with the unsaponifiable fraction of the pig diet (Rius *et al.*, 1999). Ethane and pentane are the major hydrocarbons produced by the thermal decomposition of linolenic and linoleic hydroperoxides (Dumelin and Tappel, 1977).

1.10. Issues addressed in thesis

The odour and flavour of pork, particularly that from males is formed by a complex interaction of a variety of chemicals. Hence it is not surprising there is some controversy over the exact nature of the chemical basis for taint. This uncertainty is a significant barrier to the development of chemical tests to identify tainted pork. There is a need for an objective and comprehensive method that has a correlation with the sensory evaluation of pork. Once this is achieved, the highly tainted carcasses can be removed from the slaughter line and redirected into other products less affected by taint such as processed meats. This would be a valuable contribution to the industry.

The current means by which the complete odour of a sample of fat, from a carcass, can be assessed is by human sensory analysis which is a subjective and expensive process, often leading to inconsistent results. In principle, the electronic nose is a good candidate as a possible equivalent of the human sensory analysis method of detection of off-odour and taints. This is a convenient starting point for the analysis of boar taint with potential for further adaptation for on-line analysis.

There is considerable debate as to whether androstenone or skatole is the more important contributor to taint. The purported advantage of the electronic nose is that it can detect the complete odour profile of the tainted/non-tainted fats and not just a few compounds. However, the electronic nose instruments must be rigorously evaluated for any methodological limitations. Ultimately, the response of the instrument needs to be validated by analysing the odour of tainted samples by conventional sensory methods. Complementary

technologies, such as GC analysis may also be able to produce more information on the presence of taint if a comprehensive approach is taken.

The literature is replete with sensory analysis studies which have been correlated with the analytical concentrations of androstenone and skatole. Some of these studies have been conducted with consumer and trained panels (Walstra *et al.*, 1986; Malmfors and Lundstrom, 1983; Diestre *et al.*, 1990; Patterson *et al.*, 1990; Bonneau *et al.*, 1992; Hansen Moeller and Godt, 1995). Some sensory studies have taken into account panellist sensitivity to androstenone, whilst others have not. Some GC-MS studies have been conducted which examine all the compounds detected in the odour profile of fat and not just androstenone and skatole (Viallon *et al.*, 1992). However, no study to date has: (i) conducted human sensory analysis on heated samples of fat from boars, (ii) taken into account androstenone sensitivity, and (iii) correlated these results with the GC-MS headspace analysis of the volatiles from the equivalent samples of fat. Such an approach would aim to detect all the naturally occurring compounds identified in the odour of pork including androstenone and skatole and determine the exact contribution of each compound to the overall sensory evaluation.

Key factors of such an approach should include both sensory and chemical analyses conducted at temperatures that reflect normal cooking conditions. Multiple regression between the sensory and chemical analyses of the complete odour profiles should be conducted, to determine the correlation of all the compounds with the odour of pork fat.

Chapter 2

ELECTRONIC NOSE ANALYSIS AND THE DETECTION OF TAINT

2.0. INTRODUCTION

The electronic nose consists of an array of chemical gas sensors with broad and partly overlapping sensitivity. The instrument measures the volatile compounds within a headspace emitted from a sample and combines this with a computerised multi-variate statistical data processing module (Gardner and Bartlett, 1994). Conducting organic polymers (CP), one of the most commonly used sensors, are based on two main classes of polymers, the pyrroles and the anilines (Bartlett *et al.*, 1989; Amrani *et al.*, 1995; Persuad *et al.*, 1996). Conducting polymers have the ability to detect volatiles down to ppb levels (Gardner, 1996; Shaffer *et al.*, 1998). The other main class of sensors, metal oxide, can detect volatiles at ppm levels (Gardner and Bartlett, 1994). In comparison, the odour threshold of the human nose may vary from 1000 ppm down to parts per trillion depending on the chemical compound and matrix (Belitz and Grosch, 1987). It is important to realise, however, that there are some differences between what is measured by an electronic nose and the human perception of odour or flavour. The volatile compounds measured by an electronic nose will include many that are not detected by the human nose. In humans however, smell is combined with taste to give the sensation of flavour (Haugen and Kvaal, 1998).

Electronic noses have been used in the food industry for many products and applications including: tomato quality (Sinesio *et al.*, 2000), vinegar assessment (Anklam *et al.*, 1998), apple maturity (Young *et al.*, 1999), milk products (Zondervan *et al.*, 1999), cooked chicken (Siegmund and Pfannhauser, 1999), yeast fermentation (Bachinger *et al.*, 1998), spices (Madsen and Grypa, 2000), oil quality (Bazzo *et al.*, 1998) and orange juice (Bazemore and Rouseff, 1998). In the meat industry a range of studies have been conducted and reviewed by Haugen and Kvaal (1998). An application where the electronic nose seems to have great potential is the detection of spoilage and off-flavour of meat. Boar taint is perceived as a distinctly unpleasant, perspiration or urine-like sex odour when fat or meat from some entire mature boars is heated or eaten by the consumer (Malmfors and Lundstrom, 1983). The two major compound groups considered to be responsible for causing boar taint are the gonadal 16-androstene steroids and the indoles, which are produced in the hindgut of the pig by the metabolism of tryptophan.

Annor-Frempong *et al.*, (1998) used an electronic nose with 12 conducting polymer sensors combined with linear discriminant analysis to classify tainted samples. The samples contained up to 4 ppm androstenone and 1.6 ppm skatole in model systems, and there was a respective 84% and 90% correct classification, for these compounds. Bourrounet *et al.*, (1995) used five different Taguchi metal oxide sensors, which are more resistant to moisture compared to CP sensors (Gardner and Bartlett, 1994), to measure pork fat with different levels of androstenone. An 84% successful classification of the samples was achieved according to low (<1.7 ppm) and high (>1.7 ppm) androstenone content. Berdague and Talou (1993) also utilised a semi-conductor gas sensor to analyse back fat from female and male pigs and found a significant difference in sensor response between the sexes.

The major problems related to gas sensors are poor sensitivity, instability, susceptibility to humidity and a limited lifetime. In particular, the sensors react with polar molecules and therefore will respond to water vapour. Meat and meat products have a range of water activity and therefore during analysis, a variable amount of water vapour will be generated in the headspace. This problem may be addressed in several ways. One method is to run samples of purified water at the same relative humidity as the real samples and then make a water background correction. The water response will therefore be eliminated and the signal from the analytes of interest acquired. This procedure is effective provided that the response falls within the linear range of the sensors. A variety of gas sensors may exhibit a non-linear response, so this alternative should be handled in a critical way. Addition of a dessicant, or humidifying the carrier gas, to the same humidity of the sample headspace, or to a maximum humidity, have been proposed as alternative solutions for this problem (Haugen and Kvaal, 1998).

The sensors also appear to discriminate between factors when the variation between samples is large. However, when the variation is small their ability to successfully discriminate is not as successful. In such cases linear models may not be effective. The heterogeneity of real samples will also affect the reproducibility and precision of analysis. This is particularly important with the replicates of raw meat samples that are very heterogenous in nature. It is therefore important that certain parameters such as sample treatment, sample headspace, temperature, equilibration time, sample quantity and surface area are investigated prior to analysis (Kolb, 1980; Gardner , 1996).

In this study the electronic nose (*e-NOSE*) was used to classify and quantitate boar taint. Five preliminary experiments were conducted to determine the efficacy of the *e-NOSE* in measuring tainted and untainted samples provisionally classified according to androstenone

and skatole concentrations. The first aim was to determine if the *e-NOSE* sensor readings from samples of fat from gilts, castrates and boars with high and low taint, were related to androstenone and skatole levels. These experiments showed that the *e-NOSE* sensor readings did not relate to androstenone and skatole concentration, which were well known to be odorous chemicals. The next step was therefore to conduct some method development with regard to sample quantity, surface area, sample headspace and equilibration time to refine the method of analysis. This was followed by an investigation into whether the *e-NOSE* sensor readings were related to the concentration of the androstenone pure standard, dissolved in mineral oil. To check whether there was a relationship between androstenone and the sensor scores, a cause and effect experiment was analysed whereby the samples of fat were spiked with androstenone and analysed. Further studies analysing the sensor response in the presence and absence of water to control the variation in relative humidity were also investigated.

2.1. Materials and Methods

2.1.1. Sample, collection and preparation

The samples of fat from the subcutaneous abdominal region of chilled carcasses, were collected from a commercial abattoir, 24 h post slaughter. The live weight of these Large White × Landrace pigs ranged from 80-140 kg. The samples of fat were placed in a snap lock plastic bag and were analysed on the day of collection. Approximately, 400 g of fat was collected from each animal. For each experiment the fat was removed from the fridge and the surface layer of the fat, along with any blood vessels, was discarded. For each experimental sample, 20 g of fat from one animal was cut into 5 g pieces of approximately equal shape, placed into a 500 mL borosilicate beaker *(e-NOSE* beaker) and allowed to equilibrate at room temperature, i.e. 20-22°C, for 30 min. The order of analysing the samples from each animal, was randomised prior to analysis.

2.1.2. Calibration and e-NOSE analysis

The electronic nose instrument was an *e-NOSE* ™ 4000 Sensor Array, Neotronics Scientific, *(e-NOSE)* fitted with 12 conducting polymer sensors in a modular array. The individual sensors varied slightly in carbon chain length, however, the exact composition of the sensors was proprietary information of Neotronics Scientific. The sensors though were known as Type: 258, 259, 260, 261, 262, 263, 264, 278, 283, 297, 298, and 301 which are similar to those described in Maul *et al.*, (2000). The sensor head was continuously maintained at 30°C,

and the *e-NOSE* beaker and sample (vessel) was exposed to a temperature of 30°C throughout the temperature stabilising and acquisition period. The *e-NOSE* 4000, manual states that an RH humidity sensor and a temperature sensor were in the *e-NOSE* 4000 to acquire data on the RH and the temperature of the samples. The instrument, which was placed in a room with normal air conditioning standards, also contained the *e-NOSE* 4000 Series Software, Windows 95 version, by Neotronics Scientific.

Prior to analysis the *e-NOSE* beakers were washed with 0.2% Decon and rinsed at least 6 times with distilled and deionised water (dd H_2O). The instrument was auto-calibrated using 80 mL of 1:4 (v:v) dd H_2O:1,2-propanediol (99%; Sigma, Castle Hill, NSW, Australia). A calibration was carried out on a weekly basis or prior to the commencement of an experiment.

The *e-NOSE* analysis initially involved the purging of the gas phase from the vessel and the sensor head for 7 min. The purge gas was ultra high purity nitrogen at a flow rate of 1 mL min^{-1}. The vessel and sensor head were then equilibrated for 7 min and the temperature stabilising time was 1 min. The sample acquisition lasted for 4 min and the point of analysis was then taken at several time points. At each point of the analysis, the percent change in the resistance of each of the 12 sensors over the acquisition period was automatically calculated by the *e-NOSE* 4000 series software as indicated in the *e-NOSE* 4000, Manual.

2.1.3. HPLC analysis

Androstenone and skatole were measured by the method of Hansen-Moeller (1994) with the following modifications. Samples were rendered by microwave and 200 µL samples extracted by vortex mixing with 1 mL of 95:5 (v:v) mixture of methanol (Merck, Kilsyth, Vic. Australia) and water containing 0.25 ppm androstanone (Sigma, Castle Hill, NSW, Australia) and 0.05 ppm 2-methyl indole (Sigma, Castle Hill, NSW, Australia) as internal standards. The extract was centrifuged at 2000 × g for 10 min at 0°C, and the supernatant placed in sample vials. Samples were derivatized according to the method of Hansen-Moller (1994) except that boron trifluoride and dansyl hydrazine were premixed before adding 12 µL to 20 µL of the extracted sample.

Chromatography was conducted using a Waters 2690 Separations module (Waters, Rydalmere, NSW, Australia), on a 4 × 100 mm, 3 µm particle size Hypersil ODS column (Hewlett Packard, Australia) and the peaks were detected by a Waters 474 fluorescence detector. The limits of quantitation of the assay were 0.2 to 2.4 ppm and 0.03 to 0.6 ppm for

androstenone and skatole, respectively. The intra-assay variation for the samples with low and high concentrations was 5% and 2% for skatole and 6% and 8% for androstenone, respectively. Inter-assay variation for samples with low and high concentrations was 5% and 7% for skatole and 10% and 12% for androstenone, respectively.

2.1.4. Study 1: Investigation of fat for taint compounds

2.1.4.1. Sample, preparation and treatment

The adipose tissue was collected from 20 Large White x Landrace pigs, on the day of slaughter, from a commercial abattoir. Samples were collected over two days. On the first day of collection the samples were collected from gilts (n = 3), castrates (n = 3), and entire boars (n = 6). On the second day the samples were collected from entires (n = 4) and gilts (n = 4). The samples of fat were kept at 4°C overnight, then analysed the following day. The other sample handling procedures are described in Section 2.1.1.

2.1.4.2. e-NOSE analysis

The vessel and sensor head were purged for 5 min. This is the only variation from the main method described in Section 2.1.2.

On the first day of *e-NOSE* analyses, the samples of fat from the gilts and castrates were analysed before the entire males. Thus the analyses were carried out in three batches, namely: day 1 gilts and castrates, day 1 entires, and all day 2 pigs.

2.1.4.3. Statistical analysis

The statistical analyses were conducted using GenStat® 5, Release 4.1 for all studies unless otherwise indicated. Mutiple regression analysis of each sensor score versus the androstenone and skatole concentrations was carried out. The regression was also carried out for other terms such as: (i) the relative humidity of the vessel and the instrumental environment (RH), (ii) the temperature of the vessel (vessel temperature), (iii) the temperature of the sensor module (head temperature), (iv) the differences between sex (sex), (v) the grouping according to the concentration of androstenone and skatole above the sensory thresholds (chemical grouping) and (vi) the sample batches as indicated in Section 2.1.4.2 (sample/batch variation). Interactions and quadratic responses were investigated, as appropriate (Mead *et al.*, 1993).

A statistical method known as principal component analysis (PCA), reduces a larger number of data variables to a smaller number of transformed variables This method is particularly important when a large number of variables are highly correlated due to a large proportion of redundancy in the original variables. The PCA will find the best linear combination of the data to produce the first index, which represents the largest amount of variation, the second index represents the second largest amount of variation and the third index represents the third etc, etc. (Manly, 1986).

The sensor score data were also summarised using principal components. From these principal components it was possible to group the sensors into three groups, so that the three group means, summarised nearly all of the sample variation between sensors. Multiple regression analysis, similar to that carried out for the individual sensor scores, was carried out for each of the three sensor group means.

2.1.5. Study 2: Investigation of vessel purge and equilibration times

2.1.5.1. Sample, preparation and treatment

The vessel purge and equilibration experiment investigated the effect of these two factors on the sensor response to porcine adipose tissue. The design of the experiment was a 4 × 3 factorial design with four vessel purge times of 3, 5, 7 and 10 min and three vessel equilibration times of 4, 7 and 10 min, in a three replicate randomised block design (Mead *et al.*, 1993). The samples of fat from the carcasses of entire males were collected for three days and then prepared as described in Section 2.1.1. Each block consisted of the fat from a carcass collected on a single day.

2.1.5.2. e-NOSE analysis

The *e-NOSE* method of analysis had vessel purge times of 3, 5, 7 and 10 min and vessel equilibration times of 4, 7 and 10 min according to the factorial design of the experiment. This is the only variation from the main method described in Section 2.1.2.

2.1.5.3. Statistical analysis

A factorial randomised block analysis of covariance (Mead *et al.*, 1993) with RH as the covariate, was carried out for the overall sensor mean and the mean of each of the three

sensor groups. The effect of equilibration time and its interaction with the purge time were divided into orthogonal polynomial components (Mead *et al.*, 1993). The effect of purge time and its interaction with the equilibration time was divided into a 3 min versus longer time, such as the combination of the 5, 7 and 10 min, intervals. The remaining effect of differences between the 5, 7 and 10 min purge times was then examined.

The analysis of covariance interpretation depends on the assumption that the treatments have little effect on the RH covariate. To check for this possibility, the analysis of variance was also carried out on RH, as a response variate and the results tabulated. It was found that there was little treatment effect on RH, and hence it was appropriate as a covariate (Table 2.11).

2.1.6. Study 3: Investigation of sensor response to fat amount and surface area

2.1.6.1. Sample, preparation and treatment

The mass of fat versus the surface area of the fat was investigated. The experiment was a 3 × 3 factorial design with three sample masses of 10 g, 20 g and 30 g and three surface areas of 1 g, 5 g and a whole sample, which were in three randomised blocks. Each block was associated with the fat collected from a single carcass along with the *e-NOSE* analysis carried out per day. The samples of fat from the carcasses of entire males were collected for three days. Each experimental unit was a single *e-NOSE* analysis of a sample of fat. The preparation method was otherwise as described in Section 2.1.1.

2.1.6.2. e-NOSE analysis

The *e-NOSE* analysis was as described in Section 2.1.2.

2.1.6.3. Statistical analysis

The experiment with the three surface areas by the three masses of fat, factorial, in three randomised blocks, was statistically analysed for mean sensor scores. This was carried out both by adjusting and not adjusting for the effects of relative humidity. Surface area strongly affected relative humidity and therefore the adjusted analyses were carried out using a full regression analysis rather than using the standard covariance adjustment approximation, to a balanced analysis of variance (Cochran and Cox, 1957). As there was no evidence of any surface area by mass of fat interaction on any measurement, predicted means and P-values are presented for the main effects of surface area and mass of fat. These predicted means are

adjusted for the blocking effect, which was a different sample over three days. The predicted means were also adjusted for the effect of relative humidity. A model consisting of the full factorial treatment effects, blocking effects and a relative humidity effect was developed. A residual term was constructed from deviations from the model.

2.1.7. Study 4: Investigation of androstenone in mineral oil

2.1.7.1. Sample, preparation and treatment

Androstenone dissolved in mineral oil at various concentrations was analysed. The design of the experiment was a randomised block design with four treatment levels. The method required 20 mL of the androstenone (Sigma, Castle Hill, NSW, Australia) dissolved in mineral oil (Pharmacia, Rydalmere, NSW, Australia) at a final concentration of 0, 1.0, 2.5 and 5.0 ppm, which was then poured into the *e-NOSE* beakers. The samples were randomised for concentration levels and there were two aliquots per concentration level. Each aliquot was measured four times, from which the average result from these analyses was acquired. There were 32 samples analysed in one day and samples were analysed over two days.

The same experimental procedure was also repeated in the presence of water. The only difference being that 10 mL of the androstenone solution of 0, 1.0, 2.5 and 5.0 ppm in mineral oil was poured into a 100 mL beaker which was then placed into the *e-NOSE* beaker that already contained 20 mL of dd H_2O. An equivalent number of samples as described above were analysed over the two days.

2.1.7.2. e-NOSE analysis

The *e-NOSE* analysis was as described in Section 2.1.2.

2.1.7.3. Statistical analysis

The overall sensor mean and group mean sensor scores were analysed by randomised block analysis of variance.

2.1.8. Study 5: Investigation of samples of fat spiked with androstenone

2.1.8.1. Sample, preparation and treatment

The samples of fat collected daily from gilts were spiked with increasing concentrations of androstenone and analysed. The design of the experiment was a randomised block design with three treatment levels, conducted over two days with each day being a replicate. Nineteen grams of the fat cut into 1 g pieces was placed into the *e-NOSE* beaker. One mL of androstenone dissolved in mineral oil to make the final concentration of 0, 0.5 and 2.0 ppm was added to the fat. A glass rod was used to stir the mineral oil and the fat. The other details of the collection and preparation of the samples are described in Section 2.1.1.

A second method was investigated, which differed only in that the samples were analysed in the presence of water. With this second method, 10 g of fat was cut into approximate 1 g pieces and 9.5 g of the cut fat was weighed into a 100 mL beaker. Half a millilitre of androstenone dissolved in mineral oil was added to the fat to make the final concentration of 0, 0.5 and 2.0 ppm. A glass rod was used to stir in the mineral oil. Twenty millilitre of dd H_2O was poured into the *e-NOSE* beaker and then the 100 mL beaker was also placed into the *e-NOSE* beaker. This experiment was replicated over two days.

2.1.8.2. e-NOSE analysis

The *e-NOSE* analysis was as described in Section 2.1.2.

2.1.8.3. Statistical analysis

Randomised block analysis of covariance, with RH as a covariate was carried out on the overall sensor mean and the three sensor group means at each time. The ANOVA was also carried out on RH for the second method to check that treatments had no effect on RH.

2.1.9. Investigation of water content

2.1.9.1. Sample, preparation and treatment

Fat (10 g) for each sample was weighed into a beaker and dried to a constant weight in an oven at 60° C. Once constant weight was achieved the weight loss was calculated as a percentage of the original weight. Comparisons were then made between the weight losses of the samples of fat from boars and gilts.

2.1.9.2. Statistical analysis

A Wilcoxon rank sum test was used to determine any significant differences between the water content of the samples of fat from boars and gilts.

2.2. Results and Discusssion

2.2.1. Study1: Analysis of fat samples with taint compounds

The HPLC analysis for androstenone and skatole concentrations was conducted on all samples and the results are summarised in Table 2.1. The samples were divided into four groups. The samples from the gilts (Group 1) and the castrates (Group 2) had lower concentrations of androstenone and skatole. The samples of fat from entires were further subdivided into two groups. Group 3 consisted of samples having androstenone concentrations below the sensory threshold of 0.5-1.0 ppm and skatole concentrations that were close to the sensory threshold concentration of 0.02-0.025 ppm. Group 4 consisted of samples with levels of taint compounds that were considerably above, the sensory thresholds.

Table 2.1 Summary of the androstenone and skatole concentrations in the four types of fat.

			Androstenone Concentration /ppm		Skatole Concentration /ppm	
Group	N	Fat type	Average	Range	Average	Range
1	7	Gilts	<0.2	NA	0.049	0.029-0.098
2	3	Castrates	<0.2	NA	0.043	0.041-0.044
3	3	Entire with low taint compounds	<0.2	NA	0.03	All 0.030
4	7	Entires with high taint compounds	1.188	0.043 > 2.4	0.246	0.060-0.665

2.2.2. The discrimination of androstenone and skatole concentrations

The sensor response for the samples of fat from gilts (Group 1) castrates (Group 2) entires (Group 3) and (Group 4) were analysed using the *e-NOSE*. Multiple regression analyses were used to relate the sensor scores to androstenone and skatole concentrations, and to determine how the relationship varied with relative humidity, sensor analysis, batch variation and the vessel and head temperatures.

A simple model, namely separate linear responses to RH for each of the three batches, added together with a linear response to vessel temperature accounted for most of the variation in the sensor scores. This setup was used as the base model for the discussion of the results. The Tables 2.2-2.4 show the results of the base model modified by one term at a time, with each term either classified as a term retained in the base model, or a term rejected from the base model. A summary of the results at the 1, 2 and 4 min time points is presented in Tables 2.2-2.4.

All 12 sensor scores were strongly related to relative humidity as exemplified by the strong effect of the terms involving RH on sensor scores at the 1, 2 and 4 min time points (Tables 2.2-2.4). Terms involving batch and relative humidity account for a very high percentage of the variability (typically around 80-90%), depending on the sensor and time. The response to relative humidity often differed between batches, as indicated by the statistical significance of the batch.RH interactions. The vessel temperature was also related with the mean sensor response ($P < 0.05$) with all the sensors being responsible at one time point or another, except for sensor 298. The effect of these factors: vessel temperature, the RH, the sample/batch and the batch.RH interaction, on the mean sensor response accounted for over 90% of the variation at the 1, 2 and 4 min time points (Tables 2.2-2.4).

Table 2.2 P-values for the adjustment of the terms to the model. The model used for each sensor consisted of separate linear responses to RH for each of the three batches, added together with a linear response to vessel temperature at 1 min. The parameters used in the model are classified as terms retained in the base model or as terms rejected from the base model.

Adjustment to Model	**P-value for each sensor response at 1 min**												
	Mean	**301**	**298**	**297**	**283**	**278**	**264**	**263**	**262**	**261**	**260**	**259**	**258**
Terms retained													
All terms involving RH (RH + Batch.RH interaction)	**1.2×10^{-6}**	**0.00054**	**1.8×10^{-5}**	**3.4×10^{-8}**	**2.3×10^{-7}**	**0.0024**	**1.2×10^{-6}**	**1.8×10^{-6}**	**8.6×10^{-6}**	**0.00014**	**2.6×10^{-5}**	**4.4×10^{-6}**	**5.4×10^{-5}**
Batch.RH interaction	**0.025**	0.186	0.338	0.134	0.433	**0.0034**	**0.0048**	0.062	**0.0063**	0.081	0.232	0.071	**0.012**
Vessel temperature	**0.033**	0.069	0.190	**0.014**	**0.027**	0.202	0.568	0.317	**0.0051**	0.072	**0.035**	**0.0063**	**0.015**
Terms rejected													
Androstenone	0.551	0.502	0.503	0.365	0.375	0.280	0.735	0.869	0.208	0.668	0.739	0.704	0.525
Skatole	0.965	0.856	0.754	0.738	0.594	0.324	0.574	0.970	0.849	0.936	0.717	0.662	0.989
$(RH)^2$	0.794	0.688	0.596	0.661	0.568	0.962	0.315	0.525	0.873	0.521	0.903	0.533	0.863
$(Vessel)^2$	0.195	0.729	0.726	0.977	0.797	0.363	0.230	0.071	0.063	0.171	0.097	0.140	0.185
Sex / Chemical grouping	0.926	0.958	0.813	0.742	0.741	0.463	0.802	0.705	0.701	0.978	0.745	0.942	0.906
Head Temperature	0.392	0.287	0.310	0.618	0.700	0.118	0.822	0.743	0.098	0.449	0.158	0.273	0.238
RH.Vessel Interaction	0.945	0.857	0.308	0.929	0.627	0.263	0.704	0.894	0.868	0.829	0.406	0.863	0.941
Batch.Vessel interaction	0.810	0.815	0.700	0.942	0.952	0.865	0.749	0.710	0.665	0.680	0.591	0.675	0.852
% variance accounted for by model	91.3	85.2	85.2	95.1	93.7	86.8	93.2	93.7	90.0	83.0	83.1	88.6	85.2

Table 2.3 P-values for the adjustment of the terms to the model. The model used for each sensor consisted of separate linear responses to RH for each of the three batches, added together with a linear response to vessel temperature at 2 min. The parameters used in the model are classified as terms retained in the base model or as terms rejected from the base model.

Adjustment to Model	**P-value for each sensor score at 2 min**												
	Mean	**301**	**298**	**297**	**283**	**278**	**264**	**263**	**262**	**261**	**260**	**259**	**258**
Terms retained													
All terms involving RH (RH + Batch.RH interaction)	**1.2×10^{-6}**	**0.0041**	**2.3×10^{-5}**	**2.2×10^{-7}**	**4.5×10^{-7}**	**0.0039**	**4.3×10^{-8}**	**3.2×10^{-8}**	**1.4×10^{-5}**	**0.0001**	**4.6×10^{-5}**	**1.8×10^{-5}**	**9.4×10^{-5}**
Batch.RH interaction	**0.044**	0.207	0.518	0.146	0.598	**0.024**	**0.00028**	**0.025**	0.115	0.158	0.408	0.124	**0.03**
Vessel temperature	**0.0030**	0.060	0.158	**0.0031**	**0.0089**	**0.041**	**0.015**	**0.0095**	**0.0017**	**0.012**	**0.0082**	**0.0018**	**0.0036**
Terms rejected													
Androstenone	0.259	0.551	0.316	0.311	0.093	0.665	0.190	0.695	0.119	0.282	0.447	0.504	0.345
Skatole	0.910	0.902	0.863	0.886	0.669	0.732	0.473	0.862	0.569	0.957	0.496	0.534	0.666
$(RH)^2$	0.428	0.589	0.942	0.079	0.138	0.421	0.846	0.473	0.399	0.807	0.251	0.624	0.862
$(Vessel)^2$	0.116	0.336	0.773	0.941	0.930	0.803	0.322	**0.023**	0.059	0.058	**0.028**	**0.021**	0.054
Sex / Chemical grouping	0.694	0.919	0.546	0.808	0.502	0.863	0.411	0.606	0.427	0.589	0.334	0.807	0.899
Head Temperature	0.065	0.345	0.107	0.098	**0.036**	**0.029**	0.314	0.578	**0.022**	0.197	0.067	0.111	0.082
RH.Vessel Interaction	0.374	0.364	0.583	0.344	0.896	0.309	0.723	0.352	0.117	0.284	**0.037**	0.379	0.713
Batch.Vessel interaction	0.326	0.448	0.935	0.821	0.872	0.685	0.308	0.063	0.126	0.326	0.077	0.261	0.613
% variance accounted for by the model	92.2	84.1	85.2	93.5	92.9	76.9	96.6	97.5	89.4	81.8	79.3	86.1	84.1

Table 2.4 P-values for the adjustment of the terms to the model. The model used for each sensor consisted of separate linear responses to RH for each of the three batches, added together with a linear response to vessel temperature at 4 min. The parameters used in the model are classified as terms retained in the base model or as terms rejected from the base model.

