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Sensory Characterisation Studies on Warmed-Over Flavour in Meat

Ph.D. Thesis
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When the doors of perception are finally cleansed
One will see things as they truly are, infinite.

For Vicki and my parents, Jean and Maurice

Contents

Preface

The thesis presented is composed initially of a general review of lipid oxidation in muscle foods with emphasis on the investigation of sensory quality and chemical mechanisms in relation to warmed-over flavour (WOF) in cooked, chill-stored and reheated meats. Subsequently, details of the experimental materials and methods used in a series of studies carried out as part of the thesis are presented. Next, summaries of the studies that constitute the present thesis are included, followed by general conclusions and perspectives for the future. The research articles upon which the thesis is based are included in their entirety at the end of the dissertation. Overall, the project was financially supported by the European Union Grant, Fair-97-5003. In addition, the research was linked to studies supported by Danish Research Council, at present through the LMC-Centre for Advanced Food Studies as part of the FØTEK program.

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The following are a few lines where I will attempt to express my gratitude to the many people scientific and non-scientific (all human of course!) who have stuck with me through thick and thin to bring this thesis to fruition. It is of course an impossible task to express my thanks in words but I will do my best. Magni Martens: Well what can I say, the guiding light that brought me down the long road that was this Ph.D. Thanks Magni for allowing me to land in scientific heaven, which I never knew existed. You are but an inspiration. Wender Bredie: Again what can I say, but cheers, your help and guidance was a major force in getting this thesis finished. You always pulled out all the stops to help me and for that I thank you. Lets keep up the good work in the future as it has been nothing but a pleasure so far. Harald Martens: Thanks Harald I think you helped me to understand at lot about this multivariate 'mumbo jumbo' as a friend of ours calls it, which I could never have hoped to fathom in the last 3 years. Here's to future days and more collaboration, its always FUN!. Maurice O'Sullivan: I don't have 100 pages for how this man has been great to know in the last few years. But cheers Maurice, hows about a pint?. Judith Henning: The greatest!, you need it she's got it, if she doesn't have it, it doesn't exist. Thanks Judith just for being Judith. Garmt Dijksterhuis: cheers G man lets, do some more guitar as well. Anne Marie Laustsen: Thanks for you help in the last few years with the panellists. Lisbeth Pii: Cheers for the help in recent times. I would now like to thank the remaining sensory types, Michael Bom Frøst (the office mate, cheers!) and Lisbeth Wienberg for making it a good crew to work in. Last but not least I would like to thank Vicki, she has basically kept me from starvation in recent months, I love the lasagne keep it coming. Thanks for all your help, consideration and understanding, I could not have done it with out you.

Derek Victor Byrne, September 2000

Abstract

Byrne, D. V. (2000). *Sensory Characterisation Studies on Warmed-Over Flavour in Meat*. Ph.D. thesis.

The purpose of this thesis was to characterise warmed-over flavour (WOF) from the perspectives of sensory quality, the various chemical mechanisms and sensory-chemical interrelationships involved, in the development of the off-flavour in cooked, chill-stored and reheated meats.

Descriptive sensory vocabularies were developed for the characterisation of WOF in meats with additional variation, i.e. in pork meat; WOF and pre-slaughter stress, in chicken meat; WOF and oven cooking temperature, and further in pork meat; WOF, oven cooking temperature and RN^- genotype. These vocabularies were determined to describe and discriminate the sensory variation present in the experimental samples. In addition, a concise vocabulary development methodology taking approximately one week was devised as part of these studies.

Sensory profiles were carried out using the developed sensory vocabularies to study in detail the sensory variation in the cooked and chill-stored meats. Moreover, various chemical analyses were performed to allow study of sensory and chemical interrelationships with a view to further elucidation of the mechanisms underlying WOF in meat.

In pork sensory profiling with WOF and pre-slaughter stress variation, WOF was found to involve the development of a number of lipid oxidation derived nuance off-flavours and odours, in association with a decrease in 'meaty' flavour with days of chill-storage. Loss of cooked pork meat flavour was attributed to a combination of sulfur amino acid degradation and perceptual masking by lipid oxidation products. Chemical measurements TBARS and conjugated dienes were found to be strong predictors of the sensory terms related to the lipid oxidation aspect of WOF. In addition, PUFAs in the total lipid and phospholipids were found to decrease with WOF development, reflecting their loss in lipid oxidation reactions. Overall, WOF and pre-slaughter stress appeared in general to be manifested as separate sensory dimensions in the meat samples. However, some indications of interaction were found, indicating increasing pre-slaughter stress may have reduced perceived WOF development. From a sensory viewpoint increasing pre-slaughter stress was described by a sour to sweet taste continuum. This reflected increasing measured pH which was a strong predictor of the sensory effects resulting from pre-slaughter stress in the cooked meat. Water content was also found to be a highly correlated with the sensory effects of increasing pre-slaughter stress.

In chicken sensory profiling with WOF and oven cooking temperature variation, WOF was described by increased 'rancid' and 'sulfur/rubber' sensory notes associated with a concurrent decrease in chicken 'meaty' flavour. Cooking temperature was described by increased 'roasted', 'toasted' and 'bitter' sensory notes. Roasted flavour was determined related to both WOF and cooking temperature. Analysis of volatile compounds from the chicken showed a rapid development of lipid oxidation during refrigerated storage. This was also apparent in the sensory profiling data. Changes in identified sulfide compounds were conjectured as related to the

progression of lipid oxidation. Cooking temperature increased the formation of Maillard-derived compounds, however, did not show strong effects on the prevention of WOF in the chicken patties.

