The chemical profiling of boar taint within entire male pigs in the Western Cape, South Africa

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature

Date: December 2015

Summary

The objective of this study was to investigate whether there were correlations between sections of the South African PORCUS classification system and the intramuscular fat percentage of the *Longissimus dorsi* muscle of entire male carcasses, as well as between the classification system and androstenone concentration found in the subcutaneous backfat of the carcass. Furthermore it was investigated if androstenone in the subcutaneous fat of the *Longissimus thoracis* would increase or decrease due to the use of three different cooking methodologies (pan fried, oven baked and *sous vide*).

In the first trial, the *Longissimus dorsi* of P-, O- and R-classified entire male carcasses (44 P-, 82 O- and 46 R-carcasses) underwent chemical composition- and androstenone concentration (HPLC-MS/MS-FD) analyses. It was expected that as the PORCUS classes decreased in percentage lean meat, both the intramuscular fat (determined with the use of chemical analysis) and androstenone concentration (µg/g) would increase. However in this study no differences were found in the intramuscular fat percentage of P- and O-carcasses or P- and R-carcasses ($P > 0.05$). R-classified carcasses had a higher intramuscular fat percentage than that of O-carcasses ($p < 0.001$). These results therefore indicated that there was no specific trend that the intramuscular fat percentage correlates with the lean meat percentage as predicted by the PORCUS classification. Also, there was no correlation ($r = 0.065$; $p = 0.397$) between the intramuscular fat percentage and backfat thickness (as determined by the Hennessey grading probe). It was also found that there was no significant difference between androstenone found in the intramuscular fat of P-, O- or R carcasses ($P > 0.05$). This was also contrary to what was expected. It could thus not be claimed that as the % lean meat yield according to the PORCUS classification decreases, the androstenone concentration would increase. There was, however, a significant but low correlation between increasing warm carcass weight (kg) and increasing androstenone concentrations (µg/g) ($r = 0.2674$; $p < 0.01$). Further investigation also indicated that O-carcasses had the highest percentages of carcasses (30.12%) with androstenone levels above the sensory threshold (androstenone > 0.45 µg/g).

In the second research trial, different cooking methods (pan fried, oven baked and *sous vide*) of 12 pork chops (*Longissimus thoracis*) having a high predetermined androstenone concentration were compared to determine how androstenone would react to different cooking methods. All samples used were from O-classified entire male pig carcasses as this carcass classification group was readily available and it was shown that they had higher concentrations of androstenone present within the subcutaneous fat. Androstenone concentrations in the subcutaneous fat were measured before and after preparation. Pan fried chops had a significantly lower concentration of androstenone than oven baked chops ($p = 0.046$) which could have resulted from the different

heating methods (direct dry heat and indirect dry heat, respectively) used. No difference was found in androstenone concentrations between raw samples and any of the prepared samples ($P > 0.05$). It is important to note that the means of all samples were above the sensory threshold (> 0.45 µg/g) which could result in consumers experiencing off odours whilst preparing pork. This could possibly have a negative effect on the pork industry.

Opsomming

Die doel van die studie was om die PORCUS karkas-klassifikasie-sisteem wat in Suid-Afrika gebruik word te bestudeer en te bepaal of die verwagte korrelasies tussen die klassifikasie groepe en die intramuskulêre vet, asook die konsentrasie androstenoon in die onderhuidse vet (op die *Longissimus dorsi*) van die karkasse bestaan. Verder is daar vasgestel hoe androstenoon in die onderhuidse vet van die *Longissimus thoracis* reageer met drie verskillende gaarmaakmetodes (panbraai, oondbraai en *sous vide*).

In die eerste proef was die chemiese samestelling asook androstenoon konsentrasies (HPLC-MS/MS-FD) van P-, O- en R-geklassifiseerde intakte manlike karkasse (44 P-, 82 O- en 46 R-karkasse) bepaal. Daar was verwag dat, soos die persentasie maer vleis binne die PORCUS klassifikasies afneem, beide die intramuskulêre vet konsentrasie en androstenoon konsentrasie sou toeneem. In hierdie studie was daar egter geen verskil tussen die intramuskulêre vet konsentrasies van P- en O-karkasse of P- en R-karkasse nie ($P > 0.05$). R-karkasse het wel ‘n hoër intramuskulêre vet konsentrasie gehad as die O-karkasse ($p < 0.001$). Die resultate dui daarop dat die verwagte patroon van verhoogte maervleis opbrengste soos voorspel deur die PORCUS klassifikasie nie tot laer intramuskulêre vet konsentrasies gelei het nie. Verder was daar ook geen korrelasie ($r = 0.065$; $p = 0.397$) tussen intramuskulêre vet konsentrasies en rugvetdikte gevind nie. Daar was ook geen verskil tussen die konsentrasies van androstenoon in die onderhuidse rugvet en die verskillende PORCUS groepe nie ($P > 0.05$). Dit was verwag dat soos die PORCUS klassifikasie groepe daal in persentasie maervleis, so sou die konsentrasie androstenoon styg, dit is egter nie in hierdie studie bewys nie. Daar was wel ‘n korrelasie tussen die toename in warm karkas massa (kg) en toename in androstenoon konsentrasies (µg/g) ($r = 0.267$; $p < 0.01$). Verdere ondersoeke het getoon dat O-karkasse die hoogste persentasie karkasse (30.12%) met androstenoon konsentrasies bo die sensoriese waarnemings limiet getoon het (androstenoon > 0.45 µg/g).

In die tweede proef is die effek van drie verskillende gaarmaakmetodes op die androstenoon konsentrasie teenwoordig in onderhuidse vet van 12 varktjop monsters (*Longissimus thoracis*) ondersoek. Alle monsters was afkomstig van O-geklassifiseerde intakte manlike karkasse. Androstenoon konsentrasies in die onderhuidse vet was bepaal voor en na gaarmaak. Daar is gevind dat monsters wat in die pan gaargemaak is laer konsentrasies androstenoon getoon het as monsters wat in die oond gaargemaak is ($p = 0.046$). Die resultaat is moontlik as gevolg van die verskillende maniere waarop die gaarmaakmetodes funksioneer (die pan maak gebruik van direkte droë hitte terwyl die oond indirekte droë hitte gebruik). Daar was verder geen verskil tussen rou monsters en oorstemmende gaar monsters nie ($P > 0.05$). Dit is belangrik om daarop te let dat die gemiddelde androstenoon konsentrasie van alle monsters bo die sensoriese waarnemings limiet was (androstenoon > 0.45 µg/g). Die hoë konsentrasie kan lei tot onaangename kookervarings deur

die androstenoon sensitiewe verbruiker, wat uiteindelik ’n negatiewe effek kan hê op die varkvleis industrie.

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Notes

This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. The language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable

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CHAPTER 1
General Introduction

Androstenone is a steroidal male pheromone which is produced in the testes, liver and submaxillary glands (Zamaratskaia & Squires, 2009). Due to its lipophilic properties, some of this compound accumulates in the fat and when pork is subsequently heated, an undesirable odour is released (Brooks & Pearson, 1989).

The most effective method to ensure that male pigs produce low levels of androstenone is castration. In Europe, between 80 and 100% of male pigs are castrated in order to limit the presence of androstenone (Font-i-Furnols, 2012; Bekaert *et al*., 2013). However, due to the increasing amount of pressure enforced by animal welfare groups, the European Union has banned the use of surgical castration from 1 January 2018 (Font-i-Furnols, 2012; Haugen *et al*., 2012; Meier-Dinkel *et al.*, 2013). There is therefore a need to develop new methods to prevent the presence of androstenone in entire male carcasses. Some of the methods that are currently in use include slaughtering at an earlier age or lower weight and specific breeding programs (Pieterse, 2006; Aluwé, 2012).

Research has been performed on the differences between carcass and sex characteristics of entire male pigs compared to that of castrates and gilts. Entire male pigs have been found to have a better feed efficiency than gilts (Blanchard *et al*., 1999). Entire male pigs also grow faster; have leaner meat than that of castrates and have a reduced production cost (Rius *et al*., 2005; Andersen, 2006; Pauly *et al*., 2008). However, these pigs have the tendency to be more aggressive which results in injuries and carcass bruising and of course the possibility of boar taint is high (Horgan, 2006; Pieterse, 2006; Fredriksen *et al*., 2009).

In South Africa the greater majority of male pigs are not castrated. Uncastrated pigs have positive attributes such as faster growth, lean growth and a better feed conversion ration; however farmers are negatively influences because of aggressiveness and possible boar taint. Carcasses are priced according to a number of parameters including; sex, genotype and carcass weight and the PORCUS classification system (Pieterse, 2006). Even though this classification system was established in 1992 (Government Gazette, 1992; Siebrits *et al.,* 2012), it is still used to determine the percentage lean meat of pork carcasses. This is determined with the use of either a Hennessey Grading Probe or an Intrascope® which determines the backfat thickness and also the depth of the *Longissimus dorsi* muscle (Government Gazette, 1992). The measurement that is given by the apparatus is used to calculate to which of the PORCUS categories the carcass will belong (Visser, 2004). In the South African pork industry “RCUS” categorised carcasses are not desired as the possibility of the presence of boar taint increases as the age and weight (fat) of the pigs increase (Van Oeckel *et al*., 1996; Babol *et al.*, 2002; Pieterse, 2006). Furthermore, it is expected that as the

PORCUS classifications change (and subsequently the percentage lean meat), so does the intramuscular fat content (Hoffman *et al*., 2005).

The objective of this study was to investigate whether the PORCUS classification system and some of the assumptions that are made (reflects the percentage of “lean” in the carcass) whilst using it are still viable. This was done by determining the chemical components (moisture, protein, fat and ash content) of the *Longissimus dorsi* muscle and also the androstenone concentrations in the subcutaneous fat of P-, O- and R-classified carcasses. Furthermore, the effect of different cooking methodologies on androstenone concentration in the subcutaneous fat of the *Longissimus thoracis* was analysed.

References

Andersen, J.R. (2006). Sorting criteria. Methods for on-line / at-line sorting of entire male castrated with emphasis on the Danish method based on skatole content. *Acta Veterinaria Scandinavica*, **48**, 20–22.

Babol, J., Squires, E.J. & Gullett, E.A. (2002). Factors affecting the level of boar taint in entire male pigs as assessed by consumer sensory panel. *Meat Science*, **61**, 33–40.

Bekaert, K.M., Aluwé, M., Vanhaecke, L., Heres, L., Duchateau, L., Vandendriessche, F. & Tuyttens, F.A.M. (2013). Evaluation of different heating methods for the detection of boar taint by means of the human nose. *Meat Science*, **94**, 125–132.

Blanchard, P.J., Ellis, M., Warkup, C.C., Chadwick, J.P. & Willis, M.B., (1999). The influence of sex (boars and gilts) on growth, carcass and pork eating quality characteristics. *Animal Science*, **68**, 487-493.

Brooks, R.I. & Pearson, A.M. (1989). Odor thresholds of the C_{19}-Δ^{16}-steroids responsible for boar odor in pork. *Meat Science*, **24**, 11–19.

Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: a review. *Meat Science*, **92**, 319–329.

Fredriksen, B., Font-i-Furnols, M., Lundström, K., Migdal, W., Prunier, A, Tuyttens, F.A.M. & Bonneau, M. (2009). Practice on castration of piglets in Europe. *Animal*, **3**, 1480-1487.

Government Gazette (1992). Regulation No. R. 1748. Agricultural Product Standards Act, 1990 (Act No. 119 of 1990). Regulations regarding the classification and marking of meat. Government Gazette No. 14060, 26 June 1992. Pretoria: Government Printer. South Africa.

Haugen, J.E., Brunius, C. & Zamaratskaia, G. (2012). Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods. *Meat Science*, **90**, 9–19.

Hoffman, L.C., Styger, E., Muller, M. & Brand, T.S. (2005). Sensory, physical and chemical quality characteristics of bacon derived from South African indigenous and commercial pig breeds. *South African Journal of Animal Science*, **6**, 36–48.

Horgan, R. (2006). Piglet castration and EU animal welfare legislation. *Acta Veterinaria Scandinavia*, **48**, 1–4.

Meier-Dinkel, L., Trautmann, J., Frieden, L., Tholen, E., Knorr, C., Sharifi, A.R., Bücking, M., Wicke, M. & Mörlein, D. (2013). Consumer perception of boar meat as affected by labelling information, malodorous compounds and sensitivity to androstenone. *Meat Science*, **93**, 248–256.

Pauly, C., Spring, P., O'Doherty, J.V., Ampuero Kragten, S. & Bee, G. (2008). Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. *Animal*, **2**, 1707-1715.

Pieterse, E. (2006). Effects of increased slaughter weight of pigs on pork production. PhD Dissertation, University of Stellenbosch, South Africa.

Rius, M., Hortos, M. & Garciaregueiro, J. (2005). Influence of volatile compounds on the development of off-flavours in pig back fat samples classified with boar taint by a test panel. *Meat Science*, **71**, 595–602.

Siebrits, F.K., Hambrock, B.W. & Pieterse, E. (2012), Die Suid-Afrikaanse varkkarkas-klassifikasiestelsel. *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*, **31**(1) (in Afrikaans with English abstract).

Van Oeckel, M.J., Casteels, M., Warnants, N., De Boever, J.L., Van Renterghem, R. & Boucqué, C.V. (1996). Production of entire males with Belgian Landrace and hybrid pigs: the incidence of sensory aberrations. *Journal of Animal Physiology and Animal Nutrition - Zeitschrift Für Tierphysiologie Tierernahrung und Futtermit- telkunde*, **76**, 111–121.

Visser, D.P. (2004). Structuring of breeding objectives in the pork supply chain in South Africa. PhD Dissertation, University of Pretoria, South Africa.

Zamaratskaia, G. & Squires, E.J. (2009). Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal : An International Journal of Animal Bioscience*, **3**, 1508–1521.

CHAPTER 2
Literature Review

2.1 Introduction

Boar taint is an off-flavour and/or odour in the fat of entire (uncastrated) male pigs which causes their pork and pork products to be undesirable for consumers (Brooks & Pearson, 1989). Boar taint is caused by the male pheromone androstenone (5α-androst-16-en-3-one) (Patterson, 1968), as well as skatole (3-methyl-indole) (Vold, 1970) and, to a lesser degree, indole (Garcia-Regueiro & Diaz, 1989). Due to their lipophilic properties, these compounds accumulate in the fat of entire male pigs, causing unwanted sensory attributes described as a faecal and/or urine aroma and flavour (Babol *et al*., 2002). Currently, 80 to 100% of male pigs in European Union (EU) countries are castrated in order to avoid boar taint (Bekaert *et al*., 2013). However, surgical castration without anaesthesia is deemed inhumane and thus the EU countries aim to ban this castration technique by 1 January 2018 in response to the increasing amount of pressure enforced by animal welfare groups (Meier-Dinkel *et al.*, 2013; Font-i-Furnols, 2012; Haugen *et al*., 2012). It is for this reason that it has become increasingly important to produce boar taint-free pork through alternative methods, such as the use of immunocastration or slaughtering entire male pigs at an earlier age (Aluwé, 2012).

It was found that 22.7% of consumers are highly sensitive to androstenone whereas 28.3 and 49.0% of consumers have middle and low sensitivity or insensitivity, respectively (Blanch *et al*., 2012). Up to 45% of consumers are genetically predisposed to detect androstenone (Font-i-Furnols, 2012) due to a genotypic variation in OR7D4 which allows these consumers to perceive androstenone (Keller *et al*., 2007) while others are anosmic to androstenone; however, the majority of consumers are able to detect skatole. Olson *et al.* (2003) have reported that boar taint only becomes a problem for consumers if the concentration of androstenone, skatole and/or indole exceeds defined thresholds, which indicates that a method for boar taint detection is concentration-specific. Various studies have indicated that there are differences in sensitivity between consumers from different countries towards boar taint (Lundström *et al.,* 2009; Blanch *et al.,* 2012). De Kock *et al.* (2001) performed a study in which the ability of South Africans from different ethnic groups (black, coloured and white) to detect boar odour in fat samples was evaluated. A significant interaction for gender × ethnic groups was found, such that white females were more sensitive towards samples containing boar taint than any other group. It was also reported that there was no significant difference between white and black males. Furthermore, there was a low acceptability of pork containing high levels of androstenone and skatole amongst most of the consumers (De Kock *et al*., 2001).

With the movement away from surgical castration, methods to detect tainted carcasses in the slaughterhouse is of great importance and demand within the pork industry. Research has thus focused on detecting carcasses with androstenone, skatole and indole on-line, which will aid abattoirs and processors in identifying and treating these carcasses differently (Lundström *et al*., 2009; Aluwé, 2012). Various methodologies have been developed to determine whether the carcass of an entire male pig contains boar taint, which include heating methods for detection by the human nose (Bekaert *et al.,* 2013); electronic nose (Haugen, 2006); and insect invertebrates as biosensors (Olson *et al*., 2003). However, many on-line detection methods depend on a trained individual, who is highly sensitive to boar odour, to smell a heated or singed sample of pork or pork fat and thereby determine whether boar taint is present in the sample or not (Lundström *et al*., 2009; Bekaert, 2013). These detection methods have a very low reproducibility rate as there is a high percentage of human error (Haugen, 2012). The person who is performing the on-line detection also has to undergo intensive training beforehand to accurately detect boar taint. It has however been noted that a lack of clear reference standards for the compounds responsible for the boar taint has led to inaccurate results (Haugen, 2012). The abovementioned issues have resulted in inharmonious on-line detection methods, rendering the development of a sufficient on-line detection method for the pork industry of extreme importance.

In South Africa a great majority of male pigs are not castrated and there are no trained panellists working on the slaughter line to detect carcasses with high concentrations of androstenone. Furthermore there is a tendency to slaughter heavier carcasses (Pieterse, 2006). A direct correlation between increasing carcass weight, subcutaneous backfat thickness, and increasing intramuscular fat has been reported by Beattie *et al*. (1999) and Tibau *et al. (*2003). It has also been found that as the weight of the carcass increases, so does the concentration of androstenone (Van Oeckel *et al*., 1996; Babol *et al.*, 2002). Fast growing pigs are known to be less prone to boar taint also, there is a genotypic issue with boars showing late testicular development having less androstenone than those with early testicular development.

2.2 Biochemistry of boar taint compounds

Various compounds are responsible for the occurrence of boar taint in entire male pigs. The most common compounds are androstenone (Patterson, 1968), skatole (Vold, 1970) and indole (Garcia-Regueiro & Diaz, 1989) (Fig. 2.1). Other compounds have also been found to be responsible for the occurrence of boar taint, which include androstenols (5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol) (Fischer *et al*., 2011), 4-phenyl-3-buten-2-one (Solé & García-Regueiro, 2001) and short-chain fatty acids.

The concentration of boar taint compounds which accumulate in pork fat is dependent on the differences in the rates of synthesis and clearance of these compounds and thus ultimately gene

expression. These processes, involving various metabolic pathways, are controlled by encoding enzymes, protein expression and enzyme activity (Fig. 2.2) (Zamaratskaia & Squires, 2009).

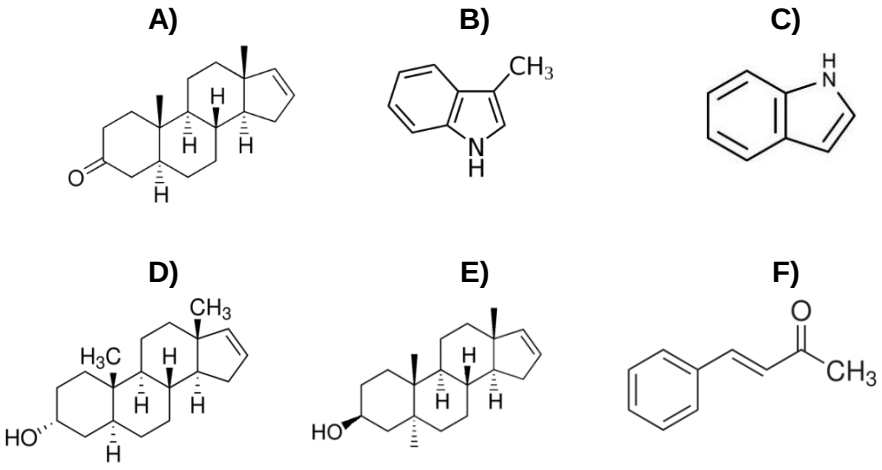

Figure 2.1 The chemical structures of A) androstenone, B) skatole C) indole, D) 5α-androst-16-en-3α-ol E) 5α-androst-16-en-3β-ol and F) 4-phenyl-3-buten-2-one.

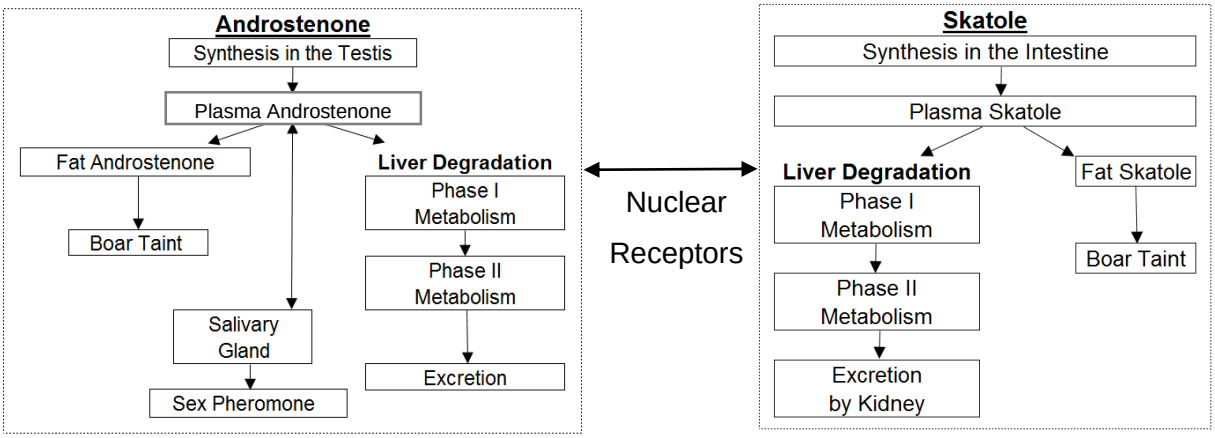

Figure 2.2 Summary of the metabolic pathways of androstenone and skatole (Zamaratskaia & Squires, 2009; Bekaert, 2013).

2.2.1 Androstenone metabolism

The testes, liver and submaxillary glands are involved in the metabolism of androstenone (Zamaratskaia & Squires, 2009) in which the enzymes 3β- and 3α-hydroxysteroid dehydrogenase are main mediators of the metabolic process (Dufort *et al.,* 2001; Doran *et al*., 2004; Sinclair *et al.,* 2005).

Sinclair and Squires (2005) reported that the high concentrations of the sulfoconjugated 5α-androstenone present in the testicular vein plasma suggested the testes are an important site with regard to the production of this steroid and also the concentration thereof that is found in the

peripheral plasma. A large amount of the androstenone is sulfoconjugated by hydroxysteroid sulfotransferase (Zamaratskaia & Squires, 2009), due to the high concentrations of sulfotransferase enzymes (hydroxysteroid sulfotransferase, HST), oestrogen sulfotransferase (EST) and phenol sulfotransferase (PST)) that are present in the Leydig cells (Sinclair & Squires, 2005). In vitro and in vivo studies indicated that androstenone is metabolised to 5α-androst-16-en-3α-ol (3α-androstenol) and 5α-androst-16-en-3β-ol (3β-androstenol) (Zamaratskaia & Squires, 2009) which are later metabolised to produce more polar conjugated steroids (Sinclair & Squires, 2005).

16-Androstene steroids such as 3β-androstenol, as well as less significant amounts of 3α-androstenol, are produced in the liver during the metabolism of androstenone by 3β-HSD and 3α-HSD enzymes (Xue & Dial, 1997; Zamaratskaia & Squires, 2009) where after androstenol undergoes reactions to form glucuronic conjugates and is further sulphated in Phase II metabolism (Sinclair *et al*., 2005). Phase II metabolism refers to the conjugation which takes place in the liver where 16-androstene steroids are subjected to glucuronidation (the addition of glucuronic acid to a substrate) (Sinclair *et al*., 2005). Xue and Dial (1997) also reported that 5β-androstenol is excreted from young boars (≤ 100 kg) through the urine as well as trace amounts in the faeces; whereas in adult boars (≥ 100 kg) mainly 5β-androstenol is eliminated through the urine. Steroidogenic capacity of individual pigs is determined by the concentration of androgens and androstenone present per testis (Oskam *et al*., 2010). Furthermore, the hepatic metabolism of this compound may also determine the concentration found in the peripheral plasma and therefore affect the accumulation of androstenone in the adipose tissue (Oskam *et al*., 2010). Sinclair *et al*. (2005) and Sinclair and Squires (2005) reported that large amounts of steroids (including 16-androstenone steroids) are present in the peripheral plasma in their sulfoconjugated forms with only 25 to 30% present in their unconjugated forms.

Sinclair *et al*. (2006) reported further that hydroxysteroid sulfotransferases such as *SULT2A1*, which is a key Phase II enzyme, and *SULT2B1* and UDP-glucuronosyltransferase may be involved (Zamaratskaia & Squires, 2009) when 16-androstene steroids are metabolised in the liver.