Adjustment to Model	**P-value for each Sensor at 4 min**												
	Mean	**301**	**298**	**297**	**283**	**278**	**264**	**263**	**262**	**261**	**260**	**259**	**258**
Terms retained													
All terms involving RH (RH + Batch.RH interaction)	**8.2×10^{-7}**	**0.0041**	**5.3×10^{-5}**	**2.1×10^{-7}**	**3.1×10^{-6}**	0.080	**2.2×10^{-7}**	**3.1×10^{-8}**	**3.5×10^{-6}**	**1.8×10^{-5}**	**2.1×10^{-6}**	**1.1×10^{-6}**	**0.00015**
Batch.RH interaction	0.068	0.188	0.446	0.304	0.907	0.462	**0.0017**	**0.0085**	0.731	0.117	0.158	0.093	0.509
Vessel temperature	**0.0012**	**0.050**	0.068	**0.0070**	0.065	**0.028**	**0.043**	**0.0016**	**0.00015**	**0.0018**	**0.0010**	**0.00012**	**0.0072**
Terms rejected													
Androstenone	0.175	0.500	0.060	0.077	**0.047**	0.507	0.104	0.536	0.223	0.382	0.666	0.471	0.334
Skatole	0.783	0.838	0.703	0.708	0.792	0.976	0.522	0.595	0.836	0.958	0.190	0.598	0.730
$(RH)^2$	0.219	0.225	0.425	0.050	0.256	0.364	0.235	0.347	0.154	0.606	0.709	0.370	0.676
$(Vessel)^2$	0.719	0.755	0.855	0.595	0.684	0.611	0.665	0.730	0.690	0.457	0.206	0.178	0.127
Sex / Chemical grouping	0.550	0.836	0.345	0.491	0.407	0.956	0.065	0.588	0.611	0.703	0.521	0.808	0.767
Head Temperature	**0.042**	0.460	0.238	0.079	0.222	0.142	0218	0.086	0.218	0.061	**0.0011**	**0.037**	**0.020**
RH.Vessel Interaction	0.872	0.583	0.568	0.966	0.493	0.387	0.844	0.966	0.896	0.699	0.818	0.695	0.747
Batch.Vessel interaction	0.179	**0.017**	0.674	0.400	0.834	0.459	0.140	**0.048**	0.484	0.154	0.311	0.089	0.255
% variance accounted for by the model	93.2	86.9	85.4	94.4	91.5	47.8	96.5	97.9	93.4	84.8	85.5	90.5	82.4

Once these effects were adjusted for, it was evident that the androstenone and skatole concentrations had no relationship with the sensor response. If any relationship was apparent, it would only, at best, account for 7-8% of the variation. There was some indication of a relationship ($P < 0.05$) at 4 min for sensor 283, but this single significance was relatively weak and isolated (Table 2.4) and therefore would not be very useful in discriminating between androstenone concentrations. Tables 2.2-2.4 show the non-existent relationship between androstenone and skatole concentration and the sensor response at 1, 2 and 4 min. It appeared as though all the variation was due to the micro-environmental effects of the vessel and sensor head temperature, the relative humidity, and the drift associated with batches. It was, however, not due to the variation in the concentration of androstenone and skatole. Schaller *et al.*, (2000) also found that the variation displayed by the sensor response of the conducting polymers, could be reduced when the effect of temperature was considered.

2.2.3. Principal Component Analysis on sensor response

The multiple regression analysis for the individual sensor score and the different concentrations of androstenone and skatole showed no correlation. Principal component analysis (Manly, 1986) was therefore conducted on the sensor response to the different types of fat, with an aim to identify if a combined sensor response correlated with androstenone and skatole concentrations. It was found that principal components 1 and 2 account for over 95 % of the variation at the 1, 2 and 4 min time points (Table 2.5).

Table 2.5 Percent variance accounted for by the first two principal components.

Component	1 min	2 min	4 min
1	84.6	84.1	86.6
2	12.9	12.2	9.2
1 + 2	97.5	96.2	95.8

The loadings (Manly, 1986) for principal component 1 on each sensor were approximately equal to each other (Table 2.6). This suggested that most of the information in the first principal component could be summarised by the mean sensor response. A simple linear regression of the first principal component on the mean sensor score showed that the mean sensor response accounts for over 97.5%, 97.7% and 98.5% of the variance of principal component 1 at the 1, 2 and 4 min time points, respectively. The relationship between the mean sensor response and principal component 1 at 1 min is displayed in Figure 2.1 and is typical of the relationship observed at 2 and 4 min. The first principal component accounts

for around 85% of the variation in the data and therefore it is reasonable to conclude that the mean sensor score accounts for the majority of the variation of the data.

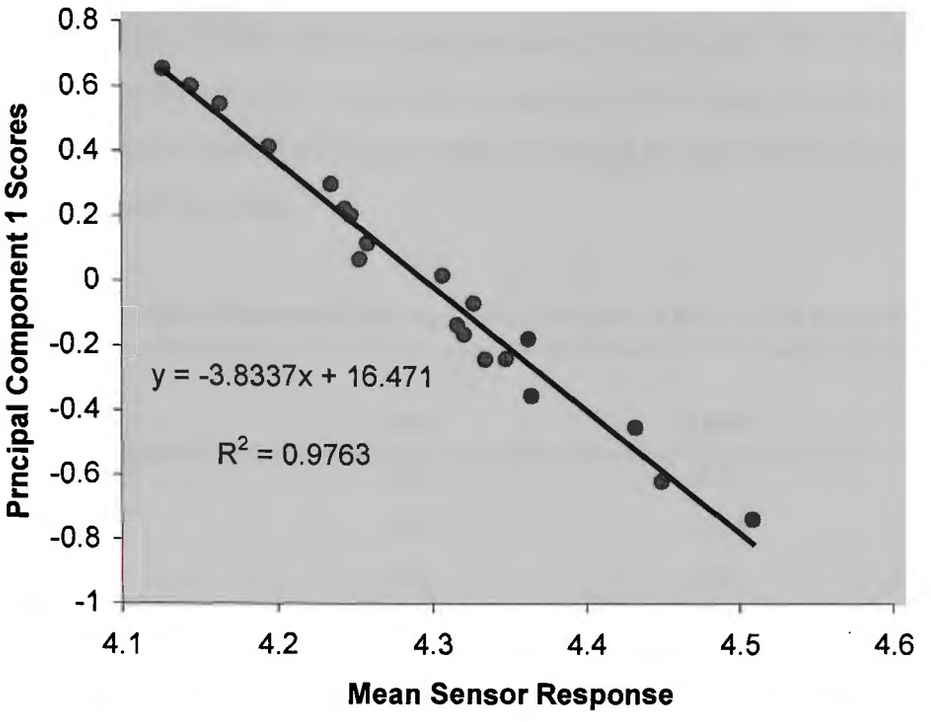


Figure 2.1. Relationship between principal component 1 and mean sensor response at 1 min which accounts for 97.5% of the variation.

Table 2.6 Principal component 1 loadings for each sensor at the varying time points.

Sensor	1 min	2 min	4 min
258	-0.15	-0.14	-0.14
259	-0.32	-0.29	-0.29
260	-0.17	-0.15	-0.15
261	-0.23	-0.21	-0.20
262	-0.16	-0.20	-0.26
263	-0.58	-0.52	-0.48
264	-0.46	-0.43	-0.41
278	-0.07	-0.04	-0.02
283	-0.17	-0.27	-0.34
297	-0.24	-0.22	-0.22
298	-0.22	-0.20	-0.19
301	-0.30	-0.41	-0.41

The loadings for principal component 2 were not as similar to each other as for principal component 1. The sensors appeared to be divided into three major groups (Table 2.7). It can be seen that at each time the loadings for sensors 258, 259, 260, 261, 262 were approximately the same and therefore designated as group 1. The sensor 263, 264 were similar and designated group 2 whilst the remaining sensors 278, 283, 297, 298, 301 were also approximately the same and compiled into group 3. This suggested that most of the information in the second principal component could be summarised by the means, defined by the three sensor groups.

Table 2.7 Principal component 2 loadings for each sensor at the varying time points.

Sensor	1 min	2 min	4 min
258	0.27	-0.31	0.25
259	0.37	-0.48	0.55
260	0.37	-0.34	0.39
261	0.42	-0.39	0.38
262	0.39	-0.12	-0.04
263	-0.43	0.50	-0.43
264	-0.31	0.30	-0.31
278	0.18	-0.22	0.25
283	-0.07	0.01	0.02
297	-0.08	-0.01	0.02
298	0.02	-0.08	0.06
301	0.06	-0.06	-0.03

To elucidate this further, the second principal component was related to the means of the three sensor groups using a simple multiple regression. The mean sensor response of the three sensor groups accounts for 99.8%, 99.7% and 99.0% of the variation in principal component 2 at the 1, 2 and 4 min time points, respectively. It is therefore possible to conclude that a large majority of the variation of principal component 2 can be described by the mean of the three main sensor groups. The overall mean sensor score can be calculated exactly from the means of the three sensor groups, and therefore it can be concluded that most of the variation of the first two principal components is associated with the means of the three sensor groups. It follows that since principal components 1 and 2 accounts for over 95% of the variation in the data, the large majority of the variation is associated with the means of the three sensor groups.

Thus the data can be summarised using: (i) the mean of the sensors 258, 259, 260, 261 and 262 (Group 1), (ii) the mean of the sensors 263 and 264 (Group 2), and (iii) the mean of sensors 278, 283, 297, 298 and 301 (Group 3). This summary will be used in the rest of this chapter.

2.2.4. The sensor group mean response

Similar multiple regression analyses for the three group mean sensor scores were carried out as for the individual scores (Tables 2.8 - 2.10). The same base model of separate linear response for RH for each batch, added to the linear response of the vessel temperature was used. The effects of sample batch, RH, batch.RH interaction and the vessel temperature at 1 min accounted for around 90% of the variation in each group (Table 2.8). As with the individual scores, after adjusting for these effects the androstenone and skatole concentrations, were unrelated to the sensor response. Once again, it appears as though all the variation was due to the micro-environmental effects of the vessel temperature, RH and batch drift.

Table 2.8 Adjustment to the model for the group mean sensor responses at 1min.

Adjustment to model	**P-value for sensors used in calculating the mean**			
	Group 1	Group 2	Group 3	ALL
Terms retained				
All terms involving RH (RH + Batch.RH interaction)	**1.4×10^{-5}**	**1.1×10^{-6}**	**4.3×10^{-6}**	**1.2×10^{-6}**
Batch.RH interaction	**0.040**	**0.017**	0.119	**0.025**
Vessel Temperature	**0.014**	0.405	0.050	**0.033**
Terms rejected				
Androstenone	0.563	0.949	0.387	0.551
Skatole	0.807	0.811	0.783	0.965
$(RH)^2$	0.721	0.409	0.652	0.794
$(Vessel)^2$	0.108	0.115	0.849	0.195
Sex / Chemical grouping	0.943	0.794	0.841	0.926
Head Temperature	0.219	0.772	0.305	0.392
RH.Vessel Interaction	0.756	0.802	0.663	0.945
Batch.Vessel interaction	0.685	0.717	0.923	0.810
% variance accounted for by the model	87.1	93.8	90.4	91.3

The results at the 2 min time point were very similar to 1 min. The one exception was an isolated significant quadratic effect by the temperature of the vessel on the group 1 sensor score. The vessel temperature was also significantly related to the sensor response for groups 2 and 3 (Table 2.9). It can be seen that at 4 min the situation was very similar to 2 min except that the temperature of the sensor head was also significantly related to the sensor response for Group 1 (Table 2.10). It would appear that as time proceeds, the effect of variation in temperature has more impact on the sensor response.

Table 2.9 Adjustment to the model for the group mean sensor response at 2 min.

Adjustment to model	**P-value for sensors used in calculating the mean**			
	Group1	Group 2	Group 3	ALL
Terms retained				
All terms involving RH (RH + Batch.RH interaction)	**2.1×10^{-4}**	**1.7×10^{-8}**	**8.6×10^{-6}**	**1.2×10^{-6}**
Batch.RH interaction	0.117	**0.0017**	0.155	**0.025**
Vessel Temperature	**0.0027**	**0.0080**	**0.014**	**0.033**
Terms rejected				
Androstenone	0.310	0.376	0.282	0.551
Skatole	0.641	0.641	0.925	0.965
$(RH)^2$	0.550	0.620	0.338	0.794
$(Vessel)^2$	**0.025**	0.083	0.682	0.195
Sex / Chemical grouping	0.667	0.588	0.741	0.926
Head Temperature	0.070	0.407	0.067	0.392
RH.Vessel Interaction	0.215	0.486	0.738	0.945
Batch.Vessel Interaction	0.202	0.110	0.832	0.810
% variance accounted for by the model	85.6	97.5	90.3	91.3

Table 2.10. Adjustment to the model for the group mean sensor response at 4 min.

Adjustment to model	**P-value for sensors used in calculating the mean**			
	Group 1	Group 2	Group 3	ALL
Terms retained				
All terms involving RH (RH + Batch.RH interaction)	**1.9×10^{-6}**	**4.8×10^{-8}**	**1.1×10^{-5}**	**8.2×10^{-7}**
Batch.RH interaction	0.108	**0.0026**	0.263	**0.025**
Vessel Temperature	**0.000040**	**0.0067**	**0.012**	**0.033**
Terms rejected				
Androstenone	0.360	0.235	0.098	0.551
Skatole	0.617	0.931	0.886	0.965
$(RH)^2$	0.428	0.261	0.128	0.794
$(Vessel)^2$	0.305	0.947	0.840	0.195
Sex / Chemical grouping	0.742	0.239	0.507	0.926
Head Temperature	**0.017**	0.125	0.132	0.392
RH.Vessel Interaction	0.884	0.930	0.804	0.945
Batch.Vessel Interaction	0.149	0.065	0.446	0.810
% variance accounted for by the model	89.6	97.5	90.9	91.3

Once the effect for the micro-environmental factors such as RH, vessel and head temperature were accounted for in all the analyses, the relationship between sensor scores and skatole or androstenone concentrations was non-existent. Schaller *et al.*, (2000) found that the reproducibility of measurements performed with an electronic nose equipped with 12 conducting polymer sensors was poor. The sensor response varied from one day to the next and even from one hour to the next. The variation could be explained in part by the temperature fluctuation on the sensor module.

Annor-Frempong *et al.*, (1998) used conducting polymer sensors combined with canonical correlation analysis to relate samples, containing up to 4 ppm androstenone and 1.6 ppm skatole in model systems, with *e-NOSE* readings. There was a respective 84% and 90% canonical correlation for these two compounds. The relationship between androstenone and skatole in real samples of fat was not as successful with 100% correct classification on a training sample and 53% correct in the cross validation of a validation sample in multiple discriminant analysis (MDA). There was no mention of controlling or correcting for the effects of relative humidity in this study.

To duplicate their androstenone results using the samples of this current study, a multiple discriminant analysis (Manly, 1986) was conducted on the sensor responses to the different fats, after adjusting for RH, but no other effect. It was found that using this analysis 100% of the samples were classified correctly and 80% were correctly classified in a cross validation analysis. These results were similar to those of Annor-Frempong *et al.*, (1998).

The MDA analysis, however cannot be considered as reliable as the regression analyses carried out in this chapter, since the MDA analyses, at least in the form used, does not involve any formal statistical inference. The results for the MDA are also likely to be spurious and caused by overfitting, involving the estimation of relatively many parameters from relatively few samples. It is well known that with MDA, plug-in error rates on training samples can underestimate true error rates by large amounts when sample sizes are small (Lachenbruch, 1977). The variation could also be attributed to the differences in water content. It can thus be concluded that the previous work of Annor-Frempong *et al.*, (1998) can be discounted and hence does not show that the *e-NOSE* is able to estimate androstenone concentrations.

2.2.5. Study 2: Analysis of vessel purge and equilibration times

The lack of correlation between the individual and combined sensor scores with androstenone and skatole concentrations indicated that further optimisation of the analysis method was necessary. Equilibration and purge times were important parameters to be optimised. The equilibration times of 4, 7 and 10 min versus the purge times of 3, 5, 7 and 10 min were investigated in this study. The results at 1 min indicated that each of the mean scores for the three sensor groups increased linearly with equilibration time (Figure 2.2, Table 2.11). The sensor response increased with equilibration time, with a greater response occurring at a purge time of 3 min (Figure 2.2). This differential response at 3 min is also demonstrated in Table 2.11 by the equilibration time × the 3 min purge versus the 5, 7 and 10 min purge times interaction. This term is abbreviated as Eq × P 3 vs P 5-10 in Table 2.11. A possible explanation for this is that at 3 min all the extraneous odour contaminants have not yet been removed and are therefore contributing to the sensor response. This is not the case at 5, 7 and 10 min. There is no other evidence of any treatment effect. Purging the vessel for longer than 3 min, designated as P 5-10 in Table 2.11, apparently has no effect on the sensor response. There is also no interaction between the longer purge times and equilibration times.

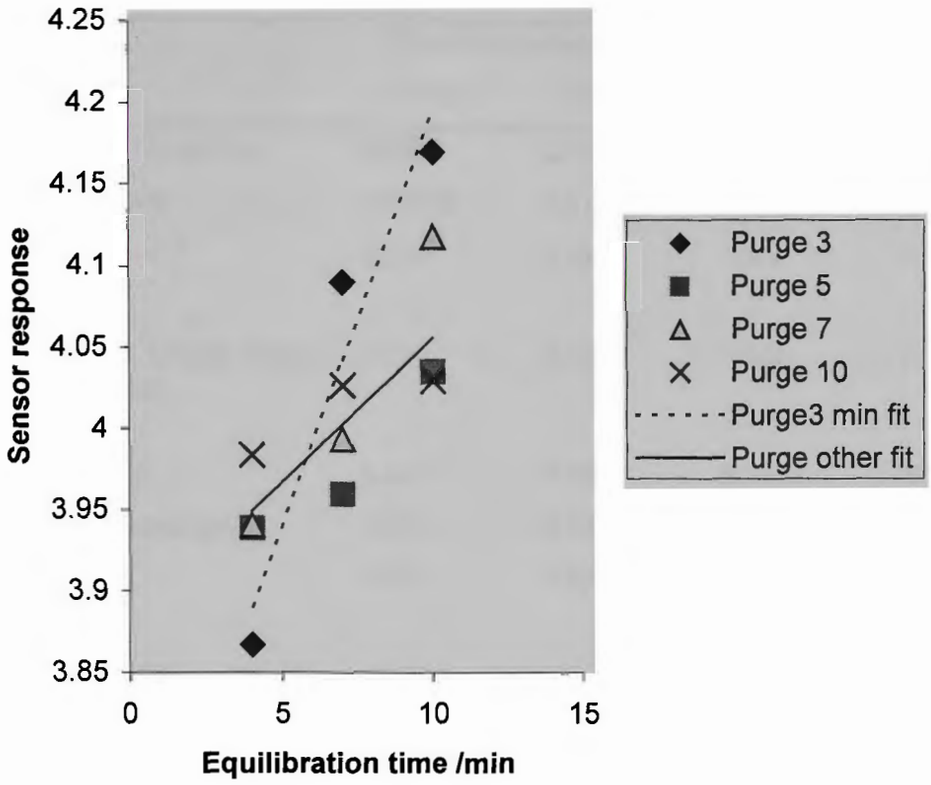


Figure 2.2. Mean sensor response over all sensors (adjusted for RH) at 1 min.

The RH covariate once again has a very strong impact on the sensor response at 1 min (Table 2.11). The treatments of equilibration and purge time however have no effect on RH. This implies that there is a large sample to sample variation in RH but RH is not affected by the equilibration or purge treatments

The sensor response increased linearly with equilibration time but the linear response was greater at a purge time of 3 min compared to a longer purge time. These observations led to the decision that in future experiments, a purge time longer than 3 min (i.e. 7 min) was to be utilised and the equilibration time was to be maintained at 7 min. The data for the 1 min time point are representative of the results acquired at the 2 and 3 min time points. The only difference is that at 2 and 3 min, the Group 3 sensor mean also has a significant linear response with increasing equilibration time (see data in Appendix 2.1 and 2.2)

Table 2.11. P-values of treatment effects equilibration and purge time ANOVA of sensor score means at 1 min.

	P-value for sensors used in calculating the mean				
Effects	**Group 1**	**Group 2**	**Group 3**	**ALL**	**RH**
Equilibration Time (Eq)	**0.0017**	**2.7×10^{-6}**	0.154	**0.00023**	0.124
Linear response	**0.00042**	**7.4×10^{-7}**	0.092	**2.4×10^{-6}**	**0.045**
Deviation (Dev) [1]	0.930	0.182	0.335	0.778	0.792
3 min purge vs 5,7 and 10min purge (P3vs5-10)	0.077	0.315	0.211	0.131	0.545
Eq × P3vsP5-10	**0.014**	0.090	**0.0044**	**0.010**	0.545
Linear response. Dev	**0.010**	**0.038**	**0.00080**	**0.0063**	0.285
Dev. Dev	0.123	0.499	0.057	0.134	0.828
5-10 min purge times (P5-10)	0.437	0.725	0.278	0.412	0.613
Eq × P5-10 interaction	0.752	0.097	0.796	0.482	0.867
Linear response. Dev	0.454	0.035	0.605	0.268	0.809
Dev. Dev	0.876	0.560	0.733	0.678	0.670
RH covariate	**5.4×10^{-6}**	**6.5×10^{-7}**	**4.3×10^{-6}**	**4.4×10^{-7}**	

[1] Deviation refers to the sensor response that has deviated from the linear response.

2.2.6. Study 3: Analysis of the sensor response to amount of fat and surface area

The equilibration versus purge experiment indicated that a purge and equilibration time of 7 min was appropriate for future experiments. These parameters were used in this experiment where the mass of fat (10, 20 and 30 g) and the surface area (1g, 5g and a whole sample) of the fat was investigated. There was no evidence that the mass of fat (Table 2.12) or an interaction of the surface area with the mass of fat (data not shown) to have an affect on any sensor mean score or the relative humdity, at 1 min (Table 2.12). The results were similar for the 2 and 3 min time points (see data in Appendix 2.3 and 2.4) except for some effect of mass of fat on RH. There were strong effects of surface area increasing sensor scores and RH, but the effects on sensor scores disappear after adjusting statistically for RH (Table 2.13). There were indications of similar effects at 2 min and 3 min (see data in Appendix 2.5 and 2.6)

although the effects were weaker. These results suggest that the surface area had a positive effect on sensor score, but that this response was purely due to an increase in the level of water exuded from the samples.

Table 2.12. The effect of mass of fat on the mean sensor scores and humidity at 1 min.

	Mass of Fat (g)				
Sensors	**10**	**20**	**30**	**SED**	**P-value**
Not adjusted for Relative Humidity					
All Sensors	4.11	4.06	4.07	0.048	0.553
Group 1	4.04	3.99	4.00	0.042	0.520
Group 2	6.62	6.53	6.54	0.087	0.510
Group 3	3.14	3.15	3.17	0.040	0.640
Relative Humidity	55.0	54.1	54.2	0.83	0.479
Adjusted for Relative Humidity					
All Sensors	4.08	4.07	4.08	0.022	0.935
Group 1	4.01	4.01	4.01	0.021-0.022	0.912
Group 2	6.56	6.56	6.57	0.034	0.917
Group 3	3.15	3.15	3.15	0.020	0.912

Table 2.13. The effect of the surface area of the fat on the sensor scores and humidity at 1 min. A small sample refers to samples cut into 1 g pieces, medium refers to 5 g pieces and whole refers to the total mass of fat left as a whole piece.

	Surface area of fat				
Sensors	**Whole**	**Medium**	**Small**	**SED**	**P-value**
Not adjusted for relative humidity					
All sensors	4.00	4.07	4.16	0.048	**0.013**
Group 1	3.93	4.01	4.08	0.042	**0.010**
Group 2	6.41	6.55	6.74	0.087	**0.0055**
Group 3	3.10	3.15	3.21	0.040	**0.049**
Relative Humidity	52.5	54.2	56.6	0.83	**0.00055**
Adjusted for relative humidity					
All sensors	4.10	4.09	4.05	0.024-0.032	0.352
Group 1	4.02	4.02	3.99	0.024-0.032	0.509
Group 2	6.59	6.57	6.53	0.037-0.048	0.473
Group 3	3.18	3.16	3.12	0.022-0.030	0.204

2.2.7. *Study 4: Analysis of androstenone in mineral oil*

The previous experiment showed that the mass of fat and the surface area did not have a significant effect on the sensor score. The equilibration and purge time however was important and indicated that a purge and equilibration time of 7 min was necessary for optimum analysis. The next experiment was to investigate the effect of increasing androstenone concentrations in mineral oil in the absence of any moisture from fat or supplemental water. The results show that in the absence of relative humidity the average sensor response was close to zero and again there was no discrimination between androstenone concentrations (Table 2.14). The same study was also conducted in the presence of supplemental water. However, once again there was no discrimination between androstenone concentrations (data not shown).

Table 2.14. Effect of the level of applied androstenone in mineral oil on average sensor scores in the absence of water.

	Time	Androstenone concentration (ppm)					
Sensors	**(min)**	**0**	**1**	**2.5**	**5**	**SED**	**P-value**
	1	-0.02	0.00	-0.01	-0.01	0.041	0.973
Group 1	**2**	0.03	0.05	0.02	0.04	0.050	0.965
	4	0.11	0.12	0.09	0.12	0.053	0.923
	1	-0.02	0.00	-0.01	0.00	0.040	0.931
Group 2	**2**	0.03	0.06	0.02	0.05	0.057	0.916
	4	0.17	0.19	0.14	0.19	0.074	0.892
	1	-0.01	0.01	0.01	0.02	0.040	0.903
Group 3	**2**	0.01	0.05	0.03	0.05	0.058	0.922
	4	0.11	0.13	0.11	0.14	0.074	0.939
	1	-0.01	0.01	0.00	0.00	0.040	0.950
ALL	**2**	0.02	0.05	0.03	0.04	0.054	0.951
	4	0.12	0.14	0.11	0.14	0.065	0.933

2.2.8. *Study 5: Analysis of samples of fat spiked with androstenone*

There was no evidence that the sensors could discriminate the different concentrations of androstenone as a pure standard dissolved in mineral oil. The observations made from the mass of fat and the surface area experiment also indicated that no treatment effect was apparent on the mean sensor score. However, a sample with a mass of 1 g was used in this experiment because visually it appeared as though the sample had a more even distribution compared to the 5 g sample. In this experiment the effect of increasing the concentration of androstenone by spiking the original sample of fat with androstenone, was investigated. However, once again there was no significant discrimination between the increasing concentrations of androstenone, at all time points (Table 2.15). The only discrimination was at 4 min for sensors 263 and 264 where the zero levels of androstenone had higher sensor scores compared to the other levels. However there was no discrimination between the 0.5 and 2.0 ppm concentrations, therefore this result was not consistent. The relative humidity covariate, however was again significantly related to the sensor response (Table 2.15).

Sensors responding to polar molecules will also respond to water vapour (Haugen and Kvaal, 1998). In most situations, when meat products are being measured, water vapour will be generated in the headspace during sampling due to the water activity in the meat. The sensors were very responsive to humidity, although the response was linear, as indicated in Table 2.11. An attempt therefore to reduce the background variation by adding an equal amount of water to each sample, was made. A correction for the response to RH was also carried out. The samples of fat spiked with androstenone in the presence of supplemental water were then analysed. This resulted in slightly higher levels of RH (Table 2.16). However, once the effect of the RH was adjusted, there was still no discrimination for androstenone concentrations (Table 2.16). The only effect that was significantly related to the sensor response was the RH. The quadratic response for the RH was also tested for but was not significant (data not shown).

Table 2.15. Effect of the level of applied androstenone on average sensor scores in the presence of fat.

Sensors	Time (min)	Androstenone concentration (ppm) 0	0.5	2	SED	P-value	P-value for RH covariate
	1	3.98	3.95	3.95	0.015	0.166	**0.00046**
Group 1	2	4.67	4.63	4.63	0.020	0.126	**0.0037**
	4	5.37	5.31	5.31	0.027	0.102	**0.018**
Group 2	1	6.78	6.77	6.78	0.032	0.929	**0.00037**
	2	7.93	7.87	7.88	0.030	0.211	**0.00042**
	4	8.75	8.67	8.67	0.028	**0.039**	**0.00050**
	1	3.27	3.25	3.35	0.012	0.219	**0.00018**
Group 3	2	4.30	4.26	4.26	0.020	0.184	**0.0045**
	4	5.08	5.03	5.04	0.024	0.212	**0.010**
ALL	1	4.15	4.13	4.13	0.015	0.329	**0.00018**
	2	5.06	5.01	5.02	0.020	0.134	**0.0015**
	4	5.81	5.75	5.76	0.024	0.089	**0.0048**
RH	1	58	59	59	1.1	0.530	-
	2	62	63	63	1.1	0.597	-
	4	67	68	68	1.1	0.604	-

Table 2.16. Effect of the level of applied androstenone on average sensor scores in the presence of water and fat.