In pork sensory profiling with WOF and oven cooking temperature and genotype variation, RN^- carriers were determined to have significantly higher thawing and cooking losses than non-carriers. TBARS, reflecting lipid oxidation, were found to significantly increase with days of storage within a genotype and cooking temperature. In addition, TBARS was found to significantly increase in carriers of the RN^- gene relative to non-carriers at similar days of storage and cooking temperature. This was probably related to an effect of lower pH increasing the activity of pro-oxidants in the samples from RN^- carriers versus non-carriers. Furthermore, TBARS also appeared to significantly decrease at similar genotype and days of storage with increasing cooking temperature. This was most likely due to the antioxidant effects of Maillard reaction products produced when meat is cooked at high temperatures. In general, WOF development displayed a pattern involving the increase of off-flavour notes with a concurrent loss of fresh 'meaty' descriptors. Cooking temperature increase was found to be described by the terms caramel-like odour, roasted like odour and bread-like flavour. While RN^- gene was related to a group of terms that included, egg/sulfur/rubber-like flavour, lactic/fresh sour like aftertaste and flavour, sour and salt tastes and metallic flavour and aftertaste. In general, the experimental design variation, WOF, cooking temperature and genotype in the meat samples presented for sensory profiling were differentiated as independent phenomena and were characterised by specific groups of sensory terms. Moreover, each of the experimental parameters differentiated in sensory profiling, WOF, cooking temperature and genotype was 'predicted' by TBARS, cooking losses and thawing losses, respectively.

Overall, in all three profiling experiments similar terms described the WOF phenomenon, i.e. sweet taste, fresh pork or chicken meat-like to linseed oil-like, rancid-like flavour and odour characteristics, indicating the loss of freshly cooked 'meatiness' as lipid oxidation proceeded. However, the additional sensory variation appeared to be characterised by a number of the sensory descriptors that were not WOF related in each vocabulary, e.g. pre-slaughter stress by sour taste, cooking temperature by roasted-like flavour and RN^- gene by lactic/fresh sour-like flavour.

Keywords: Sensory quality, meat, pork, chicken, warmed-over flavour, sensory vocabulary, descriptive sensory profiling, pre-slaughter stress, cooking temperature, RN^- genotype, TBARS, pH, fatty acids, phospholipids, thawing losses, water content, cooking losses, GC-MS, multivariate data analysis

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Introduction

What is warmed-over flavour and why is it important?

Lipid oxidation is one of the primary mechanisms responsible for quality deterioration in meat and meat products. Changes in quality are largely manifested as adverse affects on meat flavour, colour and texture (Pearson et al., 1977; Pearson & Gray, 1983; Gray et al., 1996). In addition to affecting palatability factors, lipid oxidation may also result in compounds that have harmful biological effects in humans (Frankel, 1984).

Warmed-over flavour or 'WOF' was first recognised as a sensory defect and therefore a scientific challenge in muscle foods that results from the deleterious effects of lipid oxidation on the flavour of cooked meats by Tims and Watts (1958). The term WOF was used to describe the rapid onset of off-notes that developed in cooked meat as a result of refrigerator storage (4^{o}C). Oxidised flavours in cooked meats are readily detectable after 48 hours, in contrast to the more slowly developing rancidity that becomes evident in meat only after prolonged freezer storage (Pearson & Gray, 1983). The sensory perception of WOF is also further enhanced upon reheating of cooked stored meats prior to eating (Pearson et al., 1977). Moreover, there is evidence that WOF can develop just as rapidly in raw meat that has been comminuted and exposed to air (Greene, 1969; Sato & Hagarty, 1971). WOF also appears to develop in restructured fresh meat products as a consequence of disruption of the tissue membranes and exposure to oxygen in the air, however, the off-flavour develops more slowly (Gray & Pearson, 1987).

The autoxidation of membrane phospholipids is largely accepted as causal in the formation of WOF (e.g. Pearson et al., 1977; St. Angelo et al., 1987; Asghar et al., 1988). However, there is also strong evidence that reactions involving proteins or heteroatomic compounds may be implicated in WOF, resulting in a loss in desirable meaty flavour notes (Spanier et al., 1988; St. Angelo et al., 1988; St. Angelo et al., 1990).

As a sensory phenomenon, WOF has also been studied for many years from a sensory perspective. The earliest sensory investigations proposed terms such as 'warmed-over' itself, 'stale' and 'rancid' as terms to monitor off-flavour development (Tims and Watts, 1958). Subsequent sensory research has focused more on the description of the specific sensory characteristics manifested during the development of WOF. Thus, a number of descriptive lexicons have been developed for WOF in, e.g. beef (Johnson & Civille, 1986) and chicken, (Lyon, 1987). Overall, a general pattern of sensory WOF development has been noted, involving a decrease in fresh meatiness and the development of cardboard and finally rancid/painty notes with more prolonged chill-storage.

Recognition of WOF by the consumer prior to scientific acknowledgement is very likely, as evidenced by consumers who consider reheated meats to have a more off-flavoured nature compared to when eaten just after cooking (Cross et al., 1987). The increasing production of cooked meats and their distribution through the food catering sector, e.g. fast food restaurants, hospitals and airline meals, as well as increased marketing of prepared, pre-cooked 'delicatessen' type meats and meat based meals in retail outlets, ensure the potential for WOF development is high and its

prevalence is guaranteed well into the future (Cross et al., 1987). Food companies involved in the production of any form of convenience or 'value added' pre-cooked meat products need to be knowledgeable about WOF and its potential to significantly reduce the 'shelf-life' of cooked meat products from the perspective of consumer acceptability. It is generally accepted, that apart from microbial spoilage, lipid oxidation is the primary process by which quality loss arises in muscle foods (Buckley et al., 1995). In essence cooked and stored muscle foods may become unpalatable long before they become microbiologically unsafe.

Thesis articles I-VI

The present thesis is based on the following research articles, which will be referred to by the Roman numerals, I, II, III, IV, V and VI.

I. Byrne, D. V., Bak, L. S., Bredie, W. L. P., Bertelsen, G. and Martens, M. (1999a). Development of a sensory vocabulary for warmed-over flavour: Part I. in porcine meat. *Journal of Sensory Studies, 14*, 47-65.

