The Influence of Feeding and Aging on Pork Quality

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Abstract

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The objective of the present thesis was to obtain a more basic understanding on how production factors such as feeding strategies and aging of the meat influence consumer-related pork quality attributes. Especially, *post mortem* colour characteristics of fresh pork and flavour development including warmed-over flavour in cooked pork as a function of feeding strategies have been further elucidated in the present thesis.

It was found that diet-induced progress in early *post mortem* muscle temperature development affects the colour and colour stability of pork during aging. Moreover, *post-mortem* colour development progressed differently in *M. longissimus dorsi* (LD) and in *M. semimembranosus* (SM). A gradual increase in the extent of blooming took place throughout aging in vacuum in the SM muscle; in contrast to a more complex colour progress during aging in the LD muscle. Moreover, discoloration was found to proceed faster in chops of SM compared to LD, especially after extended aging in vacuum. The observed diet and muscle effects might be coupled to the diet-induced change in *post mortem* temperature progress in the meat, which affects the activity of the inherent enzyme systems. Thus, dietary treatment and aging of the meat have a pronounced effect on colour and colour stability of pork.

Moreover, it was found that diets with different sources of vegetable oil influence the fatty acid composition in pork without any pronounced effect on overall meat quality. However, positive correlations between specific fatty acids in the phospholipid fraction of LD and single sensory traits in pork from female pigs were established with polyunsaturated fatty acids and C18:2n-6 correlating to fried meat odour and sweet odour and with monounsaturated fatty acids and C18:1n-9 correlating to piggy flavour and sourish odour in freshly cooked pork.

Finally, sensory attributes associated to warmed-over flavour and secondary lipid oxidation products formed during re-heating of cooked pork were positively correlated with gas-sensor responses (using electronic nose for detection of volatiles), and this supports the potential of e-nose systems as a quality control tool in the meat industry in the future.

Keywords: pork, diet, fresh meat colour, colour stability, aging time, fatty acid composition, meat flavour, warmed-over flavour, electronic nose

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Papers I-V

The present thesis is based on following papers, which will be referred to by their Roman numerals.

I. Tikk, K., Tikk, M., Karlsson, A.H. & Andersen, H.J. (2006). The effect of a muscle-glycogen-reducing finishing diet on porcine meat and fat colour. *Meat Science*, 73, 378-385.

II. Tikk, K., Lindahl, G., Karlsson, A. & Andersen, H.J. (2007). The significance of diet and aging time on pork colour and colour stability (submitted).

III. Tikk, K., Lindahl, G. & Andersen, H.J. (2007). The significance of slaughter weight, diet and aging time in vacuum on porcine meat colour (submitted).

IV. Tikk, K., Tikk, M., Aaslyng, M.D., Karlsson, A.H., Lindahl, G. & Andersen, H.J. (2007). Significance of fat supplemented diets on pork quality – connections between specific fatty acids and sensory attributes of pork. *Meat Science*, 77, 275-286.

V. Tikk, K., Haugen, J.E., Andersen, H.J. & Aaslyng, M.D. (2007). Monitoring of warmed-over flavour in pork using electronic nose – correlation to sensory attributes and secondary lipid oxidation products (submitted).

Kaja Tikk's contribution to the papers

I. Performed the colour measurements and chemical analysis, evaluated the results, and wrote the manuscript.
II. Performed the colour measurements and chemical analysis, evaluated the results, and wrote the manuscript.
III. Performed the colour measurements and chemical analysis, evaluated the results, and wrote the manuscript.
IV. Performed the analysis of fatty acid from back fat, tocopherols, glycogen, lactate, glucose-6-phosphate, IMP, inosine, hypoxhantine, evaluated the results, wrote the manuscript.
V. Planned the experiment, performed the sensory, chemical and electronic nose analysis, evaluated the results, and wrote the manuscript.

List of abbreviations

DFD	Dark, firm, dry
GC	Gas chromatograph
HPLC	High performance liquid chromatography
HS-GCMS	Headspace gas chromatography mass spectrometry
IMF	Intramuscular fat
IMP	Inosine 5'-monophosphate
LD	*M. longissimus dorsi*
Mb	Deoxymyoglobin
MbO_2	Oxymyoglobin
MetMb	Metmyoglobin
MetMbRA	Metmyoglobin reducing ability
MUFA	Monounsaturated fatty acids
NL	Neutral lipids
NO	Nitric oxide
NOS	Nitric oxide synthase
PL	Polar/phospholipids
PLSR	Partial least squares regression
PSE	Pale, soft, exudative
PUFA	Polyunsaturated fatty acids
RN	Rendement Napole
SFA	Saturated fatty acids
SM	*M. semimembranosus*
TBARS	Thiobarbituric acid reactive substance
WHC	Water-holding capacity
WOF	Warmed-over flavour

Introduction

Pork quality

The term "meat quality" can be defined in many ways. According to Andersen *et al.* (2005a) quality is to be considered a complex and multivariate property of meat, which is influenced by multiple interacting factors including the conditions under which the meat is produced. Production conditions include management system, breed, genotype, feeding, pre-slaughter handling and stunning, slaughter method, chilling and storage conditions. The main attributes of interest are safety, price, ethics, nutritional value, flavour, texture, water-holding capacity, colour, lipid content, lipid composition, oxidative stability and uniformity.

The most crucial quality attributes of importance for the consumer can be categorized into visible attributes e.g. overall appearance, colour and non-visible attributes e.g. flavour, texture/tenderness, juiciness (Bredahl, Grunert & Fertin, 1998). The appearance of meat is the most important factor known to determine consumer acceptability in the buying situation (Kim *et al.,* 1999) as the consumers relate the colour of the meat to appropriate quality and especially to the freshness of the meat. However, flavour and tenderness are often ranked as the important sensory attributes by consumers, and the presence of off-flavours and toughness is known to reduce the liking of pork (Bredahl, Grunert & Fertin, 1998; Bryhni *et al.,* 2002b). The development of all of these three critical quality attributes is influenced by several *ante mortem* interacting factors like age, breed, sex, nutritional status, stress level, fat content and composition and *post mortem* interacting factors like slaughter procedure, carcass handling including chilling, aging, etc. Therefore it is important to know how these mutually interacting production and slaughter factors together with storage conditions influence colour, flavour and tenderness considering optimal for control/improvement of these quality traits.

In pork production, feeding strategy is a main management factor, as it influences many of the above mentioned critical quality attributes. Consequently, strategic feeding is an appropriate quality control tool in the production of meat. Elucidation of appropriate feeding strategies and their interaction with aging time, which is known to be another critical and easily implementable quality control tool, is highly interesting in attempt to control colour, flavour and tenderness characteristics of meat, as this could contribute to higher consumer preferences for pork. This introducing part of the thesis outlines the present knowledge determined/identified to be important for these three main eating quality attributes in pork: colour, flavour and tenderness.

Pork colour

Myoglobin is the primary meat pigment responsible for meat colour, and it exists as purple red deoxymyoglobin (Mb), bright red oxymyoglobin (MbO2) or brownish metmyoglobin (MetMb). The distribution and amount of the myoglobin species Mb, MbO2 and MetMb together with internal reflectance influence the colour of pork (Ledward, 1992). Under low oxygen pressure conditions (e.g. immediately after cutting the meat or in vacuum-packed meat), the myoglobin is in the purple reduced form Mb. Very low oxygen tension (<1.4 mm Hg; Brooks, 1935) is required to maintain myoglobin in a deoxygenated state. Upon exposure to oxygen the fresh meat surface changes colour from purple to bright cherry-red. This is termed as blooming, where Mb becomes oxygenated to MbO2 (Govindarajan, 1973). As exposure to oxygen increases, the oxymyoglobin penetrates deeper beneath the surface of the meat. Depth of oxygen penetration and thickness of the oxymyoglobin layer depend on the temperature of the meat, the oxygen partial pressure, the pH and the competition for oxygen by other respiratory processes (Ledward, 1992; Mancini & Hunt, 2005). Blooming is more efficient under conditions that increase oxygen solubility and discourage enzyme activity.

Upon oxidation of Mb/MbO_2 to MetMb, discoloration takes place, as change in the redox state of the iron also change the colour from red to brownish (Satoh & Shikama, 1981; Wallace *et al.,* 1982; Shikama, 1998). Although discoloration is often referred to as the amount of surface area covered by metmyoglobin, the subsurface myoglobin forms also play a role in product appearance. This is so because metmyoglobin beneath the surface (located between superficial oxymyoglobin and interior deoxymyoglobin) gradually thickens and moves towards the surface (Mancini & Hunt, 2005). Metmyoglobin formation depends on numerous factors including oxygen partial pressure, temperature, pH, meat's reducing activity and in some cases, on microbial growth (Mancini & Hunt, 2005). However, another important factor for meat colour is the metmyoglobin-reducing activity/system (MetMbRA), which can reduce brownish MetMb back to the reduced and subsequently oxygenated red pigment. This reduction depends on the oxygen-scavenging enzymes, reducing enzyme systems and the NADH pool of the muscle, which is limited in the *post mortem* muscle and gets continuously depleted as time *post mortem* progresses. Thus, the rate of discoloration of meat is believed to depend on both oxidative processes and enzymatic metmyoglobin-reducing systems (Faustman & Cassens, 1990). Moreover, the formation/presence of aldehyde lipid oxidation products can decrease both oxymyoglobin stability and the likelihood of metmyoglobin reduction via enzymatic processes (Lynch & Faustman, 2000). Likewise, Connolly, Brannan & Decker (2002) found that peroxynitrite, formed via nitric oxide synthase (NOS) induced nitric oxide (NO), can induce rapid and extensive oxymyoglobin oxidation even at lower concentrations of peroxinitrite than that needed to catalyze lipid oxidation. It is also known that peroxynitrite promotes discoloration of oxymyoglobin at decreasing pH and increasing temperatures (Connolly, Brannan & Decker, 2002).

Production factors influencing colour and colour development

As mentioned previously, several production factors influence pork colour. In relation to fresh meat colour in pork, some of the most crucial factors are outlined below.

Breed and genetic background

In relation to the impact of the genetic background on pork colour, the recent work has focused on differences between breeds and genotypes, e.g. the influence of the Halothane and RN^- alleles. The loins from Duroc pigs have been found to be darker (Lindahl *et al.,* 2006b, d) and more red than loins from Landrace pigs, which is most likely due to a higher ultimate pH (Candek-Potokar, Zlender & Bonneau, 1998a; Lindahl *et al.,* 2006d) or a higher pigment content in pork from Duroc (Newcom *et al.,* 2004; Lindahl *et al.,* 2006b). Similarly, Edwards, Bates & Osburn (2003) found that Duroc progeny had more favourable visual colour, higher pH and redness than Pietrain-sired pigs. Breed effects on pinkness and *a** value are also noted for Duroc, Duroc/Landrace, Pietrain, Duroc/Hampshire, Large White, Hampshire and Berkshire pigs (Brewer *et al.,* 2002, 2004). Furthermore, fresh pork colour is reported to be influenced by the presence of the halothane and RN alleles. Fabrega *et al.* (2002) reported that pigs that were carriers of the halothane allele (Nn) had loins that were more red than homozygous NN pigs because of an increased oxymyoglobin concentration. Loins from carriers of the RN^- allele were more red than loins from non-carriers, and this might be associated with a slightly higher pigment content (Bertram, Petersen & Andersen, 2000). Lindahl *et al.* (2004) reported that the RN^- allele gave higher redness and yellowness, and this was explained by differences in the distribution of the myoglobin derivates, which is mainly determined by pH-related oxygen consumption rate, myoglobin autoxidation rate and MetMb reductase activity.

Gender

The effect of gender on colour characteristics has been investigated in several studies. None of these studies have been shown to have any effect of gender on the pork colour (Barton-Gade, 1987; Hammell, Laforest & Dufor, 1994; Lindahl *et al.,* 2001; Latorre *et al.,* 2004; Correa *et al.,* 2006; Mörlein *et al.,* 2007; Tikk *et al.,* 2006a, 2007a). However, Warris *et al.* (1990) reported a higher a*value and a lower hue angle, but no difference in L*-, b* value and chroma in gilts compared to castrates. This difference was related to a higher pigment concentration in gilts compared to castrates, but the variation was relatively small. Thus, the effect of gender on pork colour characteristics is not likely to be commercially important at retail level.

Muscle

The studies related to pork colour in different muscles have revealed that the differences in e.g. redness between various pork muscles can be related to the incidence of oxidative (i.e. aerobic) and glycolytic (i.e. anaerobic) muscle fibers. Thus, red muscles have high oxidative activity and white muscles high glycolytic

activity (Beecher *et al.,* 1965). Muscles generally defined as red include the shoulder muscles *supraspinatus* (SS), *infraspinatus* (IS), *triceps brachii* (TB), the ham muscle *rectus femoris* (RF) and the loin muscle *psoas major* (PM). Muscles of the pork carcass generally defined as white include the *longissimus lumborum* (LL), the ham muscles *gluteus medius* (GM) and *semimembranosus* (SM) and the lateral portion of the *semitendinousus* (ST) (Beecher *et al.,* 1965). The *biceps femoris* (BF) has been described as being red on the inside and white on the outside (Beecher *et al.,* 1965).

In relation to lightness of pork, the ham muscles GM, ST and the loin muscle LL have been found to be significantly lighter (higher L* value) than the shoulder muscles TB, SS, IS, the ham muscle RF and the loin muscle PM; the ham muscles SM and BF had intermediate values (Warner, Kauffman & Russell, 1993).

Moreover, a comparison of the pigment content of white and red muscles clearly showed that red muscles have a higher concentration of pigment (Warner, Kauffman & Russell, 1993; Lindahl *et al.,* 2001). Colour stability is generally poorer in red muscles compared to white muscles.

Feeding

Pigs are mono-gastric animals and many dietary components, including vitamins and mineral supplements, are consequently readily transferred from the feed to the muscle and fat tissues, which subsequently may affect pork quality.