Sensors	Time (min)	Androstenone concentration (ppm) 0	0.5	2	SED	P-value	P-value for RH covariate
Group 1	**1**	4.11	4.13	4.13	0.012	0.102	**0.0010**
	2	4.75	4.79	4.78	0.017	0.130	**0.0035**
	4	5.39	5.43	5.43	0.062	0.296	**0.00065**
	1	7.23	7.18	7.19	0.039	0.458	**0.000086**
Group 2	**2**	8.25	8.23	8.24	0.030	0.822	**0.000067**
	4	8.99	9.00	8.98	0.040	0.920	**0.00059**
	1	3.39	3.40	3.40	0.010	0.810	**0.00012**
Group 3	**2**	4.40	4.41	4.41	0.016	0.669	**0.00043**
	4	5.13	5.16	5.14	0.024	0.593	**0.00085**
ALL	**1**	4.33	4.34	4.34	0.013	0.891	**0.000065**
	2	5.19	5.21	5.20	0.018	0.589	**0.00039**
	4	5.88	5.91	5.90	0.026	0.563	**0.00042**
RH	**1**	62	62	63	0.7	0.573	
	2	66	66	66	0.8	0.723	
	4	71	71	71	1.0	0.782	

The results have shown that it is essential to adjust for the variation in relative humidity which may be affected by factors such as the variable water activity of the samples of fat and the immediate environment in which the sensors and samples are situated. The sensors in the *e-NOSE* are made of conducting polymers and are highly susceptible to moisture. Perhaps the metal oxide sensors which are less sensitive to moisture (Gardner and Bartlett, 1994) are more suitable for this application. Bourrounet *et al.*, (1995) could account for up to 84% of androstenone concentration using metal oxide sensors.

2.2.9. Analysis of water content

The percentage of water in the samples of fat from boars (n = 26) was found to be 2.14 compared to 1.77 in the fat from gilts (n =16). The range for the percentage of water in the fat of boars and gilts was between 1.17 and 3.19. This resulted in the samples of fat from gilts having significantly lower levels of water ($P < 0.01$) compared to the samples from boars. This variation in moisture has been found in another study (Muhl *et al.*, 2000) to affect the headspace odour profile of chicken meat when the product was heated. Specific variation in the water content between different groups of samples must be a factor taken into consideration when assessing the aroma profile of certain foods. The other implications of this result, particularly when using the *e-NOSE* with conducting polymers is that the differences in water content will result in variable relative humidity ultimately leading to a variation in the sensor response, unless corrected. The difference between the sensor analysis of the samples of fat from boars and gilts is therefore confounded if one does not correct for the effect of RH.

2.3. Summary of Findings

The sensor response was analysed by multiple linear regression on the individual sensor scores as well as grouped sensor scores as defined by the PCA. The aim was to identify individual and combined sensor relationships with androstenone and to a lesser extent, skatole concentrations. However the response of the sensors, both individually and in groups did not correlate with the variation in androstenone concentration. As this was not related to the chemistry of androstenone, it is highly probable that a similar result would have been obtained for skatole. Oil samples that were spiked with skatole were analysed using the *e-NOSE* by another member in the laboratory. The results of this experiment also presented similar problems to those observed in the current studies, however, the data is not presented. The studies by Annor-Frempong *et al.*, (1998) found a correlation between boar taint and the sensor readings. However, this was not likely to be due to the correlation with various concentrations of androstenone and skatole, but may have been due to the variation in RH, temperature fluctuation or the variation of the water content in the samples of fat.

A majority of the variation in the sensor score in this study, appeared to be due to the micro-environmental effects of the vessel and sensor head temperature, the relative humidity, and the drift associated with batches. The *e-NOSE* attempts to maintain the temperature at 30°C, however variation still occurred. The results in the current study indicate that part of the variation could be explained by the temperature fluctuation of the sample and the sensor

module. Schaller *et al.*, (2000) also found that the reproducibility of measurements performed with an electronic nose equipped with 12 conducting polymer sensors, was poor. The sensor response was found to be quite variable over short periods of time. They found that part of the variation displayed by the sensor response could be reduced by half, by accounting for the temperature fluctuations on the sensor module.

Schaller *et al.*, (2000) stated that the lack of reproducibility with conducting polymer sensors is often explained by a difference in the water content in the samples which would ultimately affect the RH. This study also found that the water content of the samples and the RH, largely affected the sensor response. Muhl *et al.*, (2000) also indicated that the moisture content was an important factor in the analysis of the aroma profile of chicken meat.

The analysis of odour/flavour should ideally measure components important to the human perception of the odour or taste in question. If not, there is a potential problem that the measures by the *e-NOSE* are based on a correlation that is not specific for the test or robust where the measure is unspecific and variable. This point is reiterated in a review by Strike *et al.*, (1999) who states that when classifying samples great attention must be paid to ensure that the classification is not based solely on a comparatively trivial parameter such as RH but rather on the odour/flavour in question.

Much further work needs to be conducted in controlling micro-environmental parameters if the *e-NOSE* can possibly be used for the rapid on-line analysis of boar taint. Roussel *et al.*, (1999) stated that despite being cited in many publications dealing with food products, electronic noses still posed methodology problems. Lack of reproducibility is one of the crucial areas that prohibit any on-line quality control measurement based on aroma profiles. This sensor technology is generally sensitive to environmental parameters such as temperature control and humidity and therefore demands careful sample preparation and an instrument able to provide a controlled and reproducible environment. These limitations inhibit the discrimination of pork with different concentrations of androstenone and skatole rendering the *e-NOSE* ineffective in the practical application of the analysis of boar taint, for the pig industry. The products to be analysed would preferably need to have a constant relative humidity and also present responses that can be easily differentiated so that the residual response variations can be neglected. However, there are few products that correspond to the above mentioned criteria. If this technology is to be developed further, improved technical control is required along with a greater understanding of the polymer and vapour interaction. The future of the conducting polymer technology as sensors for volatile compounds is substantially limited without this development.

Chapter 3

ANDROSTENONE AND SKATOLE SENSITIVTY AND ITS RELATIONSHIP TO PORK CONSUMPTION

3.0. INTRODUCTION

The most important chemical indicators of boar taint in pork are thought to be androstenone (Patterson, 1968) and skatole (Vold, 1970; Walstra and Maarse, 1970). Various studies have found that the number of people in a population anosmic to androstenone varied from 20-50% (Elsely, 1968; Claus, 1983; Gilbert and Wysocki, 1987; Weiler *et al.*, 2000). It would appear that females are also more sensitive to androstenone compared to men (Kloek, 1961; Griffiths and Patterson, 1970; Gower *et al.*, 1985). In other studies, consumers highly sensitive to androstenone scored samples with high androstenone concentrations worse than those with low concentrations. This observation however was not apparent for mildly sensitive and/or insensitive consumers. This finding was further supported by the significant interaction ($P < 0.01$) between the concentration of androstenone in the sample and the sensitivity of the consumers towards androstenone (Weiler *et al.*, 2000). These results indicate that the importance of androstenone can be underestimated for sensitive consumers. If androstenone sensitivity is not considered for these consumers, the assumed acceptance of pork from entire males is unrealistically high. This is especially important because the frequency of androstenone-tainted carcasses is much higher compared to those with high skatole levels (Weiler *et al.*, 2000). Therefore a comprehensive estimate of the consumer reaction to pork from entire males cannot be achieved accurately without taking into account androstenone sensitivity (Weiler *et al.*, 2000).

These genetic differences may partly explain the observed variability of consumer reactions to pork from entire males, found in various studies. This variability in the detection of androstenone and the tendency of women to be more sensitive to androstenone infers the necessity for the screening of panellists, especially to eliminate those individuals incapable of detecting taint compounds. The objectives of the studies described here were: (i) to determine the meat preferences of a small population, (ii) screen panellists for their sensitivity to

androstenone and skatole and (iii) identify if there were any correlations between the panellists meat preferences and their sensitivity to androstenone. The sensory thresholds of each panellist for these compounds were also determined to establish their suitability for a panel sensitive to "boar taint".

3.1. Materials and Methods

3.1.1. Questionnaire and panellists

Candidates located at Victoria University, Australia and the Victorian Institute of Animal Science, Australia were asked to complete a questionnaire in order to determine their eligibility to participate in the program which was concerned with their general health and meat preferences. A sample questionnaire is attached in Appendix 3.I. Prospective panellists were excluded if they were vegetarian or had a cultural aversion to pork consumption.

3.1.2. Test procedures for the pre-screening of compounds

3.1.2.1. Pre-screening procedure for detection of phenyl ethyl alcohol and pyridine

The two odorants pyridine and phenyl ethyl alcohol (PEA) were used. Phenyl ethyl alcohol is related to the trigeminal sense and pyridine is a compound that can be detected by most people and hence its use for detecting anosmia. Pyridine (3% w/w; Aldrich, Castle Hill, NSW, Australia) in 50 g mineral oil (Pharmacia, Rydalmere, NSW, Australia) was placed in an empty 500 mL water bottle, and a cap with a nozzle, placed on the bottle. The other odourant, PEA (0.01% w/w; Merck, Kilsyth, Victoria, Australia) also in 50 g mineral oil was placed in a similar 500 mL water bottle.

Analyses were conducted in an air-conditioned room. Panellists were escorted to a bench on which a record sheet and sniffer bottles were arranged. Here, the panellists were asked to follow the instructions on the work sheet for a two sample alternate forced choice (2-AFC) test. One panellist at a time was asked to complete the series of trials.

The panellists were always asked to detect the compound PEA prior to the detection of pyridine. For each compound two pairs of the bottles were placed on a bench. Each pair

consisted of a blank and a bottle containing the relevant compound. The order of presentation of each pair was randomised and each bottle was labelled beforehand with an arbitrary number. The panellist was asked to sniff each bottle in turn and allow at least 1 min before sniffing successive samples. To sniff the odorant, the panellist was instructed to hold the stimulus bottle about 3 cm from their nose and gently deliver three puffs of air to the nostrils. The panellist was then asked to complete the record sheet. An example of a typical record sheet is given in Appendix 3.2.

3.1.2.2. Pre-screening for detection of androstenone and skatole

The odorant skatole (Sigma, Castle Hill, NSW, Australia) at a concentration of 0.05% w/w in mineral oil, was also used in the pre-screening analysis (Moss *et al.*, 1993). The pre-screening procedure used for skatole was the same as that used for pyridine and PEA.

In a study by Laing and Oram (1999) the relationship between sensitivity to androstenone and skatole and the frequency of the consumption of pork was investigated. The sensitivity of the panellists to androstenone was determined by placing a few crystals of androstenone in a small glass container. The panellists were then asked to assess the odour according to a range of descriptors or otherwise indicate that there was no odour. In the current study a preliminary screening test to also determine the panellist sensitivity to androstenone was carried out in a similar manner. A small amount (2-3 mg) of androstenone crystals (Sigma, Castle Hill, NSW, Australia) was placed in a beaker. Some salt granules were placed in another beaker so there could not be any visual differentiation between beakers with regard to the presence of small granules. Prospective panellists were asked if they could detect a distinct odour in one of the beakers. If the answer was negative they were to state so, otherwise they were to indicate which beaker had the odour. Androstenone is described to have quite a pungent odour (by those able to detect it) and there can be little ambiguity as to whether this compound can be detected or not (Wysocki and Beauchamp, 1984). An example of the record sheet utilised in this test is in Appendix 3.3.

3.1.3. Sensory threshold levels for androstenone and skatole

The method used to determine the sensory thresholds of androstenone and skatole was a modified version of that used by Wysocki and Beauchamp (1984). A two sample forced

choice test was used to assess the sensitivity of the panellists to the odourants. A concentration series for the odourants was obtained by binary serial dilution from the most concentrated stimulus. Twelve concentrations of androstenone were prepared by dilution with white mineral oil, to provide a concentration range of 0.5 - 1000 ppm. The concentration range for skatole, also in mineral oil, was between 0.0125 - 25.6 ppm.

A 10 mL sample of each of the odourants was presented to panellists for sniffing in a 200 mL flip-top white polyethylene bottle (Nalgene, Victoria, Australia). To sniff the odorant in a bottle, the panellists were requested to hold the bottle 3 cm from their nose and lightly squeeze the bottle whilst sniffing several times. The panellists were asked to become familiar with the odorant by initially sniffing the reference sample with a medium range concentration of the sample (125 ppm for androstenone and 0.8 ppm for skatole). To avoid problems of incomplete removal of odour from the examination room that may have affected the results and to encourage better co-operation from the panellists, the androstenone standards were analysed at room temperature.

During the threshold test each panellist was given 12 pairs of bottles. Each pair contained an odorant and a blank. The 12 pairs of bottles were assembled so that they were sampled in order of increasing concentration whilst the order between the blank and odourant was randomised. Upon completing each replicate sample, the panellist had a break of 1 min. This procedure was repeated three times per panellist. An example of a typical record sheet used for this procedure is given in Appendix 3.4.

3.1.4. Analysis of data

The statistical analyses with regard to the meat preferences by the consumer were conducted using GenStat® 5, Release 4.1. The two-sided exact sign test and the Wilcoxon Rank sum with a normal approximation, (Lehmann, 1975) was conducted to determine differences between meat preferences of the consumer and to determine whether their sensitivity to androstenone influenced their purchase. The sensory thresholds for androstenone and skatole were also determined taking, for each forced choice trial, the lowest correct concentration after four consecutive, correct identifications. The group geometric means were then calculated using the individual sensory threshold values of all the panellists that detected the compounds.

3.2. Results and Discussion

3.2.1. Meats preferred by the consumer

In a population of 45 panellists it was apparent that the participants appreciate (Table 3.1) and like to eat (Table 3.2) beef, chicken, ham and bacon significantly more than pork. This agreed with the results acquired by Laing and Oram (1999) who also found people preferred to eat chicken, beef and ham rather than pork. Many studies have shown that the lower preference for pork and lamb/mutton products was due to the presence of certain aroma compounds found to be "offensive" by some consumers. The compounds considered to be offensive in pork, from entire males, are androstenone and skatole (Bonneau, 1998). Furthermore, branched- chain fatty acids and skatole have also been suspected by some cultures to cause the unacceptable flavour of sheepmeat (Prescott *et al.*, 2001). An examination of the eating frequency of pork and pork products such as sausages and smallgoods indicated that although there was a slight difference in the eating habits, this was not significant (Tables 3.1 and 3.2).

Table 3.1 The appreciation of pork compared to other meats and meat products, amongst the panellists.

Frequency of eating	Pork	Ham/ Bacon	Sausage/ Smallgoods	Lamb/ Mutton	Beef/Veal	Chicken
Like extremely	3	3	4	6	7	20
Like very much	8	17	7	8	20	14
Like moderately	18	15	18	14	9	7
Neither like or dislike	4	4	9	5	2	4
Dislike moderately	1	0	1	1	1	0
Dislike very much	2	0	1	1	0	0
Dislike extremely	2	0	0	2	0	0
N	38	39	40	37	39	45
P-value		**0.0072**	0.541	0.851	**0.001**	**0.000**

* The P-value indicates the significant difference for the appreciation of pork and other meat products

Table 3.2 Frequency of pork consumption compared to other meats and meat products, amongst panellists.

Frequency of eating	Pork	Ham/ Bacon	Sausage/ Smallgoods	Lamb/ Mutton	Beef/Veal	Chicken
3 times/day	0	0	0	0	1	1
1 times/day	3	2	2	1	2	4
3 times/week	3	10	4	5	10	14
1 times/week	15	21	21	15	22	21
1 times/month	9	6	11	9	4	1
1 times/6 month	8	1	2	7	0	0
Never	2	0	1	3	0	0
N	40	40	41	40	39	41
P-value		**0.0016**	0.136	0.690	**<0.0001**	**<0.0001**

*The P-value indicates the significant difference for the consumption of pork and other meat products. Allowances have been made for differences in N due to cultural differences in which not all respondents will consume all types of meat products.

3.2.2. Distribution of preferences for meats

There is a significantly greater appreciation for pork, ham/bacon lamb/mutton and beef/veal by males than females ($P < 0.05$). Males also eat significantly more ham/bacon and beef/veal compared to females ($P < 0.01$). There is also a trend for males to eat pork more frequently compared to females ($P < 0.10$). A large majority of the males tend to eat pork once a week or more whereas this is not the case for most females (Figure 3.1). Matthews *et al.*, (2000) also found that females were more critical of the odour and flavour of boar meat compared to men. There is however, no significant difference between the sexes, with regard to the appreciation or consumption of chicken and sausages/smallgoods ($P > 0.16$).

3.2.3. Consumption of pork related to androstenone sensitivity

Certain adults are unable to detect androstenone, even in high concentrations (androstenone specific anosmia) whereas their ability to smell other substances is normally developed (Elsely, 1968; Griffith and Patterson, 1970). The sensitivity of individuals to the two

odourants pyridine and PEA along with their sensitivity to androstenone and skatole were tested. Most people tested in the current study were able to detect pyridine, PEA and skatole but 40% of the participants were anosmic to androstenone (Table 3.3). These results agree with previous reports where anosmia to androstenone was found in 25-50% of panellists

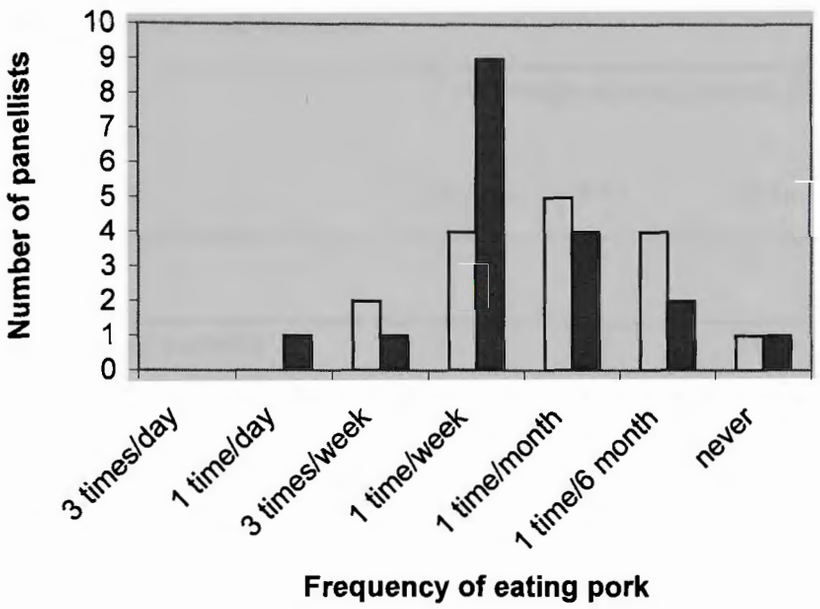


Figure 3.1. Frequency of pork consumption by (■) males and (□) females.

(Elsely, 1968; Amoore *et al.*, 1977; Claus, 1983; Wysocki and Beachamp, 1984) which is indicative of a wide diversity in the level of anosmia in various populations.

The 40% of participants in the present study who are anosmic to androstenone is comprised of an equal percentage of males and females (Table 3.3). These results fall within the range of statistics described by the literature. Anosmia to androstenone was present in 25% of healthy male and female volunteers in a study by Elsely, (1968). Kloek (1961) found 24% of females

and 46% of males to be anosmic to androstenone. Griffith and Patterson, (1970) demonstrated that 7.6% of women and 44.3% of men were unable to detect the odour of androstenone and showed that more women than men judged the smell of androstenone to be unpleasant. Finally, 70% of the women, and 15% of the men rated the smell as unpleasant in a study conducted by Gower *et al.*, (1985) implying that 30% of women and 85% of males were anosmic.

Table 3.3 Frequency of panellists with the ability to detect pyridine, phenyl ethyl alcohol (PEA), skatole and androstenone.

	Percentage of compounds detected by panellists			
	Pyridine	**PEA**	**Skatole**	**Androstenone**
N	40	40	36	36
Percentage of panellists detecting Compound	100	89	100	60
		Percentage of panellists		
	All	**Females**	**Males**	
Detect androstenone	60	29 (59)	31 (60)	
Anosmic to androstenone	40	20	20	

*The percentage of non anosmic panellists within each sex is in parentheses.

In the current study, consumer sensitivity to androstenone appeared to be correlated with the appreciation of chicken ($P = 0.02$). The consumption habits of the consumer for chicken were however not correlated to androstenone sensitivity ($P = 0.36$). Androstenone sensitivity and the consumption of beef/veal were significantly correlated ($P = 0.04$). However, once again the appreciation of beef/veal was not significantly correlated to androstenone sensitivity ($P = 0.57$). There was also a trend for a correlation with the appreciation of lamb and mutton ($P = 0.09$) and androstenone sensitivity. However, the correlation with androstenone sensitivity and the consumption habits of pork, ham/bacon and sausage/smallgoods was insignificant ($P > 0.45$).

The data indicate that the number of female panellists who like to eat pork and pork products is lower compared to males and this does not correlate with the ability to detect androstenone (P = 0.73). The results therefore indicate that, in general, the sensitivity to androstenone does not consistently predict whether people prefer to eat pork or other meats. However, a trend was observed, whereby those people who can smell androstenone, have a tendency to eat less pork compared to those people who cannot detect androstenone (Figure 3.2). Laing (1996) also found that the sensitivity to androstenone did not predict the acceptability of meats or the likelihood that consumers would purchase the meat in the future.

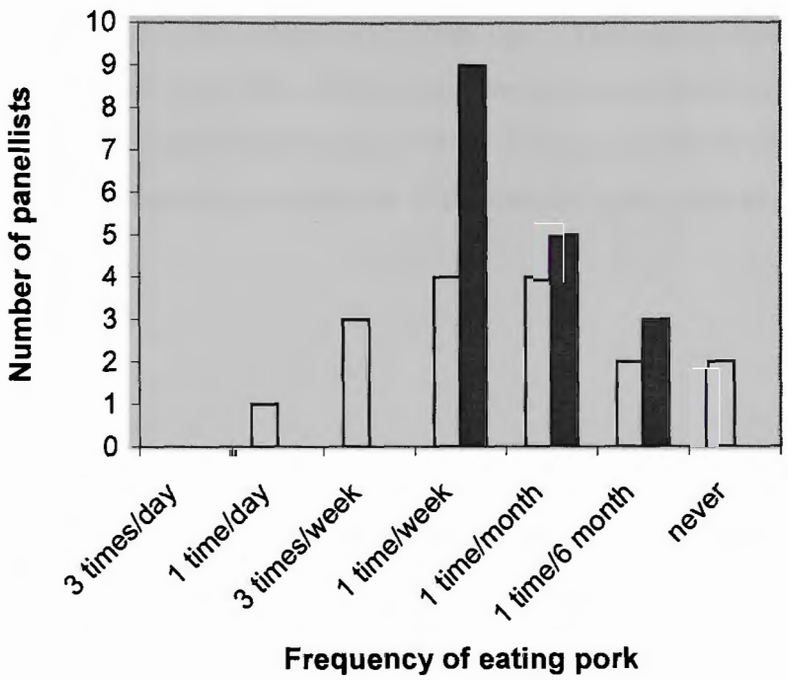


Figure 3.2. Frequency of how frequently pork is eaten between (■) non anosmic and (□) anosmic panellists.

However, Laing (1996) also found that the aroma of pork during cooking was stronger and was liked less by consumers who were sensitive to androstenone. This is further supported by the recent European study which indicates that if sensitivity to androstenone is not screened, there may be quite a substantial underestimation of the true level of taint for the androstenone sensitive consumers (Weiler *et al.*, 2000). The preference for pork is not likely to be related only to the sensitivity and detection of androstenone. It is well known that skatole also has an equal or greater influence on taint, and in addition to skatole, there may be other compounds

that may have an effect. Other studies have also indicated that compounds other than the 16-androstene steroids and skatole appear to be involved in sex taint development (Jeremiah *et al.*, 1999a; Bonneau *et al.*, 2000b). These factors may confound any analysis of the correlation between androstenone sensitivity and pork eating habits.

3.2.4. Sensory threshold for androstenone and skatole

The results from the current study indicate that the sensory threshold for androstenone is in the range of 0.5-88 ppm (Figure 3.3) with a group threshold of 6.45 ppm. Wysocki and Beauchamp, (1984) also detected the sensory threshold for androstenone in identical and fraternal twins and found it to be in the range 2-2000 ppm. The sensory threshold range for androstenone in the current study falls within a more sensitive and narrower range compared to that detected by Wysocki and Beauchamp, (1984). This may be due to the pre-screening and selection of sensitive panellists carried out in the current study, prior to determining the sensory threshold.

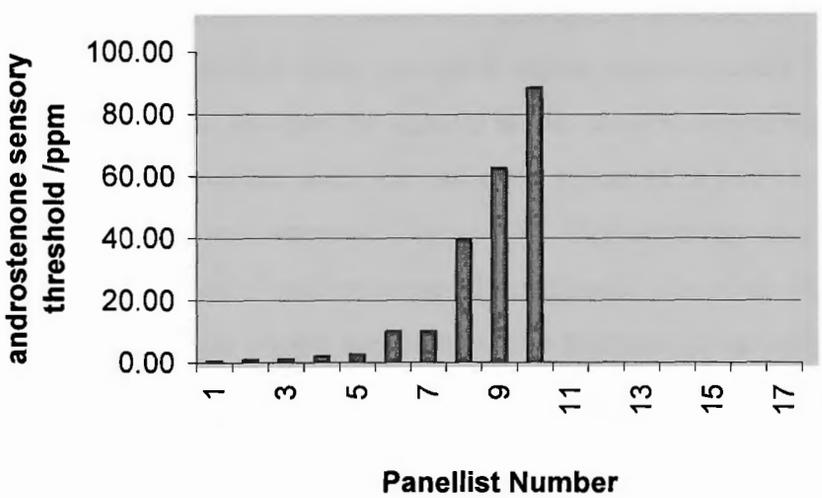


Figure 3.3. Range of sensory threshold for androstenone for panellists, after four consecutive correct detections of the compound. Panellists 11-17 were found to be anosmic.

A study by Annor-Frempong (1997a) indicated that the sensory threshold for androstenone was in the range from $1.4 \times 10^{-5} - 1.0$ ppm with a group threshold of 0.426 ppm. The samples from Annor-Frempong's study were heated at 60-65°C in a glass bottle and samples were kept warm throughout the analysis. Brooks and Pearson, (1989) found the sensory threshold for androstenone to be 0.6 ppm with confidence intervals between 0.1-1.4 ppm. In this case the samples were prepared in the presence of mineral oil and water and all samples were heated to 75°C. The threshold levels were detected at the 50% above chance level. The sensory threshold for androstenone for the panellists in the current study was approximately 10 fold higher than the sensory threshold for the panellists in the studies conducted by Annor-Frempong (1997a) and Brooks and Pearson (1989). This was to be expected given the samples in the current study were not heated and therefore the concentration of androstenone in the headspace of the samples would be lower than in the heated samples.

The sensory threshold for skatole in the current study is in the range 0.0125-1.0 ppm (Figure 3.4) and the group threshold 0.04 ppm. A study by Annor-Frempong (1997a) indicated that the sensory threshold for skatole was in the range of $8.2 \times 10^{-3} - 0.143$ ppm, with a group threshold of 0.026 ppm. The samples from the study of Annor-Frempong were heated at 60-65°C in a glass bottle and samples were kept warm throughout the analysis. The sensory threshold for skatole, in the current study was again higher, approximately 2 fold, than those reported by Annor-Frempong because the samples in this study were not heated. The skatole sensory thresholds of the panellists in the current study appear to be more consistent (Figure 3.4) than those observed for androstenone (Figure 3.3). This is perhaps due to the fact that the perception and recognition of androstenone odour appears to be more variable to that of skatole even when the anosmic people are omitted from the panel (Dijksterhuis *et al.*, 1999).

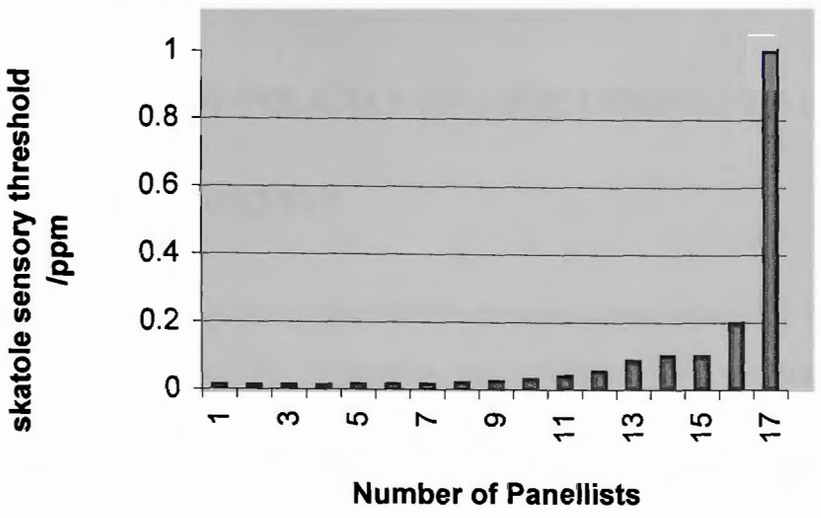


Figure 3.4. Range of sensory threshold for skatole for panellists, after four consecutive correct detections of the compound.