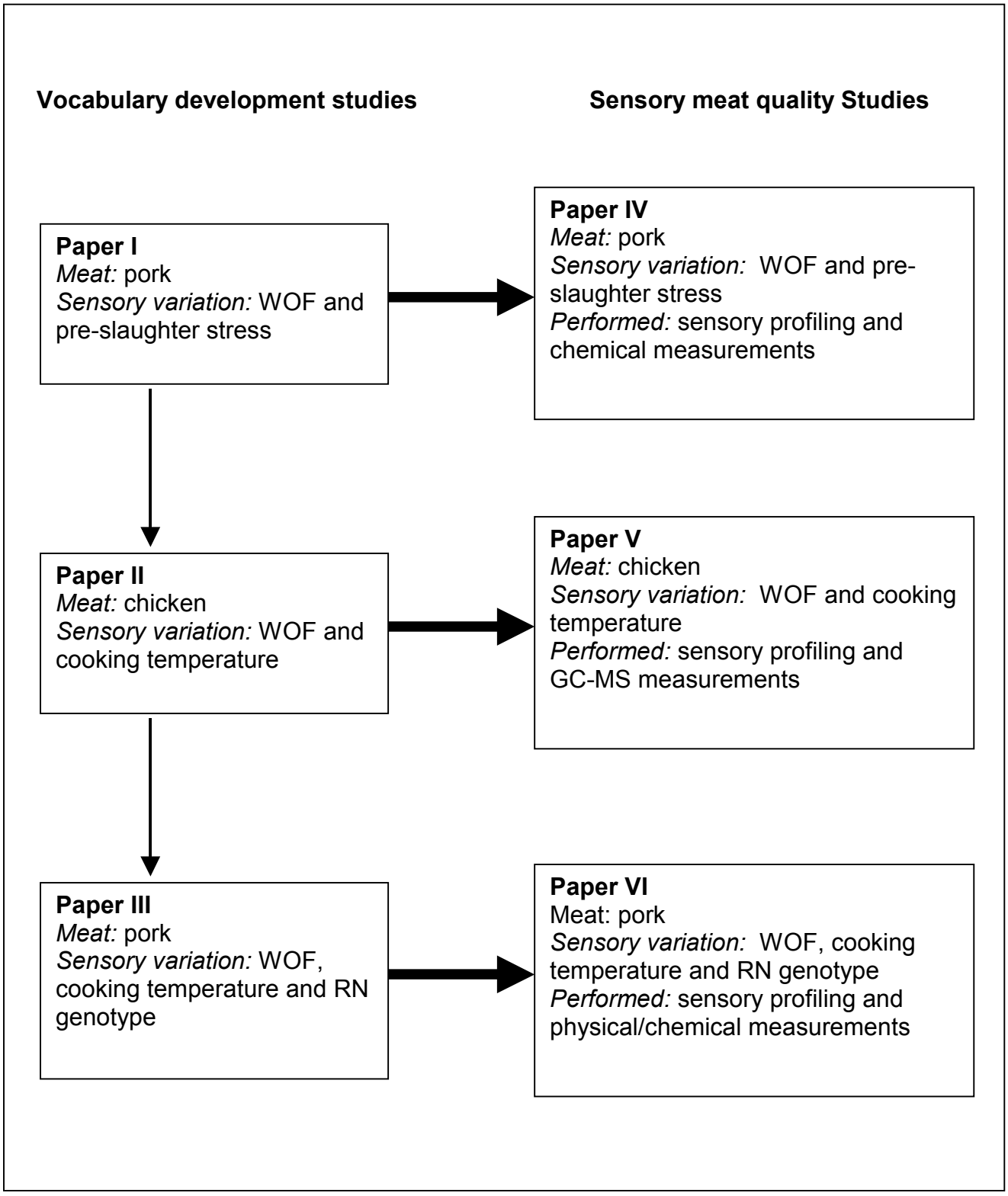
II. Byrne, D. V., Bredie, W. L. P., and Martens, M. (1999b). Development of a sensory vocabulary for warmed-over flavour: Part II. in chicken meat. *Journal of Sensory Studies, 14*, 67-78.

III. Byrne, D. V., O'Sullivan, M. G., Dijksterhuis, G. B., Bredie, W. L. P. and Martens, M. (2001). Sensory panel consistency during development of a vocabulary for warmed-over flavour. *Food Quality and Preference, 12,* 171-187.

IV. Byrne, D. V., Bredie, W. L. P., Bak, L. S., Bertelsen, G., Martens, H and Martens, M. (2001). Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Science*, *59,* 229-249.

V. Byrne, D. V., Bredie, W. L. P., and Martens, M. (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. *Meat Science, 61,* 127-139.

VI. Byrne, D. V., O'Sullivan, M. G., Bredie, W. L. P., and Martens, M. (2003). Descriptive sensory profiling and physical/chemical analyses of warmed-over flavour in meat patties from carriers and non-carriers of the RN^- allele. *Meat Science*, *63,* 211-224.



Schematic of the association of the research articles that constitute the present thesis

Objectives

The purpose of this thesis was to characterise warmed-over flavour (WOF) in cooked, chill-stored and reheated meats from a sensory perspective, using trained sensory panels. Moreover the aim was to determine the relationships that existed between sensory profiling data and relevant chemical/instrumental measurements, with a view to achieving a greater understanding of the chemical mechanisms in relation to the sensory characteristics that constitute WOF.

The aims of the thesis were:

- To develop a sensory vocabulary for the description of WOF in porcine meat patties resulting from animals subjected to different levels of natural and/or adrenaline induced pre-slaughter stress (Paper I).
- To develop a sensory vocabulary for the description of WOF in chicken meat patties that were oven cooked at different temperatures (Paper II).
- To develop a sensory vocabulary and analyse changes in panel agreement over the development sessions using multivariate data analysis, for WOF in porcine meat patties derived from carriers and non-carriers of the RN^- gene (Paper III).
- To investigate the sensory variation that resulted from WOF and pre-slaughter stress in porcine meat samples and to determine the predictive ability of chemical analyses for the descriptive terms that described this sensory variation (Paper IV).
- To determine the sensory variation resulting from WOF and different oven cooking temperatures in chicken meat patties and the relationships between the terms that described the sensory dimensions and GC-MS measured volatiles (Paper V).
- To elucidate the sensory properties of warmed-over flavour in carriers and non-carriers of the RN^- gene and relate these to chemical measurements with a view to determination of predictive and causal relationships (Paper VI).