2.2.2 Androstenone biosynthesis

Androstenone (5α-androst-16-en-3-one) is classified as a steroidal pig pheromone which is produced by the Leydig cells in the testes along with androgens and oestrogens (Babol *et al*., 1999) and was first identified by Patterson (1968). Androstenone, as well as androstenol (5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol) are classified as C_{19}-Δ^{16} steroids (Fischer *et al*., 2011) and ranges of 0 to 6.4 µg/g have been reported in the adipose tissue from entire males (Lunde *et al*., 2012). During mating activity, C_{19}-Δ^{16}-steroids are transported through the bloodstream of the pig to the salivary gland (Claus, 1994). The hormone acts as a sexual pheromone, thereby stimulating interest in female pigs and evoking the mating stance (lordis response) throughout the oestrous of

the female pig (Brooks & Pearson, 1986; Brooks *et al.*, 1986; Claus, 1994; Zamaratskaia & Squides, 2009). Claus *et al*. (1971) found that some female and castrated pigs do synthesise low levels of androstenone, which suggested that there is a possibility for androstenone to be produced by the adrenal cortex and the ovary (Claus *et al.,* 1971).

Since the testes are the site of androstenone synthesis, pigs are often castrated before sexual maturity to control the concentration of androstenone (Babol *et al*., 1999). Androstenone is derived from pregnenolone through the formation of 5,16-androstadien-3b-ol by the andien-b synthase system (Meadus *et al*., 1993; Davis & Squires, 1999). The two important cytochromes for the biosynthesis of 5,16-androstadien-3β-ol are cytochrome P450C17 (CYP17A1) and cytochrome b5 (CYB5). Cytochrome-mediated biotransformation takes place in the liver where after androstenone is excreted (Zamaratskaia & Squides, 2009). The steroid is controlled by the neuroendocrine system (hypothalamic-pituitary-gonadal axis) (Gower, 1972) and thus testicular steroids such as androstenone are reliant on the stimulatory control of gonadotropin-releasing hormone (GnRH). For this synthesis the lutenising hormone (LH) is of particular importance. Once the pig advances to its early postnatal life (approximately 2 - 4 weeks of age) the circulating testicular steroid levels increase (Zamaratskia & Squires, 2009). This is due to the transient activation of the hypothalamic-pituitary-gonadal axis (Bonneau, 1982; Schwarzenberger *et a*l., 1993; Sinclair *et al*., 2001). Androstenone is produced and released in two waves, Schwarzenberger *et al*. (1993) reported that the first wave occurs between two and four weeks of age where after the concentration of androstenone remains low (2 - 5 months of age). After this age, there is a strong increase of androstenone found in the plasma (Schwarzenberger *et al*., 1993; Claus *et al*., 1994). The biosynthesis of androstenone can be low in young pigs (Gower, 1972; Bonneau, 1982), but the levels of androstenone and other testicular steroids will progressively increase at sexual maturity. The stage at which a boar reaches sexual maturity is dependent on a variety of factors such as age, weight and the size of the secondary sex glands (Zamaratskaia & Squides, 2009).

A large amount of androstenone is metabolised and sulfaconjugated in the testes. The testicular vein releases androstenone into the bloodstream where after the steroid can accumulate in the fat (Fischer *et al*., 2011). Androstenone can be stored in the adipose tissue due to it being in the unconjugated form and due to its lipophilic characteristics. The amount of androstenone stored in the fat depends on the intensity of testicular synthesis as well as the liver and testes metabolism (Robic *et al*., 2008). The compound can again be released from the fat, but the rate of release back into the bloodstream is slower than the uptake rate (Brooks *et al.*, 1986). Androstenone has been found to be a volatile compound when heated (Font-i-Furnols *et al.*, 2003) although no data could be sourced that indicates at what temperatures this would occur; this aspect warrants further research. The odour which is released has been described by some as a ruinous, sweaty odour whereas others have described it as perfumed sandal wood (Fort-i-Furnols *et al*., 2003).

2.2.3 Skatole and Indole production

Skatole and indole are known as heterocyclic aromatic amines (Fischer *et al*., 2011) which are produced in the rumen of ruminants as well as ceca and colon of monogastric animals (Jesen *et al*., 1995) due to the anaerobic bacterial degradation of the amino acid tryptophan (Babol *et al.* 2002; Dehnhard *et al.* 1993). Only L-tryptophan is degraded to either of these two volatile lipophilic compounds (Jensen *et al*., 1995).

The occurrence of L-tryptophan in the pig is possibly due to the diet as well as the material derived from the upper part of the intestine (Jensen *et al*., 1995). When L-tryptophan is degraded, it can be directly degraded to indole or to indole-3-acetic acid where after it is converted to skatole (Lundström *et al*., 1988). These degradations are due to the presence of bacteria in the gut (Bekaert, 2013). Skatole and indole cause a faecal-like odour in pork (Claus *et al*., 1994).

The amount of skatole produced by a pig can be influenced by the environment as well as dietary conditions (Babol *et al*., 2002). Chen *et al*. (2007) found that these compounds are partially absorbed by the internal mucosa where after it is circulated in the bloodstream and finally absorbed in the adipose tissue (Fischer *et al*., 2011).

Skatole can be produced by male and female pigs and is metabolised in the liver by cytochrome P450, but non-metabolised skatole accumulates in back-fat. Zamaratskaia *et al*. (2006) found that only a few female pigs develop this compound; this could be due to sex-dependent differences during the potential metabolism of skatole. Skatole levels do increase with puberty and the increase is caused by the increase of testicular steroid levels; Zamaratskaia and Squires (2009) consider this to be due to the inhibition of hepatic skatole metabolism by androstenone and oestrogens.

2.2.4 Other compounds contributing to boar taint

Other compounds are also known to cause boar taint. These include androstenol (5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol) (Fischer *et al*., 2011), 4-phenyl-3-buten-2-one (Solé & García-Regueiro, 2001) and short-chain fatty acids. The concentrations of these compounds are much lower than those of androstenone and skatole. A comparison of the concentrations of androstenone, 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol indicate ratios of 10:1:0.5 (Fischer *et al*., 2011). Although these concentrations are low, 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol can influence a sample when occurring in higher concentrations. Their taint is especially prominent when a sample is heated before consumption (Brooks & Pearson, 1989). Gas Chromatography Mass Spectromitry (GC-MS (in SCAN mode)) has been used to detect 4-phenyl-3-buten-2-one (Solé & García-Regueiro, 2001) and it was found that the presence of 4-phenyl-3-buten-2-one depends on the concentrations of androstenone and skatole. Solé and García-Regueiro (2001) also determined

that when a fat sample is fortified with high concentrations of 4-phenyl-3-buten-2-one, this would affect the sensory attributes and panellists would pick up characteristics similar to naphthalene or mothballs. This compound can also act as enhancer for androstenone as well as skatole (Dijksterhuis *et al*., 2000). Short chain fatty acids can also attribute to the off flavours found in pork due to the oxidation of lipids. Lipid oxidation may cause off-flavours such as rancidity, pungency, sourness, fattiness, waxiness and almond-flavour (Rius *et al*., 2005).

2.3 Consumer acceptance and sensitivity to boar taint

Numerous international studies have been performed to evaluate the acceptance and sensitivity of boar taint to consumers. Consumers' reaction towards boar taint is not only dependant on country of origin, gender, age and genetic make-up (Blanch *et al.,* 2012), but also on the method of determination as a wide variety of methodologies are used to determine boar taint acceptance and sensitivity of consumers; it is challenging to compare the results of studies performed (Font-i-Furnols, 2012). The methodologies differ with regard to: analysis of boar taint compounds and -concentrations, consumer profile (gender, age, culture, etc.), product or muscle used for testing, preparation of sample (cooking method, temperature, etc.), threshold levels used, consumer sensitivity, and more (Font-i-Furnols, 2012). The diversity of consumer studies performed indicates that a harmonised method is needed.

Although there is a great deal of variation between the studies, it was clear that as the concentrations of boar taint compounds increased, consumer acceptance decreased (De Kock *et al*., 2001). This is due to boar taint compounds causing unwanted sensory attributes in pork; androstenone, with a threshold of 1.0 µg/g (Brooks & Pearson, 1989), and skatole (0.2 µg/g threshold) gives pork a sexual and faecal odour, respectively. Annor-Fermpong *et al.* (1997) however reported that consumers had a threshold of 0.2 to 1.0 µg/g and 0.008 to 0.06 µg/g for androstenone and skatole, respectively. Fort-i-Furnols *et al*. (2013) reported that between 82 and 99% of consumers are sensitive towards skatole whilst about 40% of consumers are sensitive to androstenone. Kloek (1961) (as referenced by Brooks & Pearson, 1989) reported that 46% of men and 24% of women are not able to detect androstenone. Kloek (1961) also stated that out of consumers that are able to detect androstenone, women rated the odour more intense than men. The lower percentage of androstenone sensitivity is due to genotypic variation in OR7D4; the two most common alleles are OR7D4 RT and OR7D4 WM (Keller *et al.*, 2007).

Androstenone sensitivity is gender dependent; Fort-i-Furnols *et al*. (2003) compared the acceptance of androstenone of male and female consumers and reported that 16% of men and 3% of women liked the odour of pure androstenone. This might be due to a difference in quality androstenone (eg. new, pure androstenone crystals or crystals that have been used for analysis for a substantiol amount of time) perceived between consumers. Some consumers find androstenone

to have a ruinous, sweaty odour whereas others have described it as perfumed sandal wood (Font-i-Furnols *et al*., 2003). Pearson *et* al. (1971) noted that the acceptance of androstenone may be dependent on the consumer's detection level. Furthermore, Matthews *et al*. (2000) reported that the age of consumers may also have an impact on consumer sensitivity of androstenone; older consumers are less sensitive towards androstenone than younger consumers.

2.3.1 International boar taint consumer acceptance and sensitivity comparison

A large number of studies on consumer acceptance towards boar taint have been performed in Europe. These countries include Germany, Belgium, Denmark, Spain, France, Ireland, Italy, The Netherlands, Norway, Sweden and The United Kingdom. Canada, The United States of America as well as Japan have also contributed to boar taint acceptance studies (Font-i-Furnols, 2012). From the African continent, South Africa has also performed studies. These international studies range between the 1970's and 2014.

Although various methodologies are used for consumer acceptance, the majority presented the consumer with pork, pork fat or a pork product. The consumer was then asked to evaluate the sample and rate it according to acceptance (Font-i-Furnols *et al*., 2008; Matthews *et al*., 2000). Consumers were also given questionnaires to fill in which allows analysis of consumer acceptance of boar taint. The numbers of consumers partaking in the acceptance analysis also vary; some studies have 1395 participants whilst others only have 45 (Font-i-Furnols, 2012). Blanch *et al.* (2012) determined that globally, 22.7% of consumers are highly sensitive to androstenone whereas 28.3 and 49.0% of consumers are middle and low sensitive or insensitive, respectively.

In high concentrations, androstenone would reduce the acceptability of boar meat. It was however found that medium levels of this compound could occasionally increase consumer acceptability to a point where the pork is as acceptable as that from female pigs (Blanch *et al.,* 2012). It is therefore not surprising that some authors have found that the sex of the pig does not influence the levels of androstenone or skatole acceptability for consumers. Others have reported that the acceptance of boar taint is influenced by the sex of the animal as well as the levels of boar taint compounds present (Drieste *et al*., 1990; Font-i-Furnols *et al*., 2008; Matthews *et al*., 2000).

Europe

European scientists were the first to conduct consumer studies on boar taint compounds such as androstenone, skatole and indole. The first studies published originate from France, The Netherlands and The United Kingdom (Font-i-Furnols, 2012).

Dijksterhuis (2000) performed a study where the sensory evaluation of androstenone and skatole of seven European trained panels was compared. These included UK, Danish, French, Swedish, Dutch, Spanish and German panels. It was deducted that although panellists could distinguish between androstenone and skatole, as well as between different concentrations of these substances, judges did seem to get confused between the compounds. This could be due to the fact that when samples are heated, whilst containing different concentrations of boar taint compounds; different odour descriptors can be released. This occurs particularly with skatole (Dijksterhuis, 2000). Skatole was commonly described to have a faecal odour whilst androstenone has a urine-like odour. Skatole was also described as naphthalene. It was also reported that panellists found it more difficult to effectively recall androstenone's odour (Dijksterhuis, 2000).

In a consumer study performed in different European countries, consumers from France, Germany, Spain and Sweden were critical of pork from entire male pigs. However, consumers from Denmark and Holland reacted negatively towards the odour, but not the flavour of pork from castrated male pigs. Consumers from the United Kingdom were not critical to any of the flavour or odour aspects of the pork samples (Lundström *et al.,* 2009).

Blanch *et al*. (2012) determined consumer sensitivity towards androstenone on acceptability for France, Spain and the United Kingdom. The French consumers displayed a negativity towards pork with high levels of androstenone and skatole. This correlates with the findings of Diestre *et al*. (1990) and Fort-i-Furnols *et al.* (2008). However, no differences were noted by the consumers between pork from female pigs and pork with low levels of androstenone and skatole present (Blanch *et al*., 2012). This can be explained by findings from Fort-i-Furnols *et al.* (2008) where it was reported that when the androstenone and skatole concentrations of male pigs are as low as that of gilts, the consumer will not be able to distinguish between samples; here the androstenone levels of the male and female pigs did not differ with more than 0.5 ppm.

Fort-i-Furnols *et al.* (2013) reported that between 44 and 47% of French consumers are sensitive to androstenone out of which 59.2% are women and 55.6% men. Lundström *et al*. (2009) also reported that French consumers are very sensitive towards pork containing boar taint.

Spanish consumers do not differ greatly in their acceptability towards pork with or without boar taint compounds (Fort-i-Furnols *et al*., 2003; Blanch *et al.,* 2012). Pork with higher concentrations of androstenone and skatole were given a lower acceptance rating than that of pork with low levels of androstenone and skatole as well as pork from female pigs. Fort-i-Furnols *et al*. (2003) found that consumers rejected pork samples with androstenone levels higher than 0.5µg/g

fat tissue. Other researchers (Desmoulin *et al*., 1982; Diestre *et al*., 1990) found that consumers only reacted negatively towards pork once the androstenone level is above 1.0 µg/g fat tissue. The Alternatives to Castrating and Dehorning (ALCASDE) reported that Spanish consumers prefer pork from female and entire male pigs (Olivier, 2009). The acceptance of pork from entire male pigs can be explained by the fact that the pork had relatively low levels of androstenone and skatole present.

It is estimated that between 30.9 and 47.5% of Spanish consumers are sensitive to androstenone (Font-i-Furnols *et al.,* 2013). Of these consumers, 37.3 – 53.6% are expected to be female, and 23.7 – 45.5% male. Weiler *et al*. (2000) reported that approximately 31% of Spanish consumers are highly sensitive to androstenone, a result similar to that of Font-i-Furnols *et al.* (2013).

It is reported that 47.6% of British consumers are sensitive to androstenone; of these consumers, approximately 58.9% are female and 36.5% male (Fort-i-Furnols *et al.,* 2012). Olivier (2009) found that British consumers prefer entire male and female pigs. This differed from countries such as France, the Netherlands, Germany and Italy where consumers preferred pork from female pigs or from castrated male pigs. Fort-i-Furnols *et al.* (2012) reported that British consumers ranked pork with low androstenone and skatole levels lower than pork from female pigs, but no difference was found from pork with high levels of androstenone and skatole. This is also not according to what is expected of consumer liking towards boar taint. The acceptability of the pork from entire male pigs may be due to the fact that the androstenone levels were below 0.63 µg/g fat tissue. This value is not high enough to reject pork tainted with androstenone (Olivier, 2009; Fort-i-Furnols *et al.,* 2012).

Approximately 17.6% of German consumers were reported to be highly sensitive to androstenone whereas 32.2% of consumers were sensitive towards the compound (Weiler *et al*., 2000). However, Fort-i-Furnols *et al.* (2012) reported that between 17.6 and 73.0% of German consumers are sensitive to androstenone. Lundström *et al.* (2009) reported that German consumers are critical of pork from entire male pigs.

Swedish consumers were found to be critical with respect to both flavour and odour of pork from entire male pigs (Lundström *et al.,* 2009). Hendriks and King (n.d.) reported that some New Zealand consumers found pork from uncastrated male pigs “smell/strong/unpleasant” in odour and flavour. Bekaert (2013) found that 51.1% of women and 38.3% of men are sensitive to androstenone in Flanders, Belgium.

South Africa

A limited amount of research has been performed in African countries with regard to consumer acceptability and sensitivity towards boar taint. However, De Kock *et al*. (2001) evaluated the reaction of South African consumers towards boar taint. Consumers were presented with fat samples with various concentrations of boar taint compounds, androstenone and skatole. De Kock *et al*., (2001) found that the trend for acceptance towards boar odour followed the results from

European countries; the higher the concentrations of androstenone and skatole, the more likely it is that the consumer would dislike the sample (Font-i-Furnols *et al*., 2003).

In the latter South African study the liking of three different ethnic groups (Black, Coloured and White) were also determined as was the acceptance of males and females. Overall, women would respond more negatively towards samples with detectible levels of androstenone. This correlates with the findings of Gower *et al.* (1985), Weiler *et al*. (1997), Thornhill and Gangestad (1999) and Fort-i-Furnols *et al*. (2003). De Kock *et al.* (2001) also determined that male consumers tend to like samples more that had "medium (0.5-1µg/g)" levels of androstenone present. Men tend to be more anosmic than women because men have higher levels of androstenone present in their axilla and urine (Gower *et al.*, 1985; Thornhill & Gangestad, 1999).

When comparing the selected ethnic groups, differences were found between the Black and White consumer groups. White females were more sensitive towards boar odour than White male and Black consumer groups. Furthermore, Black and White male consumers revealed the same amount of acceptance towards boar odour, whilst Black female consumers were the least critical about boar odour (De Kock *et al*., 2001). The results generated by the Coloured consumers could not be compared to these of the Black and White consumers. This was due to the samples given to the Coloured consumers not being from the same carcasses as those used for the Black and White consumer panels. Furthermore, the "High Skatole High Androstenone" sample did not meet the criteria of the androstenone concentration being higher than > 1.25 µg/g (De Kock *et al*., 2001). Although the results for the Coloured consumer panel could not be directly compared with that of the Black and White consumer panels, the trend of consumer acceptance towards fat samples with higher boar odour concentrations could clearly be seen. There was also no significant gender interaction with regard to the Coloured consumer group. As the concentration of boar odour increased, the acceptance of consumers decreased (De Kock *et al*., 2001). The differences between acceptability towards pork exhibiting characteristics of boar taint can be ascribed to the frequency pork were consumed by the consumers (Schutte, 2008) as well as consumers being anosmic (Gilbert & Wysocki 1987; Weiler *et al*., 2000). De Kock *et al.* (2001) reported further that White consumers *per capita* yearly consumption of pork is 16.2 kg whilst Coloured and Black consumers have a yearly *per capita* consumption of only 3.4 and 0.6 kg respectively. It was also found that consumers from the White consumer group were on average older than consumers from the other ethnic groups and age has been known to influence sensitivity towards boar taint (Font-i-Furnols *et al*., 2003). Furthermore, there is a possibility that due to cultural differences (Schutte, 2008), some of the consumers may even like the presence of boar taint in pork (Pearson *et al*., 1971).

2.3.2 Correlation between consumer genetics and sensitivity towards boar taint

Globally, studies have been conducted to determine consumer sensitivity towards androstenone. Blach *et al*. (2012) found that in France, Spain and the United Kingdom, 14.3 - 41.0% of consumers find an androstenone odour offensive and would reject it. Furthermore, 22.7% of consumers' worldwide are classified as highly sensitive to boar taint (Blach *et al*., 2012). Androstenone sensitivity can be determined by the genotypic variation of the OR7D4 receptor within humans. This receptor is mostly present in two alleles known as OR7D4 RT and OR7D4 WM (Keller *et al*., 2007). The reference receptor has been determined to be OR7D4 RT. This receptor will respond to androstenone whilst OR7D4 WM shows diminished response. Keller *et al*. (2007) reported that consumers with OR7D4 RT/WM and OR7D4 WM/WM genotypes were less sensitive to androstenone whilst consumers with OR7D4 RT/RT were more sensitive. The latter group was prone to describe boar taint as a more unpleasant odour. Lunde *et al*. (2012) confirmed that the OR7D4 genotype does determine the sensitivity of a consumer towards boar taint. It is also reported that consumers perceive androstenone in different ways; some distinguish/describe it as urine and sweat whilst others classified the odour as vanilla and sweet (Beets & Theimer, 1970; Wysocki & Beauchamp, 1984).

When consumers have an OR7D4 RT/WM and OR7D4 WM/WM genotype, they possibly have defective or missing molecular receptors, causing them to be anosmic (Wysocki & Beauchamp, 1984; Keller *et al*., 2007; Bekaert, 2013). It is also possible that anosmic consumers have the necessary receptors, but the receptors have a sub-threshold density (Bekaert, 2013).

2.3.3 Effect of consumer gender on boar taint sensitivity

Androstenone (3α-androst-16-en-3-one) and skatole (3-methylindole) are two of the main causes of boar taint. These compounds cause unwanted sensory odours in pork, including odours and flavours (Font-i-Furnols *et al*., 2003). Weiler *et al*. (1997) found that 99% of male and female consumers are able to perceive skatole. Sensitivity towards androstenone did however depend on the sex of the consumer (Griffiths & Patterson, 1970; Weiler *et al*., 2000; Lundström *et al*., 2009).

In all studies comparing gender sensitivity towards androstenone, it was found that women are more sensitive than men (Font-i-Furnols *et al*., 2003). Gilbert and Wysocki (1987) described percentages of anosmic women to men; in Europe (excluding the United Kingdom) it was 15.8 vs. 24.1%, 10.9 vs. 30.0% in United Kingdom, 29.5 vs. 37.2% in USA and 17.2 vs. 25.5% in Asia, respectively. Further studies have found that in Germany 66% of women and 70% of men were anosmic whereas 48 vs. 60% were anosmic in Spain, respectively (Weiler *et al*., 2000). Blanch *et al*. (2011) reported that, depending on geographic region, 11 - 66% of women and 18 - 74% of men are anosmic to androstenone. It is postulated that men might be less sensitive to androstenone

because this steroid is found in the human axilla and urine and men have higher levels thereof than women (Gower *et al.*, 1985; Thornhill & Gangestad, 1999).

It is challenging to determine the exact percentages of consumers that are sensitive towards androstenone. This is due to the great variation in methodologies used to determine whether a consumer is anosmic or not, and if not, how sensitive to androstenone the consumer truly is.

2.3.4 Relationship between cultural habits/ ethnicity and boar taint

Numerous factors effect consumer sensitivity towards boar taint. One of these factors is the different cultural habits of consumers (Bryhni *et al*., 2002; Aluwé *et al*., 2012). Mowen and Minor (1997) reported that psychological factors such as attitude (positive or negative) and honesty will also have an effect on the results given by the consumers. These factors have been found to differ between nations and consumer groups. This agrees with Fort-i-Furnols *et al*. (2003) and Bekaert *et al.* (2011) who also stated that geographic origin has an effect on androstenone sensitivity.

The population composition is also an important factor influencing cultural differences and acceptability towards boar taint products (Schutte, 2008). Demographic differences affect the food market; consumer living conditions, wealth, religion and sosio-economic status all have the ability to affect consumer preferences (Schutte, 2008). Similarly, ethnicity and religion greatly affect the consumer's demand for meat as different groups of consumers have different needs (i.e. halaal, kosher, income, education, occupational status, etc.). Income plays an important determining role in which meat products the consumer purchases. It has been reported that as the household-income of a consumer increases, the quality of meat products purchased also increases (Mainland, 1998) indicating that the consumer will start purchasing leaner and larger quantities meat of higher quality (Berry & Hasty, 1982). This could result in consumers purchasing pork and pork products, however, if the male pigs were not castrated, there is a possibility that the pork might have androstenone present.

2.3.5 The effect of consumer age on androstenone sensitivity

Font-i-Furnols *et al*. (2003) reported that consumer age does have an effect on androstenone sensitivity with regard to both odour and flavour. Consumers are sensitive up to the age of 60 years of age where after the sensitivity of consumers' decrease. Increasing sensitivity towards androstenone (until the age of 60) can be because of ostensibly anosmic consumers becoming more sensitive towards this compound. This increase has been found to be between 21.4 and 40.0% (Fort-i-Furnols *et al*., 2003). Once the consumer reaches the age of 60, it is possible that androstenone sensitivity decreases due to aging which reduces the sense of taste and odour (Russell *et al.*, 1993; Barber, 1997). Fort-i-Furnols *et al*. (2003) reported the following percentages of androstenone sensitivity depending on consumer age (Table 2.1).

Table 2.1 Percentage of androstenone sensitivity or less sensitive/insensitive consumers depending on their age (Font-i-Furnols *et al*., 2003)

Age (years)	Sensitive	Mildly sensitive	Insensitive
18 – 25	21.4	10.5	68.1
26 – 40	30.0	17.1	53.0
41 – 60	40.0	17.5	42.4
61 – 75	23.9	13.4	62.7

$\chi^2 = 57.54$ $P = < 0.0001$

2.3.6 The effect of consumer smoking habits on androstenone sensitivity

Even though smoking has been associated with a decreased sensitivity towards androstenone in the past (Bekaert, 2013), Bekaert *et al*. (2011) reported that there was no significant interaction (p = 0.720) between the consumer's androstenone sensitivity and their smoking habits.