3.3. Summary of Findings

In this small study, consumers have been found to prefer chicken, beef, ham and bacon above pork and sheep meat. Females tend to eat less pork compared to males and it is females who are generally more sensitive to the compound androstenone, although in this study it was found that equal number of males and females are sensitive to androstenone. Whilst sensitivity to androstenone does not statistically predict whether the consumer will purchase pork, the data show trends for reduced consumption of pork by consumers who are sensitive to androstenone. This inconsistency is probably due to other compounds that may interfere with the odour of pork and therefore confound the results. The studies by Babol *et al.*, (1996) and Jeremiah *et al.*, (1999a) have implied that compounds other than androstenone and skatole could be contributing to the presence of boar odour. Nonetheless screening for androstenone sensitivity in particular would appear to be quite important due to the variability of the level of anosmia found in various populations.

Chapter 4

DETECTION OF VOLATILE ORGANIC COMPOUNDS IN PORK

4.0. INTRODUCTION

The contribution of the compounds androstenone and skatole to boar taint is well established (Lundstrom *et al.*, 1980; Mortensen and Sorensen, 1984; Walstra *et al.*,1986; Viallon *et al.*, 1992; Bonneau *et al.*, 1992; Bonneau, 1993; Berg *et al.*, 1993; Andresen *et al.*, 1993; Bejerholm and Barton-Gade, 1993; Hansen-Moller and Godt, 1995). Varied indole compounds have also been thought to contribute to the odour of boar taint (Annor-Frempong *et al.*, (1997c). Some researchers (Beery *et al.*, 1971; Thompson and Pearson, 1977; Bonneau, 1982; Brooks and Pearson, 1989) have postulated that more than one of the 16-androstene steroids may contribute to the offensive nature of boar odour. Amongst these are androstadienol, androstadienone, androstenone, 5α- and 5β-androst-16-en-3-ol. A number of studies have indicated that other volatile organic compounds may be contributing to the odour profile of pork along with skatole and the 16-androstene compounds.

The levels of skatole and androstenone detected in the fat of entire male pigs, in Europe, in a study conducted by Bonneau *et al.*, (2000b) demonstrated that sorting on the basis of androstenone/skatole would reduce, but not eliminate, the differences between entire males and gilt pork. Xue *et al.*, (1996) also reported that the correlation co-efficients found between fat concentrations of androstenone and taint have ranged from 0.43 to 0.76 (Brenann *et al.*, 1986; Lundstrom *et al.*, 1988). The equivalent coefficients for skatole ranged from 0.38 to 0.65 (Lundstrom *et al.*, 1988; Bonneau *et al.*, 1992). However, the regression analysis of the combined effect of androstenone and skatole with the sensory analysis only explained 50% of the variation in boar taint (Hansson *et al.*, 1980). Again this suggests that other compounds are contributing to boar odour (Thompson *et al.*, 1972; Berg *et al.*, 1993) which could ultimately affect the values that are predicted.

Babol *et al.*, (1996) found the correlation between boar taint and the levels of 16-androstene steroids in fat to be insignificant. In a study where there was an exclusion of entire males with the highest levels of these steroids in the fat, taint was still detected. This observation suggests that, even if the increased levels of 16-androstene steroids in the fat were responsible to some degree for boar taint, other important factors are also involved. This indicates that, although both 16-androstene steroids and skatole are important they cannot completely

account for the occurrence of boar taint detected by a trained sensory panel. Other factors probably due to sexual maturity should be considered when estimating boar taint in entire male pigs.

Viallon *et al.*, (1992) conducted a study to identify all the compounds present in the volatile component of pig fat. This group utilised the purge-and-trap method coupled to GC-MS to detect the compounds that contribute to boar taint. They found that 78% of the samples were correctly classified as tainted/non-tainted according to the concentration of androstenone in the fat. However, this method not only identified androstenone but also 55 other molecules. Many of these other compounds are either an aldehyde, ketone, alkane, alkene, alcohol, lactone and/or furan which are all by-products of the lipid oxidation of fatty acids. The other biochemical compounds found were aromatic compounds, chloro compounds, pyrazines and terpenes (Viallon *et al.*, 1992).

In many food oils and fats there is a mixture of saturated and unsaturated fatty acids esterified to glycerol. The three main groups are saturated, mono-unsaturated and poly-unsaturated fatty acids, with a large proportion of these fatty acids being palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. Oxidation of saturated fatty acids is extremely slow at low temperatures, but is much more rapid at elevated temperatures, as for example during frying and roasting. In general, the main reaction involved in oxidative deterioration of food lipids is the non-enzymic reaction between oxygen and unsaturated fatty acids. This oxidation process may lead to the development of various unpleasant odours and flavours, often referred to as "rancid" in oils and lipid-containing foods, rendering them unpalatable. In some foods, such as butter, cheese, cucumber and cooked chicken, a limited degree of lipid oxidation under certain conditions, can result in the desirable characteristic flavours found, typically, in the aroma of fried foods. It should also be noted that while the primary products of lipid oxidation, known as hydroperoxides, are tasteless and odourless, it is the decomposition of the hydroperoxides and secondary volatile oxidation products that give rise to off-flavours and odours in foods containing fatty acid mixtures.

There are many factors that can increase the rate of oxidation such as the degree of unsaturation of the lipid, the presence of transition metals, water content and heat (Saxby, 1996). Other factors such as anti-oxidants, synergists and storage under an inert gas can reduce the rate of oxidation (Saxby, 1996). The effect of these factors will ultimately determine consumer choice, as the overall acceptance of meat products depends to a large extent on their flavour which is mainly determined by the taste and odour of the compounds (Ramarathnan and Rubin, 1994).

A careful selection of the method and sampling temperature is needed to distinguish between off-flavour volatiles, reflecting the actual flavour of the food lipid at the time of testing, and the breakdown of flavour precursors indicating the formation of positive or negative components. There are therefore many factors to consider when assessing the odour profile of fat, particularly from boars. Firstly, the malodour of the samples generally occurs when they are cooked at high temperatures. In addition to androstenone and skatole other compounds may contribute to the odour profile. A method that detects all volatile organic compounds (VOCs) that contribute to the odour upon cooking is required. The first aim of this study is to develop a system to produce and collect volatile compounds from the fat from boar and gilts in a manner that emulates a normal cooking process. Secondly, the identity of the main compounds present in the aroma profile of heated fat as detected by GC-MS headspace analysis, is to be established. The main compounds identified using this method can then be correlated to the results of the sensory analysis of the same samples of fat, and these results will be discussed in Chapter 5. From this analysis it will be possible to determine which compounds contribute to the odour profile of fat from boars and their relative importance.

4.1. Material and Methods

4.1.1. Animals

Subcutaneous abdominal samples of fat were collected from the carcasses of Large White × Landrace pigs. A total of 42 samples were collected on two occasions. The first set was collected from pigs of an average age of 156 days with a dress weight of about 75 kg. The second set was collected from pigs of an average age of 132 days with a dress weight of about 71 kg. The animals were fed a standard, finisher diet, which consisted of 11% wheat, 45.5% meat and blood meal and 43.5% soy bean meal as the main ingredients. Samples of fat from the first cohort were selected from 6 gilts and 9 boars. From the second cohort, samples were selected from 4 gilts, 4 boars and 1 immuno-castrate (24 samples in all). The immuno-castrate pig was subcutaneously vaccinated twice, as appropriately required for the Improvac® vaccine (Dunshea *et al.*, 2001). The samples were selected for high and low levels of androstenone and skatole in boars, as indicated by HPLC analysis, with gilts having low to undetectable concentrations of these compounds. The samples of fat (200-400 g) were stored at –20°C in sealed freezer bags. Prior to analysis the surface layer of the fat was removed and discarded and 10 g of the remaining fat that had not been exposed to the atmosphere was cut into approximately 1 g pieces for analysis.

4.1.2. Chemical analysis

4.1.2.1. GCMS Analysis

Volatile compounds acquired from the headspace of 10 g of pig fat were trapped onto a Tenax trap (Air –Met Scientific Ltd, Nunawading, Victoria, Australia). The fat was placed in a 250 mL bulb attached to a Dreschell tube. A carbon trap was attached to one arm of the tube and a supply of ultra high purity nitrogen (BOC, Chatswood, Australia) to the other arm of the tube. The fat was heated in a heating chamber (Electrothermal Engineering Ltd, Essex, England) cycling between 180-220°C. The temperature was to increase at a rate of approximately 5°C per min. The nitrogen flowed through the apparatus at a flow rate of 200 mL min^{-1} and was measured at 18°C using a digital flow meter (ADM 2000, J & W Scientific, Folsom, CA). The temperature was monitored using a thermometer and the heating chamber was then adjusted accordingly, by manual intervention. The fat was heated and the volatile compounds were purged for 1 h. After the concentration step, 0.5 mL of methanol (Merck, Kilsyth, Australia) was absorbed by a Tenax™ ampoule and the volatile compounds were then eluted with 1 mL of methanol. One microlitre of the eluant was then injected into the GC-MS for analysis.

The GC was equipped with a DB5-MS fused silica column (DB-5MS, 30 m x 0.25 mm i.d.) containing a crosslinked methylsilicone film of 250 μm thickness (Alltech Associates, Baulkham Hills, Australia). A temperature program comprising: (i) a 10 min hold at 50°C, (ii) an increase to 270°C at 5°C min^{-1} and (iii) a 1 min hold at 270°C was used. The total run time was 55 min and the injector was maintained at 270°C.

Mass spectra were recorded using a Varian Saturn 2000 ion trap mass spectrometer with an ion source temperature of 200°C, manifold temperature of 60°C and an electron multiplier energy of 1850 eV. The mass spectrometer transfer line was maintained at 250°C. The MS was automatically calibrated against the FC 43 gas (Varian, Mulgrave, Australia). The area counts of the chromatographic peaks were automatically calculated using the Saturn GC/MS Workstation 2000 software (Varian). The tentative assignment of each chromatographic peak was determined using a GC/MS mass spectral library (NIST Library, 2000).

The androstenone, androstanone and skatole standards were purchased from Sigma and were used to check the MS spectra and the retention time of these compounds identified in the samples of fat. The androstanone standard was used as an internal standard. The fat was

spiked with androstanone at a concentration of 5 ppm, prior to heating the fat

4.1.2.2. HPLC Analysis

The HPLC analyses for the 24 samples of fat analysed was carried out as described in Chapter 2, Section 2.1.3.

4.1.3. Data analysis

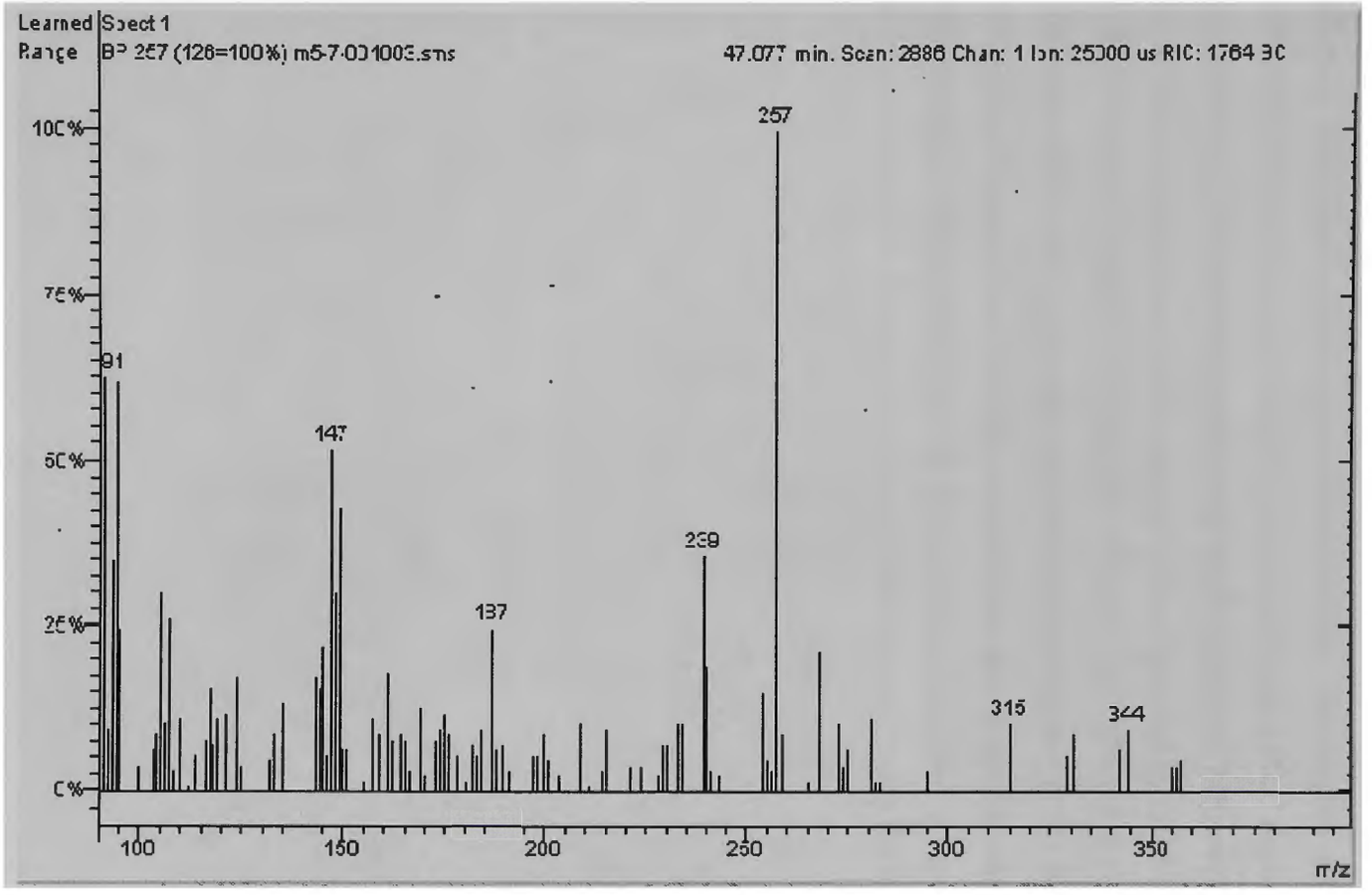
The statistical analyses were conducted using GenStat® 5, Release 4.1. A Wilcoxon rank sum test was used to determine any significant differences between the GC-MS and HPLC methods and the water content of the samples.

4.2. Results and Discussion

4.2.1. Mass spectra of the standards

Androstenone ($C_{19}H_{28}O$, MW = 272.4 g mol^{-1}, mp = 142.5 - 143°C, CAS number = 18339-16-7; de Kock *et al.*, 2001) is a known contributor to boar taint and the samples of fat from the boars were selected for the presence of androstenone using the reverse phase HPLC method as described in Section 2.1.3. Edelhaueser (1989) identified the main ions for the mass spectrum of androstenone to be m/z = 257 (100%), 272 (74%), 239 (11%) amu with the percentage in brackets representing the relative intensity of each ion. This study also found the main ions of androstenone to be m/z = 257, 272 and 239 amu. A typical mass spectrum of the androstenone isolated from the fat of boars investigated in the present study is shown in Figure 4.1. The retention times for androstenone in the samples of fat was 47.020 min and 46.956 min for the standard.

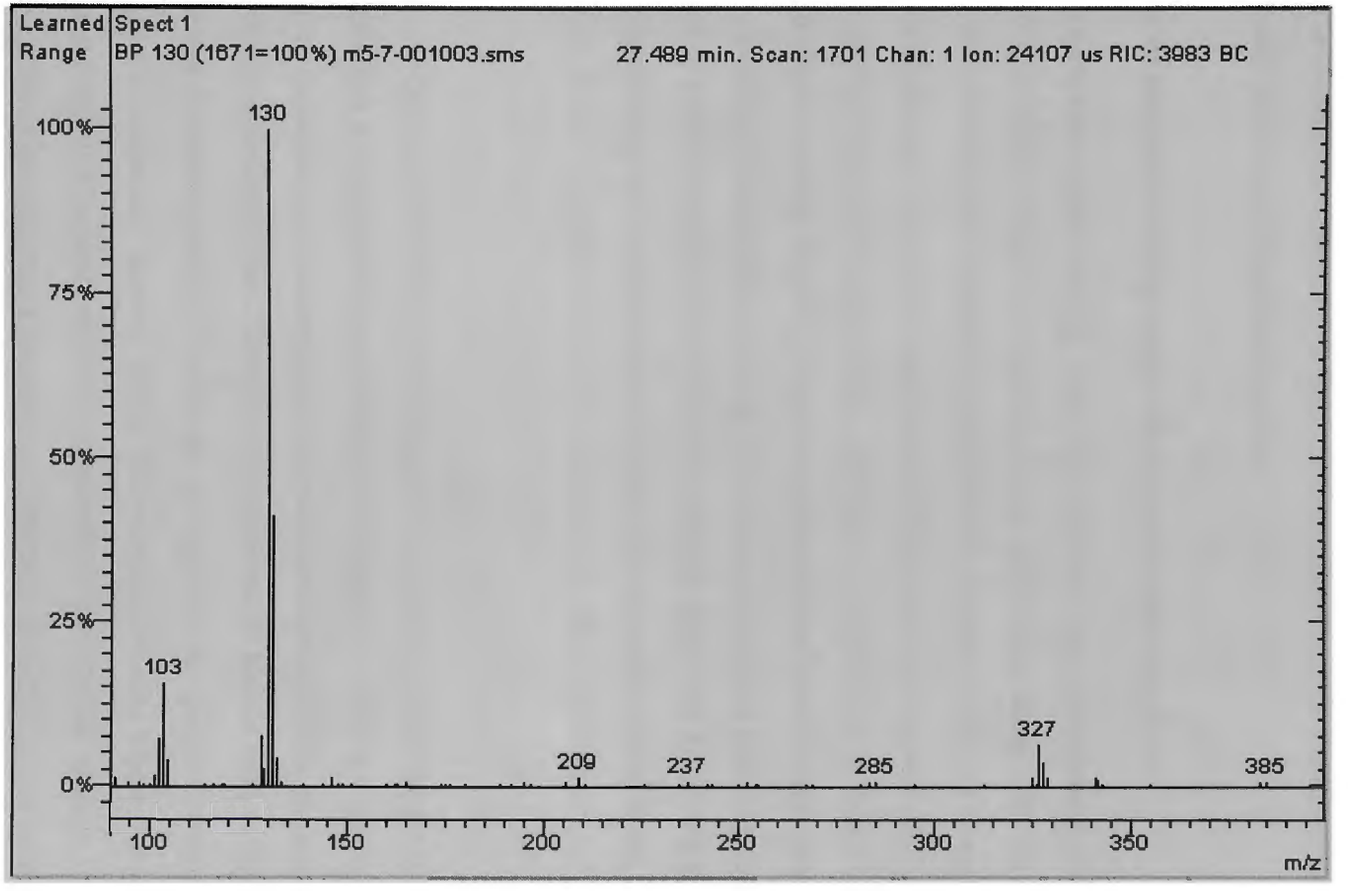
Figure 4.1. Mass spectrum for androstenone in pig adipose tissue



Androstanone, ($C_{19}H_{30}O$, MW = 274, CAS number = 1224-95-9) a closely related compound to androstenone was used as an internal standard in the GC-MS analysis. Edelhaueser (1989) found the main ions in the mass spectrum of androstanone to be m/z = 274 (100%), 259 (35%). In this study the main ions detected for androstanone were m/z = 202, 259 and 274 amu.

Skatole (C_9H_9N, MW = 131.18 $gmol^{-1}$, mp = 95-97°C, bp = 265-266 °C, CAS number = 83-34-1; de Kock *et al.*, 2001) is also a known contributor to taint. The samples of fat from the boars were selected for the presence of skatole as determined by the HPLC method as described in Section 2.1.3. The skatole standard was used to check the MS spectra and retention time of the compound found in the samples of fat as detected by GC-MS. Peleran and Bories (1985) found the main ions for skatole to be m/z = 131 (55%), 130 (100%) and 103 (10%) amu in the SIM mode. The main ions for indole were m/z = 117 (100%), 90 (46%), 89 (36%) amu. In the present study the main ion detected for skatole was m/z = 130 amu. The mass spectrum of the skatole found in the fat of boars is shown in Figure 4.2. The retention times for skatole in the samples of fat was 27.464 min and 27.428 for the standard.

Figure 4.2. Mass spectrum for skatole in pig adipose tissue.



4.2.2. Development of GC-MS pork fat odour profile assay

A modified method of Viallon *et al.*, (1992) was the starting point for this GC-MS assay. The samples of fat were heated and the VOCs collected onto a Tenax™ trap and eluted using an automated headspace analyser. However, in the current modified method (see Section 4.1.2.1) the volatile compounds bound to the Tenax™ tube were eluted with methanol and injected on the GC-MS. Both androstenone and skatole contribute significantly to boar taint and it was therefore considered important to ensure that each of these were able to be detected. Some method development to find the best conditions, for the detection of androstenone and skatole was therefore required.

4.2.3. Effect of time and cooking temperature on androstenone level

The samples of fat were heated for 0.5, 1 and 1.5 h between the temperatures 200 and 240°C. The heating of the sample of fat, trapping and elution of volatiles along with the detection of androstenone and skatole was as otherwise described in Section 4.1.2.1. The amount of androstenone and skatole detected for each treatment is plotted in Figure 4.3. This is a typical representation of the amount of androstenone and skatole extracted from numerous samples of fat for the different heating regimes. Skatole appeared to be more volatile than androstenone, and thus more easily extracted. The most appropriate time to release the largest amount of androstenone was therefore determined. This was a cooking time of 1 h, as shorter time periods may not be long enough for the androstenone to bind to the Tenax™, and over longer periods the androstenone may be displaced by other volatiles or perhaps break down.

The next experiment was performed to investigate the recovery of androstenone and skatole at various temperatures and the results are displayed in Figure 4.4. This is a typical representation of the amount of androstenone and skatole extracted from numerous samples of fat for the different temperatures. Samples were previously heated with variable times and it was evident that a cooking time of 1 h was the optimal time for maximum extraction and collection of androstenone and skatole. In the following experiment where the samples were heated for 1 h at variable temperatures it was apparent that most of the skatole and androstenone had been extracted at a temperature between 180-220°C. The lower temperature i.e.160-200°C extracted less and the 200-240°C temperature regime was too high, possibly resulting in the displacement of androstenone by other compounds or the high temperatures caused it to break down.

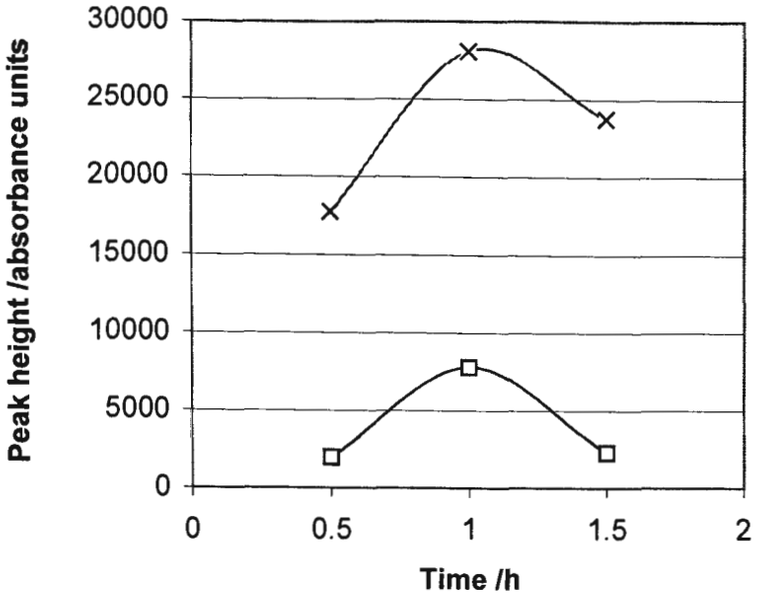


Figure 4.3. The amount of androstenone (□) and skatole (x) detected as a function of time with all samples of fat being heated between the temperatures of 200°C - 240°C.

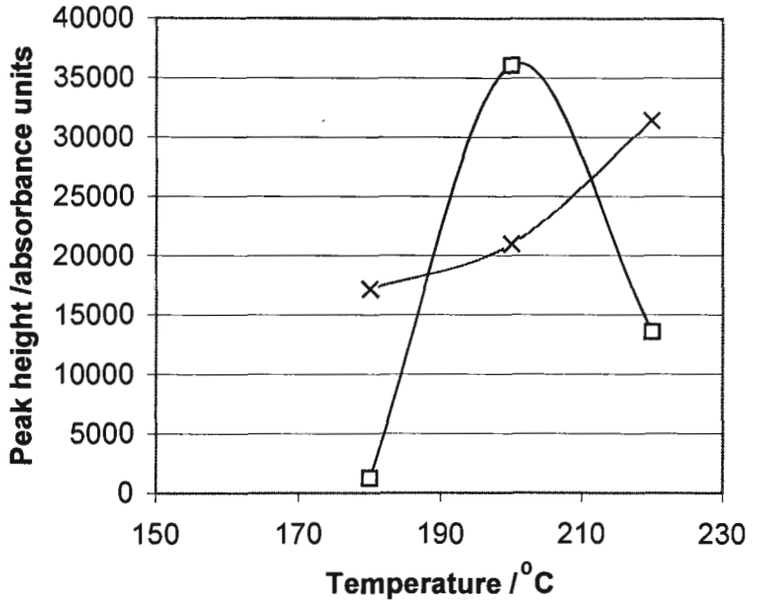


Figure 4.4. The amount of androstenone (□) and skatole (x) detected with the temperature ranging between 160-200°C, 180-220°C and 200 –220°C. Samples were incubated for a constant time of 1 h.

The time and temperature of heating selected for the final method allowed for a balance between the maximum extraction of androstenone and skatole. It was therefore concluded that a method consisting of heating the fat between the temperatures 180-220°C for 1 h was necessary. The temperature was to increase and decrease at a rate of approximately 5°C per min. These conditions were required to achieve the optimum extraction of androstenone and skatole.

4.2.4. Liquid phase extraction versus headspace analysis of taint compounds

The samples of fat from boars were analysed and selected for the presence of androstenone and skatole by liquid extraction of the fat, followed by HPLC analysis (see section 4.1.2.2). The same samples were also analysed by the GC-MS method as described in section 4.1.2.1. The correlation of the peak areas of the extraction of skatole by HPLC and the GC-MS headspace analysis of skatole was not significant ($R^2 = 0.226$, $P = 0.1003$). The correlation between the HPLC and the GC-MS methods for the extraction of androstenone indicated a trend but this was not highly significant ($R^2 = 0.278$, $P = 0.064$). There appears to be a trend in the correlation between the two methods, for the two compounds, however this is not highly significant.

There are several reasons why these correlations are not highly significant. The HPLC method uses a solvent extraction method that allows a quantitative recovery of the total amount of androstenone and skatole, while the GC method only measures the amount of the compounds volatilised by the heating conditions. The variation observed between the two methods implies a difference in the level of androstenone and skatole extracted. Androstenone compared to skatole is more lipophilic, has a higher molecular weight and a higher melting and boiling point (Windholz *et al.*, 1983). The water solubility of androstenone is 0.00023 g L^{-1} at 25°C (Amoore and Buttery, 1978) compared to that of skatole which is 0.45 g L^{-1} at 20°C (Windholz *et al.*, 1983). The water content in the samples of fat as was observed in Chapter 2 can be quite variable. The water content is also generally higher in the fat from boars than gilts. These factors and the greater solubility of skatole, compared to androstenone, in water may explain the slightly better correlation observed for androstenone compared to skatole between the two methods. The GC-MS method may be more appropriate when making comparisons between the concentrations of chemical compounds and the sensory analysis of taint, as the gas phase extraction method is more similar to the cooking process used with the sensory analysis compared to the solvent extraction used in the HPLC method.

4.2.5. Identification of volatile compounds in fat

Once the GC-MS method had been developed, the volatile compounds in the odour profile of the 24 samples of fat, were detected. A representative aroma profile of pig adipose tissue is shown in Figure 4.5. and the tentative identifications of the compounds in the odour profile are shown in Table 4.1. The four main groups of compounds identified were fatty acids, aldehydes, ketones and alcohols and are summarised in Table 4.2. The typically low levels of the compounds androstenone (peak 31) and skatole (peak 30) compared to the other compounds is indicated in this chromatogram.

Figure 4.5. Chromatogram of the peaks identified in a representative odour profile of pork fat.