1. Review: *Sensory meat quality and warmed-over flavour (WOF)*

1.1. Mechanisms, promotion and prevention of WOF in cooked meats

1.1.1. Mechanisms of lipid oxidation

Lipid oxidation is a rather complex process whereby unsaturated fatty acids react with molecular oxygen via a free radical mechanism to predominantly form fatty acyl hydroperoxides, generally referred to as peroxides or the primary products of lipid oxidation. The primary autoxidation step is followed by a series of secondary reactions which lead to the degradation of the unsaturated lipids and the development of WOF in cooked and chill-stored meats (see Asghar et al., 1988; Ladikos & Lougovios, 1990; Skibsted et al., 1998). In schematic form, Fig. 1 (Appendix A) displays the steps, reactions and the compounds that are produced as a result of lipid oxidation in cooked meats. In general, oxidation is initiated when a labile hydrogen atom is abstracted from a site on the fatty acyl chain (RH). This produces a free lipid radical (R˙), which reacts rapidly with activated oxygen to form a peroxyradical (ROO˙). The peroxyradical abstracts a hydrogen atom from another hydrocarbon chain (RH) yielding a hydroperoxide (ROOH) and a new free radical (R˙), which can propagate the chain reaction mechanism (Ingold, 1962; Tappel, 1962).

The decomposition of the hydroperoxides involves further free radical mechanisms and the formation of stable products. Homolysis of lipid hydroperoxides to hydroxyl and alkoxy radicals followed by cleavage (β-sission) of the fatty acid chain adjacent to the alkoxy radical produces low molecular weight volatile compounds, some of which have distinct aromas and can affect flavour properties (Mottram, 1998). Many of these degradation products have been attributed as causal in the sensory detection of the lipid oxidation aspects of WOF in cooked and stored meats, e.g. aldehydes, ketones and alcohols (e.g. St. Angelo et al., 1987). Lipid hydroperoxides may also condense into dimers and polymers, that subsequently oxidise and decompose into volatile breakdown products (Shahidi, 1998). Additional oxidation may also occur in the unsaturated aldehydes or in the original peroxides, which through further degradation form epoxides, cyclic peroxides and bicyclic endoperoxides (Enser, 1987). These secondary products can also break down to form additional volatiles and dialdehydes that further contribute to cooked meat flavour deterioration (Ladikos & Lougovios, 1990).

1.1.2. Decreasing meatiness as a result of WOF development in cooked meat products

The autoxidation of unsaturated fatty acids and the off-flavour compounds that result have largely been accepted as the main cause of the sensory perception of WOF in cooked and chill-stored meats (e.g. Pearson et al., 1977; St. Angelo et al., 1987; Asghar et al., 1988). However, there are reports that reactions involving the degradation of sulfur containing heteroatomic compounds, leading to a decrease in 'meatiness', may also be an integral part of WOF development (Spanier et al., 1988; St. Angelo et al., 1988; St. Angelo et al., 1990). As a result, Spanier et al. (1988) have suggested that a term such as meat flavour deterioration (MFD) may be more appropriate to describe the complex series of chemical reactions that contribute overall to the development of off-flavours and the loss of desirable meaty flavours in cooked and chill-stored muscle foods.

1.1.3. Promotion of WOF

1.1.3.1. Lipid composition, meat processing and cooking

The main unsaturated fatty acids that make up the lipids of animal tissues are oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and arachidonic acid (C20:4) (Wilson et al., 1976; Slover et al., 1987). Their autoxidation results in a number of different hydroperoxides which through the multitude of different decomposition pathways lead to the large range of volatile compounds that are involved in WOF (Mottram, 1998). The unsaturated fatty acids of phospholipids, specifically, have been shown to be the major contributors to the development of WOF in cooked meats (Ingene & Pearson, 1979). Wilson et al. (1976) demonstrated that phospholipids played a major role in the development of WOF in all cooked meats except pork, where total lipid level appeared to be the major contributor. Studies on individual phospholipids have demonstrated phosphatidyl ethanolamine in particular to be the major phospholipid involved in WOF development in cooked meat (Pearson et al., 1977; Asghar et al., 1988). The susceptibility and rate of oxidation of phospholipids depend on the level of fatty acids present and their degree of unsaturation. Autoxidation of the major fatty acids has been reported to follow the order C18:1 < C18:2 < C18:3 < C20:4 (Berlitz & Grosch, 1999). Moreover, authors such as Tichivangana and Morrissey (1985) and Rhee et al. (1996) have shown that lipid oxidation of muscle foods occurs in the order, fish > poultry (chicken and turkey) > pork > beef > lamb. This ordering is largely related to increasing levels of the more susceptible unsaturated fatty acids in each of the tissues phospholipids (Melton, 1983).

Variation in dietary lipid content is known to result in changes in the lipid composition of animal tissues (Pearson et al., 1977). Thus, a number of studies have investigated the effect of dietary fatty acid composition on the susceptibility of subsequently derived meat products to WOF. For example, it is well established that incorporation of fish oil or meal, high in unsaturated fatty acids, into the rations of animals can lead to the development of increased levels of WOF in pork (Willemot et al., 1985) and chicken (O'Keefe et al., 1995).

In terms of meat processing, any disruption of the integrity of muscle membranes by methods such as mechanical deboning, grinding or restructuring results in altered cellular compartmentalisation This facilitates the interaction of lipid oxidation catalysts with unsaturated fatty acids and results in the generation of free-radicals and propagation of the lipid oxidation mechanism (Pearson et al., 1977). Moreover, mild heating of meats, to core temperatures of 70-80^{o}C, further disrupts muscle membrane structure. Subsequently, upon chill-storage (within 48 hours) the rapid development of off-notes, that characterise WOF in cooked meat, are manifested (Asghar et al., 1988; Love, 1988). WOF has also been reported to develop in restructured fresh meat products as a consequence of the disruption of the tissue membranes however, the off-flavour develops much more slowly (Gray & Pearson, 1987).

1.1.3.2. Catalysts

There is still much confusion as to the nature of the initiation process in lipid oxidation. It is known that spontaneous lipid radical formation or direct reaction of unsaturated fatty acids with molecular oxygen is thermodynamically unfavourable (Gray et al., 1996). However, the spin restriction which prevents the direct addition of triplet state oxygen to singlet state unsaturated fatty acid molecules can be overcome by several mechanisms including photoxidation, partially reduced or activated oxidation species such as hydrogen peroxide, superoxide anion, and hydroxyl radical active oxygen-iron complexes (ferryl radical), and iron-mediated homolytic cleavage of hydroperoxides which generates organic free-radicals (Hsieh & Kinsella, 1989; Kanner, 1994). Initiation of lipid oxidation in biological systems has been widely researched and discussed. However, at present much of the evidence in support of proposed initiators is tentative rather than conclusive (Gray et al., 1996). Most researchers indicate however, that the presence of transition metals, notably iron is pivotal in the generation of species capable of abstracting a hydrogen atom from an unsaturated fatty acid (Kanner, 1994).