Supplementation with nutrients

An extensive number of studies on dietary supplementation with nutrients i.e. magnesium, vitamin D_3, vitamin E, vitamin C and creatine have been carried out in relation to pork colour (Asghar *et al.,* 1991; Monahan *et al.,* 1992, 1994; Lanari, Schaefer & Scheller, 1995; Cannon *et al.,* 1996; Jensen *et al.,* 1997; Hoving-Bolink *et al.,* 1998; Gebert *et al.,* 1999; Apple *et al.,* 2000; Berg & Allee, 2001; Hamilton *et al.,* 2002; Maddock *et al.,* 2002; Wiegand *et al.,* 2002; Geesink *et al.,* 2004; Wilborn *et al.,* 2004; Lindahl *et al.,* 2006d).

The numerous studies related to the effect of vitamin E on pork colour are not conclusive. The studies have shown that feeding supra-nutritional levels of vitamin E improves meat colour, colour stability and can extend shelf-life of pork (Asghar *et al.,* 1991; Monahan *et al.,* 1992, 1994; Lanari, Schaefer & Scheller, 1995; Hoving-Bolink *et al.,* 1998). Moreover, supplementing pig diets with at least 200 IU kg^{-1} of vitamin E reduces lipid oxidation (Jensen *et al.,* 1997) and enhances colour stability (Lanari, Schaefer & Scheller, 1995), however, several studies reported no concomitant improvements or only a limited effect on pork colour stability (Buckley, Morrissey & Gray, 1995; Cannon *et al.,* 1996; Jensen *et al.,* 1997; Zanardi *et al.,* 1999; Phillips *et al.,* 2001; Rosenvold & Andersen, 2003b; Geesink *et al.,* 2004). Jensen *et al.* (1997) suggested that the beneficial effect of added vitamin E on muscle colour depends on the relative vitamin E level in the muscles of the control animals. Where this is above a critical level (e.g. 3.5 µg/g), no beneficial effect is seen, which is in contrast to low levels, where it improves colour retention. Consequently, Lanari, Schaefer & Scheller (1995) noted that the

improvement in colour stability of pork muscle caused by dietary vitamin E supplementation was not as profound as has been reported for beef muscle.

Short term vitamin D_3 supplementation has been reported to result in a darker (Wiegand *et al.,* 2002; Wilborn *et al.,* 2004) and more red pork colour (Wiegand *et al.,* 2002). The mechanism behind was suggested to be an increased oxidative muscle metabolism and hereby a decreased rate and extent of pH decline. However, Swigert *et al.* (2004) found no effect of dietary supplementation with vitamin D_3 on pork colour.

Dietary magnesium supplementation has been reported to improve pork colour (D'Souza *et al.,* 1999; Apple *et al.,* 2000; Hamilton *et al.,* 2002). Supplementing pig diets with magnesium mica improved colour of *longissimus* by increasing *a** and chroma (hue decreased), but showed no effect on *L** or *b** value (Apple *et al.,* 2000). However, Hamilton *et al.* (2002) reported that diets with magnesium did not influence *longissimus a** and *b** value, but darkened the loin colour. This may be due to the fact that magnesium can minimize stress before slaughter, influence intracellular calcium gradients and thus decrease the incidence of PSE meat (Frederick, van Heugten & See, 2004). Other studies have not been able to confirm this improved effect of magnesium supplementation on pork colour (Geesink *et al.,* 2004; Swigert *et al.,* 2004).

In conclusion, the results obtained in supplementation studies including various nutrients are often inconclusive regarding pork colour. However, supplementing pig diets with supra-nutritional levels of vitamin E has shown some advantages regarding pork colour display life and a reduced oxidative deterioration of meat. While no negative effects of vitamin E supplementation have been reported on feed intake or the performance of the pigs, the entire production and processing sections of the meat industry might gain from including supranutritional vitamin E in the diets of pigs (Dikeman, 2007).

Strategic finishing feeding

In recent years, there has been a growing interest in controlling the muscle glycogen stores at the time of slaughter, as glycogen plays a crucial role as substrate for energy metabolism of the living muscle and as well as in the *post mortem* metabolism, during which time the muscle is converting to meat (Rosenvold & Andersen, 2003a; Rosenvold, Essen-Gustavsson & Andersen, 2003c; Andersen *et al.,* 2005a). The anaerobic metabolism of glycogen results in the formation of lactate and a simultaneous decline in pH (Hamm, 1960; Bendall, 1973; Offer, 1991; Offer & Cousins, 1992). The reason for manipulating the muscle glycogen stores at the time of slaughter is that it plays decisive role for the meat quality attributes, mainly through its effect on pH progress and thereby on water-holding capacity (WHC) and colour (Briskey, 1964; Rosenvold *et al.,* 2001a, b; Tikk *et al.,* 2007a).

Muscle glycogen stores in *M. longissimus dorsi* at the time of slaughter can be reduced by feeding the pigs with diets that are high in fat (≈17–18%) and protein (22–24%) and have a low content of digestible carbohydrates (<5%) during the last three weeks before slaughter without influencing the overall growth performance,

but improving WHC of *M. longissimus dorsi*, *M. biceps femoris* and *M. semimembranosus* (Rosenvold *et al.,* 2001a, b, 2002). In contrast, Bee (2001) showed that a minor reduction in the digestible carbohydrate content with a simultaneous iso-energetic supplement in fat has no influence on *post mortem* glycogen stores in porcine *M. longissimus dorsi* and hereby on WHC and the colour of meat. Likewise, Leheska *et al.* (2002) found no effect on *post mortem* glycolytic potential or pork quality attributes, including colour and WHC, upon feeding a low digestible carbohydrate/high protein diet during the last two weeks prior to slaughter. Hence, feed-induced reduction in muscle glycogen content, which affects $pH_{45\ min}$ and WHC in the porcine muscle, seems to claim a critical ratio between fat and digestible carbohydrate in the diet (Rosenvold & Andersen, 2003a) and the finishing feeding period before slaughter (Rosenvold *et al.,* 2001a). The minimal levels of muscle glycogen stores were achieved by feeding the finishing diets three weeks before slaughter. Upregulation to normal glycogen levels occurs above a time period of three weeks (Rosenvold *et al.,* 2001a).

Preliminary colour evaluations by means of strategic feeding have shown that strategic finishing feeding of pigs with reduced muscle glycogen stores at the time of slaughter tended to darken *longissimus* colour (Rosenvold *et al.,* 2001a, b).

Considering the possibility of controlling the levels of muscle glycogen stores at the time of slaughter by strategic finishing feeding and the subsequent effect on muscle metabolism *post mortem*, this feeding strategy can be a potential way to control colour development in pork.

Pre-slaughter handling

Several studies have shown that poor pre-slaughter handling causes stress of the animals which furthermore can cause adverse effects on pork quality (Faucitano, 1998; Warris *et al.,* 1998; van der Wal, Engel & Reimert, 1999). Pre-slaughter treatment has an effect on temperature and pH development in the muscle post mortem (Milligan *et al.,* 1998; van der Wal, Engel & Reimert, 1999; Hambrecht *et al.,* 2004a, b), which has been shown to be crucial for pork colour (Rosenvold & Andersen, 2003b; Hambrecht *et al.,* 2004a, b; Lindahl *et al.,* 2006c). Stress before slaughter induces a higher muscle temperature and a lower pH early post mortem (Lindahl *et al.,* 2006c). These changes in muscle metabolism have been reported to result in lighter (Hambrecht *et al.,* 2004a, b), more red and more yellow pork colour (Milligan *et al.,* 1998; Lindahl *et al.,* 2006c). The increase in early post mortem muscle temperature has been reported to promote denaturation of proteins involved in oxygen consumption, and it resulted in a greater surface oxygenation due to less competition for oxygen by enzymes (Rosenvold & Andersen, 2003b; Lindahl *et al.,* 2006c).

Weight of animals

Few studies have investigated the influence of slaughter weight on pork colour characteristics. Higher *a** and *b** values at carcass weights from 70 kg to 100 kg were found by Beattie *et al.* (1999). Likewise, García-Macías *et al.* (1996) found an increased pigment content and due to this increased a* and b* values by

increasing the live weight from 90 kg to 120 kg. Latorre *et al.* (2004) found that the LD from pigs slaughtered at 133 kg was significantly darker (lower L* values), redder (higher a* value) and had a significantly higher myoglobin content than the LD of pigs slaughtered at 116 and 124 kg. In contrast, Correa *et al.* (2006) did not find significant differences on colour characteristics from pigs with a slaughter weight between 107 kg and 125 kg. Therefore, most of the studies have shown some influence of weight on the pork colour. By increasing the slaughter weight, the animal age is also often increased, and this may affect the pigment content, which increases redness in pork.

Aging

Meat is often pre-packed and aged prior to retail display. With the exclusion of oxygen from the package, the colour does not change during storage; however, this storage has an effect on the subsequent blooming, resulting in an increased lightness, redness and yellowness of the pork (Zhu, Bidner & Brewer, 2001; Lindahl *et al.,* 2006a, b). This is due to the fact that enzymatic activity of meat decreases continuously as a function of time *post mortem*, which is why aging in vacuum prior to exposure to oxygen has been found to accelerate both the rate and the degree of blooming in both beef (Ledward, 1992) and pork (Zhu, Bidner & Brewer, 2001; Lindahl *et al.,* 2006b).

Moreover, during extended retail display under aerobic storage conditions, discoloration of the meat takes place and the surface of the meat changes from bright cherry-red to greyish-brown. This colour change is due to the oxidation of red oxymyoglobin (MbO_2) to brown metmyoglobin (MetMb) (Lindahl *et al.,* 2006a), and the reaction generally proceeds in parallel to rancidity. The accumulation of MetMb on the surface of the meat decreases redness, tends to increase lightness, whereas yellowness remains constant (Rosendvold & Andersen, 2003b; Lindahl *et al.,* 2006a, b, d). The discoloration rate following prior-aged meat might be higher compared to non-aged meat due to the decreased MetMb-reducing activity over time *post mortem* (Ledward, 1992; Zhu & Brewer, 1998) and to the loss of mitochondrial structure (Tang *et al.,* 2005). Even limited accumulation of the oxidized meat pigment MetMb has a pronounced impact on consumer perception as they associate such an accumulation with non-fresh products (Johnson *et al.,* 1990; Kim *et al.,* 1999).

In conclusion, these data clearly show that feeding together with biological factors and subsequent storage conditions influences meat colour. This is supported by the fact that small differences in early *post mortem* pH/temperature process have been reported to affect colour development by influencing the activity of oxygen-consuming/metmyoglobin-reducing enzymes, denaturation of proteins and enzymes, which is known to be decisive for meat colour characteristics (Zhu & Brewer, 1998; Rosenvold & Andersen, 2003b; Bekhit & Faustman, 2005; Lindahl *et al.,* 2006c; Tikk *et al.,* 2006a, 2007a, b). Thus, any factor which could alter early *post mortem* progress in meat is of great importance for obtaining the appropriate set of tools to optimize meat colour development.

Pork flavour

Flavour is one of the most important quality attributes contributing to the overall eating quality of meat. Raw meat flavour is almost negligible and can mostly be attributed to a blood-like taste. The flavour of meat first develops during the cooking process, where inherent lipid- and water-soluble raw meat components act as essential flavour precursors (MacLeod, 1986). These flavour precursors include reducing carbohydrates, fatty acids, phospholipids, peptides, vitamins, ribonucleotides e.g. inosine monophosphate (IMP) and its secondary degradation products inosine and hypoxanthine (Baltzer, Santora & Landmann, 1962; Hartman *et al.,* 1984; Guentert *et al.,* 1990; Mottram, 1991; Güntert *et al.,* 1992; Mottram & Madruga, 1994; Madruga & Mottram, 1995a, b), which all have been shown to influence the flavour development in cooked meat (Madruga & Mottram, 1995a; Farmer, Hagan & Paraskevas, 1996; Aliani & Farmer, 2001). Considering the nature of these inherent flavour components, the overall flavour of meat can be influenced by oxidative processes triggered thorough storage and cooking, lipid content, feeding/diet, post mortem pH progress, ultimate pH etc. (Calkins & Hodgen, 2007). All together this makes meat flavour an incredibly complex area**.**

In the following is given a brief introduction to how main flavour pre-cursors may contribute to pork flavour formation.

Most of the research that has been carried out to understand the chemistry of meat flavour and to determine main factors of importance for flavour quality has been carried out in model systems. Such model experiments have indicated that the reaction between amino acids and reducing sugars (Maillard reaction chemistry) is one of the main pathways in the formation of many of the aroma compounds, which have been identified in cooked meat (Salter, Mottram & Whitfield, 1988). However, thermal degradation of the individual meat components, e.g. thiamine, oxidation of lipids, reactions between Maillard reaction products and phospholipids together with other interactions between pre-cursors and reaction intermediates are also meant to have influence on the overall flavour formation in meat (Chen & Ho, 1998).

The flavour compounds found in meat include a broad array of chemical compounds. These include hydrocarbons, aldehydes, ketones, alcohols, furans, thriphenes, pyrrols, pyridines, pyrazines, sulfurus compunds and many others (Ho, Oh & Bae-Lee, 1994). Many of these are formed during the heating process, while others first become evident during subsequent storage of the meat and/or after reheating of the product (e.g. lipid oxidation products). Especially, heterocyclic compounds containing sulfur produced in Maillard reactions are important flavour compounds, as they provide savoury, meaty, roast and boiled flavours (Shahidi, 1989; Mottram & Madruga, 1994). Likewise, many lipid-associated compounds also contribute to desirable cooked meat flavour, even though many of these compounds can oxidize further and result in rancid off-flavours during storage (Mottram, 1998a).

The odour threshold of many lipid-derived compounds is generally higher than the water-soluble precursors, e.g. sulphur- and nitrogen-containing heterocyclic

compounds, why the latter dominates meat flavour (Farmer & Patterson, 1991; Mottram, 1998). However, several of the unsaturated fatty acids, such as linoleic and arachidonic acid, rapidly auto-oxidize to hydroperoxides and further degrade to e.g. 2,4-decadienal, 2-nonenal, 1-octen-3-one, 2,4-nonadienal, and 2-octenal, that have similar threshold values as the sulphur compounds and hereby contribute to meaty flavour, e.g. fatty and tallowy (Mottram, 1998b; Calkins & Hodgen, 2007).