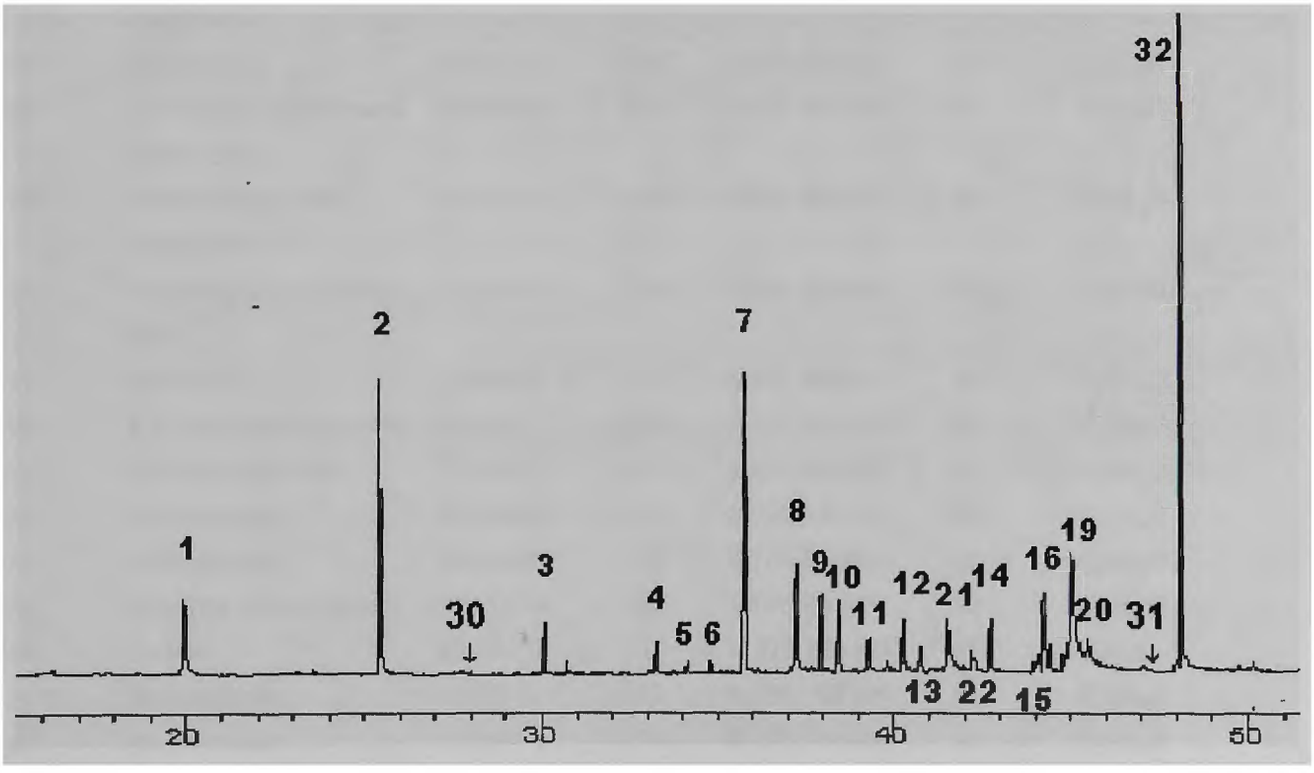


Table 4.1. Identification of the compounds in the odour profile of fat from boars and gilts.

Peak	Compound Tentative assignment	CAS No	MW	FIT[1]	Prob[2] %	Formula
1	3-nonen-1-ol	10340-23-5	142	S:783, RS:815	19	$C_{19}H_{18}O$
2 and 3	Siloxane					
4	1-undecanol	112-42-5	172	S:649, RS:725	19	$C_{11}H_{24}O$
7	2-nonadecanone	629-66-3	282	S:807, RS:878.	44	$C_{19}H_{38}O$
8	1-azabicyclo (2,2,2) octan-3-one	3731-38-2	125	S:355, RS:638.	19	$C_7H_{11}NO$
9	2-dodecanone	6175-49-1	184	S:819, RS:839.	23	$C_{12}H_{24}O$
10	Tetradecanal	124-25-4	212	S:884, RS:900	16	$C_{14}H_{28}O$
11	3-methyl-4 (3,7,7-trimethyl-2-oxa-bicyclo[3,2,0] hept-3-en-1-yl) but-3-en-2-one	-	220	S:591, RS:610	29	$C_{14}H_{20}O_2$
12	2-methyl-hexadecanal	55019-46-0	254	S:795, RS:847	11	$C_{17}H_{34}O$
13	Hexadecanoic acid methyl ester	112-39-0	270	S:866, RS:873	69	$C_{17}H_{34}O_2$
14	Pentadecanal	2765-11-9	226	S:879, RS:912	19	$C_{15}H_{30}O$
15	9,12-octadecadienoic acid methyl ester	2566-97-4	294	S:871, RS:929	19	$C_{19}H_{34}O_2$
16	9-octadecenoic acid methyl ester.	112-62-9	296	S:879, RS:894	24	$C_{19}H_{36}O_2$
18	Octadecanoic acid methyl ester	112-61-8	298	S:888, RS:892	71	$C_{19}H_{38}O_2$
19	Oleic acid	112-80-1	282	S:831, RS:843	44	$C_{18}H_{34}O_2$
20	9,12-octadecadienoic acid	60-33-3	280	S:773, RS:783	39	$C_{18}H_{32}O_2$
21	n-hexadecanoic acid	57-10-3	256	S:829, RS:843	53	$C_{16}H_{32}O_2$
22	13-octadecenal	58594-45-9	266	S:855, RS:878	20	$C_{18}H_{34}O$
23	2-tridecanone	593-08-8	198	S:779, RS:806	15	$C_{13}H_{26}O$
24	Butylated hydroxytoluene	128-137-0	220	S:909, RS:921	72	$C_{15}H_{24}O$
30	Skatole	83-34-1	131	S: 825, RS: 827	65	C_9H_9N,
31	Androstenone	18339-16-7	272	S: 798, RS 809	83	$C_{19}H_{28}0$,
32	Androstanone	1224-95-9	274	S: 836, RS: 858	95	$C_{19}H_{30}0$,

[1] Fit is the similarity in the simulation (S) and reverse simulation (RS) of the detected compound compared to those in the NIST library. The fit is between 0-1000 and over 700 is considered a good match.

[2] Probability is a % regarding the similarity of the identified compound compared to the compounds in the NIST library

Table 4.2. Identification of the family of compounds, the odour index and the peak area frequency.

Compound Family	Peak Number	Compound Tentative assignment	Peak Freq[a]	Odour Index[b] /ppm	Reference
Alcohol	1	3-nonen-1-ol	19	$10^{1.5}$-10^{4}	Hamilton-Kemp & Andersen (1986)
	4	1-undecanol	4	$10^{1.5}$-10^{4}	Timon *et al.*, 2001
Ketone	7	2-nonadecanone	24	4	Elmore *et al.*, (2001)
	9	2-dodecanone	3	4	Timon *et al.*, (2001)
	23	2-tridecanone	6	4	Hamilton-Kemp & Andersen (1986)
	8	1-azabicyclo (2,2,2)-octanone	10	4	Berdague *et al.*, (1992) Elmore *et al.*, (2001)
	11	3-methyl-4 (3,7,7-trimethyl-2-oxabicyclo [3,2,0] hept-3-en-1-yl) buten-3-en-2-one	4	4	
Aldehyde	10	Tetradecanal	23	10^{5} - 10^{7}	Elmore *et al.*, (2001) Hamilton-Kemp & Andersen (1986)
	14	Pentadecanal	20	10^{5}-10^{7}	Elmore *et al.*, (2001) Hamilton-Kemp & Andersen (1986)
	22	13-octadecenal	11	10^{5}- 10^{7}	Timon *et al.*, (2001) Young *et al.*, (1997)
	12	2-methyl-hexadecanal	18	10^{5} - 10^{7}	
Fatty acids	21	n-hexadecanoic acid	10	10^{5}	
Saturated	18	Octadecanoic acid methyl ester	21	10^{5}	
	13	Hexadecanoic acid methyl ester	21	10^{5}	
Mono-	19	Oleic acid	14	10^{5}	
Unsaturated	16	9-octadecenoic acid methyl ester.	17	10^{5}	
Poly-	15	9,12-octadecadienoic acid methyl ester	19	10^{5}	
Unsaturated	20	9,12-octadecadienoic acid	5	10^{5}	
Imidazole	30	Skatole	9	3×10^{4}	Young *et al.*, (1997)
Hormone	31	Androstenone	13		
Miscellan-eous	2 & 3	Siloxane	23		
	24	BHT	19		

[a] Peak Freq is how often the peak was identified in the fat samples.

[b] Odour Index is the vapour pressure divided by the odour recognition threshold (100% ppm)

4.2.6. Fatty acids and oxidation products

4.2.6.1. Saturated fatty acids

Peak 18 has been tentatively assigned as the methyl ester of octadecanoic acid. Octadecanoic acid is commonly known as stearic acid and is a C18:0 fatty acid. Peak 13 has been tentatively assigned as the methyl ester of hexadecanoic acid and Peak 21 tentatively assigned as hexadecanoic acid commonly known as palmitic acid (Table 4.1) which is a C16:0 saturated fatty acid. Palmitic acid is one of the most abundant fatty acids in nature and is found in the lipids of all organisms with stearic acid also found to be relatively common (Christie, 1989). The most abundant saturated fatty acids in animal and plant tissues, are the straight-chain compounds with 14,16 or 18 carbon atoms (Christie, 1989). However, all the possible odd and even numbered homologues with 2 to 36 carbon atoms have been found in nature in esterified form (Christie, 1989). The saturated fatty acids are less susceptible to lipid oxidation than the unsaturated fatty acids.

4.2.6.2. Mono-unsaturated fatty acids

Peak 16 has been designated as the methyl ester of 9-octadecenoic acid and Peak 19 designated oleic acid. The most abundant monenoic fatty acid in tissues is cis-9-octadecenoic acid which is commonly termed oleic acid and is found in many lipids of plant and animal origin (Christie, 1989). Oleic acid is one of the higher chain fatty acids which oxidises to form the shorter chain fatty acids (Piedrafita *et al.,* 2001). The *cis*-monenoic fatty acid of 18 carbon atoms or less, such as oleic acid, have melting points close to room temperature and therefore susceptible to oxidation at room temperature. The *trans* isomers have higher melting points (Christie, 1989).

4.2.6.3. Poly-unsaturated fatty acids

The tentative assignment for Peak 15 is the methyl ester of 9-12-octadecadienoic acid and Peak 20 designated 9,12-octadecadienoic acid, commonly known as linoleic acid which is a 18:2 (n-6) fatty acid. These are examples of polyunsaturated fatty acids (PUFAs) that contain from two to a maximum of six *cis* double bonds, separated by a single methylene group, known as methylene-interrupted unsaturation (Christie, 1989). The PUFAs containing one or more methylene interrupted double bonds in the cis-configuration are very susceptible to oxidation, with atmospheric oxygen (Kochhar, 1993).

Linoleic acid is the most widespread of this PUFA type and is a major constituent of complex lipids found in most animal tissue. Feed is a source of tissue linoleic acid (C18:2n-6) and α-linolenic acid (C18:2n-3) for they cannot be synthesized by the animal, however they are important for animal growth, reproduction and development (Saxby, 1996). Most vegetable oils and nuts are the principal source of these essential fatty acids (EFAs). In animal tissues linoleic acid is the precursor of a family of other fatty acids which are produced from it, by desaturation and chain elongation. The enzymes in animals are only able to insert new double bonds between an existing double bond and the carbonyl group. Linoleic acid therefore serves as the precursor of a family of fatty acids in which the terminal (n-6) structure is retained. Arachadonic acid is the most important of the linoleic acid metabolites appearing to have special functions in the membrane phospholipids of the nervous system and the eye tissue. It is also the precursor for specific prostaglandins (Christie, 1989).

Diets rich in linoleic acid appear to have an effect on the odour of meat products (Warnants *et al.,* 1998). Generally the oxidation of unsaturated fatty acids leads to the formation of saturated, mono and poly unsaturated aldehydes. These aldehydes play an important role in the flavour of cooked chicken and other meat products (Wettasinghe *et al.,* 2001).

4.2.6.4. Products of lipid oxidation

The tentative assignment for peak 12 is 2-methyl-hexadecanal, peak 10 is tetradecanal and peak 14 is pentadecanal. 13-octadecenal is the tentative assignment for peak 22. Pentadecanal along with 8,11-heptadecadienal and 8,11,14-heptadecatrienal have been thought to arise from the oxidation of palmitic, linoleic and linolenic acids respectively (Hamilton-Kemp and Andersen, 1986). These authors identified pentadecanal as a high boiling compound found as a major constituent of wheat, which is one of the components of the diet that was fed to the animals in this study. Longer chain aldehydes like 2-undecenal are good indicators of a grain diet (Young *et al.,* 2001). Pentadecanal and tetradecanal were identified in this study, and by Hamilton-Kemp and Andersen (1986) in wheat and by Elmore *et al.,* (2001) in pork

In many cases, the precursors of volatile aldehydes such as butanal, pentanal, hexanal, heptenal are the unsaturated fatty acids (Martin *et al.*, 2000). These volatiles are extremely important since they add a highly pleasing sweet or fruity character to the aroma of cured ham (Specht and Baltes, 1994) and have low perception thresholds due to their low odour index*.

*Odour index is a dimensionless term that is based upon vapour pressure/ppm divided by the odour recognition (100% ppm) threshold.

These observations indicate that their contribution to the ham aroma is much higher than their scarce presence might indicate (Martin *et al.*, 2000). Badings (1984) has mentioned octadecadienoic acid to be the precursor of the aldehyde heptenal. The 2,4–decadienals identified have been reported to have oily, deep fat frying odours. Volatile aldehydes formed from the decomposition of lipids play both positive and negative roles in lipid-rich foods (Grosch, 1987). They are probably the most interesting of the lipid derived volatiles as they have low threshold olfactory concentrations (Shahidi *et al.*, 1986) and constitute an important group of natural flavours (Martin *et al.*, 2000). In general, saturated aldehydes help strengthen and intensify aroma, while the 2-enal and the 2,4 enal compounds add sweet, fruity and fatty features to the flavour and smell of products (Hamilton, 1989). It is only when the concentrations of the aldehydes occur above a certain threshold, flavour defects will occur. Hexanal is an abundant aldehyde detected in pork products. It is an index of lipid oxidation because it is often associated with rancidity (Zanardi *et al.*, 2000). Hexanal, however was not detected in this study.

Peak 9 has been tentatively assigned to 2-dodecanone, Peak 7 was designated 2-nonadecanone and Peak 23 was designated 2-tridecanone. Aliphatic ketones are formed by the oxidation of unsaturated fatty acids and contribute to the desirable and undesirable flavours of oils and food products (Saxby, 1996). The even numbered β-keto alkanoic acids esterified in the glycerides are reported to be the precursors of methyl ketones. Such glycerides are present at a level of about 0.4% in butterfat. The methyl ketones are produced from these precursors by hydrolysis and decarboxylation and the reaction is accelerated by heat. Hamilton-Kemp and Andersen (1986) isolated a high boiling compound extracted from wheat known as 2-octa-decanone. It had a GC-MS fragmentation pattern that indicated it was an aliphatic methyl ketone exhibiting a characteristic base peak at 58 and a series of ions 14 amu apart (Heller and Milne, 1975). The fragmentation pattern for the ketones identified in this study also had a main peak at 58 amu and a series of ions 14 amu apart. The ketone decanone has been identified by a number of studies such as Elmore *et al.*, (2001) in pork, Timon *et al.*, (2001) in ham and Hamilton-Kemp and Andersen (1986) in wheat.

Nonaka *et al.*, (1967) reported the presence of 3-octanone and 5-undecanone in cooked chicken. A complex form of octanone and butenone were also identified in this study. Methyl ketones are also formed in mould ripened cheeses and in high concentrations particularly, 2-heptanone and 3-nonanone, contribute to the desirable flavours (Kinsella and Hwang,1976). These compounds along with short chain fatty acids and lactone are important flavour constituents of butter aroma, however any imbalance among these substances may cause flavour defects. Langler and Day (1964) also showed that methyl ketones at

concentrations far below their flavour threshold values interacted positively to produce unpleasant off-flavours. The thresholds of methyl ketones, (i.e. the alkan-2-ones) are significantly higher than those of their isomeric aldehydes (Seik *et al.*, 1971) and they also vary with carbon chain length. Therefore at similar concentrations they are relatively unimportant in the flavour/odour of meat products, however they can add spicy, fatty character to the product (Grosch, 1982 Molimard and Spinnler, 1996).

The production of alcohols are a result of the degradation of fat (Watanabe and Sato, 1971). The alcohols 3-nonen-1-ol and 1-undecanol were identified in the odour profiles from a number of samples in this study. Nonenol was also identified by Hamilton-Kemp and Andersen (1986) in wheat and 1-undecanol has been identified in ham by Timon *et al.*, (2001). Other alcohols such as 1-octenol have been identified in pork (Elmore *et al.*, 2001) and beef (Wettasinghe *et al.*, 2001).

4.2.6.5. Anti-oxidants

The tentative assignment for Peak 24 is butylated hydroxy toluene (BHT) which is an anti-oxidant. This compound is often added to many solvents such as methanol. There was also a significant positive correlation ($P = 0.015$) between the level of BHT remaining in the fat, after the heating process and the water content of the samples. The rate of lipid oxidation in foods depends strongly on water content (Karel, 1980). In dried foods the moisture content is low and oxidation proceeds rapidly. An intermediate level of water activity, ($a_w = 0.3$), is required to protect against the effects of lipid oxidation (Saxby, 1996). However if the water activity is too high (0.6-0.9) the rate of oxidation is accelerated again probably because of an increased mobilisation of the pro-oxidant catalysts in the foodstuffs. These results further emphasize that the rate of lipid oxidation may be dependent on the water content of the samples. Bonneau (1998) also stated that the adipose tissues of entire males had higher water content and more unsaturated fatty acid, therefore making them less resistant to oxidation that may ultimately affect the odour of the sample.

4.2.7. Skatole, androstenone and androstanone - Peak assignment

The "standard" taint chemicals, skatole and androstenone were assigned to Peaks 30 and 31 respectively. Androstanone, added as an internal standard, was designated Peak 32. The mass spectra for these compounds have been discussed in Section 4.2.1.

4.3. Summary of Findings

The odour profile of fat has been found to contain a variety of compounds that belong to the family of fatty acids, alcohols, aldehydes and ketones. This agrees with other studies conducted by Elmore *et al.,* (2001) and Viallon *et al.,* (1992). Some chemical compounds identified in this study were found to be exactly the same as those identified by these authors whilst others were not, although they belong to the same family of compounds. The compounds in this study have been extracted from heated fat, a process which is representative of the cooking process. However the extraction method was slightly different from other headspace studies, in that there was a liquid extraction of the volatiles as opposed to a gas-phase extraction. This may explain why a number of fatty acids and esters have been identified in this study and not others.

Many of the compounds with sensory effects are likely to be derived from the breakdown of triglycerides. There is a need to investigate how the various compounds correlate with the sensory attributes of the fat. Cooked meats are highly susceptible to lipid oxidation because the cooking process denatures compounds, damages cell structure and exposes membrane lipids to the environment (Ahn *et al.*, 1998). The substantial proportion of fatty acid esters and lipid oxidation products may be a result of the high temperature to which the samples of fat are subjected. The roasting process subjects the fat to high temperatures that will clearly promote triacylglycerol and fatty acid volatility resulting in more branch chain volatiles than is found with boiling or frying (Brennand and Lindsay, 1992). Esters are of particular interest because of their fruity character and their low odour threshold values. (Bruna *et al.,* 2001).

Pork has a relatively high content of PUFA and therefore oxidises more rapidly than either beef or lamb (Pearson, Love and Shaland, 1977). The most abundant fatty acid found in dry cured hams was oleic, followed by palmitic, stearic and linoleic acids which were also present in relatively large amounts (Timon *et al.,* 2001). These fatty acids have been found in the cooked fat of the animals in this study. Cooking of pork patties caused a four-fold increase in oxidation levels compared to raw patties (Mc Carthy *et al.,* 2001). Kanner (1994) showed that high temperature decreased the activation energy of the oxidation process, breaking down preformed hydroperoxides that propagate lipid oxidation. This probably accounts for the higher levels of oxidation in cooked patties compared to raw patties. The cooking process probably caused a degree of lipid oxidation, resulting in the formation of various aldehydes such as 2-methyl-hexadecanal, pentadecanal, tetradecanal, and octadecenal, ketones such as 2-nonadecanone, 2-dodecanone, 2-tridecanone, complex forms of octanone and butenone, and alcohols such as 3-nonen-1ol, 1-undecanol.

Aldehydes have very low odour threshold and may provide the most significant odour whilst alcohols have a higher odour threshold and therefore a higher concentration of these compounds is required to make a contribution to the odour. It can be seen however that there are many compounds that have the potential to make a significant contribution to the odour profile, with androstenone and skatole not likely to be the only contributors.

Chapter 5

VOLATILE ORGANIC COMPOUNDS RELATED TO SENSORY ANALYSIS OF PORK FAT

5.0. INTRODUCTION

The production of entire male pigs is more efficient than females or castrates but is restricted by the variable quality and presence of taint in the pork from boars. Studies have shown that amongst the factors that contribute to the variability, skatole and androstenone play an important role. However, the effectiveness and practicability of classification systems based on these two compounds has been low and has not generally led to a reduction in the variability of pork from boars. One important factor may be that, while many studies have been conducted on the components of boar taint, only androstenone and skatole have been consistently identified as having a significant correlation. However, the reported correlations, 0.3 to 0.8 are variable and often low.

There is general agreement within the industry that androstenone and skatole are important contributors to boar taint, however the relative importance of each is disputed. Berg *et al.*, (1993) found androstenone and skatole made similar contributions to boar taint. Bonneau *et al.*, (1992) however, demonstrated that the contribution of androstenone to boar taint was larger than that of skatole. In a number of other studies skatole has been reported to have a higher contribution to boar taint than androstenone (Lundstrom *et al.*, 1984, 1988; Mortensen and Sorensen, 1984; Walsta *et al.*, 1986; Andresen *et al.*, 1993; Bejerholm and Barton-Gade, 1993). Regardless of which compound is the major contributor, a number of studies have shown that androstenone and skatole odours act synergistically to enhance taint (Lundstrom *et al.*, 1980; Walstra *et al.*, 1986; Bonneau *et al.*, 1992). Taking into account the lack of precision inherent in the subjective assessment of odour intensity, such relationships suggest that both compounds have a significant contribution to boar taint (Bonneau, 1997).

The inconsistent nature of the results found in these studies may be due to a variety of reasons. There may be differences in the androstenone and skatole concentrations found within the animal population from which the samples are taken (Bonneau, 1998). Planning of experiments is also complicated because most studies use tissues from animals that vary in factors such as sex, feed ingredients, breed and management systems, which are not easily manipulated to produce pre-determined levels of androstenone and skatole. There are also

differences in the methodology used for the sensory assessment of odours (Bonneau, *et al.*, 2000a) including type of selection and training of the panel members, preparation and presentation of the samples, and interpretation of the results. Another factor that can affect the outcome of this type of study is the variation in individual assessor sensitivities to androstenone and skatole. The sensitivities to these and other compounds can also vary over time within an individual (Stevens and O'Connell, 1991).

von Seth *et al.*, (1995) suggested that, since only 50% of the variation in taint could be explained by androstenone and skatole concentrations, other compounds may be responsible for taint in pork. It is also important to be aware of the fact that in sensory assessment the more complex the mixture, the more difficult it is to make judgements on the relative importance of the competing compounds (Annor-Frempong *et al.*, 1997b). Laing, (1996) found that the presence of compounds, other than androstenone and skatole also contributed to the odour in pork. Studies by Annor-Frempong *et al.*, (1998) found instances where samples that were classified as "non-tainted", according to androstenone and skatole concentrations, were classified as "tainted" according to the electronic nose. This may be due to the presence of other compounds that contribute significantly to the taint (Babol et al., 1996; Xue et al., 1996a; Jeremiah *et al.*, 1999a).

These studies suggest that compounds other than androstenone and skatole may contribute to the odour of pork. This chapter reports on the examination of the odour profile from the adipose tissue of boar and gilts which was conducted by utilising sensory and GC-MS analysis. The general approach was to firstly obtain estimated sensory means for each of the twenty four samples of fat. These means were related, using multiple regression analysis, to the levels of the compounds in the sample of fat as reflected by the GC-MS peak areas, with the observational unit being a single sample of fat. The first aim was to identify how much influence androstenone and skatole have on the odour of pork fat and also to identify any other compounds that may contribute to the odour. The second aim was to identify descriptors that most adequately described boar odour.

5.1. Material and Methods

5.1.1. Animals

The samples for this experiment were collected as described in Section 4.1.1.

5.1.2. Sensory Analysis

5.1.2.1. Experimental Design

The sensory means for the 24 samples of fat were obtained using a 4 replicate, 24 treatment (i.e. 24 animals), block size 3, incomplete block design (Mead *et al.*, 1993). The blocks consisted of a single session by one panellist. The experimental plots were a single olfactory sampling presented 5 min apart, within a session. The temporal ordering of the three olfactory samplings within a session, was designated as the position of the samples. Each panellist was used for 2 or 3 sessions.

The design was obtained by first constructing an efficient resolvable incomplete design using the design computer program GENDEX (http://designcomputing.hypermars/gendex/). A resolvable incomplete design is one in which the blocks can be grouped together into subsets in which each treatment is replicated exactly once (GenStat® 5 Procedure Library Manual, Release PL10). The design was modified, using the resolvability property to ensure that the samples of fat that each person received were always from a different pig. The full design is shown in Appendix 5.1

5.1.2.2. Sensory Analysis Method

Ten grams of fat was cut into approximately 1 g pieces, placed into a 100 mL beaker and then wrapped completely with two pieces of domestic grade aluminium foil, 60 cm × 30 cm for each sample. Three samples of fat were heated simultaneously at 200°C for 1 h in an oven (Rational Combi Steamer CCC, Landsberg, Germany) under moist heat. There were 11 panellists who were pre-screened for their ability to smell androstenone and skatole as described in Chapter 3. The age and the sex of the panellist can be found in Appendix 5.7. There were 32 sessions, with three samples per session and 96 samples were tested. In each session the samples were cooked simultaneously and brought out individually, to the panellist, immediately after the cooking process. The first layer of foil covering the beaker was then unwrapped carefully and a hole, that was approximately 2 cm in diameter, was made in the second layer. The sample was brought forward until it was approximately 10 cm from the panellist's nose, whereby the odour could be sniffed and assessed according to the instructions in the record sheet, which can be found in Appendix 5.2. The panellists gave an odour rating on a continuous scale of 0-10 for the descriptors: *overall, first, lingering, roast, sweet, mothball, ammonia, musty, rancid, sweaty, urine* and *manure*. The descriptor *first,* referred to the first impression of the odour of the sample of fat as detected by the panellist.

The descriptor *lingering,* referred to the odour detected after a few minutes, when the sample had cooled down slightly and the descriptor *overall,* referred to the overall impression of the odour once an assessment for the other two decriptors had been made.

5.1.3. Chemical analysis

5.1.3.1. Analysis of Androstenone and skatole by HPLC and GCMS

The samples of fat were analysed for androstenone and skatole by HPLC as indicated in Chapter 2 Section 2.1.3. They were also analysed by the GC-MS method as indicated in Chapter 4 Section 4.1.2.1 and a tentative identification of the major peaks was made.

5.1.4. Data analysis

The statistical analyses were conducted using GenStat® 5, Release 4.1 (GenStat® Procedure Library Manual, Release PL 10). A Restricted Maximum Likelihood (REML) analysis was conducted to calculate the estimated means for the *overall, first* and *lingering* scores of each sample of fat. When the predicted means were conducted the REML analysis residuals versus the fitted graphs were examined to check that the model assumptions were appropriate and that no outliers were also present. The *overall* and *lingering* scores were adjusted for random effects of person and session within person, whilst the *first* sensory score was also adjusted for a fixed position effect. The results for the *first* sensory score indicate that the position effect was significant ($P = 0.016$) using a Wald test after adjusting both for the random and the sample of fat effects. However, it was not significant ($P > 0.1$) for the *overall* or *lingering* sensory scores. Many of the descriptor scores were zero or low, (i.e. values of 1 or 2) and given these values were fairly discrete, it was thought that the REML analyses adjusting for random effects of persons and sessions within persons would be ineffective. Thus simple arithmetic means were calculated instead. The peak area values obtained by GC-MS analysis were all $\log_{10}(y + 10{,}000)$ transformed (Snedecor and Cochran, 1980), before formal analysis, to reduce the skewness in the peak values.

The Wilcoxon Rank Sum Test with the χ^2 approximation to the P-value (Lehmann, 1975) was conducted, to identify any differences between boars and gilts (including the immuno-castrate), for the estimated means of the sensory attributes and for measurements of the GC-MS peak areas and the water content of samples. The data had many zero values on a number of variates. A non-parametric test was therefore used because the data had a high level of discreteness

Multiple linear regression analyses were conducted to relate human sensory analysis scores for the pork fat, to the GC-MS profiles. The regression analyses included examining how these relationships depended on sex, cohort and water content, and how these factors and GC-MS profiles interact. In all the analyses the immuno-castrate data fell into the same range as the data for the gilts and therefore throughout the analyses was considered as being the same as female. However, the data for the latter were continually examined to ensure that this was not an outlier, from the data range of gilts. The means for the descriptor scores were calculated as arithmetic means. Principal component analysis (PCA) was also conducted on the positive (*roast* and *sweet*) and negative (*sweaty, musty, mothball* etc) descriptors.