2.3.7 Effect of cooking method on consumer boar taint perception

Once pork has been heated, the concentrations of these androstenone and skatole will decrease due to their volatile properties. This indicates that the final internal temperature of pork products affect the consumer's perception of boar taint (Dehnhard *et al*., 1995; Wood *et al*., 1995; Lundström *et al., 2009*). In contrast, Lundström *et al.* (2009) noted that boar odour was less evident in ham (*Musculus semimembranosus, MS*) produced at 80˚C than at 65˚C indicating that these hormones had evaporated due to the increased temperature. It was also reported that pork chops have lower concentrations of androstenone and skatole at an internal temperature of 65˚C rather than at 72.5˚C and 80˚C (Wood *et al*., 1995).

A consumer survey was performed on pork from gilts and entire male pigs with various concentrations of androstenone and skatole that was prepared with the use of pan frying and oven cooking (Lundström *et al.,* 2009). It was found that pork chops with high concentrations of boar taint compounds that were prepared with the pan frying method rendered a better response from consumers than when prepared with the oven cooking method. McCauley *et al*. (1997) performed a study on the effect of different cooking methods on pork with various concentrations of androstenone and skatole. In Table 2.2 it is shown that pork with high concentrations of boar taint causing compounds (1.1 µg/g androstenone and 0.17 µg/g skatole) received significantly higher scores of boar taint in all product fields.

Table 2.2 Difference in sensory quality (assessed as boar odour by a trained sensory panel) between products from female and entire male pigs (McCauley *et al*., 1997)

Product	Female	Low Male	High Male
Dry, oven-roasted pork	2.2 [a]	3.0 [a]	5.6 [b]
Syewed, oven-roasted pork	4.9 [a]	5.4 [a]	8.6 [b]
Marinated, oven-roasted pork	1.0 [a]	1.1 [a]	3.6 [b]
Bacon	1.4 [a]	1.5 [a]	5.8 [b]
Ham, consumed cold	1.6 [a, b]	1.5 [a]	2.0 [b]
Salami, consumed cold	1.5 [a]	0.8 [a]	1.5 [b]

Low level of boar taint: 0.25 µg/g androstenone and 0.06 µg/g skatole; high level: 1.1 µg/g androstenone and 0.17 µg/g skatole

[a,b] Means with different letters indicate significantly differences between rows ($P < 0.05$).

Scale: absent = 0 strong = 10.

Muscle type is also known to influence the concentration of the boar taint causing hormones. The loin (*Musculus longissimus dorsi*) is known for its high androstenone and low skatole concentrations. This muscle group had the highest levels of androstenone odour at 80˚C (Lundström *et al.,* 2009).

2.4 Boar taint detection methods

The pork industry prefers the rearing of uncastrated male pigs as entire males grow faster on less feed when compared to castrated male pigs (Andersen, 2006). Bonneau (2000) also reported that the nitrogen output into the environment, as well as that of other pollutants from modern farming, decreases when pigs are reared that are not castrated. Furthermore, when comparing castrates to entire male pigs, the lean meat yield of entire males was 1.5-2.5% higher than that of castrated male pigs (Andersen, 2007). These factors contribute to the fact that some farmers avoid castration due to economic and environmental reasons (Bonneau, 2000; Andersen, 2006; Andersen, 2007).

Unfortunately, the abovementioned factors are not the only aspects that should contribute to the choice concerning castrating or not castrating male pigs. Boar taint compounds play a big role in the decision whether to castrate or not. Androstenone (5-α-androst-16-en-3-one) is a pheromone that is produced by the testis of male pigs (Lundström *et al*., 2009). Due to the lipophilic properties of this compound, it accumulates in the fat (Verheyden *et al*., 2007). Androstenone is one of the compounds that can be especially offensive to certain consumers, and the only way to ensure that this compound is not present in pork is with the use of castration (Font-i-Furnols *et al*., 2003). However, only a small percentage of pigs produce androstenone. Also, very few countries penalise

the producers if their intact males show signs of boar taint. One of the reasons for this lack of penalisation is that there is no rapid reliable on-line testing procedure available (Bekaert, 2013).

Two types of analysis are commonly used in the industry to detect boar taint. They are categorised as chemical and on/offline analyses. Through the years, a great variety of detection methods have been developed by the pork industry and researchers. Many chemical analysis methods have recently been established (Haugen, 2006); more than 30 chromatographic methods and approximately 10 immunological methods have been developed (Haugen, 2010). The need for these detection methods has become more important as the European Union (EU) has banned the use of surgical castration without anaesthesia as from 1 January 2018 (Aluwé, 2012).

Although there has been extensive development in terms of chemical analysis, there is still no harmonisation for on/offline boar taint detection (Lundström *et al*., 2009; Bekaert, 2013). On-/offline detection methods predominantly make use of the "human nose" method to detect boar taint. In order to use the "human nose" detection method, a panellist who is highly sensitive to boar taint (detects the compound at 0.2 ppm) is selected, trained and placed on the slaughter line. The panellist smells all carcasses and indicates whether boar odour is detected. To aid in detection, the back fat is singed with a soldering iron or pyropen (Weller®) where after the off-odour is made more prominent (Bekaert, 2013; Bekaert *et al*., 2013). In theory, this would be an adequate method to detect boar taint on the slaughter line, however a single panellist may experience "fatigue" if on the line for a whole day without being relieved by another assessor. There have also been reports that assessors struggle to distinguish between the odours of androstenone and skatole, although if the concentrations of any of the boar taint compounds are defined as high, the carcass is removed from the line and processed otherwise.

2.4.1 Chemical analysis of boar taint

Chemical or laboratory analyses can be used to determine the concentrations of androstenone, skatole and indole present in fat samples (Aluwé, 2012). These analyses include HPLC, GC, LC-MS, immunological and colorimetric methods (Zamaratskaia, 2004; Lundström *et al*., 2009; Aluwé, 2012 & Haugen *et al*., 2012). These methods have been developed in such a way that boar taint compounds can be detected either simultaneously or separately. Haugen *et al*. (2012) reported that skatole and indole are generally measured with the use of normal or reverse phase HPLC, GC and spectrophotometric methodologies, whereas androstenone is measured with HPLC, GC and immunological methods.

2.4.1.1 Sample pre-treatment

Chemical detection cannot be possible if fat samples have not undergone proper sample pre-treatment. This treatment ensures that any non-target bulk matrix interference is removed from the fat samples. Furthermore, sample pre-treatment protects the lifetime of the analytical system and increases the signal over noise which guarantees the sensitivity of the target analytes (Bekaert, 2013).

The most common extraction procedures for fat are liquid extraction and the liquid-liquid extraction. Adipose tissue is heated for the extraction where after an aliquot of liquid fat is added to a solvent (Dehnhard *et al*., 1993; Fischer *et al*., 2011). Solid-liquid extractions can also be used to perform these extractions. In this procedure fat is cut into pieces and mixed with the solvents (Hansen-Møller, 1994; Garcia-Regueiro & Ruis, 1998; Verheyden *et al*., 2007). Methanol, hexane dichloromethane, acetonitrile as well as solvent mixtures are used to extract androstenone and/or skatole. These solvents can be used for both liquid-liquid and solid-liquid extractions (Bekaert, 2013). Hansen-Møller (1994), Chen *et al*. (2007) and Verheyden *et al*. (2007) have found that methanol is very useful for the simultaneous extraction of androstenone, skatole and indole.

These steps mentioned above are critical due to the lipophilic properties of boar taint compounds. Androstenone is more lipophilic that skatole and indole, making it more difficult to extract successfully (Bekaert, 2013). Saponification can be added as an additional step in the pre-treatment process as androstenone is found in the non-saponifiable fraction of fat. This will render a more successful extraction of this compound (Thompson & Pearson, 1977). Solutions such as potassium hydroxide can be used for the saponification step. Hereafter, hexane or a quaternary mixture of water-methanol-toluene-light petroleum can be used to finish the fat extraction (De Brabander & Verbeke, 1986).

Derivatization can also be used to enhance the extraction of androstenone. This step will lower the polarity of the compound, making it more volatile. This step is usually used before gas chromatography separation. It has however been found that the use of derivatization can lead to false positives (Vanden Bussche *et al*., 2010). The use of derivatization is also a very labour-intensive process.

Sample clean-up is another important part of the pre-treatment process. When dissolving compounds in a liquid mixture, compounds can be separated from each other with the use of their physical and chemical properties. This is an example of solid phase extraction however, this method is used more often for the clean-up of liquid-liquid extractions. There are many different ways to perform these clean-ups. For example, one could make use of normal phase conditions with a Florosil column (Garcia-Regueiro *et al.,* 1986) in contrast to the use of reverse phase conditions (Hansen-Møller, 1992). Filtration of samples is also used as a clean-up technique. This will typically be done after the fat has been precipitated. Thompson and Pearson (1977) cooled fat in an ice bath

after saponification and before extraction to ensure precipitation of the fat. However, Hansen-Møller (1994) placed the samples in an ice bath after extraction to clear the extraction before detection. Garcia-Regueiro and Ruis (1998) and Verheyden *et al*. (2007) cleaned the extraction with the use of filtration.

2.4.1.2 Chemical detection techniques

Various boar taint detection methods have been developed in order to determine the concentration of androstenone, skatole and indole present in the adipose tissue of pigs. Haugen *et al*. (2012) found that the techniques most commonly used for skatole and indole detection are normal and reverse phase HPLC, GC and spectrophotometric methods whereas HPLC, GC and immunological methods are used for the quantification of androstenone.

2.4.1.3 Spectrophotometric methods

The use of spectrophotometric methods is dependent on a colour development. For this to happen, specific reagents are used (Bekaert, 2013). This method however does not give a separation between the indolic compounds, skatole and indole, and androstenone (Squires, 1990; Haugen *et al*., 2012).

2.4.1.4 Immunological methods

Immunological methods refer to the use of immunoassays in which an analyte concentration can be measured due to the reactions between the analyte, which acts as an antigen, and a complimentary antibody (Haugen *et al*., 2012). The reactions which take place are monitored with the use of labelled units such as radioactive isotopes, enzymes or fluorescence compounds which are conjugated to the antigen or antibody (Haugen *et al*., 2012; Bekaert, 2013). Immunological methods are selected based on the characteristics of the label; methods include radioimmunoassay (RIA), enzyme immunoassay (EIA) or fluoroimmunoassay (FIA). The labelled antigen/antibody complex is detected by using a suitable technique (Haugen *et al*., 2012).

However, immunological methods have certain disadvantages such as low selectivity and a possible decrease of accuracy when measuring androstenone at low concentrations due to cross-reactivity (Bekaert, 2013). Furthermore, the analysis of skatole using immunological methods is also limited as the antigen has a low molecular weight as well as the unavailability of specific antibodies (Claus *et al*., 1988; Haugen *et al*., 2012; Bekaert, 2013)

2.5.1.5 Chromatography

Chromatography is a physical separation method (Bekaert, 2013). Compounds are selectively distributed between two unmixable phases which consists of a mobile phase that flows through the

stationary phase (Bekaert, 2013). In chromatography, the stationary phase is packed in the column. Gas and liquid chromatography can be used as the mobile phase.

Chromatography includes HPLC and GC methodologies. In these techniques the boar taint components are measured when analytes are separated into two phases – the mobile and stationary phase (Haugen *et al*., 2012). For chromatographic methods it is important to note that detectors are used in order to measure compound levels. Detectors that are used for Light chromatography (LC) include UV absorption, fluorescence and mass spectrometry (MS) (Haugen *et al*., 2012). Gas chromatography (GC) uses other types of detectors. These include flame ionisation (FID), electron capture (ECD), thermionic or nitrogen/phosphorous detection (TSD or NPD), surface acoustic wave (SAW) and MS (Haugen *et al*., 2012).

2.4.1.6 Gas chromatography

Gas chromatography (GC) was the first androstenone detection method to be developed (Fuchs, 1971; Edelhäuser, 1989; Haugen *et al*., 2012). This method makes use of porcine adipose tissue and was first reported by Williams and Pearson (1965) and later in 1968, Pearson was the first to detect androstenone with the use of GC coupled with ionisation detection and olfactory examination. Methods typically use derivatization steps for androstenone detection to ensure the compound becomes more volatile (De Brabander & Verbeke, 1986). The use of GC has become a common type of analysis for the detection of boar taint compounds and many novel methods for detecting androstenone have been developed (Bekaert, 2013). A capillary gas chromatography (CGC) detection method for androstenone and its metabolites was developed by Garcia-Regueiro and Diaz (1989). The compounds were separated on a medium-polar stationary phase (column Fused Silica Open Tubular (FSOT) RSL-300 (25 m × 0.25 mm; 0.25 µm)) and detected with the use of FID and MS.

A gas chromatographic method for the detection of skatole was developed by Vold in 1970. This analysis was performed on pork backfat samples.

Currently, several GC-based methods have been developed for the detection and measurement of androstenone and skatole in the adipose tissue of pork (Haugen *et al*., 2012).

As any detection method, GC has its advantages and disadvantages. GC methods used for the quantification of boar taint compounds make use of high cost equipment and the analysis time for androstenone is long. However, the analysis time for skatole is relatively short, e.g. 12 minutes (Haugen *et al*., 2012). GC can now also be used for the simultaneous quantification of androstenone and skatole (Fischer *et al*., 2011).

2.4.1.7 Liquid chromatography

Garcia-Regueiro *et al.* (1986) was the first to describe an HPLC-based detection method coupled to UV detection for skatole analysis. The first column used for HPLC skatole detection was a normal phase column, thereafter various chromatographic columns and different compositions of mobile phases were/are used to separate indolic compounds (Haugen *et al*., 2012). Haugen *et al*. (2012) reported that indolic compounds are soluble in various organic solvents. This is due to the polar and non-polar properties of indolic compounds which allow them to be separated with normal and reverse phase chromatography.

Hansen-Møller (1994) first developed a HPLC method for the simultaneous detection of derivatized androstenone, skatole and indole. The HLPC method was coupled to florescence detection. In 2007, Verheyden *et al.* developed a simultaneous detection method with the use of LC-MS^n without the derivatization step. Ultra-high performance liquid chromatography (U-HPLC) has recently started replacing HPLC (Haugen *et al*., 2012); U-HPLC enables faster compound separation due to its sub-2 micron particle packed columns (Bekaert, 2013). Furthermore, U-HPLC also offers faster analysis speed, better resolution, increased sensitivity as well as a reduction of the matrix effect (Wille *et al*., 2012).

2.4.1.8 Mass spectrometry

Mass spectrometry (MS) is becoming a more popular choice for the detection of separate and simultaneous detection of androstenone, skatole and indole (Zamaratskaia & Jastrebova, 2006; Verheyden *et al*., 2007 & Bekaert, 2013). For this detection method, chemical compounds are ionised to generate charged molecules or molecule fragments. The compounds are then sorted and identified according to their mass-to-charge (m/z) ratio and measuring the abundance of the ions (Vandan Bussche, 2011; Bekaert, 2013). Unfortunately, the matrix effect is present when using LC-MS. Signal suppression or enhancement can take place due to co-extracted matrix constituents. This causes the spectrometric analyses to be disturbed and renders a less adequate quantification (Wille *et al*., 2012; Bekaert, 2013).

2.4.1.9 Ionisation source

Electron impact ionisation (EI) is an energetic process in which a heated filament emits electrons that are accelerated by a specific potential difference into the sample chamber. Fischer *et al*. (2011) reported that the electron impact is most commonly set to 70 eV. The positive ions emitted are also closely monitored. Ionisation of the sample takes place in the gas phase by collision of the neutral sample molecules with these energetic electrons which induces ionisation and fragmentation (Bekaert, 2013). EI is often coupled with gas chromatography. LC-MS methodologies are becoming more preferable for when boar taint compounds are analysed. This is due to the method's softer

ionisation technique. Additionally, atmospheric pressure ionisation techniques have allowed LC-MS to successfully expand its range of compound detection.

Verheyden *et al*. (2007) and Bekaert (2013) reported that atmospheric pressure chemical ionisation is the most commonly used LC-MS method for the detection of boar taint compounds. The method is able to better detect and express compounds with small non-polar and medium polar properties. The optimum molecular weight for the compounds range between 100 and 1000. This makes the method optimal for boar taint compounds as androstenone, skatole and indole have molecular weights of 272, 131 and 117, respectively.

2.4.1.10 Rapid skatole measurement

Hansen-Møller and Andersen (1994) and Bonneau (2000) described an automated colorimetric assay for rapid skatole measurement. This assay is performed in duplicate (two separate fat extractions are performed for each sample). For the skatole measurement, 0.5 g of fat was homogenised in 5 mL Tris-HCl/ acetone buffer until it was completely homogenised. The homogenate is then cooled to 5^{o}C where after it is filtered into a test tube. To 1 mL of the filtrate, 1.42 mL colour reagent (di-methyl-amino-benzaldehyde/ ethanol/sulphuric acid solution) is added. The reagent is mixed and cooled for 3 minutes. Hereafter the mixture is measured with the use of a spectrophotometer (460 – 730 nm range). Quantification is done with the absorption value at 580 nm. The absorption at this value (580 nm) is then compared to a known skatole standard curve where after the quantity of skatole is determined.

2.4.2 On/Offline boar taint detection

The on-/offline detection of boar taint refers to methods that rapidly determine in the abattoir whether a carcass has an unacceptable level of boar taint or not. Although many methods have been developed, there is still no standard which is used in pork abattoirs. Offline detection methods are performed at the abattoir but not directly on the carcass as a piece of fat is removed from the carcass for the analysis to take place, whereas online detection methods are performed on the carcass. Furthermore, some detection techniques are performed on fat whilst others are performed on the pork meat (Bekaert, 2013).

There is a great need across the world for the development of an online boar taint detection method that would deliver constant, repeatable results. For this reason many abattoirs and researchers make use of a trained sensory panel or individual to evaluate the level of boar taint present in the pig carcass. This type of evaluation is referred to as “Human nose detection” (Meinert *et al*., 2011). Aluwé *et al*. (2009) reported that in Belgium, 4% of entire male pigs have high concentrations of boar taint whilst a further 25% have moderate concentrations of boar taint. This has resulted in all entire male carcasses being deemed inferior to that of castrated male pigs.

It is important to have a trained analyst who is highly sensitive to boar taint compounds in the abattoir. The assessor would have undergone a standard sensitivity selection as well as training tests (Lunde *et al*., 2010). Claudi-Magnussen *et al*. (2011) describes the initial screening method which can be used to select panellists. Prospective assessors are selected on the bases of their ability to detect androstenone and skatole. For each of these compounds two triangle tests are performed. Two 100 mL flasks with 20 mL pure sunflower oil and one mL flask with 20 mL sunflower oil and the dissolved compound (10 ppm androstenone and 1 ppm skatole respectively) are presented to the prospective assessor. If the prospective assessor selects all the flasks containing boar taint compounds correctly, they will undergo training to detect boar taint (Claudi-Magnussen *et al*., 2011). Assessors are trained to detect combinations of androstenone and skatole at different concentrations. These combinations are used to simulate boar taint concentrations found in pork fat. The assessors are placed on-/offline in the abattoir where they use various detection techniques to determine whether a carcass is contaminated with boar taint compounds. If a carcass has levels of boar taint present that are above a pre-determined level, the carcass will be "repurposed" to ensure that the pork does not reach the consumer in its present form (Bekaert *et al*., 2013).

Meinert *et al*. (2011) reported that assessors did not appear to experience fatigue towards skatole (0.25 µg/g limit) after smelling approximately 200 samples. However, it is strongly advised that assessors work in shifts and that all assessors must be equally (highly) sensitive to both androstenone and skatole (Meinert *et al*., 2011). Another limitation that is placed on on-/offline detection methods is that a maximum of 180 samples can be analysed per hour. Furthermore, androstenone is also not properly measured (Bekaert *et al*., 2013). It should also be noted that it is important that an extraction system exists for the area in which the boar taint concentration is being determined. This would also help to ensure that no residual boar taint compounds remain in the air after the assessor has analysed the previous sample.

2.4.2.1 Online detection techniques

When performing online boar taint detection a trained specialist would always perform aroma experiments. It is extremely important to ensure that these individuals have gone through a screening process and also receive training to ensure that the prospective assessor is highly sensitive towards both androstenone and skatole and also be able to render accurate and repeatable results (as mentioned in on/inline boar taint detection techniques) (Bekaert *et al*., 2013).

2.4.2.1.1 Soldering iron/ Singeing method

The soldering iron or singeing method is the most commonly used online detection method in the Netherlands and Belgium (Bekaert *et al*., 2013). When implementing this detection method for online boar taint evaluation, a 30 W (maximum temperature of 380°C) soldering iron is used to singe the

fat on the carcass (Aluwé *et al.*, 2009). Alternatively, a gas burner with a small plate attached to it is used to singe the fat. The latter method is not frequently used within abattoirs because of the high flame temperature (approximately 1300°C) which may cause the fat to burn (Bekaert *et al*., 2013).

The pyropen (Weller®) is a new gas burner that was developed especially for the singeing of pork fat to determine the presence or absence of boar taint. The instrument is a pen-like gas burner. The pyropen has five temperature settings and is approximately 20 cm long and is filled with isobutene (Bekaert *et al*., 2013). This gas burner will be able to determine more accurately the presence of boar taint due to its temperature setting; the temperature is typically adjusted to lower temperatures more similar to those used with the soldering iron detection method (Bekaert *et al*., 2013). A further advantage to the use of the pyropen is that it does not require an electrical cord. One can also use the pyropen with an assemble plate. This plate will result in no carry-over of boar taint odour due to previous singes (Bekaert *et al*., 2013).

When comparing various detection techniques, Whittington *et al.* (2011) found that the soldering iron method was suitable for use in an abattoir. However, Bekaert *et al*. (2013) reported that neither the soldering iron nor singeing method has been subjected to scientific scrutiny.

It is important to note that the cleaning of the soldering iron significantly affected boar taint sensory scores ($P < 0.001$) in a study by Bekaert *et al*. (2013).

2.4.2.2 Offline detection techniques

2.4.2.2.1 Soldering iron

When performing the soldering iron detection method offline, fat is cubed (4 x 4 x 4 cm) and placed into a Petri dish. The fat is singed with the tip of a soldering iron (180°C) where after the assessor smells the volatile compounds (boar taint compounds) that are given off (Whittington *et al*., 2011). The tip of the soldering iron is cleaned between each application with the use of vegetable fat (Whittington *et al*., 2011). The cleaning of the soldering iron has a significant effect on the sensory score given to the pork fat sample with regard to its level of boar taint present (Bekaert *et al*., 2013).

2.4.2.2.2 Boiling/ Hot water method

This detection method is most commonly used in abattoirs in Denmark where small numbers of entire male pigs are slaughtered. A piece of pork fat is removed from the carcass and cut into smaller pieces where after it is placed into a flask of boiling water. The flask is closed with aluminium foil and is smelled by a trained assessor after two minutes. Meinert *et al*. (2011) reported that the optimal amount of fat and water is 5 g pork fat placed into 75 mL water at 80°C.

2.4.2.2.3 Cooking

Bekaert *et al*. (2013) compiled a comprehensive list of heating methods. In this list seven cooking methods are compared (Table 2.3).

Table 2.3 List of cooking methods to detect boar taint offline (adapted from Bekaert *et al*. 2013)

Temperature	Time	Heating method	Sample type	Sample size	Reference
-	3 min	1800 W Grill plate	Meat	2.5 cm	Aluwé *et al*. (2009)
180°C	2 x 4 min	-	Meat	-	Byrne *et al*. (2008)
70°C	-	Internal temp	Meat	1.27 cm^3	Coker *et al*. (2009)
150°C	2 x 3 min	-	Meat	1 cm	Hansen *et al*. (2008)
190°C	4 min	Grill plate	Meat	1 cm	Pauly *et al.* (2010)
180°C	5 min	-	Fat	50 g	Parunovic *et al.* (2010)
-	2 x 1 min	Frying	Meat	25 g	Lunde *et al.* (2010)

As seen above (Table 2.3) there are various offline detection techniques used in the industry. It is also clear that these techniques differ in temperature, time, heating method, sample type as well as sample size indicating that there is no standard method used when detecting boar taint when using a cooking method (Bekaert *et al*., 2013).

2.4.2.2.4 Microwave

Many different microwave cooking methods exist for the determination of the presence of boar taint. Aluwé *et al*. (2009) microwaved an unknown amount of fat for 50 seconds at 70 W, whereas Prusa *et al*. (2011) microwaved 20 g fat for 2 x 20 s at “High power”. Prusa *et al*. (2011) also used a method where 10 g of pork meat was microwaved for 2 x 15 s at “High power”. Whittington *et al*. (2011) microwaved 20 g of pork fat and composite (muscle and fat from the cheek and submaxillary glands) for 90 seconds at 750 W. Furthermore, Bekaert *et al*. (2012) microwaved a 2 g pork fat sample for 3 minutes in a 220 W microwave.