The concentration of flavour pre-cursors in meat has also been found to be of importance for the flavour formation in meat. Thus model studies have shown that high concentrations of ribose and glucose 6-phosphate can react during cooking and form volatiles, which increase the intensity of roasted and/or meaty odours in pork (Farmer, Hagan & Paraskevas, 1998). This was recently confirmed in fried pork, which resulted in more intense fried flavour, when it contained natural high levels of glucose and glucose 6-phosphate compared to pork with low content of these carbohydrates (Meinert *et al.,* 2007a). Likewise, has addition of inosine monophosphate (IMP) to pork shown to increase the "meaty" and "roasted" aromas (Farmer, Hagan & Paraskevas, 1996, 1998), and a recent study showed that natural high concentrations of hypoxanthine, which is a degradation product of IMP, coincided with an increased bitterness of pork (Tikk *et al.,* 2006b).

Production factors influencing flavour and flavour development

As mentioned above flavour formation of meat is highly dependent of the composition and concentration of inherent muscle components, why production factors influencing composition and concentration of these will have directly influence on flavour formation in pork. In the following are mentioned some of the production factors shown to be of importance for pork flavour formation.

Breed and genetic background

The few studies on the effect of breed on flavour are not conclusive. Pork from Duroc has been reported to have poorer flavour and being less acceptable than Landrace meat despite being more juicy (Cameron *et al.,* 1990). This was suggested to be due to the particular fatty acid composition of the subcutaneous fat. However, a study by McGloughlin *et al.* (1988) did find that meat from Duroc crosses had higher flavour and flavour intensity scores compared to meat from Large White and Landrace breeds. Moreover, has meat from Hampshire crosses carrying the RN^- allele been reported to have desirable flavour characteristics, which is meant to be explained by the higher muscle glycogen content and simultaneous formation of the glycolytic intermediates, e.g. glucose-6-phosphate (Lundström, Andersson & Hansson, 1996).

Gender

Even though the potential effect of gender on flavour characteristics has been investigated in several studies, no conclusive evidence is available from these studies (Edwards *et al.,* 1992; Wood *et al.,* 1995; Ellis & McKeith, 1995; Ellis *et al.,* 1996; Tikk *et al.,* 2007c). However, a few studies reported some minor differences, and a recent study reported that pork from females had a more

pronounced piggy odour compared to castrates (Meinert *et al.,* 2007b). Moreover, loin roasts from barrows has been reported to have superior flavour and being tenderer than roasts from gilts (Martel, Minviellc & Poste, 1988).

Muscle and weight of animals

Only few studies have been investigating potential difference in flavour between muscles. However, *M. psoas major* has been found to have higher pork flavour and overall liking scores than *M. longissimus dorsi* (Wood *et al.,* 2004). This might be explained by the fact that the metabolic activity and nature of red muscles will result in higher concentration of several flavour pre-cursors. However, more studies are needed to draw any conclusions.

Even though flavour characteristics of meat from animals with different carcass weight have been studied, none of these studies have been shown to have any effect of carcass weight on the flavour characteristics (Ellis *et al.,* 1996; Bejerholm & Aaslyng, 2003).

Feeding

Pigs are mono-gastric animals and many dietary components are consequently readily transferred from the feed to the muscle and fat tissue. Furthermore, it has been shown that feed affects muscle glycogen stores at the time of slaughter (Rosenvold *et al.,* 2001a, b) and hereby concentration and rate of formation of several flavour pre-cursors, e.g. glucose-6-phosphate and IMP and its degradation products. Consequently, feeding plays an important role in flavour formation, as any feed that influences the concentration of flavour precursors or deposits unique components in the meat and fat will affect the cooked meat flavour (Melton, 1990).

Beside feed containing components given rise to off-flavour, e.g. fishmeal (Hertzman, Göransson & Ruderus, 1988), especially the fatty acid composition of the feed has been in focus (Wood & Enser, 1997; Mourot & Hermier, 2001; Wood *et al.,* 2003).

The increased interest in recent years to manipulate the fatty acid composition of the feed and subsequently the fatty acid composition of meat has been driven by the interest in production of healthier pork. However, the fatty acid composition of meat also has an effect on taste, shelf-life (oxidative stability) and the technological quality of the pork. Saturated and monounsaturated fatty acids are *de novo*-synthesized, hence their concentrations are less readily influenced by diet. In contrast, the polyunsaturated fatty acids linoleic (C18:2n-6) and α-linolenic (C18:3n-3) cannot be synthesized *in situ*, thus tissue concentrations rapidly respond to dietary changes (Wood, 1984). The effect of fatty acids on meat flavour is mainly due to the production of volatile, flavour active lipid oxidation products during cooking and the subsequent formation of additional flavour compounds during reaction of lipid oxidation products and Maillard reaction products. Especially, unsaturated phospholipids have been shown to be important in flavour development (Mottram, 1996; Cameron *et al.,* 2000; Wood *et al.,* 2003, Tikk *et al.,* 2007c).

Most of the basic information on understanding how different fatty acids may contribute to meat flavour has been obtained in model systems (reactions between fatty acids, cysteine and/or ribose upon heating) studying the effects of the individual fatty acids on the formation of flavour active volatiles (Campo *et al.,* 2003). In general, the study has shown that odour scores are often more intense for C18:3 fatty acid than for C18:2 fatty acid, and that fatty acids C18:1 and C18:2 are contributing mainly with oily notes, while C18:3 fatty acids contribute to formation of different flavour attributes, e.g. fishy, linseed/putty (Campo *et al.,* 2003). However, meaty aromas become much more pronounced in the presence of cysteine and ribose. In general, the various reactions and interactions involved in overall flavour formation are relative complex to investigate.

Modification of the composition and concentration of unsaturated fatty acids in porcine muscle through different feeding procedures on flavour formation in cooked pork has given contradictory results. Thus improved palatability of pork has been reported upon increasing concentrations of oleic acid (C18:1) (Rhee *et al.,* 1990), while increased levels of oleic acid through feeding of peanuts to pigs also have been reported not to influence pork flavour (Myer *et al.,* 1992). Muscles containing high concentrations of C18:2 readily oxidize upon heating and result in the production of volatile compounds, including the aldehydes pentanal and hexanal, which all are known to contribute to typical oxidized flavour notes (Ladikos & Lougovois, 1990). However, several studies have not shown significant differences in the flavour of pork made from muscles with low or high concentrations of C18:2 (Hartman *et al.,* 1985; West & Myer, 1987; Larick *et al.,* 1992). Melton (1990) concluded that pork high in C18:2 is normally not recognized to give rise to off-flavour notes, as the formed oxidation products of C18:2 are considered as natural pork flavour components by consumers. Supplementing pig diets with rapeseed oil and especially linseed oil, which are good sources of C18:3, has been investigated by several researches. No effects on meat quality parameters have been observed (Leskanich *et al.,* 1997; Enser *et al.,* 2000), but solid evidence exists regarding formation of off-flavours in processed (re-heated, cured, comminuted, freezed etc.) pork from pigs supplemented with high concentrations of C18:3 (Shackelford *et al.,* 1990; Myer *et al.,* 1992). A possible explanation for these results depends on the level of C18:3 achieved in the fat or muscle tissue. Only C18:3 levels above about 3% of the total fatty acids in the porcine muscles give rise to C18:3-associated oxidation products, if the processing conditions accelerate lipid oxidation, which has an adverse impact on the flavour of cooked products (Wood *et al.,* 2003).

Cooking

Eating quality characteristics including flavour formation in pork are highly influenced by cooking conditions, e.g. final internal temperature of the meat and cooking/frying temperatures (Mottram, 1985; Simmons, Carr & McKeith, 1985; Smith, Salih & Morgan, 1987; Mottram, 1998b; Satynarayan & Honikel, 1992; Wood *et al.,* 1995; Bejerholm & Aaslyng, 2003; Støier *et al.,* 2006; Meinert *et al.,* 2007a; Tikk *et al.,* 2007c).

An increase in centre temperature in meat has been shown to increase pork flavour (Heymann *et al.,* 1990; Wood *et al.,* 1995). Likewise, the increase in pork flavour with rising frying temperatures has been found (Støier *et al.,* 2006; Meinert *et al.,* 2007a). This increase in pork flavour intensity with rising temperatures is caused by an increased formation of the Maillard reaction and associated reactions involving carbohydrates, proteins and lipids and their degradation products (Mottram, 1992). Moreover, it has been shown that the temperature-increased intensity in pork flavour also is able to mask any abnormal flavours present, e.g. flavours associated with blood (e.g. metallic notes), which are more pronounced at low cooking temperatures (Heymann *et al.,* 1990). Finally, Bejerholm & Aaslyng (2003) investigated the influence of cooking technique on the sensory attributes of pork, and the results showed that pan-frying (high surface temperature) gave a more intense fried/roasted flavour compared to roasting in an oven (low surface temperature). In general, lipid-derived volatile compounds dominate the flavour profile in pork cooked at temperatures below 100°C (Chen & Ho, 1998), while Maillard reaction products dominate the flavour profile, when meat is cooked at high surface temperatures (Whitfield, 1992).

Re-heating

Together with microbial spoilage, chemical deterioration - especially lipid oxidation - is a main factor limiting the shelf-life of muscle foods (Gray, Gomaa & Buckley, 1996). Pork is susceptible to lipid oxidation during storage. Oxidative processes, mainly due to oxidation of PUFA, are important as contributors for flavour formation (Mottram, 1996; Mottram, 1998a, b), rancidity development and warmed-over flavour (WOF) formation in cooked, cold-stored and re-heated meat (Shackelford *et al.,* 1990; Skibsted, Mikkelsen & Bertelsen, 1998).

WOF has been shown to be one of the most easily detected off-flavours in pork (Cross, Leu & Miller, 1987; Bryhni, Ofstad & Hunt, 2002a; Bryhni *et al.,* 2003), and the development in WOF has been shown to increase linearly with the formation of secondary oxidation products during cold-storage and re-heating (McMillin *et al.,* 1991; Kerry *et al.,* 1998; Jensen *et al.,* 1998b; Byrne *et al.,* 2001, 2002, 2003; O'Sullivan *et al.,* 2003; Tikk *et al.,* 2007d). Consequently, the meat industry is aware of the WOF problem due to the ever increasing production of ready-to-eat products and supply of pre-heated products to the catering sector.

WOF is a characteristic flavour defect and develops by a rapid oxidative deterioration in meat that has been pre-cooked, cold-stored and re-heated (Byrne *et al.,* 1999). WOF in meat is mainly believed to be the result of oxidation of membrane phospholipids, a process triggered by hemoproteins and other iron species during cooking (Love & Pearson 1974; Ingene & Pearson 1979; Gray & Pearson, 1987; St Angelo *et al.,* 1987). However, degradation of proteins and some minor components, e.g. Maillard-derived aroma volatiles associated with the aroma of freshly cooked meat, may also contribute to formation of WOF, as this result in the disappearance of desirable meat flavour notes present in freshly cooked meat (Spanier, Edwards & Dupuy, 1988; St Angelo *et al.,* 1988, 1990; Byrne *et al.,* 2001). Typical oxidation products are aldehydes, lactones, hydrocarbons, furans and ketones, which contribute to undesirable, rancid off-flavour notes (Ladikos &

Lougovois, 1990). In WOF, the secondary oxidation product hexanal is the most prominent volatile compound that has been found to be inversely proportional to flavour acceptability of pork (Ullrich and Grosch, 1987; Shahidi & Pegg, 1994). Dietary supplementation of vitamin E has been reported to be an effective tool in minimizing WOF formation in pre-heated pork (Asghar *et al.,* 1991; Buckley, Morrissey & Gray, 1995; Jensen *et al.,* 1997, 1998a; O'Sullivan *et al.,* 1998; Lauridsen *et al.,* 1999).

Tenderness

Tenderness is an important eating quality factor affecting consumer satisfaction (Huffman *et al.,* 1996; Miller *et al.,* 2001). Moreover, eating satisfaction often results from the interaction of tenderness and flavour. As it is the case with presence of off-flavours, toughness of meat is one of the most common issues in relation to consumer unacceptability (Jeremiah, 1982; Huffman *et al.,* 1996).

Generally, meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development and consequently sarcomere length and *post mortem* proteolysis of myofibrillar proteins (Ouali, 1992; Wheeler, Shackelford & Koohmaraie, 2000; Sentandreu, Coulis & Ouali, 2002; Maltin *et al.,* 2003; Rhee *et al.,* 2004; Koohmaraie & Geesink, 2006). Three factors that determine meat tenderness are background toughness, the toughening phase and the tenderization phase. While the toughening and tenderization phases take place during the *post mortem* storage period, background toughness exists at the time of slaughter and does not change during the storage period (Koohmaraie & Geesink, 2006).

The relative importance of proteolysis, connective tissue and sarcomere length to tenderness is muscle dependent e.g., proteolysis is the major determinant of *longissimus* tenderness while sarcomere length is the major determinant of *psoas major* tenderness (Koohmaraie & Geesink, 2006). While changes in muscle protein degradation affect meat tenderization, changes in muscle protein synthesis are not expected to affect meat tenderness. Tenderization of meat is very likely to be a result from the softening of the myofibrillar structure by the synergistic action of endogenous peptidases including mainly cathepsins (De Duve et al., 1955), calpains (Guroff, 1964) and proteasome (Wilk & Orlowski, 1980; Sentandreu, Coulis & Ouali, 2002). The individual and combined contribution of the major peptidases to the softening process is not fully identified, and therefore these mechanisms are strongly debated. However, current evidence suggests that the calpain proteolytic system is a major regulator of muscle protein degradation and for meat tenderization (Goll *et al.,* 2003; Veiseth & Koohmaraie, 2005). Particularly, μ-calpain-induced degradation of key myofibrillar proteins is the primary source of variation in tenderness of muscles (Koohmaraie & Geesink, 2006). Moreover, these studies suggest that increased focus on the mechanisms of μ-calpain activity regulation in early *post mortem* muscle and the development of methods to accelerate μ-calpain activity might influence the ability of the meat industry to produce a product that is consistently tender (Veiseth & Koohmaraie, 2005; Koohmaraie & Geesink 2006). Based on different approaches, many studies have reported that proteasome could contribute to tenderization of stored meat as

well (Matsuishi & Okitani, 1997; Otsuka *et al.,* 1998; Ouali, 1999; Lamare *et al.,* 2002; Thomas *et al.,* 2004).