5.2. Results and Discussion

5.2.1. Comparison of samples of fat from boars and gilts

5.2.1.1. Sensory analysis of samples of fat

The range of standard error of differences (SEDs) for all the estimated means of *overall, first* and *lingering* sensory scores is relatively small (Table 5.1). Hence, only the range of minimum and maximum SEDs are quoted. The differences between estimated means for the sensory scores for the three descriptors are often several times greater than the SEDs. The Wald test for a sample effect is also highly significant ($P < 0.001$). This indicates that the sensory analysis has discriminated between samples of fat from boars and gilts.

The *overall* scores, for the fat from gilts, are around 5. However, the *overall* sensory scores for the fat from some boars is substantially lower (see Table 5.1 and data in Appendix 5.3). There are similar patterns in first and lingering sensory scores, although the variation between the pigs is somewhat greater in the first sensory score.

Table 5.1. Adjusted means for the *overall, first* and *lingering* sensory scores for the 11 panellists, with the standard error of difference (SED) and significant levels of difference for the samples of fat from boars and gilts.

	Adjusted means for the descriptors by the panellists		
Sample	***OVERALL***	***FIRST***	***LINGERING***
Gilts + Immunocastrate			
Lowest adjusted mean	4.0	2.6	4.1
Median adjusted mean	5.9	4.6	5.4
Largest adjusted mean	6.7	6.5	6.0
Boars			
Lowest adjusted mean	2.0	1.4	2.0
Median adjusted mean	3.9	3.5	4.3
Largest adjusted mean	6.4	6.5	6.7
SED for boars and gilts			
Maximum	1.16	1.55	1.49
Minimum	1.10	1.05	1.11
P-value for differences between samples of fat using the Wald test from REML analysis	**5.3×10^{-7}**	**0.0166**	**7.0×10^{-4}**

5.2.1.2. Ratings of the descriptors

The panellists were asked to give an odour rating according to the following descriptors: *overall, roast, sweet, mothball, ammonia, musty, rancid, sweaty, urine* and *manure* as indicated in Table 5.2. The sensory analysis by the 11 panellists indicates that the *overall* odour score of the samples of fat from boars, that generally have high levels of androstenone and skatole is significantly lower (P = 0.01), compared to that of the odour of fat from gilts. There is no difference in the sensory score for boars and gilts according to the positive descriptors, *roast* and *sweet.* However, the sensory scores according to the negative descriptors such as *mothball, rancidity, sweaty, urine and manure,* are statistically significantly higher for boars, with the descriptors, *ammonia* and *musty* showing similar trends (Table 5.2). The data also indicate that for most of the negative descriptors the variability, as indicated by the standard deviation, is greater in boars than gilts (Table 5.2). These are undesirable outcomes for the pig industry.

Table 5.2. Mean sensory score and standard deviation for the positive and negative descriptor scores of the samples of fat, as determined by the sensory panel.

Descriptor	GILTS Mean	GILTS Standard Deviation	BOARS Mean	BOARS Standard Deviation	P Value Wilcoxon Test
Overall	5.72	0.82	4.13	1.41	**0.01**
Roast	5.47	1.54	4.42	1.57	0.16
Sweet	1.83	1.56	1.47	1.43	0.43
Mothball	0.17	0.45	0.82	0.83	**0.03**
Ammonia	1.04	0.77	1.79	1.16	0.12
Musty	0.74	1.01	1.33	0.87	0.10
Rancid	0.28	0.40	1.28	1.21	**0.02**
Sweaty	0.44	0.40	1.68	1.02	**0.00**
Urine	0.06	0.09	0.85	0.86	**0.01**
Manure	0.08	0.18	0.68	0.56	**0.00**

Laing, (1996) studied the consumer responses to loin samples from 36 animals which represented three groups of 12 animals that comprised of sows, males with low levels of boar odorants, and males with high levels of boar compounds. Panellists were asked to indicate whether the sample had horrible, through to satisfactory, to excellent attributes. This was conducted for 11 attributes regarding the pork flavour and odour, and for 8 of 11 attributes such as aroma liking, flavour liking and overall acceptability, the panel gave lower ratings to pork that had higher levels of boar flavour compounds ($P < 0.05$). Higher ratings were given to off-flavour and aroma strength that are undesirable outcomes. Thus on the basis of a wide range of sensory characteristics consumers discriminated between the pork from boars that had high levels of androstenone and skatole and the samples from sows and boars that had low levels of these odorants. These results also suggested that the pork derived from boars with high levels of androstenone and skatole was measurably poorer in a wide range of sensory qualities and that smell was a major contributor to this lowering of quality (Laing, 1996).

Verbeke *et al.*, (1999) found the percentage of off-flavours and odours in boars to be 15 and 20%, respectively whilst the percentage of off-flavour and odour for both females and barrows was around 5% or lower. In other studies conducted throughout Europe it was found that 6-10% of consumers were dissatisfied with the odour of boar meat, compared to gilt pork where 2-6% were dissatisfied with the odour (Bonneau, 1998). Grilled loin chops, bacon,

sausages and samples of fat from male pigs received significantly higher abnormal odour and flavour scores compared to female pigs ($P < 0.05$) (Sheard *et al.*, 2000; Leskanich *et al.*, 1997)

5.2.1.3. Average GC-MS peak areas

The average GC-MS peak areas for each sample of fat indicate that there are significant differences between the levels of androstenone and skatole in boars and gilts. This was to be expected, given the samples were pre-screened and selected for both skatole and androstenone concentrations by HPLC analysis (Table 5.3). The GC-MS peak areas for the compounds 1-4, 6-10, 12-23, 25-29 and 34 show no significant difference between boars and gilts and the data for the differences between these peaks can be found in Appendix 5.4. The GC-MS peak areas of the compounds other than androstenone and skatole that were present in quantities that indicate a statistically significant difference between boars and gilts, at the 10% level, are shown in Table 5.3.

Table 5.3. Comparison of the transformed mean of the peak areas of selected compounds detected by GC-MS, in the fat of boars and gilts.

	GILTS			BOARS			
Compound	**Log_{10} T-mean[1]**	**sd[2]**	**Back T-mean[3]**	**Log_{10} T mean**	**Sd**	**Back T-mean**	**P-value[4]**
1-azabicyclo(2,2,2) octan-2-one	0.11	0.19	2812	0.49	0.55	20890	**0.08**
3-methyl-4 (3,7,7-tri-methyl-2-oxa-bicyclo [3,2,0] hept-3-en-1-yl) buten-3-en-2-one	0	0	0	0.21	0.33	6212	**0.05**
2-methyl-hexadecanal	0.38	0.39	13959	0.67	0.27	37153	**0.06**
9-12-octadeca-dienoic acid methyl ester	0.61	0.33	30756	0.47	0.28	19376	**0.06**
Butylated hydroxytoluene	0.30	0.38	9857	0.80	0.32	52365	**0.00**
Skatole	0.01	0.02	120.4	0.15	0.17	3997	**0.01**
Androstenone	0	0	0	0.10	0.061	2509	**0.00**

[1]log_{10} T-mean represents $log_{10}(y + 10{,}000)$ transformed-mean

[2]sd represents the standard deviation of the $log_{10}(y + 10{,}000)$ transformed-mean

[3]Back T-mean represents the transformed mean adjusted back to the original scale of the peak areas

[4]P-value from the Wilcoxon Test with values in bold being significant at $P < 0.1$

There are indications that compound 9,12-octadecadienoic acid methyl ester is significantly higher in gilts compared to boars. Linoleic acid, (i.e. 9,12-octadecadienoic acid) is a poly-unsaturated fatty acid, that is quite susceptible to oxidation. Linoleic and linolenic acid are derived from the diet and thus are related to the feed intake. As gilts and immunocastrates have a higher feed intake per unit gain in weight than males (Dunshea *et al.*, 2001), this increase in consumption over time may result in a higher accumulation of these fatty acids in gilts compared to boars. This possibly results in higher levels of the unsaturated linoleic fatty acid, derived from feed, in gilts. However *de novo* synthesis, that is the production of saturated and mono-unsaturated fatty acids, also occurs, therefore creating a dilution effect. The metabolism and use of these fatty acids may also vary between boars and gilts and this is possibly why a higher difference is not observed. There is also a trend for the compounds 2-methyl-hexadecanal and azabicyclo (2,2,2) octan-3-one and 3-methyl-4 (3,7,7-trimethyl-2-oxa-bicyclo[3,2,0] hept-3-en-1-yl) buten-3-en-2-one to be more prevalent in boars. The variation of the levels of these compounds between the boars and gilts may contribute to the significant difference found in the odour of the fat from the different sexes as determined by human sensory analysis.

5.2.2. The GC-MS profile and the overall sensory score

When the GC-MS data from the boars and gilts are analysed there is no significant correlation with the sensory analysis (see data in Appendix 5.5). However, there is a greater variability in the overall odour score from boars as shown by the higher standard deviation in boars compared to that of gilts (Table 5.2). The data from boars was therefore analysed independently of the data from the gilts, in the initial stages of analysis.

None of the transformed GC-MS peak areas of various compounds was individually correlated to the overall sensory score ($P > 0.1$). However, this does not mean that when the GC-MS peak areas of several compounds are combined together, there is no relationship. Not deterred by the lack of individual correlations, the examination of a combined relationship of several peaks to the overall sensory score was examined using multiple regression techniques.

A multiple regression analysis between the transformed GC-MS peak area values and the *overall* sensory odour score for boars was carried out. The compounds 3-nonen-1-ol (Peak 1) and skatole (Peak 30) had some relationship with the *overall* odour score, in a multiple regression. In order to identify all the compounds that were contributing to the *overall* odour score, it was necessary to adjust for the effect of these two compounds, so that the effect of

the other compounds could become apparent. This lead to the discovery that the compounds pentadecanal (Peak 14), 2-nonadecanone (Peak 7) and androstenone (Peak 31), were also related to odour. None of the other peaks showed any statistically significant relationship with the *overall* odour score once the model based on these five peaks was adjusted for. This enabled a model based on these five peaks to be constructed, (Table 5.4). In this model, the relationship between some of the GC-MS peaks and the overall sensory scores differed between boars and gilts.

In the presentation of the figures, the *overall* score was adjusted for the peaks in the model, separately, for boars and gilts. The *overall* score for boars was adjusted to the mean of androstenone and skatole for boars. The *overall* score for gilts was adjusted to the mean of androstenone and skatole for gilts, which was zero. The parsiminous, simplest, model (Kotz *et al.*, 1985) has firstly, a common response to 3-nonen-1-ol for both boars and gilts (Figure 5.1.); and secondly a response to pentadecanal (Figure 5.2.), 2-nonadecanone (Figure 5.3.), androstenone (Figure 5.4) and skatole (Figure 5.5.) for boars only. The amount of androstenone and skatole in gilts is either zero or very small and therefore it is not possible to know if the responses of androstenone and skatole could also occur in gilts. It is well known however that androstenone concentrations in gilts are very low (Salvatore *et al.*, 1995), and therefore this is only an important issue for skatole given that significant ($P < 0.05$) correlations between skatole and taint in gilts, have been observed (Hansson *et al.*, 1980).

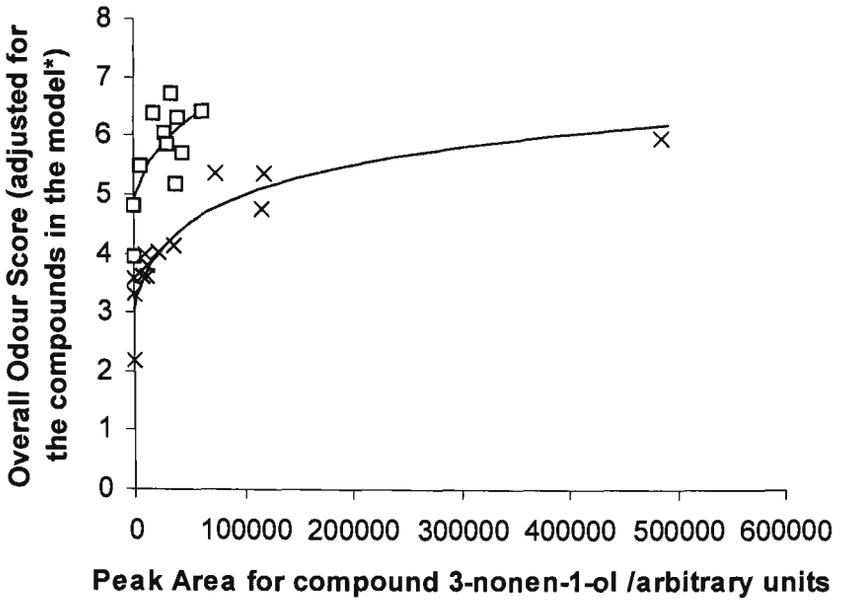


Figure 5.1. Overall fat odour score versus the peak area of 3-nonen-1-ol for (□) gilts and (x) boars. *The model refers to compounds 2-nonadecanone, pentadecanal, skatole and androstenone. The outmost sample is considered influential and not an outlier. However, its influence is less extreme in the analysis which is conducted on the log scale of peak areas and not the actual scale of peaks areas as shown in this diagram.

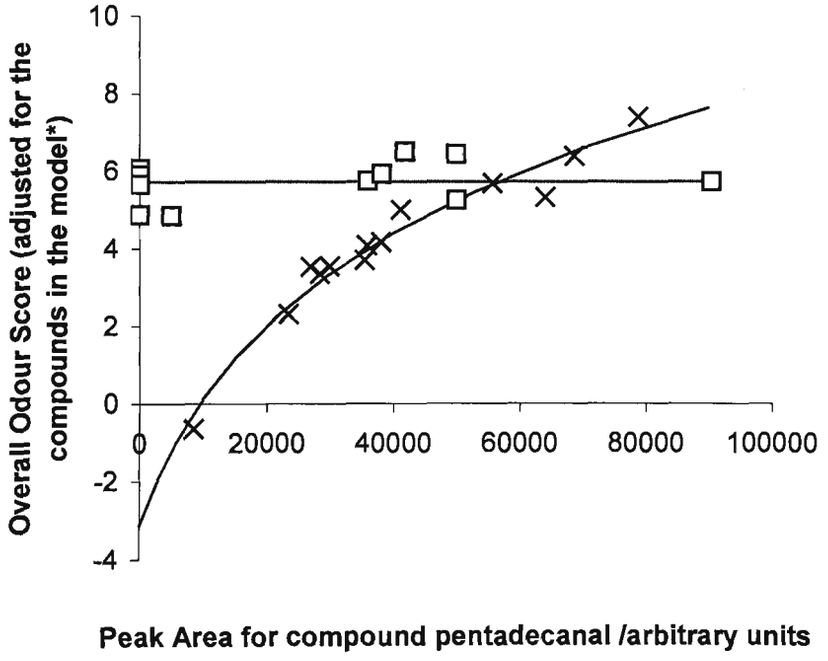


Figure 5.2. Overall fat odour score versus the peak area of pentadecanal for (□) gilts and (x) boars. The adjustment for the effects of other peaks changes the odour score value to arbitrary values hence the negative odour score values displayed which is a common outcome of this form of statistical transformation. *The model refers to compounds 3-nonen-1-ol, 2-nonadecanone, skatole and androstenone.

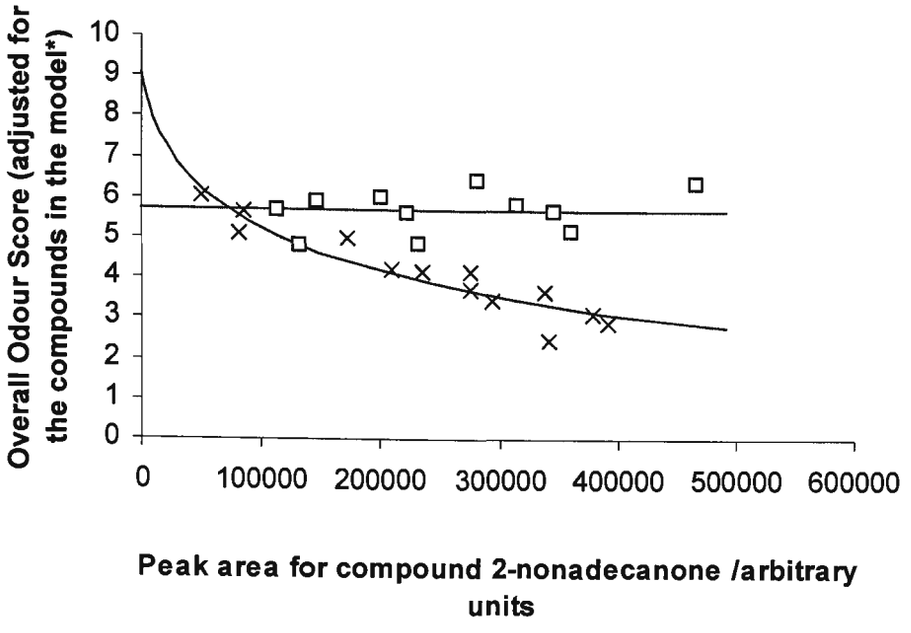


Figure 5.3. Overall fat odour score versus the peak area of 2-nonadecanone for (□) gilts and (x) boars. *The model refers to compounds 3-nonen-1-ol, pentadecanal, skatole and androstenone.

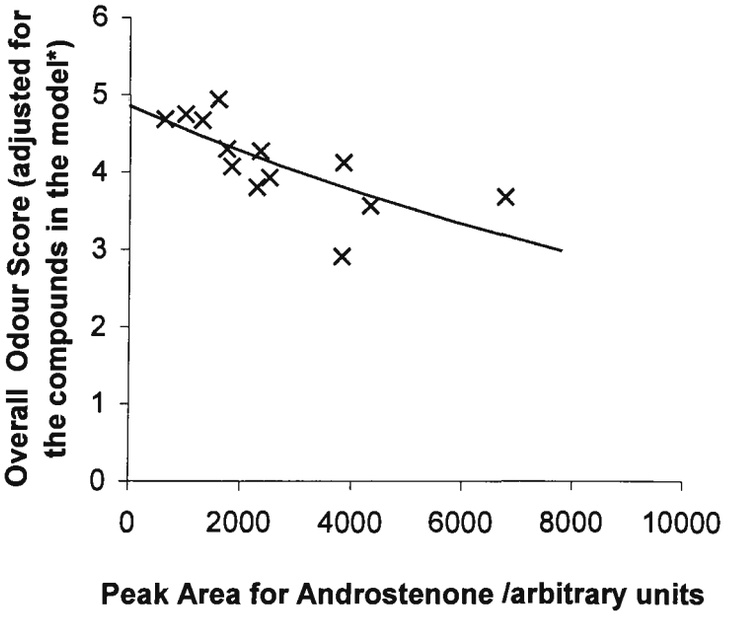


Figure 5.4. Overall fat odour score versus the peak area of androstenone for (x) boars. The mean androstenone concentration for gilts was zero and therefore not displayed. *The model refers to compounds 3-nonen-1-ol, 2-nonadecanone, pentadecanal and skatole.

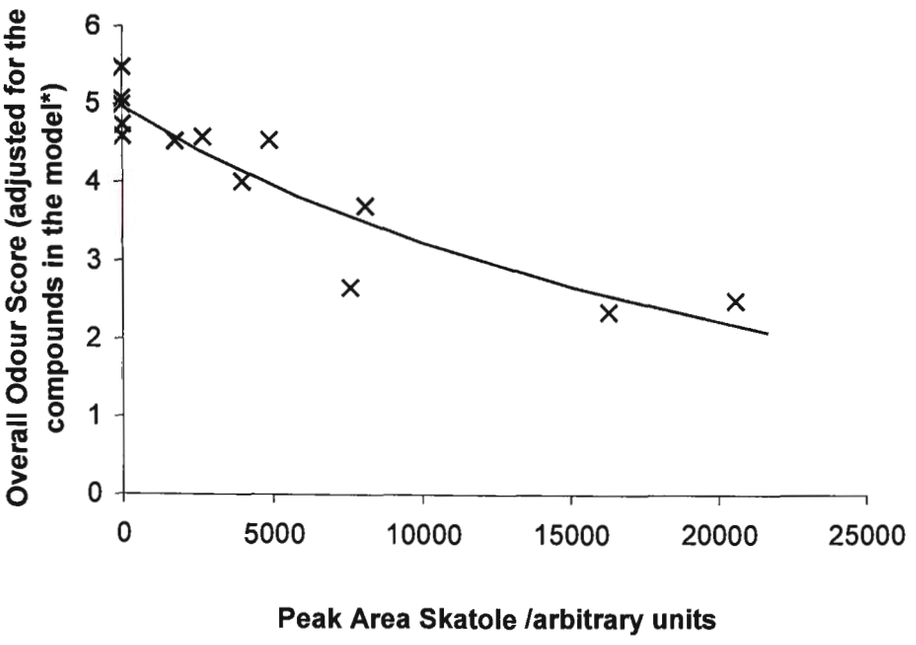


Figure 5.5. Overall fat odour score versus the peak area of skatole for (x) boars. The mean skatole concentration for gilts was zero and therefore not displayed. *The model refers to compounds 3-nonen-1-ol, 2-nonadecanone, pentadecanal and androstenone.

The model gave a fitted equation for gilts that included a term for the effect of the compound 3-nonen-1-ol. Hence the model for gilts is:

$$Overall = 4.797 + 1.869 \log (a_1 + 10{,}000) \qquad 5.1$$

The model gave a fitted equation for boars that included terms for the effects of the compounds 3-nonen-1-ol, pentadecanal, 2-nonadecanone, skatole and androstenone. Hence the model for boars is:

$$Overall = 2.332 + 1.869 \log_{10} (a_1 + 10{,}000) - 3.0704 \log_{10} (a_2 + 10{,}000) + 10.75 (a_3 + 10{,}000) - 5.70 \log_{10} (a_4 + 10{,}000) - 7.43 \log_{10} (a_5 + 10{,}000) \qquad 5.2$$

Where a_1 is the peak area of 3-nonen-1-ol, a_2 is the peak area of 2-nonadecanone, a_3 is the peak area of pentadecanal, a_4 is the peak area of skatole and a_5 is the peak area of androstenone.

According to this experimental model, pentadecanal has the strongest positive impact on odour per unit compound, given the fitted curve has the greatest slope (Table 5.4). This is followed by androstenone having the next strongest impact although negative, per unit compound, followed closely by skatole and then 2-nonadecanone both having a negative impact, and then 3-nonen-1-ol which has the least impact, albeit a positive one (Table 5.4).

Table 5.4. Terms included in the five peak model relating transformed GC-MS peak areas to the overall means.

Adjustment to Model ***Terms retained and utilised in the model***	**Impact of compound on odour score**	**Direction of impact[2]**	**P-value[3]**
Males vs Females (including immunocastrate)			$\mathbf{1.5 \times 10^{-2}}$
Lpeak 3-nonen-1-ol in boars and gilts[1]	1.87	+	$\mathbf{1.3 \times 10^{-5}}$
Lpeak 2-nonadecanone in boars	3.07	-	$\mathbf{1.5 \times 10^{-3}}$
Lpeak pentadecanal in boars	10.75	+	$\mathbf{2.5 \times 10^{-6}}$
Lpeak skatole in boars	5.70	-	$\mathbf{1.3 \times 10^{-4}}$
Lpeak androstenone in boars	7.43	-	$\mathbf{2.9 \times 10^{-2}}$

[1] Lpeak denotes log10 (magnitude of the peak area + 10,000).

[2] The + sign indicates a favourable impact on odour, the – sign indicates an unfavourable impact on odour.

[3] P-value has 1, 17 degrees of freedom.

The greater impact of androstenone compared to skatole in this study compared to other published studies may be due to the fact that panellists anosmic to androstenone, were excluded from the sensory panel. Bonneau *et al.*, (2000b) found that boar odour was correlated more with skatole than androstenone, however, he also stated that this could be attributed to the existence of anosmia towards androstenone in a substantial proportion of consumers. When the sensitivity of the panellists to androstenone, is taken into consideration, the effect of androstenone on consumers is quite substantial (Weiler *et al.*, 2000)

Matthews *et al.*, (2000) found that skatole explained more of the variation in odour score than androstenone (approximately 27% compared to 11%). However, when both skatole and androstenone were considered together there was a small improvement (29%) in the variation explained, which was significant at the 5% level. Perhaps the question is not which of either androstenone or skatole, is more important, but rather how do they interact and contribute to the overall odour?

The residual standard deviation for the model, including the five peaks, in the current study was 0.564 which accounted for 85.6% of the variation. However, if only androstenone and skatole are considered, then only 37.2% of the variation would have been accounted for. This is only 7% greater than the variation accounted for just by using a separate mean for each sex, and ignoring all the peak information.

The peak area values were log transformed to reduce the skewness of the data and these values were used to develop the model. When the actual GC-MS peak values (using the values back-transformed to the original scale of the data) were plotted according to the model, the results showed that in boars, as expected, androstenone and skatole were negatively correlated with odour (Figure 5.4-5.5). The compound 3-nonen-1-ol appeared to a have a beneficial effect on the odour of pork and this occurred in boars and gilts (Figure 5.1). Pentadecanal also had a positive effect on the odour however, this was apparent only in boars (Figure 5.2). A similar observation was made with 2-nonadecanone, but the association was a negative one in boars with no effect on gilts (Figure 5.3). This indicated that there may be a "trigger effect" occurring in the samples of fat from boars whereby the presence of another compound (androstenone perhaps) affected the perception of other compounds.

The simultaneous presence of androstenone and skatole has been known to act synergistically in the perception of taint (Walstra *et al.*, 1986; Bonneau *et al.*, 1992). Hanson *et al.*, (1980) also observed a significant interaction between levels of skatole and androstenone in boar taint. Annor-Frempong *et al.*, (1997b) found that the effect of skatole was enhanced and

dependent on the presence of androstenone, i.e. skatole was perceived as more intense, with higher concentrations of androstenone. de Kock *et al.*, (2001) have postulated a possible explanation for the phenomenon of odour synergism between androstenone and skatole. They state that one of these compounds might be behaving similarly to a fixative agent, which influences the properties of odours. Fixatives are oily or waxy materials, for example musk and civet used in the perfume industry, that have very low air-oil partition coefficients capable of retaining large proportions of odourants and subsequently release them slowly over time (Amoore, 1982). de Kock *et al.*, (2001) proposed that the differences in the volatilisation properties of skatole and androstenone along with the fixative odour retention property of androstenone may possibly further explain this increase in odour intensity of the two molecules. The odour intensity may also appear to be perceptually more intense, if it lasts longer. Beery *et al.*, (1971) and Patterson, (1968) also commented that androstenone had a long retention time, probably due to the fact that it was absorbed in the mucosal layer in the nose and due to its low odour threshold could be detected for hours.

The quantity of pentadecanal and 2-nonadecanone detected in the fat of boars and gilts was not greatly different, however the two compounds behave differently in boars and gilts. The odour of each of these two compounds is enhanced in boars. Androstenone, a compound present only in boars has odour retention properties which could be responsible for this phenomenon.

The analysis shows that the five compounds (androstenone, skatole, pentadecanal, 2-nonadecanone and 3-nonen-1-ol) have appealing and unappealing effects on the *overall* odour. However, it is only after adjusting for the effect of one or two of these compounds on the *overall* odour score, that the relationship of the other compounds that also significantly contribute to the *overall* odour score, becomes apparent. The complexity of this situation may explain why there is so much discrepancy in the literature with regard to the correlation of androstenone and skatole concentrations with boar odour as assessed by a sensory panel. When the adjustment for the five main peaks is made the remaining peaks 2-6, 8-13, 15-29 and 33-34 have little relationship to the sensory analysis in this subset of samples (see data in Appendix 5.6). The current data also suggest that there are no interactions with the five peaks of interest, that is 3-nonen-1-ol, 2-nonadecanone, pentadecanal, androstenone and skatole, in boars. There are also no quadratic effects for these peaks and the results supporting this observation can be found in Appendix 5.6. The correlation with the five peaks of interest and the *overall* sensory score is not significant within gilts. The water content, cohort (i.e. samples collected at different times) and sex are also not significantly correlated to odour and these results can also be found in Appendix 5.6.

Initially the amount of variation in the *overall* score was substantially different between boars and gilts (P = 0.01). This difference was due mostly to the larger variation in the odour scores for the fat from boars (Table 5.2). However once the adjustment for these five peaks was made the variation in the data, i.e. the scatter around the graph, as shown in Figures 5.1, 5.2 and 5.3 was more similar between boars and gilts. This information may lead to strategies for making pork from boars and gilts more similar if the presence or absence of these compounds could be manipulated.

The relationship between the transformed areas of the odorant peaks 3-nonen-1-ol, 2-nonadecanone, pentadecanal, androstenone and skatole with the other peaks was investigated using simple linear regression to identify possible precursors or break down products (Table 5.5). The effect of sex, cohort, water content and their interaction with the five peaks was examined but the analyses showed only minor effects and therefore were not reported.