Much of the information related to lipid oxidation in cooked meats deals with hydroperoxide-dependent lipid oxidation (Gray et al., 1996). Pure lipid hydroperoxides are stable at physiological temperatures, but in the presence of transition metal complexes, especially iron salts, their decomposition is greatly accelerated. The ferrous ion (Fe^{2+}) causes fission of peroxide bonds to form very active alkoxy radicals for the propagation reaction, whereas the ferric ion (Fe^{3+}) can form both peroxy and alkoxy radicals (Ingold, 1962). Sato and Hegarty (1971), presented evidence that 'free' non-haem iron and ascorbic acid were the major catalysts of lipid oxidation in cooked meat. Ingene et al. (1979) further reported that the increased rate of lipid oxidation in cooked and stored meats was due to the release of non-haem iron as a result of cooking. Lipid oxidaton in cooked meats has also been attributed to both non-haem and 'protein stabilised' or haem iron (Liu & Watts, 1970). Haem pigments have been reported to be more active as lipid oxidation catalysts with iron in the ferric state, whereas non-haem iron appears to be more active in the ferrous state in cooked uncured meats (Greene & Price, 1975; Pearson et al., 1977). Later work by Tichivangana and Morrissey (1985), Ledward (1987) and Johns et al. (1989), revealed that, in raw meat and model systems, ferric haem pigments were the main catalysts of lipid oxidation, whereas in heated meats the system was much more complex and inorganic iron played a more important role. Thus, it appears that the relative contributions of the different forms of iron in promoting lipid oxidation in cooked meats have yet to be definitively assigned (Gray et al., 1996). More detailed discussion of the iron catalysis of lipid oxidation in cooked and chill-stored meats has been presented in exemplary reviews by, Love (1987) and Decker and Hultin (1992).

Sodium chloride (NaCl), an important additive in the meat industry, has been reported to act as a powerful promoter of lipid oxidation in pre-cooked meat products (Ladikos & Lougovois, 1990; Kanner, 1994). Chen et al. (1984) for example found NaCl to induce WOF in cooked and chill-stored beef. The mechanism however, by which NaCl promotes lipid oxidation is poorly understood and requires clarification (Kanner, 1994).

1.1.4. Prevention of WOF

1.1.4.1. Environmental conditions, free radial preventers and terminaters

The susceptibility of pre-cooked ready-to-eat meat products to lipid oxidation and the development of WOF has challenged the meat scientist, processor and distributor to develop ways to extend the 'shelf life' of such products in terms of palatability to the consumer. A great variety of substances and conditions may be considered as exerting antioxidant effects in preventing WOF. According to Ladikos and Lougovois (1990) these could be classified into three areas: (1) environmental factors (such as redox compounds, e.g. cysteine and a_w regulators), physical conditions and packaging materials, (2) antioxidant free radical preventers (controlling the production of free radicals during the oxidation mechanism) such as metal complexing agents, e.g. ethylenediamine tetraacetic acid (EDTA), citric acid and phosphates, and (3) antioxidant free radical terminaters (donating hydrogen to the free radical and thus stopping the chain reaction) such as phenolic compounds, e.g. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutyl hydroquinone (TBHQ) and dietary vitamin E.

Much attention has been focused recently on packaging methods, and both vacuum packaging and modified atmosphere packaging have proved very effective in controlling WOF in meat products (Mielche & Bertelsen, 1994). The beneficial effects of vacuum packaging has been shown by for pre-cooked chill-stored sliced or ground beef (Stapelfeldt et al., 1993) and for pre-cooked chilled or freezer stored sliced turkey or sliced pork (Nolan et al., 1989). Vacuum packaging is suitable for many food items, including sliced meats, but the use of high vacuum is limited for certain products such as ready-to-eat meals containing, e.g. sauces (Mielche & Bertelsen, 1994). For such products modified atmosphere packaging is an effective alternative. Church (1994) has reviewed the most recent developments in modified atmosphere packaging materials, machinery and related sensory technology and concluded that these will improve shelf life, sensory quality, product range and safety of cooked muscle foods.

Overall, many studies have indicated that lipid oxidation can be controlled effectively or at least minimised in cooked meats or meat products by the use of antioxidants. The applications of antioxidants in meat systems to prevent WOF have been described in detail in reviews by, e.g. Simic et al. (1992); Mielche and Bertelsen (1994); Gray et al. (1996) and Skibsted et al. (1998). Overall, antioxidant compounds generally employed range from commercial synthetic phenolic antioxidants such as BHT and EDTA, nitrite, phosphates, and on to more exotic compounds isolated from natural food products. The inhibitory effects of naturally occurring antioxidants in cooked and stored meats has been extensively discussed by St. Angelo et al. (1988). Of all the antioxidants, vitamin E, as it can be incorporated into the cell membranes of animal tissues through dietary supplementation, has received most attention in recent years as a method of reducing the development of lipid oxidation in meat (Buckley et al., 1995; Skibsted et al., 1998). Vitamin E has been reported to be effective at improving the oxidative stability of cooked pork. However, this was true only at very high levels of supplementation, i.e. 700 mg α-tocopherol (Jensen et al., 1997).

1.1.4.2. Maillard reaction products

The extent of lipid oxidation and thus, the level of WOF developed in cooked meat also appears to be related to the intensity of heat treatment. As previously discussed, at 'mild' or 'normal' cooking temperatures WOF development is enhanced upon chill-storage (Asghar et al., 1988). However, when much higher temperatures (above 100^{o}C) are used in cooking, e.g. in the retorting of canned meats, WOF development is greatly reduced (Pearson et al., 1977). This effect has been attributed to the antioxidant properties of melanoidins or premelanoidins, in the presence of lipids, produced from non-enzymatic Maillard browning reactions that occur when meat is heated to such temperatures (Bailey, 1988). An early study by Sato et al. (1973) demonstrated that reductic acid, maltol and other products of the Maillard amino-sugar reaction, in extracts from retorted canned meats, were effective inhibitors of WOF in cooked ground beef. Einerson and Reineccius (1977) have reported that retorted turkey extract exhibited strong reducing properties similar to those of reductones (known intermediates of the Maillard browning reaction) and was considered to act as a primary antioxidant, interrupting the free radical mechanism. Bailey (1988) and Bailey and Um (1992) have reviewed the effects of Maillard reaction products (MRP) on WOF and concluded that they appear to be a quite effective in prevention and have great potential for preserving the desirable 'meaty' flavour of processed meats.