Whittington *et al*. (2011) determined that the microwave method was the best offline boar taint detection method due to its simplicity and speed.

2.4.2.2.5 Melting

Whittington *et al*. (2011) described a melting method where a 20 g fat sample is melted on a hot plate. The fat is diced into 5 mm^3 pieces and placed into a 400 mL glass beaker where after it is covered with a plastic film. The beaker is placed on a heating plate (185°C) until the fat is melted but not browned. It is important that for this detection method the melted sample should be analysed

within two minutes of melting (Whittington *et al*., 2011). Haugen (2010) reported that Germany makes use of a melting test in order to detect whether sexual odours are present within a carcass. This sensory evaluation of the pork fat is a rapid detection technique that can be performed within an abattoir.

2.5 Framework of this study

The PORCUS classification system, sex, genotype and carcass weight is used in South Africa in order to determine the cost of commercially slaughtered pork. In the classification system the backfat depth is determined where after the %lean meat is calculated. However, male pigs are generally not castrated in South Africa and therefore male carcasses, and especially carcasses classified as R, C, U and S, are penalised as there is a chance that androstenone could be present in the pork fat. But due to limited research on the presence of androstenone in South African pork, the question is raised whether the fat level (PORCUS classification) of male pigs have any influence on the concentration of androstenone present. Many European countries have steps in place to decrease the chance of the consumer buying pork or pork products which have androstenone present, however this is not the case in South Africa. This study also investigated whether androstenone reacts differently with the use of different cooking methods. Many researchers have focused on consumer acceptance and sensitivity towards androstenone, however very little research has been done on androstenone concentrations present in subcutaneous fat before and after cooking. The main goals of this study were thus as follows:

- Determine whether there is a correlation between the porcus classification system (%lean meat) and the percentage fat in the intramuscular fat of the *Longissimus dorsi* muscle;
- Determine whether there is a correlation between androstenone concentration in the subcutaneous fat and the PORCUS classification system;
- Determine how androstenone concentration in the subcutaneous fat connected to pork chops of the *Longissimus thoracis* reacts to different cooking methods.

2.6 References

Aluwe´ M 2012. Influence of feed and management strategies on boar taint prevalence. PhD dissertation, Ghent University, Belgium.

Aluwé, M., Millet, S., Nijs, G., Tuyttens, F.A.M., Verheyden, K., De Brabander, H.F., De Brabander, D.L. & Van Oeckel, M.J. (2009). Absence of an effect of dietary fibre or clinoptilolite on boar taint in entire male pigs fed practical diets. *Meat Science*, **82**(3), 346-352.

Andersen, J.R. (2006). Sorting criteria. Methods for on-line / at-line sorting of entire male castrated with emphasis on the Danish method based on skatole content. *Acta Veterinaria Scandinavica*, **48**(1), 20–22.

Annor-Frempong, I.E., Nute, G.R., Whittington, F.W. & Wood, J.D. (1997). The problem of taint in pork: 1. Detection thresholds and odour profiles of androstenone and skatole in a model system. *Meat Science*, **46**(1), 45–55.

Babol, J., Squires, E.J. & Lundström, K. (1999). Relationship between metabolism of androstenone and skatole in intact male pigs. *Journal of Animal Science*, **77**(1), 84–92.

Babol, J., Squires, E.J. & Gullett, E.A. (2002). Factors affecting the level of boar taint in entire male pigs as assessed by consumer sensory panel. *Meat Science*, **61**, 33–40.

Barber, C.E. (1997). Olfactory acuity as a function of age and gender: a comparison of African and American samples. *International Journal of Aging and Human Development*, **44**(4), 317–334.

Beets, M.G.J. & Theimer, E.T. (1970). Odour similarity between structurally unrelated odorants. In:, Taste and Smell in Vertebrates. (Eds. Wostenholme, G.E.W. & Knight, J.). London: J. & A. Churchill. England, pp 313–323.

Bekaert, K. (2013). Chemical and sensory detection of boar taint. PhD Dissertation, Gent University, Belgium.

Bekaert, K.M., Aluwé, M., Millet, S., Goethals, K., Nijs, G., Isebaert, S., De Brabander, D. L., Vanhaecke, L. & Tuyttens, F.A.M. (2012). Predicting the likelihood of developing boar taint: early physical indicators in entire male pigs. *Meat Science*, **92**(4), 382–5.

Bekaert, K. M., Aluwé, M., Vanhaecke, L., Heres, L., Duchateau, L., Vandendriessche, F. & Tuyttens, F. A. M. (2013). Evaluation of different heating methods for the detection of boar taint by means of the human nose. *Meat Science*, **94**(1), 125–32.

Bekaert, K.M., Tuyttens, F.A.M., Duchateau, L., De Brabander, H.F., Aluwé, M., Millet, S., Vandendriessche, F. & Vanhaecke, L. (2011). The sensitivity of Flemish citizens to androstenone: influence of gender, age, location and smoking habits. *Meat Science* **88**(3), 548–552.

Berry, B.W. & Hasty, R.W. (1982). Influence of demographic factors on consumer purchasing patterns and preference for ground beef. *Journal of Consumer Studies & Home Economics*, **6**, 351-360.

Blanch, M., Panella-Riera, N., Chevillon, P., Furnols, M.F.I., Gil, M., Gil, J.M., Kallas, Z. & Oliver, M.A. (2012). Impact of consumer's sensitivity to androstenone on acceptability of meat from entire male pigs in three European countries: France, Spain and United Kingdom. *Meat Science*, **90**(3), 572–578.

Bonneau, M. (1982). Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livestock Production Science*, **9,** 687-705.

Bonneau, M. (2000). An international study on the importance of androstenone and skatole for boar taint: I. Presentation of the programme and measurement of boar taint compounds with different analytical procedures. *Meat Science*, **54**(3), 251–259.

Brooks, R.I., Pearson, A.M., Hogberg, M.G., Pestka, J.J. & Gray, J.I. (1986). An immunological approach for prevention of boar odor in pork. *Journal of Animal Science*, **62**, 1279–1289.

Brooks, R.I. & Pearson, A.M. (1989). Odor thresholds of the C_{19}-Δ^{16}-steroids responsible for boar odor in pork. *Meat Science*, **24**, 11–19.

Bryhni, E.A., Byrne, D.V, Rødbotten, M., Claudi-magnussen, C. & Agerhem, H. (2002). Consumer perceptions of pork in Denmark, Norway and Sweden. *Food Quality and Preference*, **13**(5), 257–266.

Byrne, D.V., Thamsborg, S.M. & Hansen, L.L. (2008). A sensory description of boar taint and the effects of crude and dried chicory roots (*Cichorium intybus L.*) and inulin feeding in male and female pork. *Meat Science*, **79**(2), 252–269.

Chen, G., Zamaratskaia, G., Andersson, H.K. & Lundström, K. (2007). Effect of dietary supplement of raw potato starch and live weight on the levels of skatole, indole and androstenone measured by different methods. *Food Chemistry*, **101**, 439–448.

Claudi-Magnussen, C., Hermansen, J.E., Horsted, K. & Andersen, B.H. (2011). "Sensory evaluation of boar taint – training and attributes." *57th International Congress of Meat Science and Technology*, 7-12 August 2011, Ghent, Belgium.

Claus, R., Hoffmann, B. & Karg, H. (1971). Determination of 5-androst-16-en-3-one, a boar taint steroid in pigs, with reference to relationships to testosterone. *Journal of Animal Science*, **33**, 1293–1297.

Claus, R., Mahler, G. & Münster, E. (1988). Determination of boar taint steroid 5a-androst-16-en-3-one in adipose tissue of pigs with a rapid microtiter plate enzyme-immunoassay (MTE). *Archiv Für Lebensmittelhygiene*. **39**, 87−90.

Claus, R., Weiler, U. & Hogberg, A. (1994). Physiological aspects of androstenone and skatole formation in the boar. A review with experimental data. *Meat Science*. **38**, 289-305.

Coker, M.D., West, R.L., Brendemuhl, J.H., Brendemuhl, D.D., Johnson, D.D. & Stelzleni, A.M. (2009). Effects of live weight and processing on the sensory traits, androstenedione

concentration and 5-alpha-androst-16-en-3-one (androstenone) concentration in boar meat. *Meat Science*, **82**(3), 399–404.

Davis, S.M. & Squires, E.J. (1999). Association of cytochrome b5 with 16- androstene steroid synthesis in the testis and accumulation in the fat of male pigs. *Journal of Animal Science*, **77**, 1230–1235.

de Brabander, H.F. & Verbeke, R. (1986). Quantitative determination of androstenone in pig adipose tissue. *Journal of Chromatography*, **363**, 293–302.

De Kock, H.L., van Heerden, S.M., Heinze, P.H., Dijksterhuis, B.B. & Minnaar, A. (2001). Reaction to boar odour by different South African consumer groups. *Meat Science*, **59**, 353–362.

Dehnhard, M., Claus, R., Herbet, E. & Hillenbrand, M. (1995). (Skatole and androstenone concentrations in products from boar meat). Die Ebermast, -Fragen und Konsequenzen bei der Produktion von Schweinefleisch. Schriftenreihe des Bundesministeriums fur Ernahrung. *Landwirtschaft unf Forsten,* **449**, 44-71 (in German).

Dehnhard, M., Claus, R., Hillenbrand, M. & Herzog, A. (1993). High-performance liquid chromatographic method for the determination of 3-methylindole (skatole) and indole in adipose tissue of pigs. *Journal of Chromatography*, **616**, 205–209.

Desmoulin, B. & Bonneau, M. (1982). Consumer testing of pork and processed meat from boars: The influence of fat androstenone levels. *Livestock Production Science* **9**, 707-715.

Diestre, A., Oliver, M.A., Gispert, M., Arpa, I. & Arnau, J. (1990). Consumer responses to fresh meat and meat products from barrows and boars with different levels of boar taint. *Animal Production*, **50**, 519-530.

Dijksterhuis, G. B., Engel, B., Walstra, P., Font-i-Furnols, M., Agerhem, H., Fischer, K., Oliver, M. A., Claudi-Magnussen, C., Siret, F., Béague, M. P., Homer, D. B. & Bonneau, M. (2000). An international study on the importance of androstenone and skatole for boar taint: II. Sensory evaluation by trained panels in seven European countries. *Meat Science*, **54**, 261–269.

Doran, E., Whittington, F.M., Wood, J.D. & McGivan, J.D. (2004). Characterization of androstenone metabolism in pig liver microsomes. *Chemico-Biological Interactions*. **147**, 141–149.

Dufort, I., Soucy, P., Lacoste, L. & Luu-The, V. (2001). Comparative biosynthetic pathway of androstenol and androgens. *Journal of Steroid Biochemistry and Molecular Biology*, **77**, 223–227.

Edelhäuser, M. (1989). Eine schnelle Methode zur Bestimmung des Ebergeruchssteroids Androstenon. *Deutsche Lebensmittel-Rundschau*, **85**, 80–84 (in German).

Fischer, J., Elsinghorst, P.W., Bücking, M., Tholen, E., Petersen, B. & Wüst, M. (2011). Development of a candidate reference method for the simultaneous quantitation of the boar taint compounds androstenone, 3 α-androstenol, 3β-androstenol, skatole, and indole in pig fat by

means of stable isotope dilution analysis - headspace solid-phase microextraction - gas chromatography/mass spectrometry. *Analytical Chemistry*, **83**, 6785-6791.

Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: a review. *Meat Science*, ***92***(4), 319–329.

Font-i-Furnols, M., Gispert, M., Diestre, A. & Oliver, M. A. (2003). Acceptability of boar meat by consumers depending on their age, gender, culinary habits, sensitivity and appreciation of androstenone smell. *Meat Science*, **64**, 433–440.

Font-i-Furnols, M., Gispert, M., Guerrero, L., Velarde, A., Tibau, J., Soler, J., Hortós, M., García-Regueiro, J.A., Pérez, J., Suárez, P. & Oliver, M.A. (2008). Consumers' sensory acceptability of pork from immunocastrated male pigs. *Meat Science*, **80**(4), 1013–1018.

Font-i-Furnols, M., Guerrero, L., Serra, X., Rius, M. A. & Oliver, M. A. (2000). Sensory characterization of boar taint in entire male pigs. Journal of Sensory Studies, 15(4), 393–409.

Fredriksen, B., Font-i-Furnols, M., Lundström, K., Migdal, W., Prunier, A, Tuyttens, F.A.M. & Bonneau, M. (2009). Practice on castration of piglets in Europe. *Animal*, ***3***(11), 1480-1487.

Fuchs, G. (1971). The correlation between the 5a-androst-16-en-3-one content and the sex odour intensity in boar fat. *Swedish Journal of Agricultural Research*, **1**, 233–237.

Garcia-Regueiro, J.A., Hortos, M., Arnau, C. & Monfort, J.M. (1986). Determination of skatole and indole in back fat of pigs by HPLC. *Journal of High Resolution Chromatography and Chromatography Communications*, **9**(6), 362–363.

Garcia-Regueiro, J.A. & Diaz, I. (1989). Evaluation of the contribution of skatole, indole, androstenone and androstenols to boar-taint in back fat of pigs by HPLC and capillary gas chromatography (CGC). *Meat Science*, **25**, 307-316.

Garcia-Regueiro, J.A. & Rius, M.A. (1998). Rapid determination of skatole and indole in pig back fat by normal phase liquid chromatography. *Journal of Chromatography*, **809**, 246–251.

Gilbert, A. & Wysocki, C. (1987). Results of the smell survey. *National Geographic*, **172**(4), 514–525.

Gower, D.B. (1972). 16-Unsaturated C 19 steroids. A review of their chemistry, biochemistry and possible physiological role. *Journal of Steroid Biochemistry*, **3**, 45–103.

Gower, D.B., Bird, S., Sharma, P. & House, F.R. (1985). Axillary 5 alpha-androst-16-en-3-one in men and women: relationships with olfactory acuity to odorous 16-androstenes. *Experientia*, **41**(9), 1134–1136.

Griffiths, N.M. & Patterson, R.L.S. (1970). Human olfactory responses to 5α- androst-16-en-3-one – principal component of boar taint. *Journal of the Science of Food and Agriculture*, **21**, 4-6.

Hansen, L.L., Stolzenbach, S., Jensen, J.A., Henckel, P., Hansen-Moller, J., Syriopoulos, K. & Byrne, D.V. (2008). Effect of feeding fermentable fibre-rich feedstuffs on meat quality with emphasis on chemical and sensory boar taint in entire male and female pigs. *Meat Science*, **80**(4), 1165–1173.

Hansen-Møller, J. (1994). Rapid high-performance liquid chromatographic method for simultaneous determination of androstenone, skatole and indole in back fat from pigs. *Journal of Chromatography*, **661**, 219–230.

Hansen-Møller, J. & Andersen, J.R. (1994). Boar taint - analytical alternatives. *Fleischwirtschaft*, **74**(9), 963–966.

Haugen, J.E. (2006). The use of chemical sensor array technology, the electronic nose, for detection of boar taint. *Acta Veterinaria Scandinavica*, **48**, 1–3.

Haugen, J.E. (2010). Methods to detect boar taint. State of the art and critical review. *Norfima-Food, Fisheries and Agricultural Research*. Brussels, Belgium (2 June 2010).

Haugen, J.E. (2012). Boar taint detection methods. State of the art and critical review. *Norwegian Institute of Food, Fisheries and Agricultural Research*. Brussels, Belgium (17 December 2012).

Haugen, J.E., Brunius, C. & Zamaratskaia, G. (2012). Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods. *Meat Science*, **90**(1), 9–19.

Hendriks, W.H. & King, M.R. (n.d.). A review of the literature on boar taint for New Zealand PORK. Pp. 1-24. http://www.nzpork.co.nz/images/custom/literature_review_on_boar_taint.pdf. Accessed 09/12/2014.

Jensen, M.T., Cox, R.P. & Jensen, B.B. (1995). Microbial production of skatole in the hind gut of pigs given different diets and its relation to skatole deposition in backfat. *Animal Science,* **61**, 293-304.

Keller, A., Zhuang, H., Chi, Q., Vosshall, L.B. & Matsunami, H. (2007). Genetic variation in a human odorant receptor alters odour perception. *Nature*, **449**(7161), 468–472.

Kloek, J (1961). The smell of some steroid sex-hormones and their metabolites. Relations and experiments concerning the significance of smell for the mutual relation of the sexes. *Psychiatra Neurologia, Neurochirugica*, **64**, 309–344.

Kirsching, Á., Bázár, G., Házás, Z. & Romvári, R. (2012). Classification of meat with boar taint using an electronic nose. *Acta argiculturae Slovenica*, **3**, 99–103.

Loutfi, A., Coradeschi, S., Kumar, G., Shankar, P., Bosco, J. & Rayappan, B. (2015). Electronic noses for food quality : A review. *Journal of Food Engineering*, **144**, 103–111.

Lunde, K., Egelandsdal, B., Skuterud, E., Mainland, J. D., Lea, T., Hersleth, M., & Matsunami, H. (2012). Genetic variation of an odorant receptor OR7D4 and sensory perception of cooked meat containing androstenone. *PloS one*, **7**(5), 1-8.

Lunde, K., Skuterud, E., Egelandsdal, B., Font-i-Furnols, M., Nute, G.R., Bejerholm, C., Nilsen, A., Stenstrom, Y.H. & Hersleth, M. (2010). The importance of the recruitment method for

androstenone sensitivity with respect to accurate sensory evaluation of androstenone tainted meat. *Food Quality and Preference*, **21**(6), 648–654.

Lundström, K., Malmfors, B., Malmfors, G., Stern, S., Petersson, H., Mortensen, A.B. & Sørensen, S.E. (1988). Skatole, androstenone and taint in boars fed two different diets. *Livestock Production Science*, **18**, 55-67.

Lundström, K., Matthews, K. R., & Haugen, J. E. (2009). Pig meat quality from entire males. *Animal*, **3**(11), 1497.

Mainland, D.D. (1998). Health and the demand for food in Scotland: economic and demographic effects. *British Food Journal*, **100**, 273-277.

Matthews, K.R., Homer, D.B., Punter, P., Béague, M.P., Gispert, M., Kempster, A.J., Agerhem, H., Claudi-Magnussen, K., Fischer, K., Siret, F., Leask, H., Font-i-Furnols & Bonneau, M. (2000). An international study on the importance of androstenone and skatole for boar taint: III. Consumer survey in seven European countries. *Meat Science*, **54**(3), 271–283.

McCauley, I., Hennessy, D.P., Boghossian, V., Sali, L., Salvatore, L., Reynolds, J. & Mawson, R. (1997). Effect of methods of cooking and processing pork on the perception of boar taint. In Boar taint in entire male pigs. (Eds. M Bonneau, K Lundström and B Malmfors) Wageningen Pers., Wageningen, The Netherlands. EAAP Publication no. 92, pp. 156–160.

Meadus, W.J., Mason, J.I. & Squires, E.J. (1993). Cytochrome P450c17 from porcine and bovine adrenal catalyzes the formation of 5,16-androstadien-3 beta-ol from pregnenolone in the presence of cytochrome b5. *Journal of Steroid Biochemistry and Molecular Biology,* **46**, 565–572.

Meier-Dinkel, L., Trautmann, J., Frieden, L., Tholen, E., Knorr, C., Sharifi, A. R., Bücking, M., Wicke, M. & Mörlein, D. (2013). Consumer perception of boar meat as affected by labelling information, malodorous compounds and sensitivity to androstenone. *Meat Science*, **93**(2), 248–56.

Meinert, L., Bejerholm, C., & Støier, S. (2011). Validation of “human nose” based method for boar taint detection. *57th International Congress of Meat Science and Technology*. Ghent, Belgium.

Mowen, J.C. & Minor, M. (1997). Consumer beliefs, attitudes and behaviors. In: *Consumer behaviour*. (Ed. W. Blake). Upper Saddle River, New Jersey: Prentice-Hall., United States of America, pp. 248–249.

Oliver, A. (2009). Study on the improved methods for animal-friendly production, in particular on alternatives to the castration of pigs and on alternatives to the dehorning of cattle. *Alternative to castration and dehorning (ALCASDE) – Final report*.

Olson, D.M., Rains, G.C., Meiners, T., Takasu, T., Tertuliano, M., Tumlinson, J.H., Wäckers, F.L. & Lewis, W.J. (2003). Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors. *Chemical Senses*, **28**, 545–549.

Oskam, I.C., Lervik, S., Tajet, H., Dahl, E., Ropstad, E. & Andresen, Ø. (2010). Differences in testosterone, androstenone, and skatole levels in plasma and fat between pubertal purebred Duroc and Landrace boars in response to human chorionic gonadotrophin stimulation. *Theriogenology*, **74**(6), 1088–1098.

Parunovic, N., Petrovic, M., Matekalo-Sverak, V., Parunovic, J. & Radovic, C. (2010). Relationship between carcass weight, skatole level and sensory assessment in fat of different boars. *Czech Journal of Food Sciences*, **28**(6), 520–530.

Patterson, R.L.S. (1986). 5α-androst-16-ene-13-one: compound responsible for taint in boar fat. *Journal of the Science of Food Agriculture*, **19**, 31-38.

Pauly, C., Spring, P., O'Doherty, J. V, Ampuero Kragten, S., & Bee, G. (2008). Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. *Animal*, **2**(11), 1707.

Pauly, C., Spring-Staehli, P., O'Doherty, J.V., Kragten, S.A., Dubois, S., Messadene, J. & Bee, G. (2010). The effects of method of castration, rearing condition and diet on sensory quality of pork assessed by a trained panel. *Meat Science*, **86**(2), 498–504.

Pearson, A.M., Ngoddy, S., Price, J.F. & Larzelere, H.E. (1971). Panel acceptability of products containing boar meat. *Journal of Animal Science*, **33**, 26–29.

Prusa, K., Nederveld, H., Runnels, P.L., Li, R., King, V.L. & Crane, J.P. (2011). Prevalence and relationships of sensory taint, 5 alpha-androstenone and skatole in fat and lean tissue from the loin (Longissimus dorsi) of barrows, gilts, sows, and boars from selected abattoirs in the United States. *Meat Science*, **88**(1), 96–101.

Rius, M., Hortos, M. & Garciaregueiro, J. (2005). Influence of volatile compounds on the development of off-flavours in pig back fat samples classified with boar taint by a test panel. *Meat Science*, ***71***(4), 595–602.

Robic, A., Larzul, C. & Bonneau, M. (2008). Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: a review. *Genetics Selection Evolution*, **40**, 129–143.

Russell, M.J., Cummings, B.J., Profitt, B.F., Wysoki, C.J., Gilbert, A.N. & Cotman, C.W. (1993). Life span changes in the verbal categorization of odors. *Journal of Gerontology*, **48**(2), 49–53.

Schutte, S. (2008). Development of value added ostrich (*Struthio camelus*) meat products. MSc Thesis, University of Stellenbosch, South Africa.

Schwarzenberger, F., Toole, G.S., Cristie, H.L. & Raeside, J.I. (1993). Pigs' plasma levels androgens and estrogens from birth to puberty in male domestic pigs. *Acta Endocrinologica*, **128**, 173-177.

Sinclair, P.A. & Squires, E.J. (2005). Testicular sulfoconjugation of the 16-androstene steroids by hydroxysteroid sulfotransferase : Its effect on the concentrations of 5α-androstenone in plasma and fat of the mature domestic boar. *Journal of Animal Science,* **83**, 358–365.

Sinclair, P.A., Hancock, S., Gilmore, W.J. & Squires, E.J. (2005). Metabolism of the 16-androstene steroids in primary cultured porcine hepatocytes. *The Journal of Steroid Biochemistry and Molecular Biology*, ***96***(1), 79–87.

Sinclair, P.A., Gilmore, W.J., Lin, Z., Lou, Y. & Squires, E.J. (2006). Molecular cloning and regulation of porcine SULT2A1: relationship between SULT2A1 expression and sulfoconjugation of androstenone. *Journal of Molecular Endocrinology*, ***36***(2), 301–311.

Sinclair, P.A., Squires, E.J. & Raeside, J.I. (2001). Early postnatal plasma concentrations of testicular hormones, pubertal development and carcass leanness as potential indicators of boar taint in market weight intact male pigs. *Journal of Animal Science*, **79**, 1868–1876.

Solé, M.A., & García-Regueiro, J.A. (2001). Role of 4-phenyl-3-buten-2-one in boar taint: identification of new compounds related to sensorial descriptors in pig fat. *Journal of Agricultural and Food Chemistry*, **49**(11), 5303–5309.

Squires E.J., 1990. Studies on the suitability of a colorimetric test for androst-16-ene steroids in the submaxillary gland and fat of pigs as a simple chemical test for boar taint. *Canadian Journal of Animal Science*, **70**, 1029–1040.

Thompson, R.H. & Pearson, A.M. (1977). Quantitative determination of 5a-androst-16-en-3-one by gas chromatography-mass spectrometry and its relationship to sex odor intensity of pork. *Journal of Agricultural and Food Chemistry*, **25**, 1241-1245.

Thornhill, R. & Gangestad, S.W. (1999). The scent of symmetry a human sex pheromone that signals fitness? *Evolution and Human Behaviour*, **20**, 175–201.

Vanden Bussche, J. (2011). Analytical approaches to unravel the semi-endogenous status of thiouracil. PhD dissertation, Ghent University, Belgium.