Pentadecanal is associated with appealing odour ($P = 2.5 \times 10^{-6}$, Table 5.4). Low levels of saturated aldehydes have often been associated with desirable flavours in many foods (Saxby, 1996). Pentadecanal is also correlated with the presence of hexadecanoic acid methyl ester (P = 0.031, Table 5.5), 9,12-octadecadienoic acid methyl ester (P = 0.032) and 9-octadecenoic acid methyl ester (P = 0.047). In general, the mono-unsaturated and PUFAs are susceptible to lipid oxidation and often lead to the formation of saturated and poly-unsaturated aldehydes which are thought to contribute significantly to the odour/flavour of meat products (Wettasinghe *et al.*, 2001). However in the frying pan, saturated fatty acids can also be oxidised thereby generating by-products. Sanudo *et al.*, (2000) found a high correlation between odour and flavour intensity with fatty acid composition, in lamb. They found the profile of volatiles from sheepmeat fed a grain diet was mostly aldehyde and monoketone, which are mainly derived from linoleic acid. Badings (1984) also found octadecadienoic acid to be the precursor of the compound heptenal, which was correlated with the creamy flavour in butter. These observations also suggest that fatty acid oxidation could contribute to the presence of pentadecanal a compound associated with pleasant odour.

Table 5.5. Correlation between compounds identified in the odour profile of fat from boars and gilts and the five odourant peaks.

Peak No.	Compound Tentative assignment	*Odour Related Peaks, P-values*				
		3-nonen -1-ol	**2-nona-decanone**	**Pentadecanal**	**Androstenone**	**Skatole**
1	3-nonen-1-ol	-	0.142	0.654	0.145	0.380
2 + 3	Siloxane	**0.0024**	0.558	0.122	**0.019**	0.668
4	1-undecanol	**0.040**	0.283	0.414	0.468	0.564
7	2-nonadecanone	0.142	-	0.116	0.422	0.608
8	1-azabicyclo (2,2,2) octan-3-one	0.978	0.734	0.175	0.972	0.450
9	2-dodecanone	**0.033**	0.294	0.105	0.663	0.380
10	Tetradecanal	0.277	**0.011**	**0.0000061**	0.249	0.419
11	3-methyl-4 (3,7,7-trimethyl-2-oxa-bicyclo[3,2,0] hept-3-en-1-yl) buten-3-en-2-one	0.457	0.706	0.442	0.865	0.598
12	2-methyl-hexadecanal	0.898	**0.017**	**0.00083**	0.386	0.579
13	Hexadecanoic acid methyl ester	0.775	0.629	**0.031**	0.653	0.662
14	Pentadecanal	0.654	0.116	-	0.227	0.352
15	9-12-octadecadienoic acid methyl ester	0.874	0.714	**0.032**	0.733	0.456
16	9-octadecenoic acid methyl ester	0.584	0.856	**0.047**	0.563	0.342
18	Octadecanoic acid methyl ester	0.816	0.553	0.085	0.973	0.471
19	oleic acid	0.836	0.919	0.699	0.074	0.379
20	9,12-octadecadienoic acid	0.751	0.629	0.978	0.556	0.203
21	n-hexadecanoic acid	0.702	0.473	0.668	**0.045**	0.341
22	13-octadecenal	0.112	**0.023**	**0.00099**	0.077	0.411
23	2-tridecanone	0.460	**0.042**	0.952	0.792	0.375
24	Butylated hydroxytoluene	0.551	0.851	0.065	0.413	0.940
30	Skatole	0.475	0.490	0.132	0.651	-
31	Androstenone	0.414	0.854	0.057	-	0.651

[1] P-values for the linear relationship between the odourant and remaining compounds.

[2] Correlations conducted for androstenone and skatole were from male samples only.

It is well known that grain diets increase the proportion of unsaturated fatty acids in adipose tissue (Young *et al.*, 1997) and that many straight chain aldehydes are derived from the unsaturated fatty acids (Elmore *et al.*, 1999; Love and Pearson,1971). The volatile profile from the grain diet can result in the presence of aldehydes and monoketones many of which are derived from unsaturates like linoleic acid (Frankel, 1985). The level of linoleic acid in the pig diet appears to affect the levels of decanal in pork (Larick *et al.*, 1992). In this study the aldehyde pentadecanal appears to be derived from wheat, possibly via one of the poly-unsaturated fatty acids such as linoleic acid.

Pentadecanal is also highly correlated to the presence of tetradecanal ($P = 6.1 \times 10^{-6}$, Table 5.5) and 13-octadecenal ($P = 9.9 \times 10^{-4}$) and 2-methyl-hexadecanal ($P = 8.3 \times 10^{-4}$). Possibly the 2-methyl-hexadecanal which has a MW of 254 is contributing to the presence of pentadecanal (MW 226) whilst pentadecanal may be degraded down in further reactions to produce tetradecanal (MW 212). This is indicative of primary and secondary products of lipid oxidation being formed and further reacting during the cooking process.

The compound 2-nonadecanone is associated with unappealing odour ($P = 1.5 \times 10^{-3}$, Table 5.4). This compound is likely to be a product of lipid oxidation contributing to off-flavour. Methyl ketones are responsible for off-flavour in vegetable oils and animals fats. The presence of octanone and 5-undecanone has been found in cooked chicken (Nonaka *et al.*, 1967). A complex form of octanone has also been found in this study. Octen-3-one is responsible for metallic flavour in oxidised butter (Stark and Forss, 1962). Penten-3-one is responsible for sharp fishy odour in oxidised fats resulting from the decomposition of linoleic hydroperoxide (Stark *et al.*, 1967). Badings (1970) identified 1-octen-3-one and 1-penten-3-one from oxidised arachidonic and linolenic acid respectively. The compound 2-nonadecanone is also correlated with the presence of tetradecanal ($P = 0.011$, Table 5.5), 2-methyl-hexadecanal ($P = 0.017$), 13-octadecenal ($P = 0.023$), and 2-tridecanone ($P = 0.042$). It is perhaps possible that 2-nonadecanone which has a MW of 282 is degraded in further reactions to produce 2-methyl-hexadecanal (MW = 254) and tetradecanal (MW =198).

The compound 3-nonen-1-ol is associated with appealing odour ($P = 1.3 \times 10^{-5}$, Table 5.4). It is also correlated with the presence of Peak 2 which has been identified as methyl silicone a column component and should therefore be discounted (Table 5.5). Undecanol ($P = 0.040$) and 2-dodecanone ($P = 0.033$) are also correlated with 3-nonen-1-ol. The compound 3-nonen-1-ol has a lower molecular weight (MW 142) than either 1-undecanol (MW 172) or 2-dodecanone (MW 184) and hence could possibly be derived from these two compounds.

There is no association with the fatty acids and 3-nonen-1-ol. The alcohol, 1-octen-3-ol has a low odour threshold value of 0.1 ppm and imparts a mushroom character (Sunusen *et al.*, 2000) and also an important odour in cheese (Molimard and Spinnler, 1996). Linoleic acid is proposed as the natural precursor of 1-octen-3-ol (Tressl *et al.,* 1982) however in this study, 3-nonen-1-ol only appears to be correlated with the presence of the ketone, 2-dodecanone, a product of lipid oxidation.

An interesting point to note is that whilst androstenone is a known sex pheromone for the pig, skatole, decanal, nonenol and decanone have also been identified as sex pheromones in insects. Both decanal and decanone have been identified as chemicals that were attractive to the olivebark beetle (Szauman-Szumski *et al.*, 1998). The synthetic sex pheromone (2-hydroxy-3-decanone) of the coffee white stem borer (*Xylotrechus quadripes*), has also been shown to be effective in attracting female stem borers, in laboratory and field tests (Jayarama *et al.*, (1998). The compound 2-nonenol, also identified as the sex pheromone of *Anomala schonfeldti,* is also successful in baiting the male of this species (Hasegawa *et al.*, 1993). Finally skatole along with a synthetic oviposition pheromone is thought to influence the oviposition and behaviour of *Culex quinquefasciatus*. Perhaps these compounds also have a role in some of the behavioural aspects of the pig that is currently unknown.

5.2.3. GC-MS peak areas and descriptors: overall, start and lingering

In the remainder of this chapter, the terminology "the effects of the GC-MS peak areas" will be used. This phrase represents "the effect of the peak areas in the model, which includes the parallel linear effect of the log transformed peak area values for the compound 3-nonen-1-ol in boars and gilts (refer equations 5.1 and 5.2). It also represents the linear effects of the transformed peak area values for androstenone, skatole, 2-nonadecanone and pentadecanal in "boars only" (refer equation 5.2). The GC-MS peak areas therefore account for 85.6% of the variation in the *overall* score and the descriptors *first* and *lingering* account for 85.1% (Table 5.6). The relationship between the *overall, first* and *lingering* odour scores and the peak areas are described in Table 5.7. There are strong effects of *lingering* adjusted for the peak areas on the odour descriptor (Y-variate) *overall* (P = 0.00084) and strong effects of *overall* adjusted for peak areas on the odour descriptor (Y-variate) *lingering* (P = 0.00084). This suggests that the rating of *overall* and *lingering* by panellists is strongly correlated when the volatile components are the same (Table 5.7).

Table 5.6. Models for *overall*, *first* and *lingering* scores comparing the accounted variance.

Odour descriptor Y-variate	Independent variates	Variance accounted for %
Overall	*Peak area*[1]	85.6
	First	57.5
	Lingering	84.9
	First+Lingering	85.1
	Peak area +First	86.4
	Peak area +Lingering	93.0
	Peak area + First + Lingering	92.5
First	*Peak area*	48.3
	Overall	57.5
	Peak area +Overall	50.8
Lingering	*Peak area*	71.0
	Overall	84.9
	Peak area +Overall	85.6

[1] "Peak area" is shorthand notation for all the peak-related model terms; i.e. terms for sex, and the peak areas for 3-nonen-1-ol in both sexes and peak areas for 2-nonadecanone, pentadecanal, skatole and androstenone in males as accounted for in equations 5.1 and 5.2.

Table 5.7. Test for relationships between *overall*, *first* and *lingering* odour scores.

Odour descriptor Y-variate	Descriptor effects tested	Descriptor effects adjusted for	F-value	Degrees of freedom	P-value
Overall	*Lingering*	*Peak area*[1]	17.32	1,15	**0.00084**
	First	*Peak area*	1.80	1,15	0.20
	Lingering +First	*Peak area*	8.10	2,14	**0.013**
	Peak area	*Lingering*	4.98	6,15	**0.0054**
	Peak area	*First*	8.48	6,15	**0.00039**
	Peak area	*Lingering +First*	4.23	6,14	**0.012**
Lingering	*Overall*	*Peak area*	17.32	1,15	**0.00084**
	Peak area	*Overall*	1.17	6,15	0.37
First	*Overall*	*Peak area*	1.80	1,15	0.20
	Peak area	*Overall*	0.52	6,15	0.78

[1] "Peak area" is shorthand notation for all the peak-related model terms; i.e. terms for sex, and the peak areas for 3-nonen-1-ol in both sexes and peak areas for 2-nonadecanone, pentadecanal, skatole and androstenone in males as accounted for in equations 5.1 and 5.2.

The *overall* score measures aspects of the volatile compounds which *lingering* does not measure since the effects of peak areas adjusted for *lingering* on *overall* is highly significant (P = 0.0054). However there is no evidence that *lingering* measures aspects of volatile compounds not included in *overall* score since the effect of peak areas adjusted for *overall* on *lingering* (P = 0.37) is not significant. This implies that while *overall* and *lingering* scores are correlated, there is more information about the volatile compounds in *overall* (P = 0.0054) than in *lingering* (P = 0.37).

There is no evidence of an *overall* effect adjusted for peak areas on the odour descriptor (Y-variate) *first* (P = 0.20) or an effect of *first* adjusted for peak areas on the odour descriptor (Y-variate) *overall* (P = 0.20). This indicates that the rating of *first* and *overall* are uncorrelated when the volatile compounds are the same. In a similar vein to *lingering*, there is no evidence of an effect of peak areas adjusted for *overall* on *first* (P = 0.78) but strong evidence of an effect of peak areas adjusted for *first* on *overall* (P = 0.00039). This implies that there is more information about the volatile compounds in *overall* than in *first* (Table 5.7).

Together this suggests that *overall* is the more informative measurement of volatile compounds and, by implication the odour of the fat, than either *first* or *lingering*. However since *lingering* and *overall* are correlated measurements, even when the volatile compounds present are the same, it is possible that assessment of *lingering* helps with the assessment of *overall*.

5.2.4. GC-MS peak areas and descriptors: overall, positive and negative

Principal component analysis was conducted on the data for the positive and negative descriptors and this showed that the principal components 1 and 2 account for 81.95% of the variation. To improve interpretation, the first components were rotated using the varimax criterion (Manly, 1986). The first rotated factor had large loadings on *sweetness* and *roast* odours, while the second rotated factor had a large negative loadings on all the other descriptors. Thus the scores of the first principal component were graphed against the mean of the *roast* and *sweet* ratings (denoted "+D") of each sample (Figure 5.6). Similarly the scores of the second rotated principal components were graphed against the mean of the other seven negative descriptors, namely *sweat, ammonia, manure, musty, rancid, mothball* and *urine* (denoted "–D") and this is represented in Figure 5.7. These two graphs show a very close relationship between the rotated principal component and the appropriate mean. From this it is clear that most of the variation in the data relating to the positive and negative descriptors can be described as a linear combination of the mean of the two positive

descriptors (+D) and the mean of the seven negative descriptors (-D). Thus it was thought reasonable to use these two calculated means as summaries of the nine descriptors in further analyses.

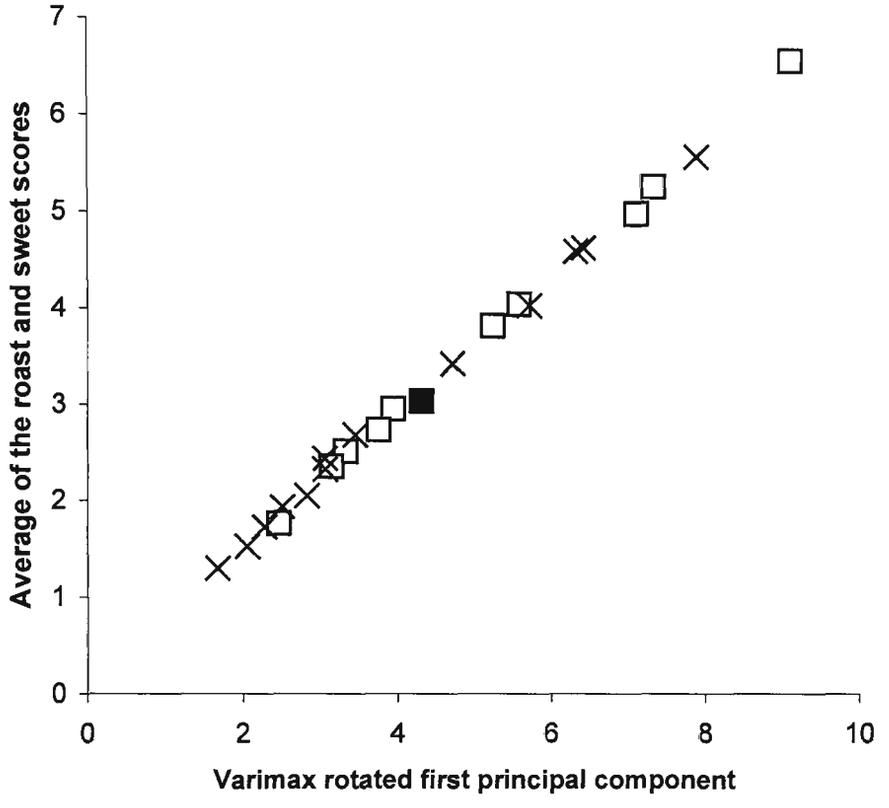


Figure 5.6. The varimax rotated principal component versus the average of the positive descriptors, such as the *roast* and *sweet*, scores for (x) boars, (□) gilts and (■) immunocastrate.

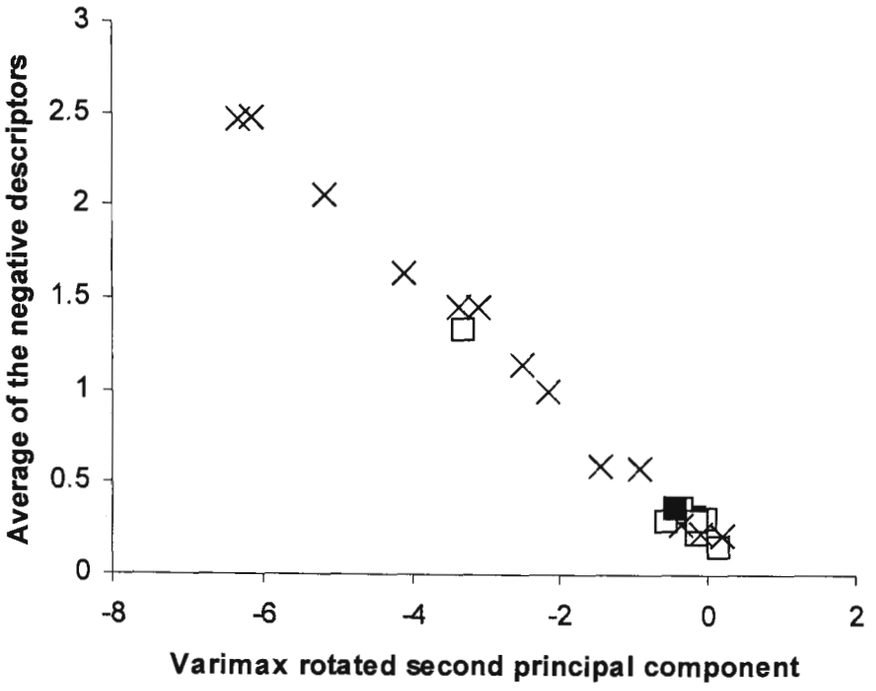


Figure 5.7. The varimax rotated principal component versus the average of the negative descriptors, such as *sweaty, urine, manure, mothball* etc, scores for (x) boars, (□) gilts and (■) immunocastrate.

The peak areas account for 85.6% of the variation within the descriptor scores as shown in Table 5.8. A representation of the effects accounted for by the various descriptors and the peak areas is given in Table 5.9. In this table it can be seen that there are strong effects of peak areas adjusted for +D ($P = 1.9 \times 10^{-5}$), strong effects for peak areas adjusted for –D ($P = 6.2 \times 10^{-4}$) and strong effects for peak areas adjusted for both –D and +D ($P = 0.019$) on *overall*. This indicates that the peak areas give more information over and above the positive or negative descriptors (Table 5.9). There is some evidence that the +D and -D provide other information on *overall* score above peak areas ($P = 0.036$) and hence to some extent are correlated measurements with *overall*. However this effect is much weaker than the corresponding result for *lingering* and *first* ($P = 0.013$, Table 5.7) indicating that *lingering* is the measurement, made by the panellist, that is most related to *overall* when the same volatile chemicals are present.

Table 5.8. Models comparing *overall*, average of *sweet* and *roast* descriptors (+D) and average of other negative descriptors (-D) and the percent variance accounted.

Odour descriptor Y-variate	Independent variates	% Variance accounted for
Overall	*Peak area*[1]	85.6
	+D	46.9
	-D	60.0
	First+Lingering	85.1
	Peak area and +D	87.8
	Peaks and –D	85.5
	+D and –D	81.6
	Peak area and +D and -D	89.5
+D	*Peak area*	21.3
	Overall	46.9
	Peak area +Overall	33.6
-D	*Peak area*	51.9
	Overall	60.0
	Peak area +Overall	52.3

[1] "Peak area" is shorthand notation for all the peak-related model terms; i.e. terms for sex, and the peak areas for 3-nonen-1-ol in both sexes and peak areas for 2-nonadecanone, pentadecanal, skatole and androstenone in males only, as accounted for in equations 5.1 and 5.2.

Table 5.9. Tests for relationships between *overall*, the average of the positive (+D) and the negative (-D) descriptors.

Odour descriptor Y-variate	Descriptor effects tested	Descriptor effects adjusted for	F-value	Degrees of Freedom	P-value
Overall	*+D*	*Peak area* [1]	4.14	1,16	0.059
	-D	*Peak area*	0.86	1,16	0.37
	+D and –D	*Peak area*	4.18	2,15	**0.036**
	Peak area	*+D*	13.33	6,16	**0.000019**
	Peak area	*-D*	7.43	6,16	**0.00062**
	Peak area	*+D and –D*	3.66	6,15	**0.019**
+D	*Overall*	*Peak area*	4.14	1,16	0.059
	Peak area	*Overall*	0.26	6,16	0.95
-D	*Overall*	*Peak area*	0.86	1,16	0.37
	Peak area	*Overall*	0.38	6,16	0.88

[1] "Peak area" is shorthand notation for all the peak-related model terms; i.e. terms for sex, and the peak areas for 3-nonen-1-ol in both sexes and peak areas for 2-nonadecanone, pentadecanal, skatole and androstenone in males as accounted for in equations 5.1 and 5.2.

There is no evidence that the peak areas give any information about appealing (+ D, P = 0.95) or unappealing odours (-D, P = 0.88) that is not in the *overall* score (Table 5.9). This suggests that the information about the peak areas, and by implication the odour of the fat, that is in +D or -D is already in the *overall* score. Since the *overall* score is univariate it can only contain one dimension of the peak area information. Thus the information regarding peak areas in +D and -D must be on the same dimension. Hence by implication, the information in +D and -D on the odour of the fat must be the same, albeit measured with different precision. It can then be implied that the differences between +D and -D means are due to the variation in the way the same aspects of odour were described by different individuals at different times.

In studies by Dijksterhuis *et al.*, (2000) the odour of androstenone was associated with the *urine*-like odour. The skatole odour was associated with the *manure*-like odour, although there was some degree of confusion with regard to attributing certain descriptors to the two compounds in the heated meat. In the current studies androstenone was also associated with *sweat* and *urine* odour and skatole was also associated with *manure*-like odour but also *sweat*-like odour (data not shown). No single descriptor was exclusively assigned to either of the compounds. Furthermore, a considered *overall* score provides more information over and above the positive and negative descriptors such as *urine, sweat, manure, sweet* etc, referred to as the +D and -D descriptors. A general conclusion from these analyses is therefore that when describing the odour of the fat, the panels need only be required to give a considered *overall* score.

5.3. Summary of Findings

Major differences have been found in the odour of fat from boars compared to that of gilts by the sensory panellists who had been screened for sensitivity to androstenone. These differences are due largely to the variation in the *overall* odour score found for the fat from entire male pigs. The results from the sensory analysis also indicate that a considered *overall* score provides more information than the more specific descriptors of appealing (*sweet* and *roast*) and unappealing (*sweaty, urine, manure, rancid, musty, mothball, ammonia, mothball*) odour.

A key finding of this chapter is that 3-nonen-1-ol and pentadecanal contribute to the appealing aspects of odour in pork fat. These compounds interact with the contribution of the

newly identified 2-nonadecanone along with androstenone and skatole which all produce unappealing odour. The balance of these contributions can be expressed in a model which describes the sensory measure of the odour of fat, in terms of the contribution of these five compounds. Within this assessment the contribution of specific compounds associated with boars can also be established.

The finding that other compounds contribute to boar taint is consistent with the recent studies of Rius-Sole and Regueiro (2001) who identified the compound 4-phenyl-buten-3-one, as a possible contributor to the off-odour in the fat of pigs. This ketone is different to the one identified in this study and a reason for this is that the samples in this study were heated prior to analysis.

The presence of androstenone in the fat from boars, is known to enhance the intensity of the skatole in the overall perception of taint (deKock *et al.*, 2001). It would also appear that in the presence of androstenone perhaps, the intensity of other compounds, such as pentadecanal and 2-nonadecanone may also be enhanced.

The literature has described the role of androstenone and skatole and has indicated that the presence of other compounds contributes to the odour of pork. However no-one has conducted a comprehensive study which has correlated sensory analysis and detailed GC-MS analysis of pig fat to provide a model for the analysis of the odour and the contributing compounds. This model is so effective that it accounts for most of the variation in sensory evaluation. When all the five compounds, androstenone, skatole, pentadecanal, 3-nonen-1-ol and 2-nondecanone, are considered as participants in the odour profile then this accounts for 85.6% of the variation in the data. No other reported study has accounted for so much of the variation of the odour score with compounds that contribute to appealing and unappealing odour. If the effect of androstenone and skatole are considered they account for only 37.2% of the variation. Finally, once the adjustment for the effect of all five compounds is made, the variation in the odour score of the fat from boars is similar to that of the gilts. This would imply that if the presence or absence of these compounds could be manipulated this would allow the odour of fat from entire boars to be more equivalent to that of the gilts, which is considered to be free of taint.

This analysis has not only confirmed that skatole and androstenone contribute to the unappealing aspects of boar taint but has also identified 3 other compounds, pentadecanal and 3-nonen-1-ol, that are perceived as appealing, along with 2-nonadecanone which produces an unpleasant odour. Furthermore the compounds interact to provide appealing and unappealing

odours that form the overall sense of the odour of pork fat. This has major implications for the approach to improving the odour/flavour of pork.

Chapter 6

6.0 Conclusion

The initial aim of this program was to develop a rapid, on-line method of analysis for the detection of boar taint. The conducting polymer *e-NOSE* typifies the "electronic nose technology" which is currently being developed for on-line analysis in the food industry and is thought to be useful for this purpose. Thus the analysis of the response of the *e-NOSE* to the complete odour profiles of tainted and non-tainted samples of fat was carried out. Androstenone and skatole are compounds well known for their contribution to boar taint and any instrument that is required to measure taint, must have a proportional response to these compounds in heated pig fat. Multiple regression analysis on the individual *e-NOSE* sensor scores as well as the combined sensor response, as defined by principal component analysis, versus the concentration of these two compounds in model systems was conducted. The data from these studies indicate there is little relationship between the *e-NOSE* sensor response and the concentrations of androstenone and skatole in pig fat.

This study shows that most of the variation of the *e-NOSE* data is due to the micro-environmental effects of the vessel and sensor head temperature, the relative humidity, and the drift associated with batches. Other researchers have also found that part of the variation can be explained by the temperature fluctuation on the sensor module. These problems limit the precision of the measurements performed with the *e-NOSE* both in the current study and in the published literature. Studies that have not taken these factors into consideration, should be interpreted carefully. These findings therefore limit the practicality of the instrument for the routine analysis of samples of pork.

In the current studies, correlations between the androstenone standard and the samples of fat spiked with androstenone, were rigorously explored. However, the variation was most likely due to the relative humidity resulting from the environmental conditions of the instrument and the different levels of water in each of the samples of fat. Statistically significant differences had been found in the water content of the samples of fat from boars compared to gilts. Attempts were made to maintain a constant, maximum relative humidity by supplying a consistent source of water with each sample, since the sensors had been observed to have a linear response to the samples of fat. However, this proved to be unsuccessful in reducing the variation in moisture levels relating to the sensor scores. This constitutes a serious problem that is inherent in the method and may be responsible for the differences between the results

obtained from the fat from male and female pigs in various published electronic nose studies. If these factors are not controlled, then confounding results may occur. Perhaps instruments based on metal oxide sensors would be more suitable for this application, because such sensors are not as sensitive to moisture or heat. The metal oxide sensors also allow for the sample to be heated to a temperature range that is closer to the domestic situation, whereby the volatile compounds are released and may facilitate a more accurate assessment of the boar odour. Alternatively, if conducting polymers are to be used as sensors, at the very least, more stringent controls need to be implemented to maintain a constant relative humidity. The *e-NOSE*™ 4000 as manufactured is therefore considered to be not suitable for on-line analysis of boar taint.

Other avenues were researched, leading to the development of a new GC-MS headspace method for the analysis of taint. The correlation between the levels of androstenone in the fat as identified by HPLC analysis and as identified by the headspace GC-MS method, was also found to be low. This correlation was even less significant for skatole. The low correlation between the two methods is indicative of a difference in the amount of the compounds detected by each method even though the samples were the same. The HPLC method involves a "cold extraction" process. The newly developed GC-MS method however requires the trapping of volatile compounds during the heating of the sample, under conditions that are similar to the domestic environment. It is believed that these factors affect the level of the compounds detected by the GC-MS method and that this will ultimately provide an improved correlation with the human sensory analysis.

Panellists who had been screened for sensitivity to androstenone and skatole analysed the overall odour of the samples of fat. These samples were also analysed by GC-MS. The sensory analysis of the samples of fat from boars appeared to be significantly different from the odour of the fat from gilts which indicated that the panellists could discriminate between the odour of fat from the two sexes. It would appear that this is largely due to the greater variation in the overall odour found in the fat from boars. The fat from male pigs appear to have higher scores for the unappealing attributes such as *sweat, manure, urine, ammonia, rancid, musty*, and *mothball* like odours. These results agree with previously published studies. This study also suggests that females tend to eat less pork and other meats, compared to males which is also in accordance with previously published literature. However, as was found in this study and other literature, consumer panellist sensitivity to androstenone does not accurately predict with confidence, the consumer-preferred choice and purchase habits that one associates with pork. A possible reason for this is the presence of other compounds contributing to the odour, that thereby confound the results.

A large variety of other compounds were also found in the aroma of pork fat as identified by this newly developed GC-MS method, with the major groups of compounds emerging as fatty acids, alcohols and aldehydes and ketones. The aldehydes, ketones and alcohols are likely to have been produced by lipid degradation. Multiple regression analysis conducted between the peak areas of the compounds detected by GC-MS and the overall sensory odour score showed that, along with skatole and androstenone, three other newly identified compounds also contributed significantly to the odour of pork. These compounds have been tentatively identified as pentadecanal, 2-nonadecanone and 3-nonen-1-ol.