Production factors influencing tenderness

Biological and other factors that determine meat tenderness have been subjects of research for a long time, particularly in beef. In the following some of the production factors of importance for pork tenderness are mentioned; however, the research in this area is scarce.

Breed and genetic background

Even though breed and genotype are known to have significant effect on tenderness, the present knowledge within the area seems to be rather scattered. Pork from Duroc has been reported to be less tender than Landrace (Cameron *et al.,* 1990). In contrast, chops from the *M. longissimus dorsi* from Duroc pigs were more tender, juicier and more desirable than those from Yorkshire pigs (Thornton, Alsmeyer & Davey, 1965). Similarly, a study by Wood *et al.* (2004) found that "modern" breeds like Duroc and Large White grew faster and had more tender meat than the two "traditional" breeds, Berkshire and Tamworth. Tenderness of meat was significantly lower in Swedish Yorkshire pigs compared with Hampshire and Swedish Landrace pigs (Essen-Gustavsson & Fjelkner-Modig, 1985). Despite the fact that meat from Hampshire crosses carrying the RN^- allele has been reported to have detrimental effect on other pork quality traits (Hamilton *et al.,* 2000; Houde, Godbout & Gariepy, 2001), it may have a positive effect on tenderness. However, in some studies, no difference between breeds or genotypes in relation to tenderness has been found (Lundström, Andersson & Hansson, 1996; Wood *et al.,* 1996). For the proper evaluation of the impact of various crossbreeding programs on overall meat quality, it is important to know and understand breed differences for several quality traits including tenderness.

Gender

The influence of gender in relation to tenderness has been investigated in few studies. Generally, it has been concluded that either there is no difference (Skelley & Handlin, 1971; Ramsey *et al.,* 1990), or that meat from castrates is more tender compared to females (Martel, Minviellc & Poste, 1988; Latorre *et al.,* 2003). The explanation for more tender meat from castrates has been suggested to be the faster growth and higher intramuscular fat content compared to females (Latorre *et al.,* 2003). However, in a study by Kristensen *et al.* (2004), an interaction between gender and treatment was found, suggesting that a compensatory growth strategy, where the growth is accelerated immediately before slaughter, increased pork tenderness of gilts compromising neither slaughter weight nor percentage of meat. Contradictory, in barrows, compensatory growth had no effect on *longissimus* tenderness; however, other muscles may respond to compensatory growth.

Muscle

Within any species, there is a considerable variation in tenderness among muscles. Generally, *Longissimus dorsi* (LD) and *Psoas Major* (PS) are considered to be more tender compared to several ham and shoulder muscles e.g. *Semitendinousus*. Moreover, the PS muscle has been found to have higher tenderness, juiciness, flavour and overall liking scores than LD (Wood *et al.,* 2004). One source of muscle-to-muscle variation in tenderness is the amount of stretch or tension applied to each muscle, while the carcass is being chilled. Muscles which are prevented from shortening (e.g. by using pelvic suspension) during *rigor mortis* are generally more tender (Møller & Vestergaard, 1986; Taylor, Perry & Warkup, 1995b). Likewise, pelvic suspension has been reported to lengthen the sarcomeres in the LD muscle and hereby result in a more tender LD compared to conventional carcass suspension (Møller & Vestergaard, 1986). However, while muscles usually differ in the amount of connective tissue, fatty acid composition, marbling fat, fibre type, oxidative capacity etc., variations in these might explain some differences in regard to tenderness (Essen-Gustavsson & Fjelkner-Modig, 1985; Ramsey *et al.,* 1990; Maltin *et al.,* 2003; Aaslyng & Støier, 2004), but contradictory results have been reported (van Laack, Stevens & Stalder, 2001).

An improved knowledge with regard to tenderness differences between muscles could lead to a more effective use of the carcass, i.e. selecting muscles with low tenderness for processed products.

Feeding

Several feeding studies have evaluated the response on pork tenderness. Strategic finishing feeding resulting in low muscle glycogen content at slaughter has been reported to result in less tender meat (Rosenvold *et al.,* 2001a, b). In an earlier study, pigs fed a reduced energy diet resulted in less tender and juicy meat compared to normally fed pigs (Thornton, Alsmeyer & Davey, 1965). Low protein diet produced more tender and juicy meat, although pork flavour and flavour liking were reduced (Wood *et al.,* 2004).

Moreover, tenderness development and *post mortem* proteolysis in meat can be manipulated by the use of various feeding strategies e.g., restricted feeding, *ad libitum* access to feed, and compensatory growth (Jones *et al.,* 1990; Kristensen *et al.,* 2002). Restrictive feeding has been reported to give rise to inferior eating quality including reduced tenderness compared with *ad libitum* feeding in pigs (Ellis *et al.,* 1996; Danielsen *et al.,* 2000; Kristensen *et al.,* 2002; Bee *et al.,* 2005). However, recently it has been shown that a feeding strategy allowing compensatory growth, i.e., restrictive feeding followed by *ad libitum* feeding of pigs, may increase tenderness of pork compared to pork from only *ad libitum*-fed animals (Kristensen *et al.,* 2002; Therkildsen *et al.,* 2002). Compensatory growth is believed to increase protein turnover and thereby the proteolytic potential at the time of slaughter, leading to faster tenderization rates of meat (McMeekan, 1940; Millward *et al.,* 1975; Prince, Jungst & Kuhlers, 1983; Jones *et al.,* 1990; Oksbjerg, Sorensen & Vestergaard, 2002).

Because of the importance of several feeding strategies to different quality attributes in pork (including colour, flavour, tenderness etc), research should continuously focus on developing new feeding strategies to improve or control several quality attributes. Moreover, the implementation of compensatory growth could be a way to optimize the tenderness development in meat-producing animals due to its effect on growth rate and protein turnover.

Weight of animals

In practise, lack of tenderness in pork as a result of animal weight or age at slaughter is hardly ever encountered, because compared to other species pigs are slaughtered at a relatively young age. However, some studies have reported that the increase in slaughter weight and age is associated with a significant reduction in tenderness in pork (Ellis *et al.,* 1996; Candek-Potokar *et al.,* 1998b). This is due to the fact that as an animal matures the connective tissue in the muscle gets less soluble. In contrast, Bejerholm & Aaslyng (2003) found that meat (*longissimus dorsi*) from pigs with a higher carcass weight (>90 kg) was more tender than meat from the lower carcass weight group (<65 kg). Likewise, Bee *et al.* (2005) reported porcine muscles to be less tender when slaughter weight was lower, even though the pigs were at the same age. Thus, young, rapid growing animals will have the most tender meat (Maltin *et al.,* 2003). Although age and weight might be a factor in pork tenderness, it obviously plays a less important role than factors such as muscle location, etc., because meat from older animals is normally processed further e.g. into sausages.

Pre-slaughter and post-slaughter handling

Transportation time of pigs before slaughter may influence final pork quality. Thus, 8 h of transport compared to 0.5 h has been found to improve tenderness due to the reduced glycolytic potential at the time of slaughter and the subsequent higher ultimate pH (Leheska, Wulf & Maddock, 2003).

Moreover, chilling rate *post mortem* has been found to influence tenderness development in meat. Too rapid or too slow chilling rates result in inferior tenderness development. Thus, electrical stimulation *post mortem* might be a tool to produce tender pork, when rapid chilling of carcasses is used (Taylor, Nute & Warkup, 1995a; Taylor, Perry & Warkup, 1995b; Taylor & Martoccia, 1995; Bowker *et al.,* 1999; Maribo *et al.,* 1999).

Aging

It is well established that storage of muscles at a low temperature for a reasonable length of time is a prerequisite for the development of tenderness (van Laack, Stevens & Stalder, 2001; Channon, Kerr & Walker, 2004). Aging pork loin steaks in vacuum bags for 7 days improved tenderness, flavour and overall liking compared with steaks aged for 2 days post-slaughter (Channon, Kerr & Walker, 2004). Moreover, tenderization has been found to continue in pork through 14 days of aging (van Laack, Stevens & Stalder, 2001). Dransfield, Jones & MacFie (1980) and Rees, Trout & Warner (2002) found that improvements in tenderness due to aging were rapid in the first 2 days post-slaughter, with 80 and 90% of the

observed change in tenderness occurring within 4 and 6 days *post* slaughter, respectively. Finally, as a result of extensive research, the importance of *post mortem* storage to the ultimate tenderness is now believed to be 5 to 7 days for pork (Ouali *et al.,* 2006).

Pork tenderness is a quality characteristic that is influenced by many factors. The mechanisms behind the conversion of muscle into meat in general and the contribution of the *post mortem* development to the eating quality are not fully understood. Thus, further research on determinants and mechanisms of pork tenderness and pork tenderization is needed, before production of consistently tender pork will be possible, particularly because of the large biological variability.

Objectives

The objective of the present thesis was to obtain more basic understanding on how two major production factors - feeding strategy and aging of the meat - can control critical pork quality attributes. Particularly, colour characteristics of fresh pork and the flavour development in cooked pork were the main focus in the present thesis. As it appears from the above introduction, pork quality is to a large extent influenced by several mutually interacting factors. An understanding of how these factors influence meat production is urgent, if these production factors subsequently are going to be used in the control of several meat quality attributes.

The specific aims of the thesis were:

- To elucidate the effect of a muscle-glycogen-reducing diet containing a high ratio of rapeseed and grass meal on fat colour and the degree of blooming of the pork chops during 1, 2, 4, 8 and 15 days of aging *post mortem* compared with a control diet (Paper I).
- To investigate the effect of a strategic finishing feeding (low content of digestible starch), which is reported to affect *post mortem* muscle metabolism, on meat colour and colour stability. Pork colour was determined as the extent of blooming of chops from *M. longissimus dorsi* and *M. semimembranosus* after 1, 2, 4, 8 and 15 days *post mortem* and colour stability during subsequent air storage for up to 6 days (Paper II).
- To investigate the effect of slaughter weight on diet-induced changes in *post mortem* muscle metabolism and subsequent colour progress (blooming) of meat from the *M. longissimus dorsi* and *M. semimembranosus* 1 day after slaughter, and subsequently after 1, 3, 7 and 14 days of aging in vacuum (2, 4, 8 and 15 days *post mortem*, respectively) (Paper III).
- To investigate the influence of diets containing two different fat sources, characterized by either an increased level of saturated (palm oil) or unsaturated fatty acids (rapeseed oil) on meat quality including fatty acid profiles, several flavour precursors and sensory characteristics of the cooked meat from both castrates and females. Moreover, to explore potential relationships between specific fatty acids and meat flavour characteristics (Paper IV).
- To monitor the warmed-over flavour development in cooked, cold-stored (at 4°C for 0, 2 and 4 days) and re-heated meatballs from pork muscles *M. longissimus dorsi* and *M. semimembranosus* using a gas sensor system (electronic nose) and correlate the obtained data with sensory attributes and traditional secondary lipid oxidation product measurements to substantiate the potential of electronic noses as potential future quality control tools in the meat industry (Paper V).

Materials and Methods

Animals, feed, slaughtering and sampling

Paper I

The pigs were reared at the experimental farm at the Danish Institute of Agricultural Sciences, Research Centre Foulum. Forty crossbreed slaughter pigs (Duroc boars and Danish Landrace × Danish Yorkshire sows) originating from 20 litters, with one female and one castrate in each litter, were included in the study. Twenty control pigs were given a standard grower-finishing diet (control diet), which mainly consisted of barley (55%), soybean meal (20%), wheat (20%), and sugar beet molasses (1%), and 20 experimental pigs were given a diet with a low content of digestible starch (experimental diet), which consisted of high levels of grass meal (24%), rape seed cake (36%), dried sugar beet pulp (25%), soybean meal (7%), animal and vegetable fat (6%). The experimental diet was offered to the experimental group at a live weight of approximately 65 kg with an initial 1-week adaptation period gradually changing from the standard grower-finishing diet to the experimental diet. Control pigs were given a standard grower-finishing diet during the whole experiment until slaughter. All pigs were slaughtered at approximately 90 kg live weight with an initial fasting period of 48 h for experimental pigs, during which time the animals had free access to water.

On the day of slaughter, the pigs were transported (200 m) from the stable to the slaughterhouse where they were rested for minimum 30 min before they were individually brought up to the stunner. The pigs were stunned by 80% CO_2 for three min, exsanguinated, scalded at 62 °C for three min, cleaned and eviscerated within 30 min. Subsequently, the carcasses were placed in a chill room at 4 °C.

Paper II

Forty crossbreed slaughter pigs (Duroc boars and Danish Landrace x Danish Yorkshire sows) originating from 20 litters, with one female and one castrate in each litter, were reared at the experimental farm at the University of Aarhus. All pigs were slaughtered at approximately 110 kg live weight after feed withdrawal for 48 hours for experimental pigs, during which time the animals had free access to water. Diet composition and slaughtering procedure is described in Paper I.

Paper III

Eighty crossbred slaughter pigs (Duroc boars and Danish Landrace x Danish Yorkshire sows) originating from 20 litters, with two females and two castrates in each litter, were reared at the experimental farm at the Aarhus University. Forty control pigs were given a standard grower-finishing feed (control feed), and forty experimental pigs were given a feed with a low content of digestible starch (experimental feed). The composition of the feeds, feeding details and slaughtering procedure are described in Paper I. In addition, two pigs from each litter were slaughtered at high slaughter weight (live weight of 110 kg) and two pigs at low slaughter weight (live weight of 85 kg). This resulted in a 2 (feed) x 2 (sex) x 2

(slaughter weight) experimental design. The littermates were distributed equally according to sex and slaughter weight, but not as regards feed.