Moreover, it must be noted that many MRPs are distinctly meaty in nature, e.g. 1-(2-methyl-2-thienyl)-ethanethiol or 2-methyl-3-(methylthio)-furan. Thus, not only do MRPs help prevent lipid oxidation they are also largely responsible for the production of desirable cooked meat flavour (Mottram, 1998). Furthermore, as previously mentioned, the loss of such meaty compounds in conjunction with the development of lipid oxidation derived off-flavour compounds is considered an additional aspect of WOF development (e.g. Spanier et al., 1988).

1.2. Additional factors influencing cooked meat quality

1.2.1. Pre-slaugher stress

The relationship between pre-slaughter stress and the general quality of meat has been studied by a number of authors (Lewis et al., 1989; Fernandez & Tornberg, 1991; Warris et al., 1994; Watanabe et al., 1996). It has been shown that the amount of pre-slaughter stress imposed on animals leads to variation in pH decline post-mortem (e.g. Henckel et al., 2000). Much work has shown that the rate and extent of post-mortem pH decline has major implications for meat quality attributes such as, water holding capacity and colour stability (e.g. Briskey, 1964; Bendall & Swatland, 1988). According to Bendall, (1973) the characteristics of post-mortem pH decline are determined by the physiological conditions that reside in the muscle at the time of slaughter and can be related to lactate production, or more specifically, to the capacity of the muscle to produce adenosine triphosphate (ATP). Capacity for ATP production is reduced by the depleting effects of pre-slaughter stress on muscle glycogen reserves. The result is limited post-mortem glycogenolysis and glycolysis, resulting in meat with an above normal ultimate pH (pH_u). (Warris et al., 1989).

A related area of study has been on the application of chemical and biochemical substances pre-slaughter to animals and their subsequent effects on meat quality (Fernandez et al., 1992). It has been known since the early results of Cori and Cori (1928a), and Cori and Cori (1928b) that pre-slaughter injection of epinephrine depletes muscle glycogen leading to an elevated pH_u in the raw meat. Thus, producing a similar effect to natural or exercise induced pre-slaughter stress. Epinephrine is known to enhance the glycogenolytic activity by activation of phosphorylase and phosphofructokinase, in both cases via cyclic adenosine monophosphate (AMP) (Sutherland & Cori, 1951). The ability of epinephrine to deplete glycogen levels and produce an increased pH_u has been demonstrated in the rabbit (Bendall & Lawrie, 1962) and pig (Hatton et al., 1972; Henckel et al., 2000).

The effect of pre-slaughter stress on WOF development in cooked and chill-stored meat has not been reported per se. However, authors such as Tichivangana and Morrissey (1985) have found through TBARS measurements of minced cooked pork muscle systems that lipid oxidation was most pronounced at low pH (5) and reduced significantly by increasing pH up to pH 7. Thus, above normal pH_u due to pre-slaughter stress, may be postulated to reduce the development of lipid oxidation and thus WOF in cooked meats. The authors further suggested that the effect of increasing pH on lipid oxidation was mainly through its effect on reducing the activity of pro-oxidants.

1.2.2. The RN^- gene

The RN^- gene, was discovered in Hampshire and Hampshire cross pigs in the mid 1980s. A bimodel distribution was found for yield of cured cooked ham, so called 'Paris ham', and animals carrying the dominant allele (RN^-) gave meat with a lower processing yield (Le Roy et al., 1990). A laboratory method, termed 'Napole' (from the names of the researchers who devised the test Naveau, Pommeret and Lechaux) was devised to estimate the yield of cured cooked muscle (Naveau et al., 1985), and gave the RN^- gene its name (French: Rendement Napole, Napole yield). Carriers of the dominant RN^- allele have been found to have higher muscle glycogen density (up to 70% higher) and water content, but no difference in lipid content relative to other breeds (Monin et al., 1987; Monin et al., 1992). The higher glycogen, found mainly in the glycolytic muscles of crossbred RN^- carriers leads to a lower post-mortem ultimate pH (pH_u) when compared to non-carriers (Monin et al., 1987; Le Roy et al., 1995; Lundström et al., 1996; Enfält et al., 1997a). This pH_u results in reduced water holding capacity and higher cooking losses in RN^- carrier meat (Naveau et al., 1986; Lundström et al., 1998). The biochemical differences present in the meat of RN^- carriers vis-à-vis Hampshire non-carriers may be expected to have an influence on the sensory properties of such meats once cooked. However, studies on the development of WOF in meat from carriers of the RN^- allele have not been previously reported. It has, however, been reported that cooked meat from RN^- allele pigs has elevated taste and flavour intensities (Lundström et al., 1996; LeRoy et al., 1996). More recently, RN^- meat has been described as having a more acidulous taste and an enhanced meaty flavour (Enfält et al., 1997b; Johansson et al., 1999).

1.3. Measurement of WOF from a chemical perspective

1.3.1. Background

As lipid oxidation is a very complex process one would imagine that there are many ways in which to measure the extent of the oxidative changes that occur. Of course there indeed are a multitude of methodologies, some more reliable and accepted than others (Melton, 1983). In the following section an overview of the chemical/instrumental methods that have been used and have the potential to be used to monitor lipid oxidation, specifically in cooked, chill-stored and reheated meat products is presented.

In determining the usefulness of a particular chemical/instrumental analytical procedure for lipid oxidation measurement, a number of questions must be addressed. These include the following: (1) does the parameter measured adequately represent the extent of lipid oxidation?, (2) is the method specific for that particular parameter?, and (3) would the parameter measured arise under any circumstances other than lipid oxidation? (Gray and Monahan, 1992). In addition, the suitability of a method is dependent on the type of product and the way it has been processed and stored (Coxon, 1987). Also, of paramount importance is the degree of correlation the method has with sensory analysis where lipid oxidation or WOF is assessed (Spanier et al., 1998).