Vanden Bussche, J., Verhaecke, L., Deceuninck, Y., Verheyden, K., Wille, K., Bekaert, K., Le Bizec, B. & De Brabander, H.F. (2010). Development and validation of an ultra-high performance liquid chromatography tandem mass spectrometry method for quantifying thyreostats in urine without derivatisation. *Journal of Chromatography A*, **1217**(26), 4285-4293.

Verheyden, K., Noppe, H., Aluwé, M., Millet, S., Vanden Bussche, J. & De Brabander, H.F. (2007). Development and validation of a method for simultaneous analysis of the boar taint

compounds indole, skatole and androstenone in pig fat using liquid chromatography-multiple mass spectrometry. *Journal of Chromatography A*, **1174**(1-2), 132–137.

Vold, E. (1970). Fleischproduktionseigenschaften bei Ebern und Kastraten. IV. Organoleptisch und gaschromatografische Untersuchungen wassedampfflüchtiger Stoffe des Rückspeckes von Ebern. *Meldinger Nordlandbruckhoegskole*, **49**, 1-25 (in German).

Weiler, U., Fisher, K., Kemmer, H., Dobrowolski, A., & Claus, R. (1997). Influence of androstenone sensitivity on consumer reactions to boar meat. In: *Boar taint in entire male pigs*. Bonneau, M., Lundström, K., Malmfors, B. (Eds.), EAAP Publication, **92**, 147–151.

Weiler, U., Font-i-Furnols, M., Fischer, K., Kemmer, H., Oliver, M., Gispert, M., Dobrowolski, A. & Claus, R. (2000). Influence of differences in sensitivity of Spanish and German consumers to perceive androstenone on the acceptance of boar meat differing in skatole and androstenone concentrations. *Meat Science*, **54**(3), 297–304.

Whittington, F.M., Zammerini, D., Nute, G.R., Baker, A, Hughes, S.I. & Wood, J.D. (2011). Comparison of heating methods and the use of different tissues for sensory assessment of abnormal odours (boar taint) in pig meat. *Meat Science*, **88**(2), 249–255.

Wille, K., Brabander, H.F., De Wulf, E., Van Caeter, P., Janssen, C.R. & Vanhaecke, L. (2012). Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment. *Trac-Trends in Analytical Chemistry*, **35,** 87-108.

Williams, L.D. & Pearson, A.M. (1965). Fat components, unsaponifiable fraction of pork fat as related to boar odor. *Journal of Agricultural and Food Chemistry*, **13**, 573-577.

Wood, J.D., Nute, G.R., Fursey, G.A.J. & Cuthberston, A. (1995). The effect of cooking conditions on the eating quality of pork. *Meat Science*, **40**, 127–135.

Wysocki, C.J. & Beauchamp, G.K. (1984). Ability to smell androstenone is genetically determined. *Proceedings of the National Academy of Sciences of the United States of America*, **81**, 4899-4902.

Xue, J. & Dial, G.D. (1997). Raising intact male pigs for meat: Detecting and preventing boar taint. *Swine Health and Production*, **5**(4), 151–158.

Zamaratskaia, G. (2004). Factors involved in boar taint; Influence of breed, age, diet and raising conditions. PhD Dissertation, Swedish University of Agricultural Sciences, Uppsala.

Zamaratskaia, G. (2009). Cause and possible ways to eliminate boar taint in pork. *Tehnologija Mesa*, **50** (1-2), 43–47.

Zamaratskaia, G., Chen, G. & Lundström, K. (2006). Effects of sex, weight, diet and hCG administration on levels of skatole and indole in the liver and hepatic activities of cytochromes P4502E1 and P4502A6 in pigs. *Meat Science*, **72**(2) 331–338.

Zamaratskaia, G. & Jastrebova, J. (2006). Application of LC–MS for determination of indole and 3-methylindole in porcine adipose tissue. *Chromatographia*, **64**, 435–439.

Zamaratskaia, G. & Squires, E.J. (2009). Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal : An International Journal of Animal Bioscience*, **3**(11), 1508–1521.

CHAPTER 3

Correlation between PORCUS fat classification and intramuscular fat percentage of the *Longissimus dorsi* and the concentration of androstenone present in commercially slaughtered entire male pigs

Abstract

An investigation with 176 pigs of three PORCUS carcass classification groups (P, O and R) was conducted wherein the focus was quantifying the correlation between the PORCUS classification and intramuscular fat and also the androstenone concentration. It was expected that as the fat depth as indicated by the PORCUS classification increased, both the intramuscular fat and the androstenone concentration (µg/g) would increase. However, there was no difference between the intramuscular fat concentrations of the P- and O-carcasses or between the P- and R-carcasses which had the higher concentrations of intramuscular fat ($P > 0.05$). This indicated that the intramuscular fat did not increase/change with the POR classification. Furthermore, no differences ($P > 0.05$) were found between the P-, O- and R-classified carcasses with regard to androstenone concentration demonstrating that there is no valid reason for the industry to discriminate against the fatter and R-classified carcasses on the grounds that they may contain more boar taint. It was however noted that the androstenone concentration increased ($p < 0.01$) with the increase of warm carcass weight (kg). These results would seem to indicate that the correlation between the intramuscular fat concentrations and subcutaneous fat depth (as determined by the South African carcass classification system) needs to be revisited.

3.1 Introduction

Within the European Union (EU), approximately 80% of male piglets are castrated in order to prevent boar taint in pork meat as well as to reduce aggressive behaviour among pigs (Horgan, 2006; Fredriksen *et al*., 2009). Boar taint is an offensive odour or flavour that is present in pork adipose tissue which has been described as undesirable by the greater majority of consumers (De Kock *et al*., 2001; Zamaratskaia & Squires, 2009). It is caused predominantly by two compounds: a sexual pheromone, androstenone (5α-androst-16-en-3-one) (Patterson, 1968) and a heterocyclic amide, skatole (3-methylindole) (Vold, 1970). Due to issues raised by animal welfare groups, the EU has decided that the castration of pigs, without the use of anaesthesia was rendered unlawful in January 2012 (Haugen, 2006; Bekaert, 2013). Furthermore, surgical castration of pigs must cease as of 1 January 2018

(Meinert *et al*., 2011; Haugen *et al*., 2012; Mörlein, *et al*., 2012; Bekaert, 2013). Countries such as Belgium have decided that all pork from entire male carcasses will be deemed inferior to that of castrated male carcasses; this is due to the fact that 4% of entire male carcasses are strongly and 25%, moderately tainted with boar taint compunds (Bekaert, 2013).

Pork is the most important meat product globally (Hugo & Roodt, 2007; Stupka *et al*., 2008), however, pork is not that readily consumed in South Africa as it has been found that consumers tend to see pork as an unhealthy source of protein due to its nutritional value and fat content (Hoffman *et al*., 2005). In South Africa, pigs are generally not castrated (Pieterse, 2006) as entire male pigs have shown to be faster growing animals with leaner carcasses and a reduced production cost (Rius, 2005). However, if pigs are not castrated there is a possibility that boar taint may be present in higher concentrations in the adipose tissue (Annor-Frempong *et al*., 1997) and also that the pigs might act more aggressively as they reach sexual maturity (Xue & Dial, 1997; Pauly *et al*., 2008; Zamaratskaia, 2009). Fredriksen *et al*. (2009) reported that pork carcass weights increased in Europe over the past 40 to 50 years, as has also happened in South Africa (Pieterse *et al*., 2009; Siebrits *et al.,* 2012) and that if pigs weren't castrated; there is a strong possibility that boar taint levels found in pigs would have increased as well.

In recent years South African consumers have become more aware of standards that need to be upholded in the international pork industry. These standards include pig production practices and the demand for boar taint free products.It is therefore becoming vital to deliver pork as well as pork products that have levels of boar taint below the detection limits (0.5 µg/g androstenone and 0.02 µg/g skatole) (Brooks & Pearson, 1989; Annor-Fermpong *et al.*, 1997; Kirsching *et al*, 2012).

In South Africa, the PORCUS classification system, sex, genotype and carcass weight are used in order to determine the cost payed to the farmer per carcass of commercially slaughtered pork (Pieterse, 2006). In this classification system, the %lean meat is determined with a Hennessey Grading Probe or an Intrascope® which is used to determine the backfat thickness (Government Gazette, 1992). The fat depth (and *Longissimus dorsi* muscle depth when the Hennessey grading probe is used) that is measured between the 2nd and 3rd last rib, 45 mm in from the mid-line is used to determine to which of the P, O, R, C, U, or S categories the carcass will belong (Visser, 2004; Government Gazette, 1992). In the South African pork industry "RCUS" categorised carcasses are not desired as these pigs are fatter and the possibility of the presence of boar taint increases as the age and weight (fat) of the pigs increase (Pieterse, 2006). Aluwé *et al*. (2011) and Pieterse (2006) reported that the presence of boar taint is influenced by slaughter weight, genetics, dietary ingredients and hygienic circumstances on farms. However, the presence of androstenone in pork fat has been mainly influenced by sexual

development, age and live weight of the pig (Aluwé *et al.,* 2011). Babol *et al.* (2002) and Van Oeckel *et al*. (1996) reported a low to moderate correlation between androstenone concentration and the live weight of the pig of $r = 0.43$ and $r = 0.57$, respectively. Pigs are therefore slaughtered at a lower weight to possibly prevent high concentrations of androstenone (Aluwé *et al.,* 2011) although the effectiveness of this tactic is still not clear.

In the current study, samples of the *Longissimus dorsi* muscle and subcutaneous fat of entire male pigs were utilised for the determination of muscle chemical composition and androstenone detection. The aim of the study was to determine whether a correlation between the percentage intramuscular fat and the PORCUS classification given to a carcass exists. It was also aimed to determine whether a relationship exists between the concentration androstenone present in the adipose tissue and the PORCUS classification of the carcass. Skatole and indole are heterocyclic aromatic amines (Fischer *et al*., 2011) which also contribute to boar taint. The presence of these compounds were also analysed to determine whether they fall within their detection threshold levels or not.

3.2 Materials and Methods

3.2.1 Abattoir measurements

All measurements done at the time of slaughter; farm of origin, warm and cold carcass weight (kg), lean meat average (kg), fat thickness (mm) and PORCUS classification were performed at the commercial abattoir according to standard protocol.

The farm of origin of each pig was recorded as soon as the pigs arrived at the abattoir lairage pens. The warm carcass mass was recorded after the carcass had been eviscerated (skin, trotters and head were included in this weight) and the cold carcass weight was calculated as 96.9% of the warm carcass weight. The Hennessey Grading Probe (HGP) was used to determine the fat thickness between the 3rd and 4th lumbar vertebrae (L3/4), 45 mm from the dorsal mid-line (Pieterse, 2006). This position is known as the P2 position. The HGP measures the fat thickness as well as depth of the eye muscle. Average %lean meat was calculated with the use of the following formula (Government Gazette, 1992):

%Lean meat = 72.5114 – (0.4618 x fat thickness (mm)) + (0.057 x eye muscle thickness)

Finally, the PORCUS classification was determined by correlating the %lean meat with carcass classes (Table 3.1).

Table 3.1 PORCUS classification table (Government Gazette, 1992)

Classes for pork carcasses	P2 subcutaneous fat thickness (mm)	Estimated % lean meat in the carcass
Weaner		**
P	≤ 12	≥ 70
O	13 – 17	68 - 69
R	18 – 22	66 - 67
C	23 – 27	64 - 65
U	28 – 32	62 - 63
S	> 32	≤ 61
Sausage		**
Rough		**

** The lean meat content for these classes are not specified

A weaner is classified as a carcass which weighs 20 kg or less. Carcasses which fall into the PORCUS classification weigh between 21 and 100 kg. These carcasses are however further divided into porkers (21 – 55 kg carcass mass) and baconers (56 – 100 kg carcass mass). Lastly, sausage pigs weigh more than 100 kg and a rough carcass classification is given when a carcass is descendent from a (old) boar, has a carcass confirmation score of 1, shows obvious genetic inferiority, is excessively thin, skin appears thick and coarse or the fat of the carcass is excessively oily.

3.2.2 Sample preparation

Pork loin samples of the left lumbar area (from the last rib towards the last lumbar vertebrae) of 180 entire male pigs with three different fat classifications (45 P, 82 O and 49 R) were sourced from a commercial abattoir. The subcutaneous fat was removed, vacuum packed and placed into the -20°C freezer until needed for chemical analysis. The loin (*Longissimus dorsi et lumborum* muscle) was removed from the pork chop where after the sinew was removed and the muscle homogenised. The homogenised sample was thereafter vacuum packed and placed into the -20°C freezer until it was needed for chemical analysis. All analysis where performed in duplicates.

3.2.3 Proximate analysis

Frozen packets of homogenised pork loin were removed from the -20°C freezer and placed in the 4°C refrigeratorfor 12 h to defrost prior to proximate analysis.

Homogenised pork loin samples (2.5 g) were weighed out into a crucible and placed into a 100°C oven for 24 hours. The moisture content (% wet weight) was determined according to the official Association of Official Analytical Chemists (AOAC, 2002b) method 934.01.

The Dumas combustion method 992.15 (AOAC, 2002a) was used to determine the percentage crude protein (% wet weight) present in the pork loin samples. Defatted, dried and ground meat samples (0.1 g) were weighed out into Leco foil sheets and analysed with a Leco Nitrogen/Protein analyser (FP – 528, Leco Corporation). Prior to sample analyses (and after every 12 samples) the Leco was calibrated with ethylene-diamine-tetra-acetic acid (EDTA) to ensure the accuracy and recovery rate of the method. As results were obtained in % nitrogen, this value was multiplied with a factor of 6.25 in order to convert it to % total crude protein.

The chloroform/methanol extraction gravimetric method as described by Lee *et al*. (1996) was used to determine the total lipid content (% wet weight) within 5 g of homogenised pork loin samples. For this chemical analysis however, 2:1 (v/v) chloroform/methanol was used as the samples were expected to have more than 5% intramuscular fat.

To determine the ash content, 2.5 g pork loin samples were weighed out in crucibles and placed into the furnace at 500°C for 6 h according to the method 942.05 (AOAC, 2002).

3.2.4 HPLC-MS/MS-FD Analysis

Sample preparation

Samples were analysed according to an adapted version of Verheyden *et al*. (2007) and Bekaert *et al*. (2012)'s method. A liquid chromatographic multiple mass spectrometric (LC-MS) method was used which allowed for the simultaneous analysis of androstenone, skatole and indole.

Subcutaneous fat samples were removed from the freezer (-20°C) and allowed to thaw 12 h prior to analysis. Two grams of fat was weighed off, cut into small cubes and placed into fat beakers. These beakers were covered and placed into a 700 W microwave oven for 3 min on maximum power setting. Once removed from the microwave ,samples were allowed to stand for 1 minute where after a 150 µL aliquot (melted fat) and 750 µL methanol (containing 200 $\mu g.kg^{-1}$ internal standard, 2-methylindole) where transferred into a 2 mL Eppendorf container and vortexed (10 minutes at 14,000 rpm). The containers were placed in a heating block at 60°C for 1 hour and subsequently transferred to a -20°C freezer for another 60 minutes. Samples were removed from the freezer and centrifuged at 14,000 rpm for 10 minutes. Finally, 500 µL of the supernatant was transferred to HPLC vials and analysed.

Samples were analysed using a Waters Xevo TQ triple quadruple mass spectrometer and a Waters Acquity UPLC and fluorescence detector with a Phenomenex Kinetix C18, 2.6 µm, 150 x 2.1 mm column and using two solvents; 7.5% formic acid and 49:49:2 methanol:acetonitrile:isopropanol plus 7.5% formic acid. Column temperature was set at 40°C. Androstenone was analysed with mass spectrometry whereas skatole and

indole were analysed using fluorescence detection; 10 µL of each sample was injected for each analysis. The calibration range and limit of quantification for androstenone was 0.01 to 13 ppm and 0.02 ppm, respectively whereas the calibration range and limit of quantification for both skatole and indole was 0.008 to 0.08 ppm and 0.004 ppm, respectively.

The skatole and indole concentrations in the subcutaneous fat of 120 entire male carcasses were also determined. In order to determine these values, a HPLC-MS/MS-FD analysis was performed as described by Verheyden *et al*. (2007).

3.2.5 Statistical Analysis

One-way ANOVA was used to compare means of various measurements between the three pork classifications. For post hoc testing, Fisher least significant difference testing (LSD) was used. In some cases where normality assumptions appeared to be violated, non-parametric bootstrap was used to verify the ANOVA results (bootstrap results not reported). Relationships between the various measurements were investigated using Pearson correlation analysis. The androstenone concentrations were also categorized in low, medium and high, before being analysed using cross tabulation and the Chi-square test.

3.3 Results

In the South African pork industry, the warm carcass weight, PORCUS classification and sex of the pig are the main factors that determine the market value of pork meat. In this trial, focus was placed on the correlation between the warm carcass weight, fat thickness (as determined by HGP), carcass classification (P, O and R) and androstenone concentration. The weights of the carcasses ranged between 44.3 and 100.9 kg (Table 3.2).

Table 3.2 Means (± s.e.) of backfat thickness (mm) and warm carcass weight (kg) with regard to carcass classification

Carcass classification	Number of carcasses	Warm carcass weight (kg)	P2 – Backfat thickness at slaughter (mm)
P	44	$74.4^{a} \pm 1.54$	$11.5^{a} \pm 0.37$
O	82	$81.9^{b} \pm 1.13$	$14.8^{b} \pm 0.27$
R	46	$74.7^{a} \pm 1.51$	$16.1^{c} \pm 0.36$

^{a,b,c} Values in columns with different superscripts differ significantly ($P \leq 0.05$)

s.e. – Standard error

As expected, the backfat thickness of the carcasses differed ($P \leq 0.05$) between the different carcass classifications (Table 3.2). However, the warm carcass weight (kg) of O-

classified carcasses were heavier (P ≤ 0.05) than that of P- and R-classified carcasses. Also, the intramuscular fat of the O-carcasses was lower (P ≤ 0.05) than that of R-carcasses but similar to that of the P-carcasses (Table 3.3). These unexpected differences could possibly be due to the pigs having different levels of maturity. Furthermore, the intramuscular fat of the P-carcasses did not differ from R-carcasses (P > 0.05). There were not many unexpected differences found in the chemical composition analysis of moisture (%), protein (%) or ash (%) (Table 3.3) between the various PORCUS classifications in the *Longissimus dorsi* muscle. As pertaining to the moisture content (%) of the muscles, the R-carcasses had a lower % moisture (P ≤ 0.05) than the P- and O-carcasses, although this difference was only ~1%. For protein (%), there were no differences (P > 0.05) between P- and R-carcasses or between P- and O-carcasses. Lastly, no differences (P > 0.05) were found for the ash percentage of different carcass classifications.

A positive, moderate correlation (r = 0.452; p < 0.01) between the subcutaneous fat thickness of the *Longissimus dorsi* muscle (mm) and the warm carcass weight (kg) was noted (Figure 3.1). On the other hand, there was no correlation (r = -0.124; p = 0.105; Figure 3.2) between the intramuscular fat percentage and the warm carcass weight (kg). Therefore, as the warm carcass weight (kg) increases, the fat thickness (mm) increases, but the intramuscular fat percentage of the *Longissimus dorsi* muscle decreases (Figure 3.2). In Figure 3.3, an extremely weak positive correlation (r = 0.0650; p = 0.3970) was found between fat thickness (mm) and the intramuscular fat percentage of the *Longissimus dorsi* muscle, indicating that the intramuscular fat percentage does not necessarily increase with the increase of fat thickness (mm). This would also indicate that as the carcass receives higher (thicker) readings from the HGP, and therefore receive increased PORCUS classifications readings, the intramuscular fat of the carcass does not does not necessarily increase.

Table 3.3 The chemical composition (mean ± s.e.) of pork loin samples (*Longissimus dorsi*) of pig carcasses according to their PORCUS classification

PORCUS	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
P	$73.1^{a} \pm 0.20$	$22.8^{ab} \pm 0.29$	$3.3^{ab} \pm 0.33$	$1.2^{a} \pm 0.049$
O	$72.7^{a} \pm 0.15$	$23.3^{a} \pm 0.21$	$2.8^{a} \pm 0.24$	$1.3^{a} \pm 0.036$
R	$72.0^{b} \pm 0.19$	$22.4^{b} \pm 0.27$	$4.2^{b} \pm 0.31$	$1.3^{a} \pm 0.047$

^{a,b,c} Values in columns within groups with different superscripts differ significantly ($P \leq 0.05$)

s.e. – Standard error

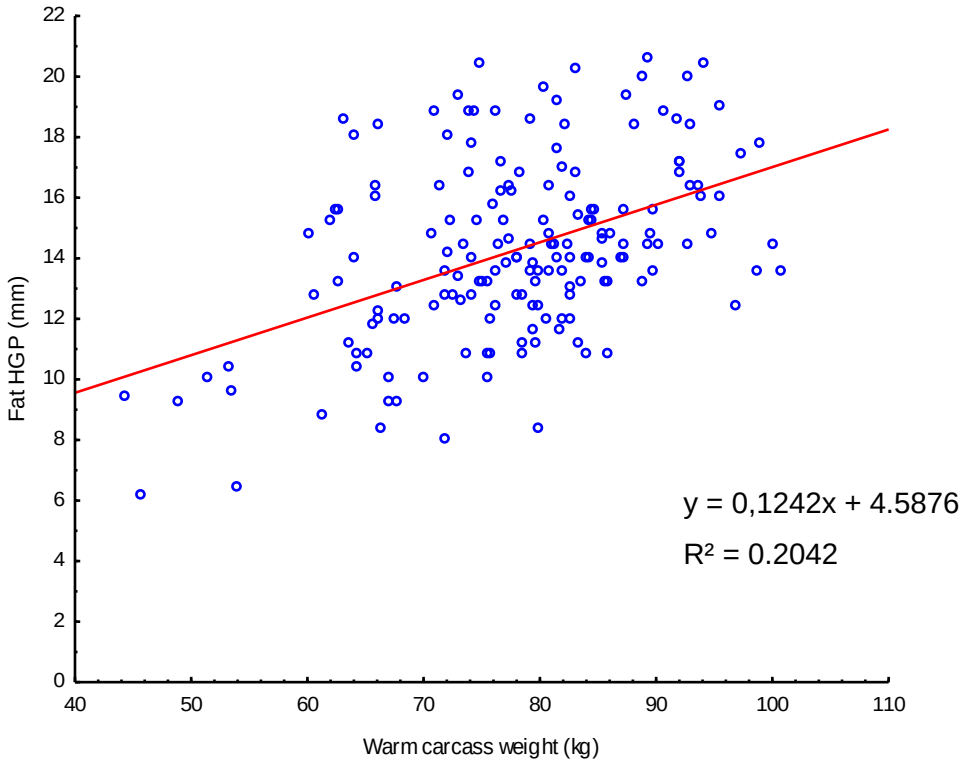

Figure 3.1 Correlation between subcutaneous P2 fat thickness (mm), as determined with the use of the Hennessey grading probe, and warm carcass weight (kg) ($r = 0,452$; $p < 0.001$).

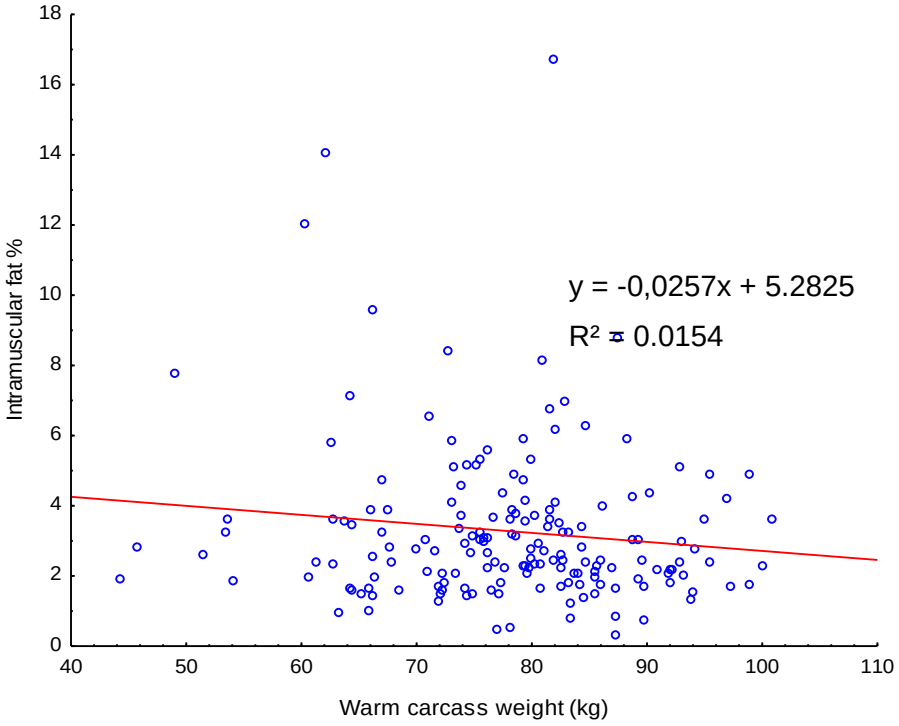

Figure 3.2 Correlation between intramuscular fat% of the *Longissimus dorsi* muscle (as determined with chemical analysis) and the warm carcass weight (kg) (r = -0,124; p = 0,105).