Paper IV

The pigs were reared at the experimental farm at the University of Aarhus. Forty crossbreed pigs (Duroc boars and Danish Landrace × Danish Yorkshire sows) originating from 10 litters with two females and two castrates in each litter were included in the study. The littermates were distributed uniformly between the diets and genders. The diet of the pigs was based on barley, wheat, soybean meal and vegetable fat. Twenty pigs were given a diet including 3% palm oil, and 20 pigs were given a diet including 3% rapeseed oil. All pigs were slaughtered at approximately 110 kg live weight with a fasting period of 24 h, during which time the animals had free access to water. The slaughtering procedure is described in Paper I.

The day after slaughter, for chemical analysis, a 5 cm thick sample (30–35 cm from the last rib in the cranial direction) was cut out from LD, vacuum-packed and stored for 2 days at 4°C before freezing at −20°C. The first layer of backfat from the top of LD (1–5 cm from the last rib in the cranial direction) was removed and frozen for determination of fatty acid composition. For sensory analysis, a 25 cm thick sample (5–30 cm from the last rib in the cranial direction) was cut from the LD, trimmed to a 3 mm fat layer, vacuum-packed and stored for 2 days at 4°C before freezing at −20°C.

Paper V

The pigs (Duroc boars and Danish Landrace x Danish Yorkshire sows) were reared at the experimental farm at the University of Aarhus, Faculty of Agricultural Sciences. The animals were fed a standard diet supplemented with tocopherol (200 mg/kg feed), where the fat source was either 3% of palm oil or 3% of rapeseed oil. The slaughtering procedure is described in Paper I. Meat from the two muscles *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM) was used. In the present experiment, the meat from the five female animals from the same feeding group was minced, and three batches from each group were formed for each muscle.

Methods

Papers I, II and III

pH and temperature

pH ($pH_{45\ min}$) and temperature ($T_{45\ min}$) were measured 45 min *post mortem* and 24 h *post mortem* ($pH_{24\ h}$ and $T_{24\ h}$) inside M. *longissimus dorsi* (LD) at the last rib and in the deep portion of M. *semimembranosus* (SM). The temperature was measured with a Testo 110 thermometer, Testo Gmbh 6 Co, Germany, and the pH was measured with a PHM201 pH Meter, Radiometer, Denmark, equipped with Methrom LL combined pH penetration electrode, Switzerland. The pH electrode was calibrated in pH 4.01 and 7.00 IUPAC buffers equilibrated at 35°C for the measurements on the warm carcasses at 45 min post-mortem and at 4°C for the measurements on the cold carcasses at 24 h post-mortem.

Water-holding capacity (WHC)

Water-holding capacity as drip loss was measured from the LD sample taken 5 cm from the last rib in the cranial direction. This was done by weighing the approximately 100 g muscle sample taken out from the LD, hanging it in a net and suspending the net in a plastic bag for 24–72 h after slaughter at 4°C (Honikel, 1998). Drip loss is expressed as a percentage of the initial weight.

Muscle glycogen

Muscle glycogen stores were determined in LD samples taken out 1 min after slaughter. The samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis. Glycogen content was determined in duplicate in 50 mg muscle samples, heated in a sealed test tube with 5 ml of 1 M HCl at 100 °C for 2 h. Glycogen was analysed as glucose residues according to Passonneau & Lowry (1993) and expressed as μmol of glucose residues per gram of muscle (wet weight).

Colour measurements

The colour of freshly cut back fat and stripped bacon fat was measured the day after slaughter (Paper I). Moreover, LD (12 cm of the loin, 19–31 cm from the last rib) and SM were removed from the carcass, and a 2 cm thick sample from each LD and SM was cut and bloomed for 1 h at 4°C with no surface covering prior to colour measurement (1 day post-mortem). The remaining part of each LD and SM was vacuum-packed and stored at 4°C for 1, 3, 7 and 14 days (2, 4, 8 and 15 days *post mortem,* respectively) before measuring the colour according to the procedure described above (Paper I, II and III). The colour after aging in vacuum was always measured on a newly cut and bloomed surface. Moreover, colour stability of the meat during 6 days of storage in air after each vacuum-packed aging period was evaluated. After the first measurements at each aging period the samples were wrapped with oxygen permeable film and stored at 4°C for 2, 4 and 6 days prior colour measurements (Paper II). Five colour measurements were carried out across the individual sample surfaces, and mean values were used for statistical analysis. Chroma (saturation) was calculated as $(a^{*2} + b^{*2})^{1/2}$ and hue angle as arctan b^*/a^* (Wyszcecki & Stiles, 1982). Colour was measured using a Minolta Chroma Meter CR-300 (Osaka, Japan) calibrated against a white tile ($L^* = 93.30$, $a^* = 0.32$ and $b^* = 0.33$). The aperture was 8 mm, illuminant D65 and 10° Standard Observer.

Paper IV

pH/temperature and water-holding capacity (WHC) measurements are described in Paper I.

Colour measurement

The colour of the meat was measured on LD and SM samples. LD and SM were removed from the carcass, and a 2 cm thick sample across the fibre direction from each LD (30–32 cm from the last rib in the cranial direction) and SM was cut and

bloomed for 1 h at 4°C. Five colour measurements were carried out across the individual sample surfaces, and mean values were used for statistical analysis.

Intramuscular fat content (IMF)

Intramuscular fat content (%) was determined by the gravimetric method according to SBR (Schmid–Bodzinski–Ratzlaff, NMKL No. 131, 1989). The method was modified to Soxtec equipment (Foss, Hillerød, Denmark).

Fatty acid composition

Fatty acids of the intramuscular fat (IMF) of LD were separated into neutral (NL) and polar/phospholipids (PL) fractions and analysed according to a slightly modified method of Kalunzy *et al.* (1985). Minced lean meat of LD (2 g) was homogenised in 4 g methanol. Subsequently, 0.6 g homogenate was mixed with 4 g of chloroform, 1 g of methanol and 2 g of water and homogenised. After centrifugation (10 min at 3000 rpm) the lipids were obtained from the chloroform phase by evaporation of the chloroform using a steam of nitrogen. Subsequently, the lipid fraction was dissolved in 1.2 ml heptane and applied to an amino-propyl column pre-conditioned with heptane under vacuum. The neutral lipids were eluted from the column with 11 ml of chloroform–propanol (2:1), phospholipids were eluted with 12 ml of methanol and charged unesterified fatty acids with 6 ml 2% acetic acid in diethylether, which were discarded. Finally, the NL and PL fractions were redissolved in chloroform and dried under the nitrogen. The extracted fat was methylated in 1 ml natrium methylat. After methylation, 4 ml saturated NaCl and 1 ml heptane were added, and the samples were centrifuged for 10 min at 3000 rpm. Heptane phase containing fatty acids was applied to the GC.

Fatty acids in backfat were analysed according to a slightly modified method described by Jakobsen *et al.* (1995). Backfat (300 mg) was placed in an oven at 50°C for 24 h (to facilitate the homogenisation) and subsequently added to 4 g of chloroform, 4 g of methanol and 3 g of water before homogenisation. The homogenate was centrifuged for 10 min at 3000 rpm. Then the extracted fat fraction was obtained by evaporation of the chloroform phase under a steam of nitrogen. 10 mg of the extracted fat was methylated in 1 ml sodium methylat. After methylation, 4 ml saturated NaCl and 1 ml heptane was added, and the samples were centrifuged 10 min at 3000 rpm. The heptane phase containing the fatty acids was applied to the GC.

The composition of fatty acids was determined by gas chromatography using a FFAP column (25 m × 32 × 5 µm; Hewlett–Packard). One µl was applied to the column using splitless injection, helium as carrier gas (8 ml/min), an injector temperature of 275°C and a detector temperature of 300°C. The initial column temperature was 50°C, which was kept for 2 min, whereafter the temperature was raised by 10°C/min to 240°C and then maintained for 15 min. Quantification was based on comparison of retention times and peak areas with external standards (Supelco™ 37 Component FAME Mix, Supelco PUFA-1 Mix), and calculations were carried out in the included software (HP Chemstation).

Tocopherols

The concentration of tocopherols in lean meat was analyzed using a slightly modified HPLC method described by Jensen, Engberg & Hedemann (1999). One g of a finely ground meat was suspended in a mixture of 2 ml ethanol with ascorbic acid, 1 ml of water and 0.3 ml of saturated KOH. The mixture was saponified for 1.5 hour at 70 °C in the dark and cooled in cold water. Three ml heptane and 1 ml of water were added and after centrifugation the mixture containing tocopherols were filtered before injection of 30 µl on the HPLC column. The HPLC column used for the determination of tocopherols was a 4.0x125 mm Perkin Elmer HS-5-Silicia column (Perkin Elmer, Gmbh, D-7770 Überlingen, Germany), and the mobile phase constituted helium degassed heptane: 2-propanol in the ratio of 98:2, at a flow of 1 ml/min. Fluorescence detection was carried out with an excitation wavelength of 290 nm and an emission wavelength of 327 nm. Identification and quantification of the tocopherols were obtained by comparison of retention time as well as peak areas with external standards and the results were expressed as µg/g of muscle (wet weight).

Inosine 5′-monophosphate, inosine and hypoxanthine

Inosine 5´-monophosphate (IMP), inosine and hypoxanthine were determined according to Henckel *et al.* (2002), however, with a five-fold increase in sample size and extraction volumes. Pork samples (50 mg) were homogenized for 10 sec in 3 ml of ice-cold 0.6 M perchloric acid (PCA) containing a pH indicator (0.004% bromthymolblue and 0.004% phenolphthalein). The samples were left on an ice for 15 min before neutralization with 2.7 ml ice-cold 0.8 M KOH and addition of 0.125 ml ice-cold KH_2PO_4 buffer. Subsequently the mixtures were mixed for 10 sec, and the pH was adjusted to 7-8 using either KOH or PCA. Finally, the mixtures were centrifuged at 4000 rpm for 10 min at 4°C, and 1 ml supernatant was transferred to an Eppendorf vial and frozen at -80°C until further analysis.

Before high performance liquid chromatography (HPLC), the samples were thawed and centrifuged at 10000 rpm for 5 min at 4°C and the supernatants were transferred to cold HPLC vials and placed in a thermostatted auto sampler (1-2°C). A 10 µl sample was injected on the column (Lichrospher 250 x 4 mm RP18, Germany) from which the three compounds were separated by isocratic elution using a solvent based on a buffer containing 10 mM tetrabutylammonium hydrogen sulfate and 215 mM KH_2PO_4 to which 7.5 ml methanol/l was added. The following flow gradient was used to obtain optimal separation: 0.5 ml/min for 5 min, increasing to 1.5 ml/min during 1 min and keeping this flow for 9 min before a final decrease to 0.5 ml/min in 0.5 min. Analysis was carried out by a Hewlett-Packard HPLC system (Series 1100 Germany) using UV detection (210 nm). Quantification was based on standard curves using external standards and calculations carried out in the included software (HP Chemstation), the results were expressed as nmol/mg.

Muscle glycogen, lactate and glucose-6-phosphate

Muscle glycogen, lactate and glucose-6-phosphate content were determined in duplicates in 50 mg muscle samples according to a slightly modified method of Passonneau & Lowry (1993). For glycogen analysis, the samples were heated in a

sealed test tube with 5 ml of 1 M HCl at 100°C for 2 hours to hydrolyse the glycogen to glycose units. Glycogen was analysed spectrophotometrically as glucose residues and expressed in µmol of glucose residues per gram of muscle (wet weight). For lactate analysis, the samples were incubated for 30 min on ice in vials containing 1.2 ml of 3 M perchloric acid. The extraction procedure was stopped by adding 2 ml of 2 M $KHCO_3$ to the vials, and the samples were centrifuged at 3000 rpm for 10 min at 4°C. For glucose-6-phosphate analysis, the samples were incubated for 1 h on ice in vials containing 750 µl of 0.6 M perchloric acid. The extraction procedure was stopped by adding 1.25 ml of 0.4 M $KHCO_3$ to the vials, and the samples were centrifuged at 3000 rpm for 10 min at 4°C. Lactate and glucose-6-phosphate were measured spectrophotometrically as quinoneimina at 500nm and as NADPH at 340 nm, respectively, and the results were expressed as µmol per gram of muscle (wet weight).

Thiamine

Thiamine determination was carried out according to a slightly modified method by Hägg (1994). Five grams of minced muscle was hydrolyzed with 30 ml of 0.1 M HCl for 30 min at 121°C, and cooled to ambient temperature. The pH was adjusted to 4.0–4.5 with approximately 2.5 ml of sodium acetate (2 M), and 5 ml of 6% freshly prepared clara-diastase solution was added before incubation at 50°C for 3 h. Subsequently, the protein was precipitated by addition of 1 ml of 50% trichloracetic acid and heating in waterbath at 100°C for 15 min. Subsequently, the samples were cooled to ambient temperature, and the sample volume was brought up to 50 ml with water before centrifugation at 3000 rpm for 5 min. Five milliliters supernatant was mixed with 2.5 ml potassium ferricyanide in 15% sodium hydroxide, and mixed for 10 s to ensure derivatisation of thiamine to thiochrome. Subsequently, the derivatisation solution was brought up to 10 ml with 3.75 M HCl before sample clean-up with C18 solid phase extraction columns and analysis of thiochrome using the method of Sims & Shoemaker (1993). Finally, 10 µl of thiochrome sample was injected into a ZORBAX Exlipse XDB-C18 USP L1 Column 4.6 × 150 mm, particle size 5 µm from which thiochrome was separated by isocratic elution using a buffer solution (72% of 0.005 M NH_4OAC and 28% of methanol, pH 5.0) at a flow rate of 1.5 ml/min. Thiamine as the thiochrome derivative was determined with a fluorescence detector at an excitation wavelength of 370 nm and 435 nm emission wavelength. Quantification was based on a standard curve using external standards and calculations carried out in the included software (HP Chemstation), and the results were expressed as mg/100 g of muscle (wet weight).