The compound pentadecanal could be derived from wheat grain, a constituent of the pig diet. It could also be derived from the oxidation of saturated and unsaturated fatty acids in the presence of heat. Possible precursors of this compound are hexadecanoic fatty acid methyl ester, 13-octadecenoic acid, 9,12-octadecadienoic acid and 13-octadecanal. Other studies have also suggested that the unsaturated fatty acids are the precursors of many lipid oxidation products. Pentadecanal however, also appears to degrade to produce smaller molecules such as tetradecanal, as lipid oxidation reactions proceed. The ketone, 2-nonadecanone may also be degraded to produce tetradecanal and 2-tridecanone.

When the contribution of the compounds pentadecanal, 3-nonen-1-ol, 2-nonadecanone, androstenone and skatole are taken into consideration, this accounts for most (85.6%) of the variation in the overall odour score in the fat from boars. However, if only the effects of androstenone and skatole are taken into consideration, this accounts for a much smaller 37.2% of the variation.

An additional important finding of this study is that the overall odour of fat can be determined by accounting for compounds that are either pleasant or offensive. This has a significant impact on the debate as to whether androstenone or skatole is more important in determining the level of taint. It appears that the question whether androstenone or skatole has more impact, is not as important as how they interact with the other compounds, as part of the overall perception of the odour.

Of the compounds that have been identified tentatively, 3-nonen-1-ol contributes to pleasant odour in the fat from boars and gilts. Pentadecanal also contributes to the appealing nature of the odour whilst 2-nonadecanone reduces the appeal of the odour. Interestingly, pentadecanal and 2-nonadecanone only contribute to odour in boars, although the quantities of these compounds in boars and gilts are not significantly different. It appears that androstenone may

enhance the odour profile obtained from boars since it has also been reported to intensify the odour of skatole in several studies. In boars it was observed that pentadecanal made the greatest contribution to an appealing odour. This was followed by androstenone and then skatole both having a negative effect on the odour. The compound 2-nonadecanone was the next most effective having an unpleasant impact and lastly 3-nonen-1-ol had a small pleasant effect on the odour. The appealing and unappealing aspects of odour both need to be considered in the assessment of the complete odour profile of pork. If this is not considered, as in the past where only the negative effects of the compounds such as androstenone and skatole have been taken into account, there will be confusion with regard to the true effect of the compounds, resulting in inconsistencies in the data.

One may consider that because the amount of these newly identified compounds does not vary within gilts and boars the compounds androstenone and skatole may still be considered as the main contributors to boar taint. However, the enhancement effect possibly due to the presence of androstenone causes the odour of two of these compounds pentadecanal and 2-nonadecanone to contribute only to the odour of samples from boars. Therefore one can consider that these two compounds do contribute to the problem of boar taint.

In this study, the panellists were also selected for sensitivity to androstenone and those who were anosmic were removed from the panel. This is possibly why, in this study, androstenone is observed to have a greater impact compared to skatole. This observation was also made in the latest European study in which it was found that if androstenone sensitivity was not taken into account, skatole appeared to be the more important compound affecting boar taint. However, when sensitivity to androstenone is taken into account, then androstenone is the compound of greater importance. Hence the screening of a panel for sensitivity to androstenone is important when interpreting the findings of a trained sensory panel that is engaged in assessing the effects of boar taint.

The odour of androstenone is often described to have *urine* and *sweat*-like attributes. Similarly, the descriptor *manure* tends to be associated with skatole. Trained panellists can detect the difference between the two substances, if appropriate descriptors are assigned although some degree of confusion can still occur. The descriptor *sweat* can also be partially attributed to skatole, as a single descriptor is not reserved exclusively to describe the odour of only one compound. The use of *sweat* as a descriptor for skatole may be due to an inability to discriminate between the odour of androstenone or skatole among the other compounds in the complex odour profile of fat. Part of the confusion may also be explained by the fact that

different concentrations of skatole, in particular, can impart different odour qualities in heated meat.

A successful outcome of this study has been the finding that one approach in the detection of taint, is to ignore off-flavour and their sensory correlates altogether and define pig meat quality solely in terms of consumer acceptance. It was found that a considered, overall score, provided more information than the appealing or unappealing descriptors such as *urine, sweat, roast*, etc. It is therefore likely that an appropriate approach to boar taint characterisation is to allow panellists to detect whether they like or dislike the odour of the pork fat, without taking into consideration the effects of the odour contributed by androstenone and skatole. The effects of these compounds can then be determined by statistical correlations between the GC-MS profile and sensory analysis, thus allowing the panellists to make a less complex, but more accurate judgement of the odour of pork. However, consideration needs to be given to the level of anosmia in the designated population and the level of anosmia of the sensory panel when making these interpretations.

6.1. Further studies

Future work may involve the confirmation of the identification of the compounds (pentadecanal, 3-nonen-1-ol and 2-nonadecanone) that contribute to the odour profile. The source of the compounds, i.e. whether they are present in the fat, or are produced by the heating process also needs to be established.

The effect of combinations of the five compounds (pentadecanal, 3-nonen-1-ol, 2-nonadecanone, androstenone and skatole) on the perceived odour of pork might be determined. These compounds and their interactions could be studied by incorporating them at pre-determined levels in pork fat to examine their influence on the perceived quality of pork. The compounds may also be studied in a model system that does not involve their incorporation into fat, to determine if one of the five compounds is causing the trigger effect.

A better understanding of the nature, source and interactions of these compounds may allow for the manipulation of their levels in pork and ultimately enable improved management of pork production, for the optimisation of odour quality and greater customer satisfaction.

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Appendix 2.1. P-values of treatment effects equilibration and purge time ANOVA of sensor score means at 2 min.

	P-values for sensors used in calculating the mean				
Effects	**Group 1**	**Group 2**	**Group 3**	**ALL**	**RH**
Equilibrium Time (Eq)	**0.00039**	**1.6×10^{-6}**	**0.010**	**0.000059**	0.277
Linear response	**0.000093**	**4×10^{-7}**	**0.0029**	**0.000013**	0.114
Deviation (Dev)	0.692	0.238	0.544	0.658	0.894
3 min purge vs 5,7 and 10 min purge (P3 vs 5-10)	0.452	0.635	0.606	0.532	0.361
Eq × P3 vs P5-10 interaction	**0.038**	0.134	**0.0094**	**0.031**	0.683
Linear	**0.031**	0.064	**0.0084**	**0.021**	0.479
Dev. Dev	0.143	0.448	0.084	0.172	0.615
5-10 min purge times (P5-10)	0.519	0.762	0.440	0.547	0.466
Eq by P5-10 interaction	0.762	0.140	0.670	0.475	0.796
Linear response. Dev	0.569	0.064	0.759	0.342	0.733
Dev. Dev	0.710	0.479	0.417	0.511	0.605
RH covariate	**0.0043**	**0.0023**	**0.00055**	**0.0011**	

Appendix 2.2. P-values of treatment effects equilibration and purge time ANOVA of sensor score means at 4 min.

	P-values for sensors used in calculating the mean				
Effects	**Group 1**	**Group 2**	**Group 3**	**ALL**	**RH**
Equilibrium Time (Eq)	**0.000078**	**7.5×10^{-7}**	**0.0026**	**0.000018**	0.552
Linear response	**0.000017**	**1.8×10^{-7}**	**0.00068**	**3.8×10^{-6}**	0.281
Deviation (Dev)	0.696	0.278	0.659	0.666	0.989
3 min purge vs 5,7 and 10 min purge (P3 vs 5-10)	0.500	0.648	0.680	0.580	0.391
Eq × P3 vs 5-10 interaction	**0.021**	0.092	**0.0061**	**0.018**	0.749
Linear response	**0.013**	**0.042**	**0.0039**	**0.010**	0.469
Dev. Dev	0.178	0.424	0.118	0.198	0.837
5-10 min purge times (P5-10)	0.342	0.648	0.350	0.407	0.395
Eq by 5-10 interaction	0.607	0.163	0.617	0.435	0.783
Linear response. Dev	0.496	0.083	0.824	0.361	0.685
Dev. Dev	0.531	0.451	0.335	0.418	0.623
RH covariate	**0.021**	**0.018**	**0.0029**	**0.0078**	

Appendix 2.3. The effect of humidity on the mean sensor score at 2 min with varying amounts of fat.

	Mass of Fat g				
Sensors	**10**	**20**	**30**	**Sed**	**P-value**
Not adjusted for Relative Humidity					
All Sensors	4.94	4.85	4.90	0.059	0.159
Sensors					
Group 1	4.67	4.59	4.63	0.053	0.180
Group 2	7.59	7.44	7.53	0.091	0.098
Group 3	4.14	4.08	4.11	0.052	0.209
Relative Humidity	59.4	57.6	58.7	0.90	**0.0033**
Adjusted for Relative Humidity					
All Sensors	4.90	4.89	4.89	0.032-0.044	0.245
Sensors					
Group 1	4.64	4.63	4.63	0.032-0.040	0.305
Group 2	7.53	7.52	7.51	0.045-0.057	0.210
Group 3	4.11	4.11	4.12	0.029-0.036	0.255

Appendix 2.4. The effect of humidity on the mean sensor score at 3 min with varying amounts of fat.

	Mass of Fat g				
Sensors	**10**	**20**	**30**	**Sed**	**P-value**
Not adjusted for Relative Humidity					
All Sensors	5.37	5.27	5.30	0.066	0.137
Sensors					
Group 1	5.04	4.98	4.95	0.060	0.122
Group 2	8.02	7.90	7.86	0.100	0.090
Group 3	4.63	4.58	4.56	0.059	0.206
Relative Humidity	62.3	61.1	60.7	0.92	**0.0017**
Adjusted for Relative Humidity					
All Sensors	5.33	5.31	5.30	0.041-0.053	0.207
Group 1	5.01	4.99	4.97	0.042-0.053	0.326
Group 2	7.95	7.92	7.90	0.059-0.053	0.156
Group 3	4.60	4.59	4.58	0.036-0.046	0.142

Appendix 2.5. The effect of humidity and the surface area of the fat on the sensor scores at 2 min. A small sample refers to samples cut into 1 g pieces, medium refers to 5 g pieces and whole refers to the total mass of fat left as a whole piece.

	Surface area of fat				
Sensors	**Whole**	**Medium**	**Small**	**SED**	**P-value**
Not adjusted for relative humidity					
All sensors	4.86	4.87	4.94	0.059	0.159
Group 1	4.60	4.60	4.69	0.053	0.180
Group 2	7.45	7.46	7.64	0.091	0.098
Group 3	4.08	4.09	4.17	0.052	0.209
Relative Humidity	57.3	57.7	60.7	0.90	**0.0033**
Adjusted for relative humidity					
All sensors	4.92	4.91	4.86	0.032-0.040	0.245
Group 1	4.66	4.64	4.59	0.032-0.040	0.305
Group 2	7.56	7.54	7.46	0.045-0.057	0.210
Group 3	4.14	4.13	4.08	0.029-0.036	0.255

Appendix 2.6 The effect of humidity and the surface area of the fat on the sensor scores at 3 min. A small sample refers to samples cut into 1 g pieces, medium refers to 5 g pieces and whole refers to the total mass of fat left as a whole piece.

	Surface area of fat				
Sensors	**Whole**	**Medium**	**Small**	**SED**	**P-value**
Not adjusted for relative humidity					
All sensors	5.24	5.32	5.38	0.066	0.137
Group 1	4.92	5.00	5.05	0.060	0.122
Group 2	7.80	7.94	8.04	0.100	0.090
Group 3	4.54	4.58	4.65	0.059	0.206
Relative Humidity	59.4	61.1	63.5	0.92	**0.0017**
Adjusted for relative humidity					
All sensors	5.35	5.33	5.26	0.041-0.053	0.207
Group 1	5.02	5.01	4.94	0.042-0.053	0.326
Group 2	7.98	7.95	7.84	0.059-0.075	0.156
Group 3	4.63	4.59	4.54	0.036-0.046	0.142

Appendix 3.1

SENSORY ANALYSIS - Prescreening Questionaire

NAME : _____

SURNAME FIRST NAME TITLE (MRS, MR ETC)

TELEPHONE NUMBER : BH_____ AH: _____

SEX: MALE_____ FEMALE: _____

AGE: Less than 18 years _____

18-34 years _____

35-55 years _____

Over 55 years _____

Marital status: _____

Nationality: _____

Do you smoke: YES_____ NO _____

How often do you smoke: _____

Smoke 1-10 times per day: _____

Smoke more than 10 times a day: _____

Do you have any medical condition that might affect your ability to smell?

Hay fever YES_____ NO _____

Sinus trouble YES_____ NO _____

Colds/Flu YES_____ NO _____

Any other ?_____

Are you a vegetarian, if so please indicate reason below

_____Dislike meat

_____Other

IF YOU ARE A NON-VEGETERIAN PLEASE PROCEED

ATTITUDE TOWARDS MEAT

Are there any foods you do not eat for medical and/or religious reasons

Pork	YES_____	NO _____
Lamb/Mutton	YES_____	NO _____
Beef/Veal	YES_____	NO _____
Chicken	YES_____	NO _____
Fruit/Vegetable	YES_____	NO _____
Dairy products	YES_____	NO _____
Cereals/Baked products	YES_____	NO _____
Eggs	YES_____	NO _____

Any other please describe: _____

Please indicate your preferences below:

Any other please describe:	**Pork**	**Ham/Bacon**	**Sausages Small Goods**	**Lamb/Mutton**	**Beef/Veal**	**Chicken**
Like extremely						
Like very much						
Like moderately						
Like slightly						
Neither Like or Dislike						
Dislike moderately						
Dislike very much						
Dislike extremely						

How often do you eat the following types of meat?

	Pork	**Ham/Bacon**	**Sausages Small Goods**	**Lamb/Mutton**	**Beef/Veal**	**Chicken**
Three times a day						
Once a day						

Three times a week						
Once a week						
Once a month						
Once every six months						
Never						

How is the meat usually cooked

	Pork	**Ham/Bacon**	**Sausages Small Goods**	**Lamb/Mutton**	**Beef/Veal**	**Chicken**
Grilled						
Pan fried						
Roasted						
Stir fried						
Stewed						

How well do you usually have your meat cooked?

_____ well done

_____ medium - well done

_____ medium

_____ medium – rare

_____ rare

How is the meat you eat usually seasoned?

_____ Anglosaxon / Lightly seasoned

_____ Mediterranean / Italian / Greek

_____ Asian

_____ Hot / Indian / Mexican

Appendix 3.2

SENSORY ANALYSIS

SAMPLE CODE : _____

PANELLIST CODE : _____

Date:_____

INSTRUCTIONS

- In front of you are pairs of sniff bottles.
- Evaluate from left to right the samples in pairs.
- You may retest the samples.
- To evaluate samples please proceed with the following steps:

1. Hold the sniff bottle in the middle of the bottle and swirl its contents for several seconds.
2. Raise the nozzle.
3. Hold the nozzle of the bottle about 3 cm from your nose.
4. Gently deliver three puffs of air to the nostrils.
5. Wait 1 min prior to analysing the next sample of the pair.
6. Wait 1 min and then assess the next pair of bottles

- Circle the code of the sample that has an odour.
- You may choose only one of the two samples in each pair.

- Thank you for your co-operation.

Sample Code

1. _____ _____
2. _____ _____

Appendix 3.3

SENSORY ANALYSIS

SAMPLE CODE : _____

PANELLIST CODE : _____

Date:_____

INSTRUCTIONS

- In front of you are two beakers.
- Evaluate the samples starting from left to right.
- To evaluate the samples please proceed with the following steps.

1. Carefully remove the alfoil cap on the beaker.
2. Take the beaker by and bring it forward until it is approximately 3 cm away from the nose.
3. Assess the odour.
4. Wait 1 min prior to analysing the next beaker.
5. Circle the code of the sample which has the odour.
5. You must choose only one sample.
6. If you find there is no difference between the samples please place a tick inside the box. ☐

Thank you for your co-operation

Sample Code

1. _____ _____

Appendix 3.4

THRESHOLD TEST SENSORY ANALYSIS

SAMPLE CODE :_____PANELLIST CODE : _____

Date: _____

INSTRUCTIONS

- In front of you are 12 pairs of sniff bottles.
- Evaluate from left to right the samples in pairs.
- To evaluate samples please proceed with the following steps.

1. Hold the sniff bottle in the middle of the bottle and swirl its contents for several seconds.
2. Raise the nozzle.
3. Hold the nozzle of the bottle about 3 cm from your nose.
4. Gently deliver three puffs of air to the nostrils.
5. Wait 1 min prior to analysing the next sample of the pair.
6. Circle the code of the sample which has an odour.
7. You may retest the samples
8. You must choose only one of the two samples in each pair.
9. Wait 1 min and then assess the next pair of bottles

Thank you for your co-operation.

Sample Number:

1. _____ _____
2. _____ _____
3. _____ _____
4. _____ _____
5. _____ _____
6. _____ _____
7. _____ _____
8. _____ _____
9. _____ _____
10. _____ _____
11. _____ _____
12. _____ _____

Appendix 5.1. Experimental design of the sensory analysis

REP	BLOCK	Position	Sample
1	1	1	5
1	1	2	1
1	1	3	3
1	2	1	8
1	2	2	24
1	2	3	18
1	3	1	6
1	3	2	7
1	3	3	19
1	4	1	13
1	4	2	20
1	4	3	9
1	5	1	15
1	5	2	21
1	5	3	22
1	6	1	23
1	6	2	4
1	6	3	16
1	7	1	17
1	7	2	2
1	7	3	12
1	8	1	14
1	8	2	10
1	8	3	11
2	1	1	2
2	1	2	4
2	1	3	15
2	2	1	11
2	2	2	20
2	2	3	23
2	3	1	22
2	3	2	12
2	3	3	7
2	4	1	5
2	4	2	21
2	4	3	18
2	5	1	8
2	5	2	17
2	5	3	9
2	6	1	6
2	6	2	16
2	6	3	10
2	7	1	3
2	7	2	14
2	7	3	19
2	8	1	13
2	8	2	24
2	8	3	1
3	1	1	11
3	1	2	24
3	1	3	12
3	2	1	22
3	2	2	8
3	2	3	10
3	3	1	1
3	3	2	23
3	3	3	17
3	4	1	6
3	4	2	20
3	4	3	21
3	5	1	3
3	5	2	9
3	5	3	15
3	6	1	7
3	6	2	5
3	6	3	4
3	7	1	19
3	7	2	2
3	7	3	13
3	8	1	16
3	8	2	18
3	8	3	14
4	1	1	11
4	1	2	7
4	1	3	9
4	2	1	1
4	2	2	22
4	2	3	4
4	3	1	13
4	3	2	5
4	3	3	10
4	4	1	23
4	4	2	8
4	4	3	19
4	5	1	18
4	5	2	20
4	5	3	2
4	6	1	24
4	6	2	15
4	6	3	6
4	7	1	21
4	7	2	17
4	7	3	14
4	8	1	16
4	8	2	3
4	8	3	12

Appendix 5.2. Instruction to the record sheet for the sensory analysis experiment.

Name:_____

Date:_____ Time_____

SENSORY ANALYSIS FOR BOAR TAINT

THE FOLLOWING IS A DIFFERENCE TEST

INSTRUCTIONS

1. In front of you is one sample of three

2. Evaluate the samples in order of presentation

3. Unwrap the first layer of alfoil and use your pen to make a hole in the second layer of alfoil, across the diameter of the beaker. Use the first layer to re-wrap the sample when required.

4. Proceed to smell the sample; hold the sample approximately 10 cm from your nose. Be careful the sample is hot.

5. Determine whether you like or dislike the smell

6. Complete section A of the record sheet for that sample

7. Then proceed with Section B i.e. the descriptors of the sample

8. Ignore a descriptor if it is not relevant

9. You may retest the sample

Sample

SECTION A - OVERALL PREFERENCE

PLACE AN X ON THE LINE AT A POINT WHICH INDICATES YOUR LEVEL OF PREFERENCE OR DISLIKE FOR THE SAMPLE

First Impression

0----------5----------10

Dislike Satisfactory Excellent

Lingering odour

0----------5----------10

Dislike Satisfactory Excellent

OVERALL IMPRESSION

0----------5----------10

Dislike **Satisfactory** **Excellent**

Section B – Descriptors

Place an X on the line at a point, which indicates how well the ***descriptor represents*** *the* ***odour****.* (0 = not relevant, 10 = highly relevant)

ROAST PORK/CRACKLING

0----------5----------10

SWEET

0----------5----------10

MUSTY

0----------5----------10

MOTHBALLS

0----------5----------10

RANCID

0----------5----------10

AMMONIA

0----------5----------10

SWEATY/BODYODOUR

0----------5----------10

MANURE

0----------5----------10

URINE

0----------5----------10

OTHER

0---------- ----------5----------10

Appendix 5.3. Adjusted means for the response variates, *overall, first* and *lingering* for the 11 panellists and the overall mean sensory score for fat from boars and gilts.

	Adjusted means for the descriptors by the panellists		
Sample	**OVERALL**	**FIRST**	**LINGERING**
Female 14	5.49	5.55	4.72
Female 15	6.42	6.17	5.98
Female 28	5.19	4.97	5.00
Female 30	6.74	4.39	5.39
Female 31	3.96	3.97	4.10
Female 32	4.80	2.62	4.94
Female 33	6.06	4.57	5.73
Female 34	5.70	3.51	5.21
Female 4	6.39	4.05	5.84
Female 9	6.31	6.51	5.73
IMC13	5.86	6.20	5.91
Overall mean female+IMC	5.72	4.77	5.32
Male 1	2.62	1.37	1.98
Male 10	4.59	4.43	5.10
Male 11	5.00	3.50	4.80
Male 14	3.02	3.32	2.41
Male 2	5.85	6.54	5.89
Male 21	6.07	4.84	6.59
Male 22	6.40	5.66	6.67
Male 25	3.40	3.21	3.98
Male 3	3.80	3.23	4.35
Male 4	2.00	2.42	2.83
Male 5	2.60	2.45	2.21
Male 6	3.90	4.26	5.66
Male 7	4.50	4.36	3.95
Overall mean score male	4.10	3.82	4.34
SED for male and females-			
Maximum	1.16	1.55	1.49
Minimum	1.10	1.05	1.11
P-value for differences between sexes using the Wald test from REML analysis	**5.3×10^{-7}**	**0.0166**	**7.0×10^{-4}**

Appendix 5.4. Comparison of the mean and standard deviation of peak heights between the boars and gilts.

Lpeak	FEMALE Log10 (y + 10,000) transformed mean	Standard deviation	Back transformed mean	MALE Log10 (y+ 10,000) transformed mean	Standard deviation	Back transformed mean	P-value (Wilcoxon Test)
1	0.49	0.30	21167	0.56	0.52	26207	0.93
2	1.17	0.30	139888	0.97	0.47	83211	0.26
3	0.51	0.31	22095	0.38	0.30	14148	0.26
4	0.07	0.16	1735	0.05	0.14	1250	0.79
5	0	0	0	0.12	0.21	3263	**0.05**
6	0.31	0.26	10158	0.32	0.26	11061	0.98
7	1.39	0.19	234160	1.34	0.27	206881	0.84
8	0.11	0.19	2812	0.49	0.55	20890	0.08
9	0.11	0.25	2845	0.06	0.22	1469	0.51
10	0.59	0.24	29266	0.64	0.25	33888	0.40
11	0	0	0	0.21	0.33	6212	**0.05**
12	0.38	0.39	13959	0.67	0.27	37153	0.06
13	0.75	0.36	45669	0.62	0.35	31731	0.31
14	0.44	0.40	17266	0.68	0.18	37346	0.34
15	0.61	0.33	30756	0.47	0.28	19376	0.06
16	0.79	0.67	0.52164	0.80	0.53	52470	0.84
17	0.08	0.19	2055	0.07	0.19	1846	0.86
18	0.55	0.26	25587	0.472	0.26	19191	0.37
19	0.65	0.57	34739	0.36	0.45	12677	0.23
20	0.24	0.42	7315	0.11	0.27	2833	0.44
21	0.40	0.54	15000	0.22	0.33	6571	0.42
22	0.14	0.23	3684	0.31	0.29	10467	0.14
23	0.14	0.20	0.3949	0.06	0.14	1447	0.22
24	0.2979	0.38	9857	0.80	0.32	52365	**0.00**
25	0.17	0.24	4839	0.11	0.21	2888	0.49
26	0.14	0.33	3730	0.09	0.18	2343	0.90
27	0.10	0.18	2577	0.06	0.22	1479	0.26
28	0.03	0.10	729.6	0.09	0.23	2211	0.58
29	0.10	0.18	2682	0.03	0.10	645.4	0.20
30	0.01	0.02	120.4	0.15	0.17	3997	**0.01**
31	0	0	0	0.10	0.061	2509	**0.00**
34	0.14	0.25	3838	0.09	0.22	2240	0.54

Appendix 5.5. Non- adjusted Model for Overall Means in boars and gilts.

Peak	P-values Not Adjusted	Peak	P-values Not Adjusted
1	0.105	18	0.497
2	0.301	19	0.456
3	0.458	20	0.457
4	0.568	21	0.196
5	0.611	22	0.666
6	0.344	23	0.449
7	0.718	24	0.493
8	0.801	25	0.231
9	0.220	26	0.563
10	0.418	27	0.878
11	0.335	28	0.230
12	0.839	29	0.281
13	0.629	Skatole	0.189
14	0.436	Androstenone	0.244
15	0.445	34	0.354
16	0.796	Study	0.928
17	0.689	$\%H_20$	0.602

Appendix 5.6. Adjustments to Model for Overall Means. Peaks were $\log_{10}$ (y + 10,000) transformed.

Adjustment to Model	**F-value**	**Degrees of freedom**	**P-value**
Terms retained			
Males vs Females(incl IMC)	7.2	1,17	**0.015**
Lpeak1	36.7	1,17	**0.000013**
Lpeak7 in males	14.4	1,17	**0.0015**
Lpeak14 in males	47.7	1,17	**2.5×10^{-6}**
Lpeak skatole in males	24.2	1,17	**0.00013**
Lpeak androstenone in males	5.7	1,17	**0.029**
Terms rejected			
Lpeak2	0.24	1,16	0.63
Lpeak3	1.48	1,16	0.24
Lpeak4	0.68	1,16	0.42
Lpeak5	0.39	1,16	0.54
Lpeak6	0.32	1,16	0.58
Lpeak8	0.01	1,16	0.92
Lpeak9	1.06	1,16	0.32
Lpeak10	0.00	1,16	0.95
Lpeak11	0.00	1,16	0.95
Lpeak12	1.53	1,16	0.23
Lpeak12a	1.80	1,16	0.20
Lpeak13	0.12	1,16	0.74
Lpeak15	0.31	1,16	0.59
Lpeak16	0.17	1,16	0.69
Lpeak16b	0.78	1,16	0.39
Lpeak17	0.31	1,16	0.59
Lpeak18	0.29	1,16	0.60
Lpeak19	0.00	1,16	0.97
Lpeak20	0.49	1,16	0.49
Lpeak21	0.00	1,16	0.96
Lpeak22	0.02	1,16	0.90
Lpeak23	0.05	1,16	0.83
Lpeak24	0.79	1,16	0.39
Lpeak25	0.00	1,16	0.95

Lpeak26	0.67	1,16	0.43
Lpeak28	1.02	1,16	0.33
Lpeak29	0.63	1,16	0.44
Lpeak34	0.69	1,16	0.42
IMC vs female	0.01	1,16	0.92
Cohort	0.08	1,16	0.39
Water content	0.00	1,16	1.00
Sex(M vs F) by Lpeak1interaction	0.14	1,16	0.72
Lpeak7 within females	1.29	1,16	0.28
Lpeak14 within females	1.25	1,16	0.28
Lpeak30s within females	2.13	1,16	0.16
Square of lpeak1	1.06	1,16	0.32
Square of Lpeak7 within males	2.23	1,16	0.16
Square Lpeak14 within males	0.94	1,16	0.35
Square of Lpeak skatole within males	0.13	1,16	0.72
Square of Lpeak androstenone within males	2.69	1,16	0.12
Lpeak1.Lpeak7withinmales	0.09	1,16	0.77
Lpeak1.Lpeak14 within males	0.01	1,16	0.91
Lpeak1.Lpeak30s within males	1.67	1,16	0.22
Lpeak1.Lpeak31within males	0.56	1,16	0.47
Lpeak7.Lpeak14-all within males	2.23	1,16	0.16
Lpeak7.Lpeak30-all within males	0.04	1,16	0.85
Lpeak7.Lpeak31-all within males	0.33	1,16	0.57
Lpeak14.Lpeak30-all within males	0.02	1,16	0.89
Lpeak14.Lpeak31-all within males	0.02	1,16	0.90
Lpeak*30.Lpeak31-all within males	1.39	1,16	0.26

* Lpeak30 denotes $\log_{10}$(magnitude of the first peak + 10,000)

Appendix 5.7. The age and sex of the sensory panel members.

Panel Number	SEX	AGE
1	F	40-50
2	F	40-50
3	F	20-30
4	F	40-50
5	M	20-30
6	F	20-30
7	F	40-50
8	M	40-50
9	F	20-30
10	F	30-40
11	M	30-40