Overall, the chemical/instrumental methods available to measure lipid oxidation in cooked meat can be divided into those that measure: (1) primary changes and (2) secondary changes (Table 1, Appendix B). Under the following heading methods that are commonly used, have the potential to be employed, or those that are not widely used for WOF measurement are presented.

1.3.2. Measurement of primary changes

Methods that measure primary changes can be classified as those that quantify the loss of reactants, e.g. polyunsaturated fatty acid (PUFA) or oxygen, or those that determine the formation of primary lipid oxidation products, e.g. hydroperoxides and conjugated dienes. Of these, fatty acid composition and conjugated dienes represent two of the more promising methods available to indirectly monitor WOF development.

1.3.2.1 Fatty acid composition

Changes in fatty acid composition to measure the susceptibility of meat lipids has been used by a number of authors to monitor lipid oxidation indirectly in meat and model meat systems. In cooked pork meat. Willemot et al. (1985) reported decreases in PUFAs, particularly linoleic acid (C18:2) and arachidonic acid (C20:4), with increasing days of chill-storage and WOF development. Similar results have been reported by Ingene and Pearson (1979) and Ingene et al. (1980) in beef and poultry meat model systems. Ingene and Pearson (1979) further reported that the PUFAs of the phospholipids, particularly phosphatidyl ethanolamine (PE), are largely causal in lipid oxidation and WOF development in cooked meat. Overall, the use of chromatographic methods such as Gas Chromatographic (GC) coupled with Mass Spectroscopy (GC-MS) and High Performance Liquid Chromatography (HPLC) for separation, quantification and identification of individual

phospholipids and fatty acids, present fast and accurate methods for assessing the extent of lipid oxidation and WOF in cooked and stored muscles foods (Bolton et al., 1985).

1.3.2.2. Conjugated dienes

Conjugated dienes, trienes and tetraenes, referred to as total conjugated products of oxidation, have been determined in total lipid extracts of turkey meat stored at 4^{o}C and –18^{o}C by Sklan et al. (1983). The content of conjugated products resulting from lipid oxidation was found to increase at both temperatures, using absorbance spectroscopy at approx. 233-235 nm. In a study involving oxidation of PUFA mixtures, Corongu and Milia (1983) also found increasing levels of conjugated diene double bond structures, arising from oxidation of linoleic acid (C18:2), using second derivative (233-235 nm) spectroscopy (Schmitt, 1977). Corongu and Milia (1983) reported that when the derivative of the spectrum was taken, additional resolution and discrimination resulted in the sample spectra. Thus, for conjugated diene analysis derivative spectroscopy was more useful than the absorbance spectrum for describing small differences between similar spectra. More recently, Nielsen et al. (1997) used conjugated dienes to monitor the development of lipid oxidation in raw and cooked pork meat, also with derivative spectroscopy. In general, however, the conjugated diene or total conjugated products methods have not been routinely used by meat scientists, although they are popular methods for measuring lipid oxidation end products in human body fluids (Gutteridge & Halliwell, 1990). As these methods have been proven to be reliable in the measurement of lipid oxidation in other areas, they may also offer a practical procedure for assessing lipid oxidation in meat and meat products.

1.3.3. Measurements of secondary products

In cooked and chill-stored meats lipid oxidation occurs at a rapid rate and primary products (e.g. hydroperoxides) quickly decompose to stable secondary products. Thus, it is more appropriate to measure secondary products as a method to directly monitor WOF development. It is apparent from the literature that the 2-thiobarbituric acid (TBA) test, quantification of hexanal, and fluorescence measurement have been used most effectively to monitor the secondary products of lipid oxidation in cooked and chill-stored meat and meat products (Gray & Monahan, 1992).

1.3.3.1. TBA test

One of the oldest and most frequently used tests for assessing lipid oxidation in muscle foods is the TBA test (see Tarladgis et al., 1960; Melton, 1983; Gray & Monahan, 1992, Fernández et al., 1997). The extent of lipid oxidation is reported as ‘TBA number’, ‘TBA value’ or ‘TBA-reactive substances’ and is expressed as mg or μg of malonaldehyde equivalents per kg sample or per kg dry matter. Malonaldehyde is a relatively minor lipid oxidation secondary product formed during the oxidation of PUFAs, and reacts with TBA to produce a coloured complex with an absorbance maximum at 530-532 nm. This absorbance is usually measured using UV-visible spectroscopy. The intensity of the colour complex found on reacting the TBA reagent with lipid-containing foods,

food extracts or steam distillates of foods was originally believed to be a measure of malonaldehyde concentration (Tarladgis et al., 1960). However, several confounding factors can also affect the colour complex. For example, alkenals and 2,4-alkadienals may also react with TBA to form a coloured complex with absorption at 532 nm (Marcuse & Johansson, 1973; Kosugi et al., 1987 and 1988). For this reason, Gray and Pearson (1987) suggested that the TBA method should be considered to assess the general extent of lipid oxidation, rather than to quantify malonaldehyde, and the term 'thiobarbituric acid-reactive substances' (TBARS) is now commonly used in place of TBA number or TBA value.

Numerous authors have determined the TBA or TBARS test to be a strongly correlated with WOF development in cooked and chill-stored meat and meat products, including, chicken (Ingene et al., 1979; Lyon et al., 1988; Ang & Lyon, 1990; Lyon & Ang, 1990; Lai et al., 1995), turkey (Wu & Sheldon, 1988), pork (Willemot et al., 1985; Poste et al., 1986; Satyanarayan & Honikel, 1992) and beef (St. Angelo et al., 1990; Stapelfeldt et al., 1992). Thus, the TBA/TBARS test is widely considered an index of lipid oxidation and WOF in cooked meats. In addition, all of these authors also reported strong correlations between the TBA or TBARS test and sensory scores for WOF development. As an overall conclusion on the use of the TBA or TBARS test in such studies, Stapelfeldt et al. (1992) indicated that due to the simplicity of the method and the excellent correlation with sensory analysis it should be considered one of the first methods of choice for the objective assessment of WOF in meat.