Sensory analysis

Two cm thick pork chops from the left LD were fried in a pan at 160°C and turned every 2 min, until an end point temperature of 70°C was reached. The temperature was determined in each chop by a handheld thermometer (Testo 926, Testotherm, Buhl and Bundsoe, Virum, Denmark). The whole roasts from the right LD were cooked in roasting bags in a convection oven at a 90°C, until the end point temperature inside the roast was 65°C. For sensory assessment, the meat was sliced in 1½ cm thick slices. From both cooking methods, two slices each measuring

5 × 5 × 1½ cm were served to each assessor immediately after cooking on hot plates with a three-digit number in a randomized design.

The panel for the sensory assessments had received a basic training based on ISO 4121, ASTM-MNL 13, DIN 10964. All assessors were familiar with assessment of pork. For evaluation of the fried chops, the panel consisted of four assessors – two males and two females aged between 44 and 59 years. The fried chops were evaluated on a 15 cm unstructured scale from nothing to very intense for the following attributes: fried meat colour (surface), fried meat odour, sweet odour, sourish odour, sourish taste, bitter taste, sweet taste, metal taste, piggy flavour, fried meat flavour, hardness at first bite, crumbleness, fibrousness, tenderness and juiciness after 3–4 chews. For evaluation of the oven roasts, the panel consisted of eight assessors, all females and aged between 48 and 63 years. The oven roasts were evaluated on a 15 cm unstructured scale from nothing to very intense for the following attributes: boiled meat odour, sweet odour, sourish odour, piggy odour, pores, boiled meat flavour, piggy flavour, sourish taste, metal taste, rancid flavour in the fat layer, hardness at first chew, juiciness, fibrousness, crumbleness and tenderness after 3–4 chews.

Paper V

Preparation of meatballs

From each batch, meatballs with a weight of 18 ±3g were formed. One third of the raw meatballs were vacuum-packed and frozen until the sensory assessment in order to use them as the freshly cooked control meatballs. Two thirds of the meatballs from the batches were cooked in an oven (convection oven) at an oven temperature of 190ºC, until the end point temperature inside the meat ball was 75-80ºC (first cooking). The temperature was determined by a handheld thermometer (Testo 926, Testotherm, Buhl and Bundsoe, Virum, Denmark). After cooling, the meatballs were placed in a tray and covered with oxygen permeable plastic film and cold-stored at 4ºC for up to 4 days. Two- and four-day cold-stored meatballs were re-heated in an oven (convection oven) at a temperature of 190ºC, until the end point temperature inside the meatball was 75-80ºC (re-heating). Consequently, the overall experimental set up resulted in 12 different combinations of meatballs.

Sensory analysis

In the sensory assessment, each assessor was served two meatballs on hot plates from the same group immediately after cooking, and the plate was given a three-digit number in a randomized design. The eight-member sensory panel had received basic training according to ISO 4121, ASTM-MNL 13, DIN 10964. All assessors were familiar with assessment of pork and particularly with detection of rancidity and warmed-over flavour in pork. The meatballs were evaluated on a 15 cm unstructured scale from zero to very intensive for the following attributes: roasted meat odour, boiled meat odour, piggy odour, sweet odour, sourish odour, warmed-over odour, roasted meat taste, boiled meat taste, piggy taste, sourish taste, metal taste, bitter taste, rancid taste, warmed-over taste, sourish after-taste, metallic after-taste, warmed-over after-taste, juiciness after 3-4 chews. The result from one

of the assessors was removed during data analysis, as this person was found not to be able to detect warmed-over flavour development in meatballs.

Fatty acid composition

The fatty acid composition of the raw meatballs was determined using gas chromatography (GC). Minced lean meat (2 g) was homogenised in 4 g methanol. Subsequently, 0.6 g homogenate was mixed with 4 g of chloroform, 1 g of methanol and 2 g of water solution and homogenised. After centrifugation (10 min at 3000 rpm), the lipids were obtained from the chloroform phase upon evaporation of the chloroform using a steam of nitrogen. Subsequently, the lipid fraction was dissolved in 1.2 ml heptane and applied to an amino-propyl column pre-conditioned with heptane under vacuum. Ten mg of the extracted fat was methylated in 1 ml sodium methylat. After methylation, 4 ml saturated NaCl and 1 ml heptane was added, and the samples were centrifuged 10 min at 3000 rpm. The heptane phase containing the fatty acids was applied to the GC. The GC procedure is described in further detail in paper IV.

TBARS

Secondary lipid oxidation products from cooked meat were determined according to the method described by Young *et al.* (2003). Approximately 200 mg of the meat was incubated in 1 ml 50% trichloroacetic acid containing 1.3% (wt/vol) thiobarbituric acid (dissolved at 60°C), homogenised and heated to 60°C for 1 h, followed by determination of absorbance of the supernatant at 532 nm. Tetraethoxypropane (Sigma, St. Louis, MO), which decomposes spontaneously in an aqueous environment to form malondialdehyde (MDA), was used as a standard, and absorbance was expressed as MDA equivalents. The MDA equivalents were calculated after subtraction of blank (water), correction for turbidity measured at 650 nm and dilution of the TBA reagent from water contained in the meat.

Headspace GC/MS

In order to identify and quantify volatile organic compounds, a gas chromatography analysis was carried out on cooked meat samples according to the method described by Olsen *et al.* (2005). Fifteen g of homogenised sample (the samples were analysed in duplicate) was distributed evenly in 250 ml Erlenmeyer flasks. The samples were heated to 70°C in a water bath and purged with 100 ml/min nitrogen through a Drechsel-head for 30 minutes. Volatile compounds were adsorbed on Tenax GR (mesh size 60/80). The water was removed from the tubes by nitrogen flushing (50 mL/min) for 5 minutes in the opposite direction of sampling. Trapped compounds were desorbed at 250°C for 5 minutes in a Perkin Elmer Automatic Thermal Desorption System ATD400 and transferred to an Agilent 6890 GC System with an Agilent 5973 Mass selective detector, which is a quadropole, operated in electron impact (EI) mode at 70eV. The scan range was from 33 to 300 amu. The compounds were separated on a DB-WAXetr column from J&W Scientific/Agilent (0.25 mm i.d., 0.5 µm film, 30 m). Helium (99.9999%) was used as carrier gas. The temperature program started at 30°C for 10 minutes, increased 1°/min to 40°C, 3°/min to 70°C, 6.5°/min to 160°C, 20°/min to 230°C with a final hold time of 4 minutes. Integration of peaks and tentative

identification of compounds were performed with HP Chemstation (G1701CA version C.00.00, Agilent Technologies), Wiley 130K Mass Spectral and NIST98 Mass Spectral. Comparison of retention times and mass spectra of the sample peaks with those of pure standards confirmed identities of several of the components. Heptanoic acid ethyl ester was used as internal standard. System performance was checked with blanks and standard samples before, during and after the sample series, and the selected major compounds (80-100%) on a peak area basis were included in the data analysis. The selected volatile compounds were hexanal, pentanal, pentanol, nonanal and 1-octen-3-ol.

Gas sensor measurements – electronic nose system

The gas sensor array instrument used was an AppliedSensor AB, model 3320, consisting of 10 MOSFET (metal oxide semiconductor field effect transistor) sensors with a thin catalytic metal layer on top, 12 MOS (metal oxide semiconductor) sensors consisting of a metal oxide layer on top of a semiconductor and an IR-based CO_2-senor. Charcoal-filtered humidified ambient air was used as reference gas, and 1% ethanol in distilled water was used as calibration gas. The analysis was carried out on cooked meat samples according to the method described by Haugen *et al.* (2006). For the gas sensor analysis, 2 gram of the cooked meat samples (the samples were analysed in triplicate) were transferred to 30 ml glass vials with preheated teflon/silicon septa and screw cap. A stream of nitrogen flushed the vials before they were capped to prevent oxidation during incubation. The temperature of the samples was conditioned to room temperature at 22°C, before they were placed in the autosampler of the electronic nose. Thereafter the samples were incubated at 60°C for 20 minutes each, before a headspace gas was pumped into the sensor chamber for 10 sec at a flow-rate of 150 ml/min. Recovery time for the sensors was 120 seconds (flushing with reference air). The samples were analysed in random order, and the calibration samples were measured after every tenth sample in order to monitor possible sensor drift.

Statistical analysis

Paper I

The Proc Mixed in SAS/STAT Software, Version 8.2 (SAS Institute Inc., Cary, NC, USA) was applied when calculating least squares means (LSM) and standard errors (SE) of the LSM's. The statistical model for bacon fat and backfat colour parameters included diet and sex as fixed effects, and litter and slaughter date as random effects. The model used for analysing LD and SM colour included fixed effects of diet, sex and aging, and random effects of litter and slaughter date. When comparing differences between muscles and type of fat, animal nested within litter, sex, diet and slaughter date was included as random effect. Two-way interactions were included when significant. The model for temperature, pH and glycogen included fixed effects of diet and sex, and random effects of litter and slaughter date. The differences were considered to be significant at $p < 0.05$.

Paper II

The Proc Mixed in SAS/STAT Software, Version 8.2 (SAS Institute Inc., Cary, NC, USA) was applied when calculating least squares means (LSM) and standard errors (SE) of the LSM's. Degrees of freedom were estimated with the Satterthwaite method. The model used for analysing LD and SM colour included fixed effects of diet, sex, aging time in vacuum and stability (storage time in air), warm carcass weight was included as covariate. Slaughter date, litter within diet, and animal within litter, diet, sex and slaughter date were included as random effects. Two- and three-way interactions were included when significant. The model for temperature, pH and glycogen included fixed effects of diet and sex, and random effects of litter within diet and slaughter date. When comparing differences between muscles, muscle was added as fixed effect as well and animal nested within sex, diet, litter and slaughter date was included as random effect. The differences were considered to be significant at $p < 0.05$.

Paper III

The Proc Mixed in SAS/STAT Software, Version 8.2 (SAS Institute Inc., Cary, NC, USA) was applied when calculating least squares means (LSM) and standard errors (SE) of the LSMs. Degrees of freedom were estimated with the Satterthwaite method. The model used for analysing the colour parameters of LD and SM included fixed effects of feed, sex, slaughter weight and aging time *post mortem*. Litter within feed and animal within feed, sex, slaughter weight, litter and slaughter date were included as random effects. Two- and three-way interactions were included when significant. The model for temperature, pH, drip loss and glycogen included fixed effects of feed, sex and slaughter weight, and random effects of litter within feed and slaughter date. The differences were considered to be significant at $p < 0.05$.

Paper IV

The statistical analyses were carried out with the statistical analyses system version 8.2 (SAS Institute Inc., Cary, NC, USA). The MIXED procedure was applied when least-squares means (LSM), and standard errors of the LSMs (SEM) of all variables were calculated. Least-squares means were considered to be significantly different if $p < 0.05$. The statistical model for analysing all meat quality attributes and chemical data included fixed effects of diet and gender as well as their interaction and the random effect of slaughter date and litter. The results of the sensory profile were analysed in a MIXED model as well, with gender, diet and their interaction as fixed effects, and assessor and animal as random effects. In order to determine significant correlations, the CORR procedure was applied, where both gender and cooking methods were analysed separately. The set of the variables included all fatty acid (PL and NL fraction) and sensory variables of the fried chops and oven roasts. The correlations were considered significant if $p < 0.05$ and as a tendency if $p < 0.1$.

Moreover, to visualise the results and to get an overall overview of potential connections between fatty acids and sensory attributes, a multivariate analysis was

performed using the Unscrambler 9.1 software (Camo ASA, Oslo, Norway). Partial least square regression (PLSR) was used to investigate the ability to predict the sensory data from the chemical data. A full cross-validation and an uncertainty test were performed where *X*-variables contained fatty acids (not standardised) and *Y*-variables sensory attributes (standardised). Female and castrate pigs were analysed separately in the exploration of possible correlations and differences between specific fatty acids and sensory attributes in pork from two genders.

Paper V

The sensory data and chemical data were initially assessed by the Proc Mixed in SAS/STAT Software, Version 8.2 (SAS Institute Inc., Cary, NC, USA) when calculating least squares means (LSM) and standard errors (SE) of the LSM's. Degrees of freedom were estimated with the Satterthwaite method. The model used for analysing sensory data included fixed effects of feed, muscle and storage time. Assessor and session were included as random effects. Two- and three-way interactions were included when significant. The model for analysing fatty acids, tocopherols and volatiles included fixed effects of feed, muscle and storage, and a random effect of batch. Two-way interactions were included when significant. The differences were considered to be significant at $p < 0.05$. The sensor responses from the electronic-nose system were defined as average raw values from the sensor array for multivariate analysis; however, for evaluation of significance of sensors between cold-storage days, the model included muscle, feed and storage time as fixed effects and batch as a random effect.

Moreover, to get an overall overview of potential connections between chemical data, gas sensor responses and sensory attributes, a multivariate analysis was performed using the Unscrambler 9.1 software (Camo ASA, Oslo, Norway). Partial Least Square Regression (PLSR) was used to investigate the ability to predict the sensory data from the chemical and gas sensor response data. PLS1 and PLS2 prediction models were calculated for comparison, as PLS1 shows only one response variable at a time, and PLS2 handles several responses simultaneously. An equal weighing of the gas sensor responses, chemical oxidation parameters and sensory data by auto-scaling (1/sd) was used, which gives all the variables the same variance. A full cross validation (leaving one sample out at a time) was applied to the regression models. An uncertainty test was performed, where the approximate uncertainty variance of the regression coefficients was estimated by modified jack-knifing (Westad, & Martens, 2000), the significance level at $p<0.05$. For prediction of the oxidation products from gas sensors, the X-variables contained gas sensor responses, and the Y-variables contained chemical oxidation products. For predictability of the sensory descriptions, the X-variables contained gas sensor responses and oxidation products, and Y-variables contained sensory attributes. Indicator variables 0/1 for the muscle, feed and storage time were in some cases included in the X-matrix to allow them to appear in the subsequently derived loadings plot.