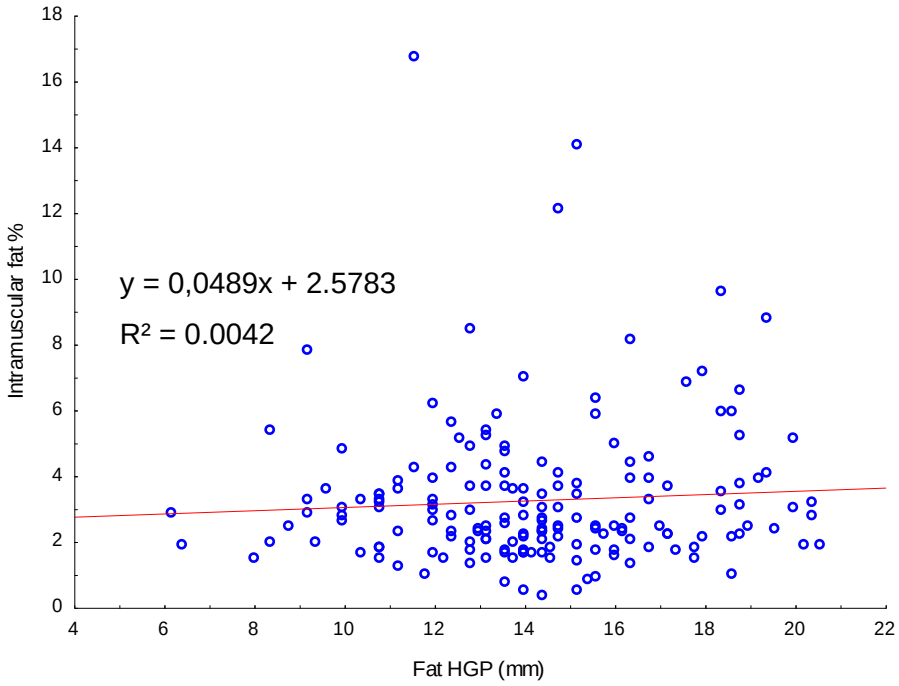

Figure 3.3 Correlation between intramuscular fat % of the *Longissimus dorsi* muscle (as determined with chemical analysis) and the subcutaneous P2 fat thickness (as determined with the Hennessey grading probe) (r = 0.065; p = 0.397).

As expected, as the warm carcass weight increases, there was a tendency for the concentration androstenone in the subcutaneous fat to increase (r = 0.267; p < 0.01; Figure 3.4). Interestingly, a large number of the androstenone readings (µg/g) were found in the Low category (< 0.31 µg/g).

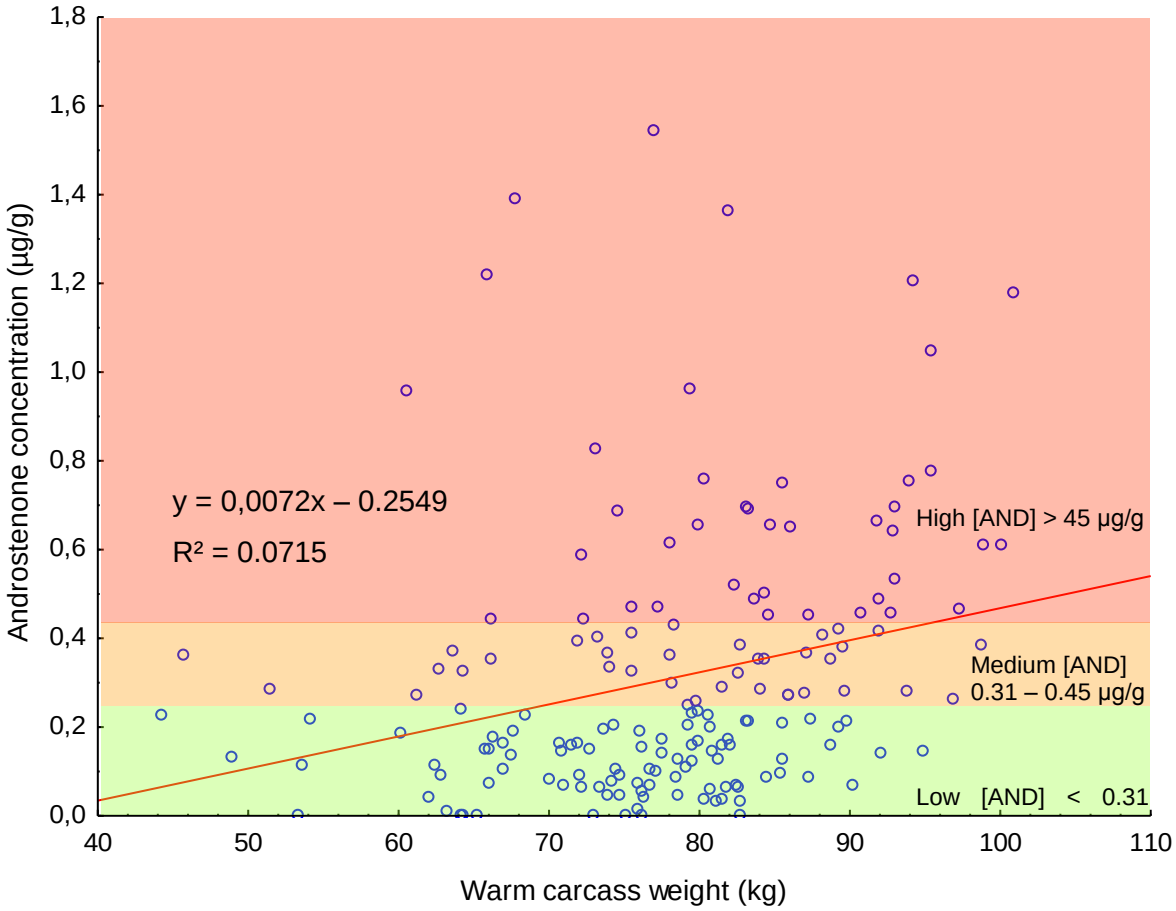

Figure 3.4 Correlation between androstenone (µg/g sample) in subcutaneous backfat and warm carcass weight (kg) with androstenone threshold overlays.

A slight negative correlation (r = -0.148; p = 0.049) was found between androstenone concentration (µg/g) and the intramuscular fat percentage of the *Longissimus dorsi* muscle (

Figure **3.5**). This result, combined with that of Figure 3.4 which indicated an increase of androstenone concentration (µg/g sample) as the warm carcass weight increases (Figure 3.2) with the slight negative correlation (r = -0.124) andTable 3.3, which shows that O-classified carcasses have a lower intramuscular fat percentage, forms a trend that O-carcasses may have higher concentrations of androstenone (µg/g) present. However, there were no differences (p = 0.341) in androstenone concentrations (µg/g) between the P-, O-, and R-classified carcasses. None the less,

it was interesting to see how the levels (low, medium and high) differed in each of the selected carcass classification groups.

Figure 3.7 The O-classified carcasses had the highest percentage (30.12%) of carcasses possessing androstenone in levels high enough to be easily detected by consumers who are sensitive to this compound (Figure 3.7). When comparing these results to that of Table 3.2, it can be seen that as the weight of the carcass increases, so does the concentration androstenone (µg/g) found in the subcutaneous fat. It is however important to note that the P-value of the Chi-square test was p = 0.131, therefore rendering these results statistically insignificant.

The average skatole and indole concentrations present in the subcutaneous fat were also determined and were found to be 0.0471 and 0.0142 µg/g, respectively. There was however no further analysis performed and reported on these compounds as they did not differ between any of the main effects.

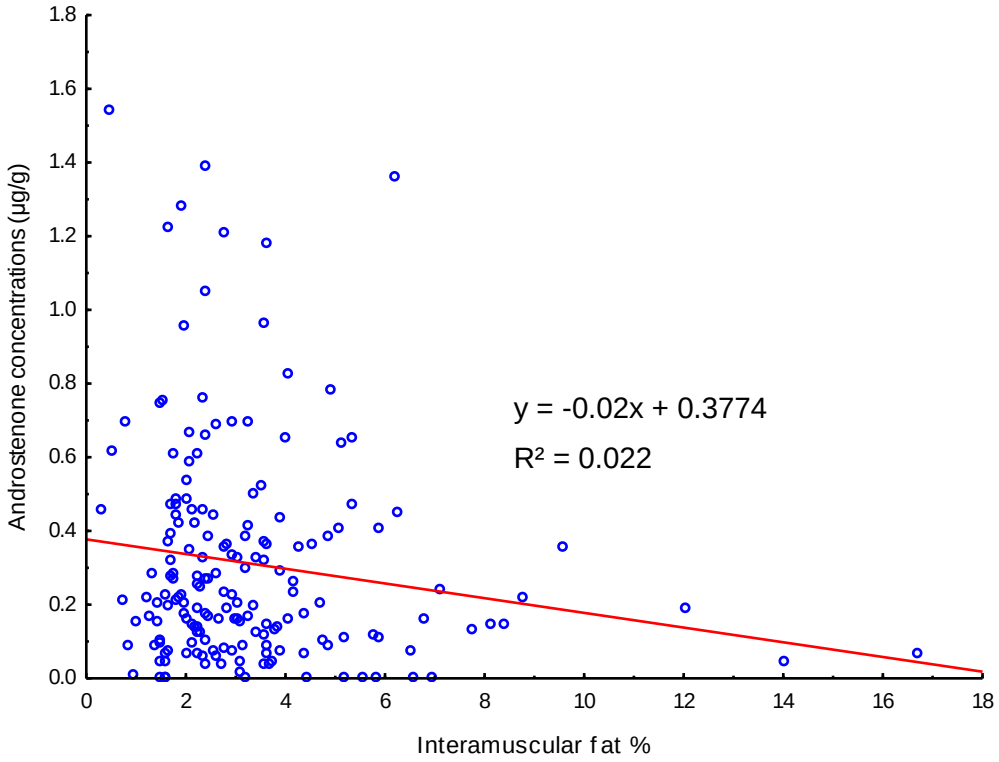

Figure 3.5 Correlation between androstenone concentrations (µg/g sample) in subcutaneous fat and the percentage intramuscular fat of the *Longissimus dorsi* muscle (as determined with chemical analysis).

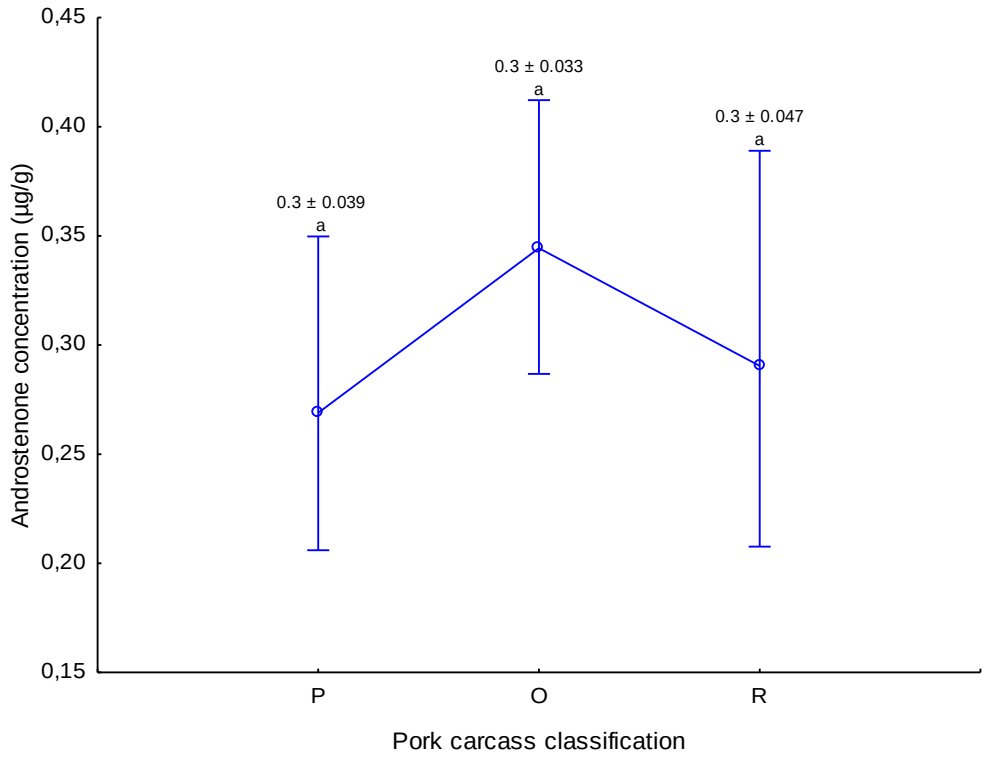

Figure 3.6 Mean (± s.e.) androstenone concentration (µg/g sample) in the intramuscular fat compared to the POR carcass classifications (p = 0.341).

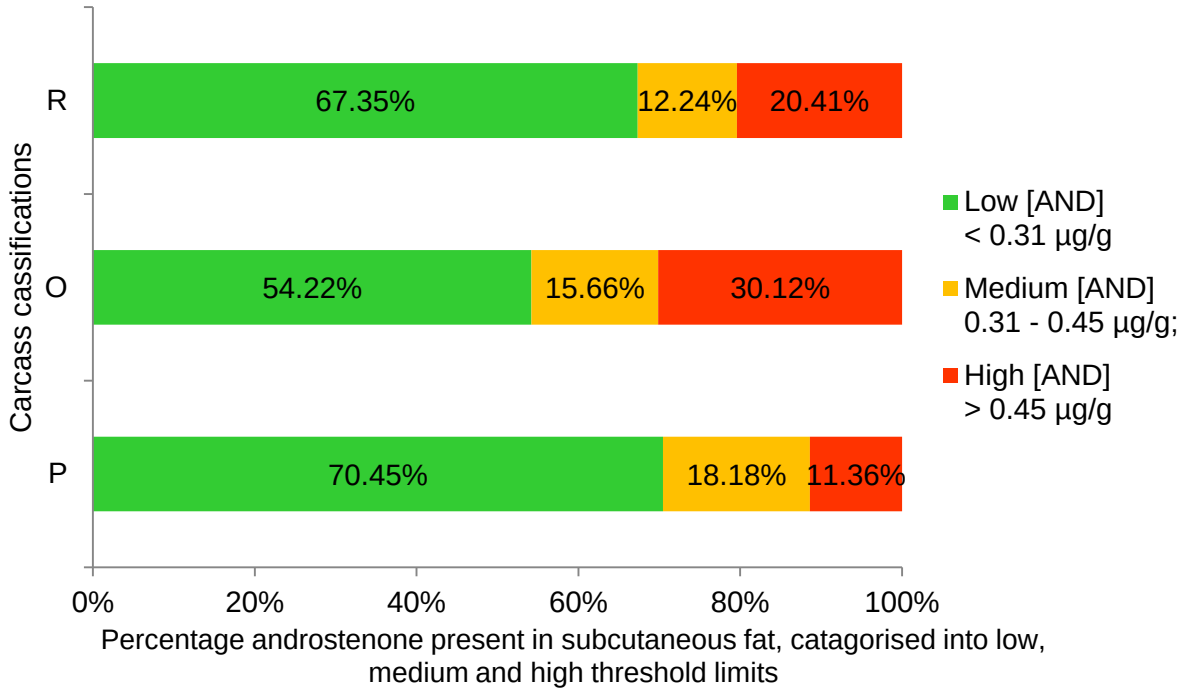

Figure 3.7 Subdivisions of percentage androstenone (µg/g) (low, medium and high threshold levels) present in various carcass classifications.

3.4 Discussion

It is of extreme importance to ensure that the PORCUS classification system used in South Africa is functioning to its full potential. An optimised system will ensure that pork and pork products of top quality are constantly delivered whilst pig producers and abattoirs receive optimum prices for their pork and pork products. The current classification system was developed in 1992 and has not been updated since, except for a carcass weight of saussage pigs increase from 90 kg to 100 kg (Siebrits *et al.,* 2012). This could lead to erroneous classifications within abattoirs as carcasses have increased in weight yet yielded leaner meat in recent years (Siebrits *et al.,* 2012). Another factor to be noted is that the PORCUS classification system categorises carcasses into specific lean meat yield classes whilst it is well known that the amount of lean meat yielded is typical of non-categorical data due to the numerous factors that could influence the yield.

The great majority of male pigs are no longer castrated in South Africa, resulting in possible higher feed efficiency than that of gilts (Blanchard *et al*., 1999), faster growth and leaner meat than castrates (Andersen, 2006; Pauly *et al*., 2008). However, boars have been found to be more aggressive which may result in injuries and carcass bruising (Pieterse, 2006). Furthermore, there is the possibility that boars produce the sexual pheromone, androstenone, when sexual maturity is reached (Babol *et al*., 1999), resulting in undesirable sensory attributes (Brooks & Pearson, 1989).

In South Africa's classification system, emphasis is placed on the sex of a pig carcass. The value of a carcass is calculated with various factors, including slaughter weight and the PORCUS classification whilst the gender of the pig indirectly accounts for the possible presence of androstenone. The R-classified carcasses (or higher) are automatically penalised in price because the lean meat score decreases. As a consequence, it is also anticipated, although not predicted by the PORCUS classification system, that the intramuscular fat percentage increases with the increase of subcutaneous fat thickness. However, conflicting results have been found for the interaction between backfat thickness and the percentage intramuscular fat. Beattie *et al*. (1999) reported that as the carcass weight increased, so did the subcutaneous fat percentage and therefore the intramuscular fat also increased. From Table 3.2 and 3.3, it is clear that the South African pork carcasses followed similar trends as described by Beattie *et al*. (1999). For example, the R-classification carcasses had the thickest layer of subcutaneous fat and also a higher ($P \leq 0.05$) percentage intramuscular fat than that of O-carcasses. However, these results do not explain the difference in intramuscular fat of the O-classified carcasses as shown in Table 3.2 and Table 3.3. Although the South African carcass classification regulation notes that the fat depth of the subcutaneous fat should be measured between the 3rd and 4th lumbar vertebrae, the whole lumbar region of the muscle was sampled and homogenised for chemical analyses and this may have resulted in some variation in results as Faucitano *et al*. (2004) noted a variation in the % fat in the *Longissimus* muscle. Faucitano *et al*. (2004) determined the intramuscular fat percentages throughout the whole *Longissimus* muscle, from the 5th thoracic rib until the 3rd last lumbar vertebra; it was found that the intramuscular fat percentage was the highest in the middle section of the thoracic region and in the middle-caudal section of the lumbar area.

The remaining chemical composition results (Table 3.3) (moisture (%), protein (%) and ash (%)) of the different PORCUS classification categories were as expected.

Evaluating only the warm carcass weight, and not the PORCUS classifications, a positive but moderate correlation ($r = 0.452$; $p < 0.01$) between the weight and the subcutaneous fat thickness was displayed. This is in accordance with results found by Beattie *et al*. (1999). Beattie *et al*. (1999) and Tibau *et al.* (2003) also reported that as carcass weight increases the intramuscular fat percentage also increased; this trend was however not shown in this investigation (Figure 3.3). The variation in intermuscular fat along the *Longissimus* found by Faucitano *et al*. (2004) could again explain why there was no correlation ($r = 0.065$; $p = 0.397$) between the fat thickness and the intramuscular fat percentage (Figure 3.3), however Pieterse (2006) reported that no significant difference was found in boars when fat thickness measurements were taken between the 2nd and 3rd last rib, 5th and 6th lumbar vertebrae or the 3rd and 4th lumbar vertebrae.

As expected, there was a positive but low correlation ($r = 0.267$; $p < 0.01$) between the increasing concentration of androstenone (µg/g) and warm carcass weight (Figure 3.4). It has been reported that higher levels of androstenone are found in heavier pigs (Aldal *et al*., 2005; Nicolau-Solano *et al.,* 2007; Zamaratskaia & Squires, 2009), however Chen *et al.* (2007) found no increased levels of androstenone in heavier pigs. The results of these studies indicate that using slaughter weight to predict whether androstenone would be present in a carcass is not a precise method. Also, if the slaughter weight were to be used to predict whether androstenone is present, this would have a negative impact on the pork prices for the pig producers in South Africa as the economics indicate that it is more profitable to slaughter heavier pigs (Pieterse *et al,* in press).

When comparing the concentration of androstenone (µg/g) with the intramuscular fat percentage (

Figure **3.5**), a slight negative correlation was found ($r = -0.148$; $p = 0.049$). One would expect the concentration of androstenone to increase because of the positive correlation shown between increasing androstenone concentrations and increasing warm carcass weight (Figure 3.4) and the reported correlation between an increased carcass weight and increasing intramuscular fat percentage (Beattie *et al*., 1999), but no correlation ($r = -0.124$; $p = 0.105$) between warm carcass weight and intramuscular fat percentage was found in the current study (Figure 3.2).

A large percentage (62% of 176 carcasses; Figure 3.7) of pig carcasses were found to have androstenone in the Low category (< 0.31 µg androstenone /g sample); 15% of all carcasses fell into the moderate androstenone category (0.31 – 0.45 µg/g), whilst 22% of carcasses were highly tainted by androstenone (> 0.45 µg/g). Although these results were not significant ($p = 0.131$) it was expected as only a minority of entire male carcasses would have started producing androstenone by the time the pigs were slaughtered (Bekaert *et al*., 2012). Aluwé *et al*. (2009) found that in Belgium, 4% of entire male carcasses have high levels of boar taint present whilst 25% of carcasses are moderately tainted. It is however clear that the pigs used in the current study exhibited higher concentrations of androstenone than the ones reported by Aluwé *et al*. (2009). This difference may be due to different genotypes being analysed. It was interesting to note that O-carcasses had the highest percentage of androstenone in the highly tainted category (Figure 3.7), again indicating that as the weight of the carcass increases (Table 3.2), so does the androstenone concentration (Aldal *et al*., 2005; Nicolau-Solano *et al.,* 2007; Zamaratskaia & Squires, 2009). However, when analysing the androstenone concentrations when divided into their PORCUS classifications (Figure 3.6), no difference ($p = 0.341$) in androstenone concentrations (µg/g) between the P-, O-, and R-classified carcasses were found.

The average skatole and indole concentrations present in the subcutaneous fat were also determined and were found to be 0.0471 and 0.0142 µg/g, respectively. Indole is not a major contributor to boar taint, however skatole is. Annor-Fermpong *et al.* (1997) reported that consumers had a threshold of 0.008 to 0.06 µg/g for skatole whereas Lundström *et al*. (2009) reported threshold levels of 0.2 – 0.25 µg/g. Furthermore, 82 – 99% of consumers are sensitive to wards this compound (Fort-i-Furnols *et al*., 2013). The average skatole value detected in the present study (0.0471 µg/g) is worrying when comparing it to the threshold levels reported by Annor-Fermpong *et al.* (1997) as they lie within the threshold range. It is consequently important to do further investigation as to why the value is so high.

3.5 Conclusions

This study has shown that many assumptions are made when classifying a pork carcass in South Africa. One would have expected that as the carcass weight increases, so would the in intramuscular fat percentage and also the concentration of androstenone found in the subcutaneous fat. Therefore, R-classified carcasses were expected to have the highest warm carcass weight and therefore the highest levels of intramuscular fat and also the highest percentage androstenone above the sensory threshold (>0.45 µg/g), making them the most undesirable of the three classification groups examined. It was therefore very interesting to find that O-carcasses had the highest carcass weight ($P \leq 0.05$) and also the highest percentage of carcasses with androstenone above the sensory threshold (>0.45 µg/g). Furthermore, a large overall percentage (15 and 22%, respectively) of carcasses were found to have androstenone of moderate (0.31 – 0.45 µg/g) and high (>0.45 µg/g) levels present. Again, these results were not significant, however carcasses in this current study seem to contain higher concentrations of boar taint than those previously reported.

Upon examining the results as a whole, there were not many areas where one could see a clear difference between carcasses in the P-, O- and R-classifications. However, it must be borne in mind that these analyses were conducted on a limited number of carcasses and a larger sample size would be required before it can be concluded that a revision of the PORCUS classification system is needed. Furthermore, the high percentage of carcasses portraying moderate and high concentrations of the boar taint compound androstenone is worrying as this could result in consumer dissatisfaction with pork and pork products. This aspect also warrants further research as De Kock *et al*. (2001) found that South Africa consumers differ in their sensitivity towards boar taint compounds; the different responses to these boar taint compounds was also confounded by the interactions between gender and different ethnic groups.

3.6 References

Aldal, I., Andresen, O., Egeli, A.K., Haugen, J.E., Grodum, A., Fjetland, O. & Eikaas, J.L.H. (2005). Levels of androstenone and skatole and the occurrence of boar taint in fat from young boars. *Livestock Production Science*, **95**, 121–129.

Aluwé, M., Millet, S., Nijs, G., Tuyttens, F.A.M., Verheyden, K., De Brabander, H.F., De Brabander, D.L. & Van Oeckel, M.J. (2009). Absemce of an effect of dietary fibre or clinoptilolite on boar taint in entire male pigs fed practical diets. *Meat Science*, **82**(3), 346-352.