Summary of presented papers

I. The effect of a muscle-glycogen-reducing finishing diet on porcine meat and fat colour

The objective of the present study was to elucidate the significance of a muscle-glycogen-reducing finishing diet containing a high ratio of rapeseed and grass meal on fat colour and pork colour compared with a control diet. Pork colour was determined as the extent of blooming of *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM) after 1, 2, 4, 8 and 15 days of aging, while fat colour was measured on back fat and stripped bacon the day after slaughter. The muscle-glycogen-reducing diet significantly decreased the glycogen content measured 1 min after slaughter in LD. This was reflected as decrease in early post-mortem temperature, as well as a tendency to higher initial pH in both muscles. Moreover ultimate pH was significantly higher in LD from strategically fed pigs compared to the control group and the same tendency was found in SM. Independent of muscle and time of aging, the colour of bloomed pork from pigs fed the control diet had higher chroma and L^*, a^* and b^* values compared with pork from the pigs fed the muscle-glycogen-reducing diet with the effect being most pronounced in LD. This can be explained by the slightly higher $pH_{45\ min}$ in the muscles from the pigs fed the muscle-glycogen-reducing finishing diet, which sustain the metmyoglobin reductase activity and the oxygen consumption potential in the muscle and hereby minimise the degree of blooming. The more pronounced influence of the experimental diet on the degree of blooming in LD compared to SM may be explained by the lower $T_{45\ min}$ in LD, which minimise denaturation of the enzymatic processes. This clearly shows that the diet composition can be used to control the extent of blooming in pork. Finally, despite the high content of grass meal in the muscle-glycogen-reducing finishing diet, this diet had negligible influence on the colour of the back fat and stripped bacon fat.

II. The significance of diet and aging time on pork colour and colour stability

The objective of the present study was to investigate the effect of a strategic finishing feeding on meat colour and colour stability. Pork colour was determined as the extent of blooming of *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM) after 1, 2, 4, 8 and 15 days *post mortem* and as colour stability during a subsequent storage period in air for 6 days. Compared to the control diet, the strategic feeding resulted in a significantly lower *post mortem* muscle temperature. Independent of feeding strategy, the extent of blooming decreased during the first 2 to 4 days *post mortem* in LD, however, the effect was more pronounced in meat from strategically fed pigs. This effect was not seen in SM, where a gradual increase in blooming took place throughout storage. The colour stability was found to be superior in aged pork from strategically fed pigs with a faster discoloration rate in SM compared to LD. The observed effects can most probably be explained by a feed-induced change in *post mortem* temperature progress in the meat, which

affects the activity of the inherent enzyme systems. In conclusion, the present study clearly shows that the diet composition can be used as a tool to control meat colour and colour stability in pork.

III. The significance of slaughter weight, diet and aging time in vacuum on porcine meat colour

The objective was to investigate the effect of slaughter weight (110 kg vs 85 kg live weight) on diet-induced changes in *post mortem* muscle temperature and subsequent colour development (blooming) in two pork muscles (*M. longissimus dorsi* (LD) and *M. semimembranosus* (SM)) aged in vacuum for up to 14 days. The experimental diet (containing low content of digestible starch) significantly ($p<0.05$) lowered the temperature 45 min *post mortem* (1°C) in both muscles. Moreover, high slaughter weight resulted in a higher temperature *post mortem* in LD ($p<0.05$). The extent of blooming measured as redness on the surface of LD decreased initially, being most pronounced in LD from heavy pigs given the experimental diet. Subsequent aging resulted in increased surface redness of bloomed LD from pork from experimental group, while redness did not change further in pork from control group. A steady increase in redness of bloomed SM was noticed throughout aging. It can be concluded that the weight of the animals in combination with diet is one of several mutually interacting factors to be considered in relation to colour development in pork, due to its influence on *post mortem* metabolic activities reflected in the early temperature progress.

IV. Significance of fat supplemented diets on pork quality – connections between specific fatty acids and sensory attributes of pork.

The influence of two diets with different fatty acid compositions has been studied with regard to overall pork quality and significance of specific fatty acids on sensory attributes in fried chops and oven roasts. Twenty castrates and 20 females were in a balanced experimental set-up fed with a standard diet supplemented with α-tocopherol (200 mg/kg feed) where the fat source was either 3% of palm oil or 3% rapeseed oil. After slaughter, despite differences in lipid composition and sensory attributes, no significant difference in overall meat quality parameters and flavour precursors was found. Comparison of the two diets showed that supplementation with rapeseed oil resulted in a significantly higher content of C18:3n-3 (polar lipid (PL), neutral lipid (NL)), C18:2n-6c (NL) and C20:2 (NL) in LD and C18:1n-9c, C18:2n-6c, C18:3n-3, C20:3n-3, C22:5n-3 in backfat, while supplementation with palm oil resulted in a higher content of C16:0 (NL), C16:1 (PL), C18:1n-9t (NL) in LD and C16:0, C17:0, C18:0, C16:1, C20:4n-6 in backfat. A positive and significant correlation between the contents of C18:2n-6c, C20:3n-6 in the PL fraction and the sensory attributes fried meat odour and sweet odour were found in fried pork chops from female pigs. Likewise, positive correlations were seen between the content of C18:1n-9c in the PL fraction and sensory attributes such as sourish odour, piggy odour and piggy flavour in whole oven roasts. These

data substantiate the view that specific fatty acids in the PL fraction influences flavour attributes in pork.

V. Monitoring of warmed-over flavour in pork using electronic nose – correlation to sensory attributes and secondary lipid oxidation products

Sensory analysis of meatballs was carried out to monitor the warmed-over flavour (WOF) development in cooked, cold-stored (at 4°C for 0, 2 and 4 days) and reheated meatballs derived from *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM) of pigs fed a standard diet supplemented with either 3% of rapeseed oil or 3% of palm oil. This was performed in combination with measurement of volatile compounds using a solid-state-based gas sensor array system (electronic nose) and gas chromatography/mass spectrometry together with measurement of thiobarbitoric acid reactive substances (TBARS). Subsequently, to elucidate the relations and predictability between the obtained data, the gas sensor responses were correlated with chemical (volatile and non-volatile secondary lipid oxidation products) and sensory data (flavour and odour attributes), using partial least-squares regression modelling (PLSR). The TBARS, hexanal, pentanal, pentanol and nonanal all correlated to the sensory attributes associated to WOF formation. Moreover, the responses from eight of the MOS (metal oxide semiconductor) sensors within the electronic nose proved to be significantly related to WOF characteristics detected by both sensory and chemical analysis, while six of the MOSFET (metal oxide semiconductor field effect transistor) sensors were related to freshly cooked meat attributes determined by sensory analysis. The obtained results show the potential of the present gas sensor technology to monitor WOF formation in pork.

General discussion

Meat quality of pork is a complex term consisting of critical attributes of importance for the consumer like colour, flavour and texture (Bredahl, Grunert & Fertin, 1998).

In recent years strategic-finishing feeding has been shown as a potential tool to improve the technological quality of meat (Rosenvold *et al.,* 2001a,b). The mechanism behind these observations has been related to either a diet-induced reduction in muscle glycogen stores (Rosenvold *et al.,* 2001a) or a diet-induced change in glycogen composition (Rosenvold, Essen-Gustavsson & Andersen, 2003c), which changes the progress in *post mortem* muscle metabolism (Bendall & Swatland, 1988; Rosenvold *et al.,* 2001a, 2003c). To further elucidate the potential of strategic feeding as a pork quality tool, the effect of a diet that is low in digestible carbohydrates in the finishing period was investigated in relation to the development in colour and colour stability of fresh pork (Papers I-III).

The diets used in the colour studies were composed of a standard grower-finishing diet (control diet), which mainly consisted of barley (55%), soybean meal (20%) and wheat (20%), and a diet with a low content of digestible starch (experimental diet), which consisted of high levels of grass meal (24%), rape seed cake (36%) and dried sugar beet pulp (25%). The diets were given to both pigs slaughtered at a high weight (live weight of 110 kg) and pigs slaughtered at a low weight (live weight of 85 kg). The results showed that the muscle glycogen stores were reduced in the pigs with low slaughter weight in contrast to the pigs with high slaughter weight; probably due to the extended feeding period for the group with high slaughter weight (3-5 weeks). The muscle-glycogen-reducing effect achieved by feeding has been reported to be relatively short-term with optimal effect after a period of three weeks, after which time adaptation may occur (Rosenvold *et al.,* 2001a). However, a clear diet effect on *post mortem* temperature progress in muscles was found, where the pigs fed an experimental diet had a significantly lower *post mortem* muscle temperature (approx 1°C) compared to pigs fed the control diet. These data are in agreement with previous studies by Rosenvold *et al.,* (2001a, b). Finally, the experimental diet resulted in a significantly higher $pH_{24\,h}$ in LD for the group with low slaughter weight.

Meat colour was measured in two muscles: the superficial carcass muscle *M. longissimus dorsi* (LD) and the deeper proportion of the ham muscle *M. semimembranosus* (SM). The extent of blooming as a function of aging in vacuum increased for up to 8 days *post mortem* in SM, with a simultaneous increase in redness, yellowness and chroma, hereby improving pork colour. Moreover, this effect was more pronounced in pork from the control group compared to the experimental group. Aging of the meat in vacuum has been reported to increase the blooming ability of the meat due to progressive inactivation of the inherent oxygen-consuming enzyme systems (Ledward, 1992) without substantial weakening of the MetMb-reducing ability (MetMbRA) of the meat (Bekhit & Faustman, 2005). In contrast, the effect of time of aging on the extent of blooming in LD showed a different picture. In LD, an initial decrease in redness and chroma

was found to take place within the first two to four days of aging dependent on the used feeding regime. Subsequently, the extent of blooming again became more pronounced for up to 15 days of aging in pigs fed the experimental diet, while this was not the case in pork from the control group. These differences are most obviously due to the early *post mortem* temperature effect on the inherent enzyme systems in the muscles. It is hypothesized that the higher muscle temperature during the first hour results in a more pronounced protein denaturation, which leads to early inactivation of oxygen-consuming enzymes (OCE) (Rosenvold *et al.,* 2003b; Lindahl *et al.,* 2006c). This would explain the slightly higher redness of pork from the control group compared to the muscle from the experimental group, where the enzyme activity is more preserved for up to 4 days *post mortem*. This can furthermore be explained by the fact that the a* value is very sensitive to early *post mortem* temperature changes in muscle compared to the L* and b* values (Lindahl *et al.,* 2006c) and even a small difference in *post mortem* muscle metabolism can influence colour characteristics of fresh pork (Lindahl *et al.,* 2006c).

Paper III showed that the above mentioned extent of blooming as a function of the first days of aging was dependent on slaughter weight. After one day of aging, the blooming was significantly lower in LD chops from pigs fed the experimental diet and with low slaughter weight, and significantly lower in LD chops from heavy pigs fed the experimental diet during the first three days of aging in vacuum compared to the control group. Independent of slaughter weight, LD chops from pigs given the control diet did not regain their ability to bloom after the first day of aging in vacuum. Increasing slaughter weight of the animals has been shown to affect pork colour characteristics in other studies (García-Macías *et al.,* 1996; Beattie *et al.,* 1999; Latorre *et al.,* 2004). By increasing the slaughter weight, the animal age is also often increased, and this may affect the content of pigment. However, another very important factor in relation to increased slaughter weight is the slower cooling rate, which is almost certain to affect colour development. In conclusion, slaughter weight in combination with diet is one of the mutually interacting factors to be considered in relation to colour development in pork, and it should be considered in the development and implementation of future slaughter lines through the introduction of differentiated cooling of the individual carcasses.

In order to be able to evaluate the rate of blooming in the present studies, the colour should have been measured directly after cutting the meat and again after blooming. However, previous studies have shown that the rate of blooming between cutting and after 1 hour of blooming did not differ between treatment groups (Rosenvold *et al.,* 2003b; Lindahl *et al.,* 2006b). Blooming progresses most rapidly during the first 30 min of exposure to oxygen, and it continues for up to 24 hours (Zhu, Bidner & Brewer, 2001; Lindahl *et al.,* 2006b).

The effect of diet-induced changes on subsequent colour development after aging in vacuum (colour stability) was evaluated in Paper II. The observed increase in colour values during air storage after aging in vacuum indicated a remaining blooming ability, where oxygenation of Mb takes place after up to 7 days in vacuum. In other studies of pork, a remaining blooming ability during the first day

of display in air has been shown in meat aged up to 9 days *post mortem* in vacuum (Lindahl *et al.,* 2006a, b).

The expected initial increase of the a* value in pork from pigs fed the experimental diet did not take place after one day *post mortem* in LD, instead oxidation, shown by a decreased redness, occurred. This unexpected fast oxidation to MetMb might be hypothesized to be an effect of the presence of the highly reactive peroxynitrite ($ONOO^-$), which has been shown to induce oxidation of reduced myoglobin (Connolly, Brannan & Decker, 2002; Connolly & Decker, 2004). The production of $ONOO^-$ proceeds through the interaction of H_2O_2 and nitric oxide (NO), with the latter being formed via nitric oxide synthase (NOS) (Koppenol, 1999). Such a formation of $ONOO^-$ in *post mortem* tissue has been suggested to be possible during the first 24 to 48 hours *post mortem* by Brannan & Decker (2002). Considering the high reactivity of peroxynitrite, this species most probably also oxidizes other reductive substances in the *post mortem* muscle, which potentially further accelerates the discoloration processes. The slightly lower $T_{45\ min}$ found in LD from strategically fed pigs compared to LD from control-fed pigs must be expected to decrease the inactivation of enzymes in the *post mortem* muscle of strategically fed pigs. Consequently, NOS induced NO production, and a potential increase of peroxynitrite formation could be expected in muscles from experimentally fed pigs and thus explain the accelerated oxidation of MbO_2.