Aluwé, M., Millet, S., Bekaert, K.M., Tuyttens, F.A.M., Vanhaecke, L., De Smet, S. & De Brabander, D.L. (2011). Influence of breed and slaughter weight on boar taint prevalence in entire male pigs. *Animal*, ***5***(8), 1283–1289.

Andersen, J.R. (2006). Sorting criteria. Methods for on-line / at-line sorting of entire male castrated with emphasis on the Danish method based on skatole content. *Acta Veterinaria Scandinavica*, **48**(1), 20–22.

Annor-Frempong, I.E., Nute, G.R., Whittington, F.W. & Wood, J.D. (1997). The problem of taint in pork: 1. Detection thresholds and odour profiles of androstenone and skatole in a model system. *Meat Science*, ***46***(1), 45–55.

AOAC (2002). AOAC Official Method 942.05. In: Official methods of analysis. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

AOAC (2002a). AOAC method 992.15. In: Official methods of analysis. Arlington, Virginia, USA: Association of Analytical Chemists Inc.

AOAC (2002b). Official Method 934.01. In: Official method of analysis. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

Beattie, V.E., Weatherup, R.N., Moss, B.W. & Walker, N. (1999). The effect of increasing carcass weight of finishing boars and gilts on joint composition and meat quality. *Meat Science*, **52**, 205-211.

Babol, J., Squires, E.J. & Gullett, E.A. (2002). Factors affecting the level of boar taint in entire male pigs as assessed by consumer sensory panel. *Meat Science*, **61**, 33–40.

Bekaert, K.M., Aluwé, M., Millet, S., Goethals, K., Nijs, G., Isebaert, S., De Brabander, D.L., Verheyden, K., De Brabander, H.F., Verhaecke, L. & Tuyttens, F.A.M. (2012). Predicting the likelihood of developing boar taint: early physical indicators in entire male pigs. *Meat Science*, **92**(4), 382–385.

Bekaert, K. (2013). Chemical and sensory detection of boar taint. PhD Dissertation, Gent University, Belgium.

Blanchard, P.J., Ellis, M., Warkup, C.C., Chadwick, J.P. & Willis, M.B., (1999). The influence of sex (boars and gilts) on growth, carcass and pork eating quality characteristics. *Animal* Science, **68**(3), 487-493.

Brooks, R.I. & Pearson, A.M. (1989). Odor thresholds of the C_{19}-Δ^{16}-steroids responsible for boar odor in pork. *Meat Science*, **24**, 11–19.

Chen, G., Zamaratskaia, G., Andersson, H.K. & Lundström, K. (2007). Effect of dietary supplement of raw potato starch and live weight on the levels of skatole, indole and androstenone measured by different methods. *Food Chemistry*, **101**, 439–448.

De Kock, H.L., van Heerden, S.M., Heinze, P.H., Dijksterhuis, B.B. & Minnaar, A. (2001). Reaction to boar odour by different South African consumer groups. *Meat Science*, **59**, 353–362.

Faucitano, L., Rivest, J., Daigle, J.P., Lévesque, J. & Gariepy (2004). Distribution of intramuscular fat content and marbling within the *longissimus* muscle of pigs. *Canadian Journal of Animal Science*, **84**, 57-61.

Fischer, J., Elsinghorst, P. W., Bücking, M., Tholen, E., Petersen, B. & Wüst, M. (2011). Development of a candidate reference method for the simultaneous quantitation of the boar taint compounds androstenone, 3 α-androstenol, 3β-androstenol, skatole, and indole in pig fat by means of stable isotope dilution analysis - headspace solid-phase microextraction - gas chromatography/mass spectrometry. *Analytical Chemistry*, **83**, 6785-6791.

Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: a review. *Meat Science*, ***92***(4), 319–29.

Fredriksen, B., Font-i-Furnols, M., Lundström, K., Migdal, W., Prunier, A, Tuyttens, F.A.M. & Bonneau, M. (2009). Practice on castration of piglets in Europe. *Animal*, **3**(11), 1480-1487.

Government Gazette (1992). Regulation No. R. 1748. Agricultural Product Standards Act, 1990 (Act No. 119 of 1990). Regulations regarding the classification and marking of meat. Government Gazette No. 14060, 26 June 1992. Pretoria: Government Printer. South Africa.

Hoffman, L.C., Styger, E., Muller, M. & Brand, T.S. (2005). Sensory, physical and chemical quality characteristics of bacon derived from South African indigenous and commercial pig breeds. *South African Journal of Animal Science*, ***6***(1), 36–48.

Haugen, J.E. (2006). The use of chemical sensor array technology, the electronic nose, for detection of boar taint. *Acta Veterinaria Scandinavica*, ***48***, 1–3.

Horgan, R. (2006). Piglet castration and EU animal welfare legislation. *Acta Veterinaria Scandinavia*, **48**(1), 1–4.

Hugo, A. & Roodt, E. (2007). Significance of Porcine Fat Quality in Meat Technology: A Review. *Food Reviews International*, ***23***(2), 175–198.

Kirsching, Á., Bázár, G., Házás, Z. & Romvári, R. (2012). Classifications of meat with boar taint using an electronic nose. *Acta Argiculturae Slovenica*, ***3***, 99–103.

Lundström, K., Matthews, K.R. & Haugen, J.E. (2009). Pig meat quality from entire males. *Animal*, ***3***(11), 1497–1507.

Meinert, L., Bejerholm, C., & Støier, S. (2011). Validation of "human nose" based method for boar taint detection. *57th International Congress of Meat Science and Technology*. Ghent, Belgium.

Mörlein, D., Grave, A., Sharifi, A. R., Bücking, M. & Wicke, M. (2012). Different scalding techniques do not affect boar taint. *Meat Science*, ***91***(4), 435–440.

Nicolau-Solano, S.I., Whittington, F.M., Wood, J.D. & Doran, O. (2007). Relationship between carcass weight, adipose tissue androstenone level and expression of the hepatic 3b-hydroxysteroid dehydrogenase in entire commercial pigs. *Animal*, **1**, 1053–1059.

Patterson, R.L.S. (1986). 5α-androst-16-ene-13-one: compound responsible for taint in boar fat. *Journal of the Science of Food Agriculture*, **19**, 31-38.

Pauly, C., Spring, P., O'Doherty, J.V, Ampuero Kragten, S. & Bee, G. (2008). Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. *Animal*, ***2***(11), 1707-1715.

Pieterse, E. (2006). Effects of increased slaughter weight of pigs on pork production. PhD Dissertation, University of Stellenbosch, South Africa.

Pieterse, E., Swarts, I.C. & Hoffman, L.C. (2009). The effect of porcine somatotropin (pST) and gender on production parameters and tissue yield of pigs slaughtered at 135 kg live weight. *South African Journal of Animal Science*, **39**, 286-295.

Pieterse, E., Hoffman, L.C., Siebrits, F., Gloy, E. & Polawska, E. (in press). The effect of slaughter weight on the carcass characteristics of pork with sex type as co-variable. *Animal Production Science*.

Rius, M., Hortos, M. & Garcia-Regueiro, J. (2005). Influence of volatile compounds on the development of off-flavours in pig back fat samples classified with boar taint by a test panel. *Meat Science*, **71**(4), 595–602.

Siebrits, F.K., Hambrock, B.W. & Pieterse, E. (2012), 'Die Suid-Afrikaanse varkkarkas-klassifikasiestelsel', *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*, **31**(1), 1-5 (in Afrikaans with English abstract).

Stupka, R., Čítek, J., Šprysl, M., Okrouhlá, M., Kureš, D. & Líkař, K. (2008). Effect of weight and sex on intramuscular fat amounts in relation to the formation of selected carcass cuts in pigs. *Czech Journal of Animal Science*, **53**(12), 506–514.

Tibau, J., Gonzalez, J., Soler, J., Gispert, M., Lizardo, R. & Mourot, J. (2003). Influence of pig slaughter weight (range 25 to 140 kg) on the chemical composition of the carcass: effect of genotype and sex. *Journees de la Recherche Porcine, sous l'egide de l'Association Franccaise de Zootechnie*, **5-7**,121.

Van Oeckel, M.J., Casteels, M., Warnants, N., De Boever, J.L., Van Renterghem, R. & Boucqué, C.V. (1996). Production of entire males with Belgian Landrace and hybrid pigs: the incidence of sensory aberrations. *Journal of Animal Physiology and Animal Nutrition - Zeitschrift Für Tierphysiologie Tierernahrung und Futtermit- telkunde*, **76**, 111–121.

Verheyden, K., Noppe, H., Aluwé, M., Millet, S., Vanden Bussche, J. & De Brabander, H.F. (2007). Development and validation of a method for simultaneous analysis of the boar taint compounds indole, skatole and androstenone in pig fat using liquid chromatography-multiple mass spectrometry. *Journal of Chromatography A*, **1174**(1-2), 132–137.

Visser, D. P. (2004). Structuring of breeding objectives in the pork supply chain in South Africa. PhD Dissertation, University of Pretoria, South Africa.

Vold, E. (1970). Fleischproduktionseigenschaften bei Ebern und Kastraten. IV. Organoleptisch und gaschromatografische Untersuchungen wassedampfflüchtiger Stoffe des Rückspeckes von Ebern. *Meldinger Nordlandbruckhoegskole*, **49**, 1-25 (in German).

Xue, J. & Dial, G.D. (1997). Raising intact male pigs for meat: Detecting and preventing boar taint. *Swine Health and Production*, **5**(4), 151–158.

Zamaratskaia, G. (2009). Cause and possible ways to eliminate boar taint in pork. *Tehnologija Mesa*, **50**(1-2), 43–47.

Zamaratskaia, G. & Squires, E.J. (2009). Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal : An International Journal of Animal Bioscience*, **3**(11), 1508–1521.

CHAPTER 4

Effect of different cooking methods on the concentration of androstenone within the *Longissimus thoracis* subcutaneous fat of boars

Abstract

This study determined how androstenone concentrations within the intramuscular fat reacts to different cooking (pan frying, oven baking and *sous vide*) methodologies. Twelve pork loins (*Longissimus thoracis*) of O-classified entire male carcasses were cut into four 2.5 cm thick chops. The chops were divided into four treatment groups, namely; raw, pan frying, oven baking and *sous vide.* The androstenone concentration of the subcutaneous fat of the raw sample was determined with the use of HPLC-MS/MS-FD. The three remaining chops were cooked according to the cooking methodologies where after the androstenone concentrations of the subcutaneous fat were again evaluated. The pan fried chops had a lower ($p = 0.046$) concentration of androstenone than the *oven baked* chops. This could be due to the effect of the different heating methodologies used to prepare the pork chops; pan frying makes use of direct dry heat, and *sous vide* makes use of direct wet heat. The direct dry heat could have caused more of the androstenone to evaporate due to the compound's volatile properties. There was no difference between the androstenone concentrations of any of the prepared samples to that of the raw sample ($P > 0.05$). Furthermore, all cooked samples were still above the sensory threshold (> 0.45 µg/g) which could result in consumers experiencing off odours whilst preparing pork which effectively could have a negative effect on the pork industry.

4.1 Introduction

Boar taint is on offensive off-flavour or off-odour that is present in pigs (Lundström *et al*., 2009). Androstenone is found in a minority of sexually matured, entire male pigs (Bekaert *et al*., 2012) whilst consumer sensitivity towards androstenone differs depending on their OR7D4 genotype (Keller *et al*., 2007) which has been reported to also be affected by the consumers' geographical region (Lundström *el al*., 2009). Keller *et al.* (2007) reported that consumers with OR7D4 RT/WM and WM/WM genotypes were less sensitive or anosmic to androstenone than consumers with OR7D4 RT/RT genotypes. OR7D4 RT/RT consumers tend to be more likely to describe androstenone odour and/ or flavour as undesirable (De Kock *et al*., 2001). Gilbert and Wysocki (1987) performed a worldwide sensory trial to determine the consumer's ability to detect and identify various compounds, including androstenone. In this study it was found that women were more sensitive to this compound

than men. Furthermore it has been found that, for both men and women, as the concentration of androstenone increases, consumers tend to dislike the odour and/ or flavour more (Blanch *et al*., 2012; Font-i-Furnols, 2012). Consumers experience boar taint odours once pork and pork products are heated due to the compounds' volatile properties. Androstenone has been described as ruinous, sweaty and sexual (Font-i-Furnols *et al.*, 2003).

Across the world, efforts are made to ensure that pork and pork products from male pigs are not contaminated with androstenone. This has been made more challenging when the European Union (EU) announced that the use of surgical castration without the use of anaesthesia of male pigs will be unlawful from 1 January 2018 (Meinert *et al*., 2011; Haugen *et al*., 2012; Mörlein, *et al*., 2012; Bekaert, 2013). Countries have therefore started using strategies such as earlier slaughter and immunocastration to help ensure low levels of androstenone in male pigs. The Meat Hygiene Regulation 842/2004/EC of the EU states that if meat has a "pronounced sexual odour", the meat is no longer fit for human consumption. This is determined in abattoirs with the use of sensory evaluations on subcutaneous fat by trained individuals with the use of boiling water or, microwave heating and/or soldering irons (Whittington *et al*., 2011). However, in South Africa these detection techniques are not used and thus there is no discrimination against boar taint although the pork industry is well aware of the potential damage that the presence of this taint could have on pork consumption. Furthermore very few male pigs are castrated locally and therefore boar taint could possibly be present (Pieterse, 2006).

Cooking of pork and pork products is a very common practice in most parts of the world. Studies determining how consumers respond to various cooking processes of pork and also the flavour and/ or odour of the pork prior, during or after the cooking process have been performed where it was determined that various cooking methodologies could have an effect on the perception of boar taint found in pork (Font-i-Furnols *et al.* 2012). Font-i-Furnols *et al.* (2003) reported that in pork loins, the flavour was preferred to the odour. This could be due to androstenone and skatole not being very volatile compounds when not heated (Font-i-Furnols *et al.*, 2003). A percentage of these compounds do evaporate during cooking, causing boar taint sensitive consumers to complain about the repulsive odour during the cooking process (Claus *et al*., 1985). Babol *et al.* (2002) also reported that pork with high (mean androstenone 0.76 µg/g; mean skatole 0.24 µg/g) and medium (mean androstenone 0.37 µg/g; mean skatole 0.22 µg/g) concentrations of boar taint compounds received the lowest scores for liking of "cooking aroma" as well as "flavour".

De Kock *et al*. (2001) performed a sensory study on the acceptability of boar taint towards consumers of different ethnic groups found in South Africa. It was reported that as the concentration of boar taint compounds increased, the acceptance of the pork fat decreased. These results correlate with those of Pauly *et al.* (2008), Whittington *et al*. (2011), Font-i-Furnols *et al.* (2003) and

Bonneau *et al*. (1992) where consumers from different countries were evaluated. Pork with boar taint levels above the detection threshold tends to have sensory odour descriptors such as “faecal”, “boar”, “urine” and “perspiration” (Bonneau *et al*., 1992).

It is clear that many studies have been performed on consumer acceptance towards androstenone. It is also clear that when preparing pork, some sensitive consumers find the odour released from the pork unacceptable. However, research is limited on what happens to the concentration of boar taint within the subcutaneous fat during and after the cooking process. The aim of this study was therefore to determine how three commercial cooking processes (pan frying, oven baking and *sous vide*) affect the concentrations of boar taint within the intramuscular fat of pork chops (*Longissimus thoracis*). Preparing pork with a pan or in the oven are the two most popular methods of cooking this type of meat (Font-i-Furnols *et al.* 2012). However, the *sous vide* method is a relatively new preparation method which is generally used in the hotel industry. For this process, the meat is typically vacuum packed where after it is placed in a waterbath at a specific temperature for a predetermined amount of time.

The objective of this study was to determine how androstenone concentrations in the subcutaneous fat (in contact with the *Longissimus thoracis*) would react to different cooking methodologies (pan fries, oven roast and *sous vide*).

4.2 Materials and Methods

Prior to the preparation of the pork chops, HPLC-MS/MS-FD was performed on the subcutaneous fat of raw chops to select samples containing sufficient androstenone concentrations (Chapter 3). Hereafter, the 12 samples with the highest concentrations of androstenone were selected to prepare with the different cooking methodologies.

4.2.1 Sample preparation

All measurements done at the time of slaughter (farm of origin, warm- and cold carcass weight (kg), lean meat average (kg), fat thickness (mm) and PORCUS classification) were performed at the commercial abattoir according to a standard protocol. .Pork loins from the cranial (P2 area) towards the lumbar area (between 13th and 14th lumbar vertebrae) of 30 O-classified (13 – 17 mm subcutaneous fat depth; 68 – 69% lean mass in the carcass; < 100 kg) (Government Gazette, 1992) entire male pigs were taken from a commercial abattoir. The loins were labelled and frozen at -20°C and thereafter cut (with the use of a bandsaw) from the cranial towards the lumbar area into four 2.5 cm chops. The chop preparation allocation can be seen below in Table 4.. Pictures were taken of each chops in order to determine the volume and area of the subcutaneous fat as well as the contact area between the subcutaneous fat and the *Longissimus thoracis*. The pork chops were individually

packaged, vacuum packed and stored at -20°C until needed for chemical analysis or cooking. All chemical analysis where performed in duplicates.

Table 4.1 Chop preparation methodology allocation

Chop position	Chop 1	Chop 2	Chop 3	Chop 4
Preparation method	Raw	Pan fried	Oven roast	*Sous vide*

4.2.2 Contact area (cm^2) between subcutaneous fat and *Longissimus thoracis* determination and subcutaneous fat volume (cm^3) and area (cm^2) determination

Pork chops (2.5 cm thickness) were cut with a bandsaw from the cranial (P2 area) towards the lumbar area. After the chops were cut, pictures of each individual chop with a ruler was taken in order to determine the subcutaneous fat volume (cm^3) and area (cm^2) as well as the contact area between subcutaneous fat and *Longissimus thoracis.* Pictures were uploaded to ImageJ (1.36b) and analysed.

First, the scale was set using the ruler included in the picture to give the length of 1 cm. The *polygon selection* icon was selected where after the subcutaneous fat and the *Longissimus thoracis* muscle were individually measured for their areas. In order to determine the subcutaneous fat volume (cm^3), the area was first determined where after this value was multiplied by the thickness of the chop (2.5 cm). The contact area between the subcutaneous fat and *Longissimus thoracis* muscle were determined by selecting the *segmented line sections* icon. A line was then drawn where there was contact between the subcutaneous fat and *Longissimus thoracis* muscle.

4.2.3 Preparation methodologies

Pan frying

The pan was heated to a medium to high temperature (EUROGAS Stove, UN6611WI, Eikestad Furnishers, 5 Plein Street, Stellenbosch, Western Cape, 7600) where after the chop was sprayed with *Spray & Cook* anti-stick cooking spray, placed in the pan and covered with a lid. The chop was turned approximately every 60 s. Small amounts of water (approximately 5 mL) were added when the chop was turned to ensure that it did not burn. The chop was removed once a core temperature of 70 – 72°C (measured with a thermocouple probe which was attached to a digital temperature monitor (Hanna Instruments, Bellville, South Africa)) was reached where after it was allowed to cool for 20 min before being placed into individually numbered packages.

Oven roasting

An EUROAS Oven (UN6611WI, Eikestad Furnishers, 5 Plein Street, Stellenbosch, Western Cape, 7600) was heated to 150°C using both elements. Aluminium foil was folded into individual trays – this was done to ensure that the fluids of the pork chops do not come into contact with each other. The chops and 10 mL water was placed into the marked trays and placed into the oven. The temperatures of the chops were moderated and were removed once the core temperature reached 65 – 68°C (measured with a thermocouple probe which was attached to a digital temperature monitor (Hanna Instruments, Bellville, South Africa)). The chops were removed from the oven and were allowed to cool for 20 min before being placed into individually numbered packages.

Sous vide

A waterbath (SMC Waterbath; 200 Hz; 50/8 AMPS) was heated to 65°C. Vacuum packed pork chops were placed in the waterbath for 1 hour. Thereafter the temperature was increased to 70°C. After another hour the temperature was increased to 72°C and removed after 30 minutes and placed in an icebath to cool down for 10 min before being placed into individually numbered packages.

4.2.4 Proximate analysis

For Chop 1, the subcutaneous fat was removed, vacuum packed and placed into the -20°C freezer until needed for chemical analysis. The loin was cleared of all surrounding sinew where after the sinew was removed and the loin homogenised. The homogenised sample was thereafter vacuum packed and placed into the -20°C freezer until it was needed for chemical analysis. However, Chops 2 – 4 were first selected based on the fat's androstenone concentration (µg/g) and then prepared with the selected cooking methods (Table 1). After cooking, the surrounding sinew was thereafter removed and the pork meat was homogenised before the proximate analysis preparations could be performed.

The proximate (moisture, protein, lipid and ash) analysis of the pork meat and HPLC-MS/MS-FD (androstenone) analysis of the subcutaneous fat were conducted as described as described in Chapter 3.

4.2.5 Statistical Analysis

Mixed model repeated measures ANOVA was used to test for differences in preparation methods. The preparation methods were treated as main effects, and the individual pigs as random effect (random selection of pigs from a larger population). Fisher LSD was used for post hoc testing. In further analyses, a fixed farm effect was also included to test for possible differences between farms. Pearson correlation analysis was used for testing relationships between measurements.

4.3 Results

The changes in the chemical composition of the *Longissimus thoracis* after the different cooking methods are depicted in Table 4.4. As expected, the raw sample had the highest moisture content which differed from that derived from the different cooking methods used ($P \leq 0.05$). There was no difference between the moisture content of pan fried and *sous vide* chops which were as the two cooking methods resulting in the lowest moisture contents ($P > 0.05$). Samples that were oven baked had intermediate moisture content (67.0%) that differed from the other cooking methodologies used ($P \leq 0.05$). After cooking, the protein content of the *Longissimus thoracis* increased significantly. There was no difference in protein content between pan fried and *sous vide* or oven baked and *sous vide* methods ($P \leq 0.05$). The percentage fat in the *Longissimus thoracis* also increased significantly after cooking, however, none of the post-cooking values differed from each other ($P > 0.05$). The ash percentage of the raw-, pan fried- and *sous vide* cooking methods did not differ whilst that from the raw-, oven baked- and *sous vide* cooking methods also did not differ from each other ($P > 0.05$).

Table 4.4 The chemical composition and androstenone concentrations (of subcutaneous fat) (µg/g) (mean ± s.e.) of pork loin samples (*Longissimus thoracis*) prepared by different cooking methods

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Androstenone (µg/g)
Raw	$72.5^{a} \pm 0.39$	$24.9^{c} \pm 0.43$	$1.6^{b} \pm 0.23$	$1.2^{ab} \pm 0.038$	$0.75^{ab} \pm 0.107$
Pan fried	$64.9^{c} \pm 0.61$	$29.7^{a} \pm 0.61$	$3.6^{a} \pm 0.32$	$1.7^{a} \pm 0.22$	$0.49^{b} \pm 0.179$
Oven baked	$67.0^{b} \pm 0.53$	$28.2^{b} \pm 0.52$	$3.1^{a} \pm 0.48$	$1.2^{b} \pm 0.063$	$0.85^{ab} \pm 0.230$
Sous vide	$65.6^{c} \pm 0.39$	$29.2^{ab} \pm 0.39$	$3.6^{a} \pm 0.35$	$1.6^{ab} \pm 0.20$	$0.86^{a} \pm 0.335$

^{a,b,c} Values in columns within groups with different superscripts differ significantly ($P \leq 0.05$)

As pertaining to androstenone (µg/g) concentrations, there were no differences ($P > 0.05$) between raw, oven baked or *sous vide* samples or between raw, pan fried and *sous vide* methods (Table 4.4). However, there was a difference ($p = 0.046$) between pan fried and oven baked samples. Even though the majority of samples had no difference between androstenone concentrations, all methodologies exhibited androstenone concentrations above the threshold levels (> 0.45 µg/g).

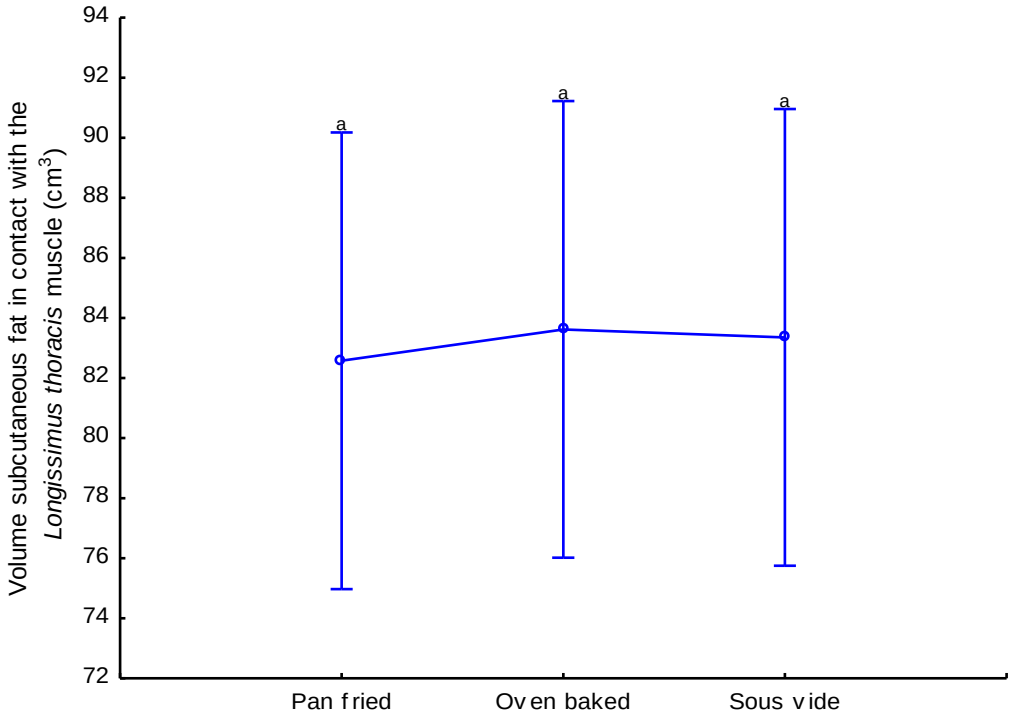

Figure 4.8 Comparison between the preparation methods used and the volume of the subcutaneous fat (cm^3) in direct contact with the *Longissimus thoracis* muscle.