The higher lightness (L* value) on the surface of pork from control-fed pigs compared to the experimentally fed pigs after extended *post mortem* aging and subsequent retail display can be explained by a more pronounced protein denaturation due to the higher early *post mortem* temperature as also reported by Rosenvold & Andersen (2003b) and Lindahl *et al.* (2006c). Likewise the initially higher $T_{45\ min}$ in the control group must be expected to result in a more pronounced inactivation of the oxygen-consuming enzymes and MetMbRA. This will allow the oxygen to penetrate deeper into the meat surface and explain the observed more red surface colour in bloomed pork from the control group as also reflected in the higher chroma and lower hue angle. Moreover, the discoloration in meat aged for a comparatively long time must be expected to proceed faster, if the MetMbRA has been reduced early in the storage period, as seen in the pork from the control group.

The subsequent discoloration rate of prior aged meat has been reported to be higher compared to non-aged meat due to loss of reducing activity over time *post mortem* (Ledward, 1992). The fact that discoloration was more pronounced in SM compared to LD can partly be explained by the different pH and temperature profiles early *post mortem* in these muscles. In SM, the temperature remains higher for a longer time period, which in combination with the simultaneous pH decline must be expected to weaken the enzyme-driven MetMb-reducing activity of the muscle more readily than in LD.

In the buying situation the consumers make their decision based on overall colour appearance, i.e. both muscle and fat colour. Interestingly the colour of the pork fat has got relatively less attention, even though this contributes to overall product appearance (Ringkob, 2003). In contrast, several colour studies on beef and lamb meat have reported that a high content of grass meal in the diet can have

an adverse influence on fat colour (Daly *et al.,* 1999; Priolo *et al.,* 2002). Considering the high grass meal content in the diet used in our studies, we decided to include investigations of fat colour. However, Paper I shows that despite the high content of grass meal in the experimental diet, this diet had only negligible influence on the colour of the backfat and stripped bacon fat. This is important in relation to the used strategy; however, it cannot be concluded from the present study whether the used finishing feeding can give rise to inferior fat colour, if the finishing period is further extended.

Our data (Paper I-III) clearly show that pork colour and colour stability is influenced by a number of production and slaughter factors, and that these factors consequently can be used to obtain specific qualities. In conclusion, diet, muscle and slaughter weight of the animals in combination with different aging times are all mutually interacting factors to be considered in relation to the control of colour development in pork. However, our studies could have been further strengthened if quantification of specific myoglobin redox species and proposed enzyme activities were quantified and combined with sensory colour analysis, as this would have made it possible to establish a more basic understanding of the effect of diet-induced mechanisms in *post mortem* muscle on colour development and perception.

The flavour development in meat mainly depends on the composition of inherent constituents, e.g. fat composition, peptides, glycogen concentration, vitamin content, especially thiamine and vitamin E, etc., and the type of heat treatment of the product (Andersen, Oksbjerg & Therkildsen, 2005b). In Paper IV, the influence of two diets with different sources of vegetable fat in the animal feed was studied with regard to overall pork quality and flavour development in cooked meat.

The diets were expected to cause differences in fatty acid composition of the meat. The standard diets were supplemented with α-tocopherol (200 mg/kg feed), and the fat source was either 3% of palm oil, which was expected to increase the level of saturated fatty acid or 3% of rapeseed oil, which was expected to increase the level of unsaturated fatty acids in the meat. Despite the obtained differences in lipid composition, no significant difference in overall meat quality parameters like pH, temperature, colour and water-holding capacity was found. Moreover, the flavour precursors inosine monophosphate (IMP), inosine, hypoxanthine, thiamine and glucose-6-P were not influenced by the diet. In a way this was an expected result as neither of the diet compositions differed to such an extent that differences in those precursors were anticipated. Many studies on the contribution of flavour precursors to cooked meat flavour have been carried out in model systems or by addition of potential flavour precursors to the meat at a concentration of 2 to 4 times that of the natural concentration in meat (Madruga & Mottram, 1995b; Farmer, Hagan & Paraskevas, 1998; Aliani & Farmer, 2005). While in Paper IV only freshly cooked pork without any addition of these precursors was studied, further analysis is needed to determine the exact conditions and concentrations that would favour the contribution of these flavour precursors to the flavour in pork.

As expected, the fatty acid composition of the meat was influenced by diet, with the changes in the PL fraction being minor. The feed containing rapeseed oil significantly increased the proportion of PUFA in backfat and in the NL fraction of

LD. Moreover, feeding pigs with a rapeseed oil diet raised the concentration of all the C18 unsaturated fatty acids, particularly the α-linolenic acid (C18:3). This is in line with the previous findings by Wood & Enser (1997) and Leskanich *et al.* (1997). In boiled, lightly grilled or roasted meat, thermal degradation of lipids have been reported to contribute to flavour formation by producing several dominating lipid-derived volatiles (Mottram, 1985, 1992, 1998b). Especially the phospholipids contribute to aroma development in meat, whereas triglycerides do not appear to be as important (Mottram & Edwards, 1983; Mottram, 1996).

In Paper IV, the dietary treatment resulted in no notable differences in the formation of the registered flavour attributes in the sensory results, except the higher scores for sourish odour from pigs fed rapeseed oil compared with palm oil. Instead some minor gender effects were found, and they were more pronounced in relation to texture attributes than to flavour. The fact that the two diets resulted in no notable differences between flavour attributes determined in sensory analysis might be explained by the relatively small difference obtained in the fatty acid composition using the two diets in combination with the high level of vitamin E. High antioxidant levels within the muscles limit the progress in oxidative reactions and hence prevent the formation of oxidation products (Lauridsen *et al.,* 1999). A limited effect between the diets supplemented with different fat sources to flavour attributes determined by a sensory analysis is in agreement with other studies (Leskanich *et al.,* 1997; Sheard *et al.,* 2000; Nuernberg *et al.,* 2005).

Moreover, no significant increase in rancid taste scores was found from freshly cooked oven roasts from pigs fed rapeseed oil compared to palm oil. Shackelford *et al.* (1990) have previously found that off-flavours were increased when feeding rapeseed oil to pigs compared with other high oleic acid dietary products. These off-flavours were attributed to the increase in 2-pentenal and 2.4-heptadienal, derivatives of linolenic acid. However, only C18:3 levels above about 3% of the total fatty acids in the porcine muscles give rise to C18:3-associated oxidation products that have an adverse impact on the flavour of cooked products (Wood *et al.,* 2003). In our study, the concentrations of C18:3 in meat from rapeseed-fed animals were approx. 2% in PL fraction, 1% in NL fraction and 3% in backfat.

In contrast to small diet-induced differences in sensory attributes, the cooking method influenced the intensity of sensory scores in Paper IV. Pan-fried pork developed a more intense, sweet odour compared with oven-prepared pork, which can be related to the formation of Maillard reaction-derived compounds, and the more intense piggy flavour and sourish taste in oven-roasted pork can be related to lipid oxidation-derived compounds (Mottram, 1985).

Finally, some sensory attributes correlated to the fatty acids in the PL fraction. Interestingly, correlations in pork from female pigs were more evident compared to castrates independent of cooking method. The polyunsaturated fatty acids and C18:2n-6c, which were found in higher levels in the PL fraction compared with the NL fraction of LD, were found to be significantly and positively correlated with the sensory attributes fried meat odour and sweet odour, but not associated to piggy flavour. In contrast, C17:0 and C18:1n-9c were significantly, positively correlated with piggy flavour and sourish odour and inversely associated with sweet odour and fried meat odour in female pigs. This is partly in agreement with the study by

Cameron *et al.* (2000), who found positive correlations between C18:2n-6, C20:4n-6, C22:4n-6 and with pork flavour, flavour liking and overall acceptability in the PL fraction of LD, and hereby it is consistent with data found in aqueous model systems showing that PLs are associated with meaty/pleasant, cooked chicken aroma (Farmer & Mottram, 1990, 1992). Thus specific fatty acids in the PL fraction might be associated with positive sensory attributes, while this connection could not be established between NL fatty acids and specific sensory attributes. However, more extensive research is needed to determine the role of each specific fatty acid and threshold levels for those fatty acids for contribution to cooked meat flavour. Low odour threshold values are likely to contribute to meat flavour (Mottram, 1992).

Increasing consumer/customer demands for pre-cooked and "ready-to-eat meat" are ever-increasingly stressing the need for minimizing and controlling the formation of warmed-over flavour (WOF), which readily develops during re-heating of cold-stored meat (Byrne *et al.,* 2001, 2002). To elucidate the consequence of typical fat sources in feed on the development of WOF in pork, the development of WOF in cooked, cold-stored and re-heated meatballs from porcine LD and SM was evaluated (Paper V). The study showed in alignment with previous studies (McMillin *et al.,* 1991; Kerry *et al.*, 1998; Jensen *et al.,* 1998b; Byrne *et al.,* 2001, 2002, 2003; O'Sullivan *et al.,* 2003) that the development in WOF-associated sensory attributes and most of the registered secondary oxidation products increased linearly during cold storage. Likewise, the increase in WOF development was more pronounced early in the storage period (day 0 to 2) than in the late part of the cold storage period (day 2 to 4) as also shown in other studies (Willemot *et al.,* 1985; Jensen *et al.,* 1998b; Byrne *et al.,* 2002).

Paper V also revealed that WOF formation determined by chemical analysis in the SM muscle was more pronounced as a function of cold-storage compared to the LD muscle, which can be explained by the higher percentage of PUFAs in SM, whereby this muscle is more susceptible to oxidation (Wood *et al.,* 2003). Moreover, the formation of TBARS differentiated pork samples according to the feed given to the pigs, as pork from pigs given rapeseed oil formed higher concentrations of TBARS during cold-storage and re-heating than pork from pigs fed the diet containing palm oil. However, these differences in differentiated WOF development as a function of muscle type and feed composition were not clearly demonstrated by the sensory panel, and several interactions were found to sensory attributes e.g. 'roasted' and 'boiled', which must be expected to affect the overall WOF perception.

Traditionally, many of the volatile lipid oxidation products have been monitored by headspace gas chromatography mass spectrometry (HS-GCMS), which like sensory analysis is an expensive and cumbersome method for detection of off-flavour formation. Recently, electronic nose systems containing an array of chemical gas sensors have been shown to have a high sensitivity towards volatile lipid oxidation products, e.g. aldehydes and ketones in meats (Grigioni *et al.,* 2000; O'Sullivan *et al.,* 2003; Haugen *et al.,* 2006). In Paper V we found that a gas sensor device can clearly separate pork samples according to the degree of WOF formation measured by both sensory analysis and chemical analysis (volatiles and

TBARS). Moreover, analysis of the individual gas sensors within the used gas sensor device clearly showed that different sensors within the gas sensor device responded selectively to volatiles associated to fresh meat and cold-stored, re-heated pork. The used gas sensor device resembles in this way the selectivity of other available electronic nose system sensors to specific volatiles (Haugen & Undeland, 2003; Olafsdottir *et al.,* 2005). Thus, electronic nose systems are to be considered a potential, rapid and economically feasible method, which could be used as a more rapid technique in detection and monitoring of off-flavours in foods and hereby substitute traditional expensive chemical and sensory methods (Haugen & Undeland, 2003; Haugen *et al.,* 2006; Olafsdottir *et al.,* 2005).

Our data (Paper IV-V) clearly show that there is a need to identify essential flavour precursors in fresh pork and to correlate these with flavour attributes in cooked pork to gain a better understanding of factors that influence specific flavour attributes in order to produce flavourful and consistent products. Combining results from chemical analysis with trained panels can give valuable information about if and to what extent consumers can taste differences, and which treatments are the most important for flavour development. Consequently, as the amounts of palm oil and rapeseed oil in the given diets did not give rise to inferior quality attributes in freshly cooked pork, they are both suitable fat sources that can be recommended for inclusion in diets, at these levels, with no detrimental effects on meat quality. This is furthermore supported by the fact that this used feed composition only seemed to have a minor effect in relation to warmed-over flavour development. Finally, for development of more successful strategies for pork production, information on the effects of production factors (e.g., breed and diet) and inherent factors (e.g., muscle type) on meat traits is further needed.

Conclusions

- Diet-induced change in the progress of early *post mortem* muscle temperature development affects the colour and subsequent colour stability of pork during aging. Thus, diet composition can be used as a tool to control meat colour and colour stability.

- Blooming progressed differently in chops from *M. longissimus dorsi* and *M. semimembranosus* during aging in vacuum.

- Grass meal content (<24%) has negligible influence on the colour of back fat and stripped bacon fat.

- Gender (female & castrate) does not have considerable effect on colour development during storage.

- Slaughter weight and diet are some of several mutually interacting factors that influence the colour development in pork.

- Diets with different sources of vegetable oil (rapeseed oil & palm oil; inclusion <3%) influence the fatty acid composition in pork, but have negligible effect on overall meat quality characteristics including sensory quality in freshly cooked pork.

- Polyunsaturated fatty acid and C18:2n-6 correlate positively to fried meat odour and sweet odour; monounsaturated fatty acid and C18:1n-9 correlate positively to piggy flavour and sourish odour in freshly cooked pork.

- The electronic nose (AppliedSensor AB, model 3320) is able to register volatiles that contribute to WOF, and this shows the potential of e-nose systems as quality control tools in the meat industry in the future.

Future research

The investigations performed in relation to the present thesis have shown the potential of the production factors feeding and aging of the pork, as control tools of several critical meat quality attributes. Future studies are suggested to focus on the following:

In relation to colour:

- Considering that small differences in *post mortem* pH/temperature progress affect colour characteristics via potential influence on the activity of inherent enzyme systems in the meat, more research should be focused on this area and on the underlying mechanisms to confirm the present results.

- An improved understanding of how several mutually interacting intrinsic and extrinsic factors influence colour characteristics is needed to develop guidelines for controlling pork colour and colour stability.

- For a further understanding of how colour and particularly colour stability during storage influence consumer perception, a visual colour evaluation should be included in addition to instrumental methods.

In relation to flavour:

- More extensive research is needed to determine the importance and role of each specific flavour precursor, including fatty acids, which are naturally present in meat in order to elucidate their effect on flavour development in pork.

- Threshold values for specific fatty acids and their contribution to specific flavour attributes would be necessary to examine and thus to further understand their role in flavour development of cooked pork.

- More research should focuse on the differences among various breeds, muscles, cooking methods, degree of doneness, storage conditions and their interactions on the subsequent flavour profile.

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