As Table 4.4 indicated a difference between pan fried and oven baked methodologies ($p = 0.046$), it was investigated whether there was a difference in the fat volume (cm^3) of the samples used (

Figure **4.8**) as this could have influenced the amount of androstenone that could migrate from the subcutaneous fat into the *Longissimus thoracis* muscle due to the heat the hormone was exposed to and its lipophilic properties. However, there was no difference between the volume of the subcutaneous fat directly in contact with the *Longissimus thoracis* muscle ($p = 0.692$) indicating that fat volume had no influence on androstenone concentration (µg/g). It is also argued that androstenone can could transfer from the subcutaneous fat to the pork meat during preparation, however, no correlation was found in

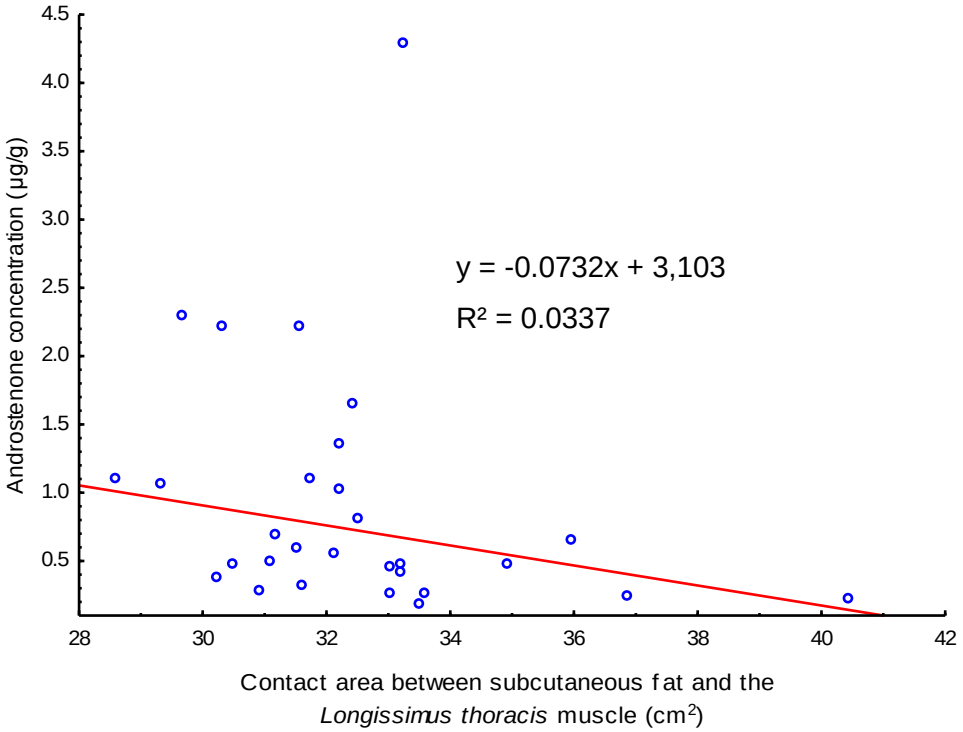

Figure 4.9 Correlation of the contact area (cm^2) between the subcutaneous fat and the *Longissimus thoracis* muscle (r = -0.184 p = 0.284).

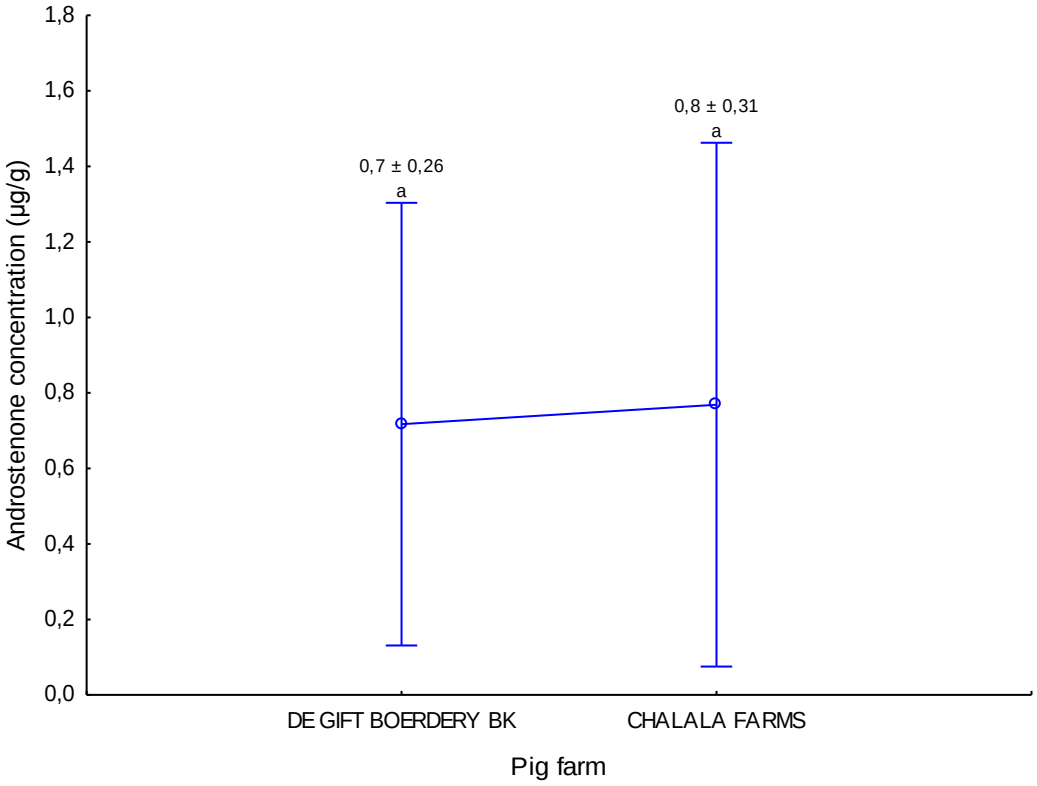

Figure 4.10 Comparison between mean (± s.e.) androstenone concentrations (µg/g) and the farms the pigs originated from (P ≤ 0.05).

It was also investigated whether the farm where the 12 selected pigs were produced on (and therefore possibly the genotype of the pig) had an influence on androstenone concentration (µg/g) (Figure 4.10). There was no difference between the concentrations (µg/g) of the farms that were compared ($p = 0.902$). This would indicate that there is probably no difference between the genotypes on the farms.

4.4 Discussion

Evaluating the chemical composition of the pork chops (*Longissimus thorasic*) and how these components reacted to different cooking methods (Table 4.4), it was interesting to find that oven baked chops had a lower moisture content than that of *sous vide* chops ($p < 0.001$). One would have expected higher moisture percentages from the *sous vide* chops because of the wet heat preparation methodology which allows for the chops to essentially be cooked in its own juices. It is possible that the water that was added to the moisture trays for the preparation of oven baked chops significantly added to, or decreased the loss of the moisture content of these chops. As expected the protein percentage of the prepared samples were higher than that of the raw samples ($P \leq 0.05$). The results indicated that the prepared pork chops seemed to follow the same inverse trend as the moisture percentages. The protein percentage increased as the moisture (%) decreased ($r = 0.668$; $P \leq 0.05$). Similarly, the fat percentage increased as the moisture (%) decreased ($r= 0.475$; $P \leq 0.05$).

Dehnhard *et al*. (1995) reported that as androstenone is a volatile compound, the concentration could reduce after the preparation process. This was however not found in the current study (Table 4.4). Furthermore, one would have expected that due to the moisture loss of samples (Table 4.4) after cooking, the androstenone concentration would have increased, but no clear trend for this was found. It was only established that pan frying resulted in a lower androstenone concentration when compared to oven baking ($p = 0.046$) (Table 4.4). This could have resulted due to the different heating methods; pan frying making use of direct dry heat via conduction whilst oven baking utilises indirect dry heat via convection. It is possible that due to the direct heat provided by pan frying (and thus the development of high temperatures between the pan surface and that of the meat), androstenone was more volatile and was thus lost to the surroundings. This would have possibly resulted in a more pungent odour which would still negatively affect consumers' perceptions (Font-i-Furnols *et al.*, 2003).

Another argument for the lower level of androstenone present in the subcutaneous fat post pan frying is that due to androstenone's lipophilic properties, under the heat the androstenone could have moved into the intramuscular fat in the *Longissimus* muscle. However, overall there were no major differences between the intramuscular fat percentages of the cooked pork (Table 4.4) ($P >$

0.05). Lundström *et al*. (2009) reported that the concentration of androstenone within the *Longissimus dorsi* muscle's intramuscular fat could be dependent on the lean meat content of the carcass.

If the androstenone is transferred into the muscle during the cooking process, two factors may have influenced the percentage of transference; the subcutaneous fat volumes (cm^3) which will influence the amount of androstenone present and the total subcutaneous fat area (cm^2) in contact with the *Longissimus thoracis* used for the different cooking methods. However, no differences or correlations were found for either fat volume (p = 0.692) (

Figure 4.8) or fat area (r = -0.184; p = 0.284) (

Figure 4.9) with androstenone concentrations. This indicates that there was no difference in the volume of fat (cm^3) of the samples used between the different cooking methods which could have influenced the differences in androstenone concentration found between the pan fried and oven baked preparation methods (p = 0.046) (Table 4.4). This further indicates that the decrease in androstenone concentration in pan fried pork is a direct result of the cooking method and not due to transference of androstenone from the subcutaneous fat into the muscle. It was also determined that the androstenone concentration in oven baked and *sous vide* pork increased from that of the raw sample (although this increase was not significant) (Table 4.4), this tendency could possibly be as a result of moisture loss.

It is important to note that the androstenone concentrations from all of the various preparation methods were all still above the sensory threshold level (> 0.45 µg/g). This would result in pork that is undesirable for androstenone sensitive consumers (De Kock *et al*., 2001; Font-i-Furnols, 2012). This undesirable experience would have been exasperated during the cooking process as some of the volatile androstenone could also have been given off as a gas resulting in an undesirable odour (Font-i-Furnols, 2003). Furthermore, consumer panel studies have clearly indicated a trend that when pork or pork fat contains higher concentrations of androstenone; consumers are likely to have a higher level of disliking towards the product (De Kock *et al*., 2001; Font-i-Furnols *et al*., 2009; Blanch *et al*., 2012; Font-i-Furnols, 2012).

4.5 Conclusions

It is extremely important to ensure pork and pork products with low levels of androstenone (< 0.31 µg/g) reach the market. This would help ensure that when products are prepared (in a restaurant or at home) the consumer always has a pleasant experience doing so.

In this study pork chops from the *Longissimus thoracis* was prepared with three different preparation methods namely; pan frying, oven baking and *sous vide*. The higher moisture content of the oven baked samples could be due to the addition of water into the aluminium trays resulting in either water uptake or less water being lost from the pork chop during cooking. It has been reported that when pork fat is heated, androstenone is released due to its volatile properties. However, limited research has been performed on the effect that various cooking methods have on androstenone levels. In the current study however, there was only a difference between pan fried and oven baked samples which could be due to the difference in the preparation heat that was applied; pan fried chops underwent a direct dry heat method which could have caused more androstenone compounds to evaporate and/or drip out from the fat than with the oven baking preparation method (indirect dry heat). However, this cooking method would have resulted in consumers experiencing off odours whilst preparing pork which effectively could have a negative effect on their perception towards pork.

4.6 References

AOAC (2002). AOAC Official Method 942.05. In: Official methods of analysis. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

AOAC (2002a). AOAC method 992.15. In: Official methods of analysis. Arlington, Virginia, USA: Association of Analytical Chemists Inc.

AOAC (2002b). Official Method 934.01. In: Official method of analysis. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

Babol, J., Squires, E.J. & Gullett, E.A. (2002). Factors affecting the level of boar taint in entire male pigs as assessed by consumer sensory panel. *Meat Science*, **61**(1), 33–40.

Bekaert, K.M., Aluwé, M., Millet, S., Goethals, K., Nijs, G., Isebaert, S., De Brabander, D.L., Verheyden, K., De Brabander, H.F., Verhaecke, L. & Tuyttens, F.A.M. (2012). Predicting the likelihood of developing boar taint: early physical indicators in entire male pigs. *Meat Science*, **92**(4), 382–385.

Bekaert, K. (2013). Chemical and sensory detection of boar taint. PhD Dissertation, Gent University, Belgium.

Blanch, M., Panella-Riera, N., Chevillon, P., Furnols, M.F.I., Gil, M., Gil, J.M., Kallas, Z. & Oliver, M.A. (2012). Impact of consumer's sensitivity to androstenone on acceptability of meat from entire male pigs in three European countries: France, Spain and United Kingdom. *Meat Science*, **90**(3), 572–578.

Bonneau, M., Denmat, M., Vaudelet, C., Veloso-Nunes, J.R., Mortensen, A.B. & Mortensen, H.P. (1992). Contribution of fat androstenone and skatole to boar taint I. Sensory attributes of fat and pork meat. *Livestock Production Science*, **32**, 63–80.

Claus, R., Schopper, D., Wagner, H.G. & Weiler, U. (1985). Photoperiodic influences on reproduction of domestic boars. 1. Light influences on testicular-steroids in peripheral- blood plasma and seminal plasma. *Zentralblatt Für Veterinarmedizin Reihe a Journal of Veterinary Medicine Series a-Animal Physiology Pathology and Clinical Veterinary Medicine*, **32**(2), 86–98.

De Kock, H.L., Van Heerden, S.M., Heinze, P.H., Dijksterhuis, B.B. & Minnaar, A. (2001). Reaction to boar odour by different South African consumer groups. *Meat Science*, **59**, 353–362.

Dehnhard, M., Claus, R., Herbet, E. & Hillenbrand, M. (1995). (Skatole and androstenone concentrations in products from boar meat). Die Ebermast, -Fragen und Konsequenzen bei der Produktion von Schweinefleisch. Schriftenreihe des Bundesministeriums fur Ernahrung. *Landwirtschaft unf Forsten,* **449**, 44-71 (in German).

Font-i-Furnols, M., Gispert, M., Diestre, A., & Oliver, M. A. (2003). Acceptability of boar meat by consumers depending on their age, gender, culinary habits, sensitivity and appreciation of androstenone smell. *Meat Science*, **64**, 433–440.

Font-i-Furnols, M., Gispert, M., Guerrero, L., Velarde, A., Tibau, J., Soler, J., Hortós, M., García-Regueiro, J.A., Pérez, J., Suárez, P. & Oliver, M.A. (2008). Consumers' sensory acceptability of pork from immunocastrated male pigs. *Meat Science*, **80**(4), 1013–1018.

Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: a review. *Meat Science*, **92**(4), 319–329.

Gilbert, A. & Wysocki, C. (1987). Results of the smell survey. *National Geographic*, **172**(4), 514–525.

Government Gazette, (1992). Regulation No. R. 1748. Agricultural Product Standards Act, 1990 (Act No. 119 of 1990). Regulations regarding the classification and marking of meat. Government Gazette No. 14060, 26 June 1992. Pretoria: Government Printer. South Africa.

Haugen, J.E., Brunius, C. & Zamaratskaia, G. (2012). Review of analytical methods to measure boar taint compounds in porcine adipose tissue: The need for harmonised methods. *Meat Science*, **90**(1), 9–19.

Keller, A., Zhuang, H., Chi, Q., Vosshall, L.B. & Matsunami, H. (2007). Genetic variation in a human odorant receptor alters odour perception. *Nature*, **449**(7161), 468–472.

Lee, C.M., Trevino, B. & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. Journal of AOAC International, **79**.

Lundström, K., Matthews, K.R. & Haugen, J.E. (2009). Pig meat quality from entire males. *Animal*, **3**(11), 1497-1507.

Meinert, L., Bejerholm, C., & Støier, S. (2011). Validation of "human nose" based method for boar taint detection. *57th International Congress of Meat Science and Technology*. Ghent, Belgium.

Mörlein, D., Grave, A., Sharifi, A. R., Bücking, M. & Wicke, M. (2012). Different scalding techniques do not affect boar taint. *Meat Science*, **91**(4), 435–440.

Pauly, C., Spring, P., O'Doherty, J.V, Ampuero Kragten, S. & Bee, G. (2009). Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac®) and entire male pigs and individually penned entire male pigs. *Animal: An International Journal of Animal Bioscience*, **3**(7), 1057–1066.

Pieterse, E. (2006). Effects of increased slaughter weight of pigs on pork production. PhD Dissertation, University of Stellenbosch, South Africa.

Verheyden, K., Noppe, H., Aluwé, M., Millet, S., Vanden Bussche, J. & De Brabander, H.F. (2007). Development and validation of a method for simultaneous analysis of the boar taint

compounds indole, skatole and androstenone in pig fat using liquid chromatography-multiple mass spectrometry. *Journal of Chromatography A*, **1174**(1-2), 132–137.

Whittington, F.M., Zammerini, D., Nute, G.R., Baker, A., Hughes, S.I., & Wood, J.D. (2011). Comparison of heating methods and the use of different tissues for sensory assessment of abnormal odours (boar taint) in pig meat. *Meat Science*, **88**(2), 249–255.

CHAPTER 5
General Conclusions and Recommendations

Boar taint is an offensive off-flavour and/or odour which is caused by androstenone, (5α-androst-16-en-3-one) (Patterson, 1968), skatole (3-methyl-indole) (Vold, 1970) and, to a lesser degree, indole (Garcia-Regueiro & Diaz, 1989). Androstenone, a male steroidal pheromone, is produced in the testes, liver and submaxillary glands (Zamaratskaia & Squires, 2009). This compound is not desired in male pigs because once the pork is heated, an androstenone sensitive consumer could experience unpleasant odours typically described as “faeces” and/ or “urine” (Babol et al., 2002). The most effective method to ensure androstenone is not present in entire male pigs is by the use of castration (Pieterse, 2006). Uncastrated male pigs have advantages such as a higher feed conversion, decreased production cost, as well as being faster growing with leaner meat (Blanchard *et al*., 1999; Rius *et al*., 2005; Andersen, 2006; Pauly *et al*., 2008), but these pigs also have their disadvantages. Uncastrated male pigs have been found to be more aggressive which could lead to injuries and carcass bruising, but there is a much greater chance that the carcasses will also have androstenone present at elevated levels (Horgan, 2006; Pieterse, 2006; Fredriksen *et al*., 2009).

In South Africa male pigs are generally not castrated and carcasses are priced according to sex, genotype and carcass weight and the PORCUS classification system which was established in 1992 (Pieterse, 2006). Presently, male carcasses are given a lower price than that of female carcasses due to, among others, the possible presence of androstenone. The study therefore aimed to determine whether the assumptions made by the PORCUS classification system are still warranted. Furthermore, a great deal of research has been performed on consumer acceptability and sensitivity towards androstenone, however, research is limited when analysing the reaction of this pheromone towards heating methods.

In the first research chapter, the chemical composition (moisture, protein, lipid and ash) of the *Longissimus dorsi* and androstenone concentration of the subcutaneous backfat of 172 male carcasses (44 P, 82 O and 46 R) were determined. The chemical composition was determined in order to find whether the intramuscular fat percentage increases with the increasing PORCUS classification, and therefore the calculated carcass lean meat percentage. Androstenone concentration was also determined to investigate whether the concentration thereof increases with increasing PORCUS classification. The results of this study showed that when chemically analysing the pork and pork fat, not many differences were found between the P-, O- and R-classified carcasses that were in line with the assumptions made in order to price pork carcasses. There was generally no significant differences between the chemical compositions of P- and O-carcasses (for moisture, protein, lipid or ash), P- and R-carcasses (for protein, lipid or ash), this being the main

indication that the assumptions made by the pork industry need to be revised. It was however interesting that the O- and R-carcasses differed (for moisture, protein and fat) from each other. O-carcasses were also found to be significantly heavier than that of the other carcasses. The differences in carcass weight could be due to differences in breeding, feeding or genotype differences. No differences were found between androstenone concentration for the P-, O- or R-classification groups. These results indicated that the PORCUS classification system could not penalise R-carcasses for obtaining a higher lean meat percentage than P- and O-carcasses. In fact, it was found that the androstenone concentration in the subcutaneous fat increases with carcass weight.

The results of the first study made it clear that the assumptions made in order to price a pork carcass are no longer applicable. A new carcass classification and pricing system is needed that will ensure that pig farms and abattoirs alike receive fair compensation for pork and pork products.

In the second trial it was examined how androstenone concentrations in the backfat above the *Longissimus thoracis* react to different preparation methodologies (pan fried, oven baked and *sous vide*). Androstenone is a volatile compound and is released, resulting in an off-flavour and/ or odour, when heated. This was done to emphasise how important it is to determine what consumers could be exposed to whilst cooking or ordering a meal in a restaurant.

It was very interesting to find that there was no significant difference between the androstenone concentrations of raw pork samples and prepared pork samples. But, there was a significant difference in androstenone concentrations between pan fried and oven baked chops. Pan frying makes use of direct dry heat whilst oven baking utilises indirect dry heat. The direct dry heat (pan frying) could have resulted in more of the compound evaporating due to its volatile propertied and a higher temperature being reached where the meat and pan makes direct contact.. Consumers that prepare pork with the use of pan frying could therefore possibly experience more off-odours than when making use of oven baking because more of the hormone is possibly lost into the air when using pan frying. It is also very important to note that the mean concentrations of androstenone stayed above the sensory detection level (> 0.45 μg/g) for all of the prepared samples. It was expected that the androstenone concentrations would have decreased in all samples due to its volatile properties. Therefore, it is possible that the concentrations remained high due to moisture loss in the subcutaneous fat, resulting in what appears to be still high concentrations of androstenone.

References

Andersen, J.R. (2006). Sorting criteria. Methods for on-line / at-line sorting of entire male castrated with emphasis on the Danish method based on skatole content. *Acta Veterinaria Scandinavica*, **48**(1), 20–22.

Blanchard, P.J., Ellis, M., Warkup, C.C., Chadwick, J.P. & Willis, M.B., (1999). The influence of sex (boars and gilts) on growth, carcass and pork eating quality characteristics. *Animal* Science, **68**(3), 487-493.

Babol, J., Squires, E.J. & Gullett, E.A. (2002). Factors affecting the level of boar taint in entire male pigs as assessed by consumer sensory panel. *Meat Science*, **61**, 33–40.

Fredriksen, B., Font-i-Furnols, M., Lundström, K., Migdal, W., Prunier, A, Tuyttens, F.A.M. & Bonneau, M. (2009). Practice on castration of piglets in Europe. *Animal*, ***3***(11), 1480-1487.

Garcia-Regueiro, J.A. & Diaz, I. (1989). Evaluation of the contribution of skatole, indole, androstenone and androstenols to boar-taint in back fat of pigs by HPLC and capillary gas chromatography (CGC). *Meat Science*, **25**, 307-316.

Horgan, R. (2006). Piglet castration and EU animal welfare legislation. *Acta Veterinaria Scandinavia*, **48**(1), 1–4.

Patterson, R.L.S. (1986). 5α-androst-16-ene-13-one: compound responsible for taint in boar fat. *Journal of the Science of Food Agriculture*, **19**, 31-38.

Pieterse, E. (2006). Effects of increased slaughter weight of pigs on pork production. PhD Dissertation, University of Stellenbosch, South Africa.

Pauly, C., Spring, P., O'Doherty, J. V, Ampuero Kragten, S., & Bee, G. (2008). Performances, meat quality and boar taint of castrates and entire male pigs fed a standard and a raw potato starch-enriched diet. *Animal*, **2**(11), 1707.

Rius, M., Hortos, M. & Garciaregueiro, J. (2005). Influence of volatile compounds on the development of off-flavours in pig back fat samples classified with boar taint by a test panel. *Meat Science*, ***71***(4), 595–602.

Vold, E. (1970). Fleischproduktionseigenschaften bei Ebern und Kastraten. IV. Organoleptisch und gaschromatografische Untersuchungen wassedampfflüchtiger Stoffe des Rückspeckes von Ebern. *Meldinger Nordlandbruckhoegskole*, **49**, 1-25 (in German).

Zamaratskaia, G. & Squires, E.J. (2009). Biochemical, nutritional and genetic effects on boar taint in entire male pigs. *Animal : An International Journal of Animal Bioscience*, **3**(11), 1508–1521.