1.3.3.2. Hexanal and other aldehydes

Other degradation products of lipid hydroperoxides have also been quantified and used as indices of lipid oxidation in meats (Gray, 1978; Melton, 1983). Hexanal, one of the major secondary products formed during the oxidation of linoleic acid (C18:2) (Frankel et al., 1981), and other aldehydes, usually analysed by GC-MS, have been successfully used to follow lipid oxidation in meat products. Overall, hexanal has been used to evaluate the oxidative state, particularly, of red meat from different species (e.g. Shahidi et al., 1987; St. Angelo et al., 1987; Drumm & Spanier, 1991; Nielsen et al., 1997). Shahidi et al. (1987) reported a linear relationship between hexanal content, sensory scores and TBA numbers in cooked and chill-stored ground pork. Drumm & Spanier (1991) and St. Angelo et al. (1987) established similar correlations for cooked and chill-stored beef. Also in agreement, Nielsen et al. (1997), found hexanal analysed by solid-phase micro-extraction (SPME) coupled with GC-MS verification, to increase in heat-treated and chill-stored minced pork. In terms of other aldehydes, St. Angelo et al. (1987) proposed that pentanal and 2,4-decadienal could be considered for use as marker compounds to follow the development of WOF and its associated rancid flavours in cooked meats. Therefore, analysis of aldehydes, particularly hexanal, appears to be sensitive and reliable method for evaluation of the oxidative state of cooked and chill-stored meats. Also, the fact that linoleic acid (C18:2) occurs to some degree in all muscle foods means that measurement of hexanal, in particular, can provide a useful method for evaluation of the oxidative status of muscle foods.

Furthermore, hexanal, specifically, has a distinctive odour described as 'green' or 'grassy' (Ullrich & Grosch, 1987; Gasser & Grosch, 1988). It also has a very low odour threshold concentration and is detectable at levels as low as 100 ppm (Schieberle & Grosch, 1987). These odour characteristics have also been exploited successfully in a number of studies involving Aroma Extract Dilution Analysis (AEDA) by GC/Olfactometry (GC/O), where hexanal increase has been used to monitor the development of WOF, e.g. in boiled chicken (Grosch et al., 1992) and ground beef (Kerler & Grosh, 1997).

1.3.3.3. Fluorescence

The measurement of fluorescent oxidation products by spectroscopy has been introduced as a method for studying lipid oxidation in muscle foods (Kamarei & Karel, 1984). More recently, the method has been employed successfully in monitoring increases in WOF in cooked and chill-stored sliced beef (Stapelfeldt et al., 1992) and cooked and warmed-over pork patties (Brøndum et al., 2000).

1.3.3.4. Additional methods that have not been commonly used for WOF measurement

Measurement of the uptake of oxygen as described by the AOCS (1989), has been used to assess the susceptibility of muscle tissue homogenates to lipid oxidation (Lee et al., 1975; Silberstein & Lillard, 1978). Although oxidation of proteins may contribute to the absorption of oxygen in muscle tissue lipids, existing differences in their rates of oxidation makes effective utilisation of the method viable (Rhee, 1978). Oxygen uptake has however, not been used widely in the evaluation of the oxidative state of WOF in cooked meat (Pearson et al., 1977; Melton, 1983).

Hydroperoxides are a major primary product of lipid oxidation in meat and may be measured by a variety of methods on extracted lipids (Gray, 1978). The most common method for measuring the content of hydroperoxides is the 'peroxide value' determination, which employs an idometric technique, generally based on the method described by the AOAC (1989), in which the peroxide value is reported as milliequivalents (meq) iodine per kg/fat or kg/total lipid. Measurement of the peroxide value has been used to estimate lipid oxidation in a number of meat products, generally in frozen uncooked meats. Bailey et al. (1973) have used peroxide value for evaluation of pork fat quality during frozen storage, and its use to determine the oxidative state of beef muscle tissues during 10 weeks of frozen storage has been reported by Owen et al. (1975). However, the decomposition of peroxides to secondary lipid oxidation products can result in an underestimation or decrease in the peroxide value during storage of meat (Awad et al., 1968; Noble, 1976). Due to the latter consideration, peroxide value has not been used extensively in the evaluation of the oxidative state of cooked meat and meat products, where measurement of secondary changes is more important (Pearson et al., 1977; Melton, 1983).

A number of other tests also exist for the measurement of lipid oxidation in muscle foods. These include the Kries test, Anisidine value and Totox value, which is a combination of peroxide value and Anisidine value and gives a measure of total oxidation (Shahidi, 1998). These methods are not

usually employed in the evaluation of lipid oxidation in meat and meat products, particularly in relation to WOF assessment.

Formation of short-chain hydrocarbons, particularly ethane and pentane, from the oxidation of lipids has also been documented (Horvat et al., 1964; Evans et al., 1967). Measurement of pentane, another breakdown product of linoleic acid has been used as an indicator of lipid oxidation in freeze dried muscle foods (Seo & Joel, 1980). However, the use of alkanes as markers of lipid oxidation has been superseded in recent years by the measurement of other secondary oxidation compounds, e.g. aldehydes.

1.3.3.5. Recent developments to measure and investigate WOF

Lipid oxidation processes in meat and meat products have conventionally been studied by analysis of primary and secondary lipid oxidation products. However, advances in for example pulse radiolysis (Simic, 1980) and electron spin resonance (ESR) (Schaich & Borgi, 1980) have enabled the detection and study of short-lived free radical intermediates. Although, the application of ESR to study lipid oxidation in biological systems is commonplace (Davies, 1987), its use to study food systems is a relatively new development (e.g. Pegg et al., 1996). In the future, ESR spectroscopy of cooked meat systems may offer a means of elucidating further the free radicals involved in the formation of WOF flavour compounds and in studying the role of antioxidants in the prevention of lipid oxidation.

A number of other spectroscopic techniques have also recently been utilised in the study of lipid oxidation in foods (e.g. Sedman et al., 1997; Brøndum et al., 2000). Near infrared (NIR), near infrared Fourier transform Raman (Raman) and low field-nuclear magnetic resonance spectroscopy (LF-NMR) were used by Brøndum et al. (2000) to investigate days of WOF development in meats derived from pigs subjected to different pre-slaughter stress treatments. Of these techniques, only LF-NMR was found to differentiate the time over which WOF developed at the different pre-slaughter stress levels. The interference of water was cited as responsible for a lack of differentiation in the NIR and Raman techniques. In addition, Sedman et al. (1997) has reviewed the application of Fourier transform infrared (FTIR) for monitoring lipid oxidation in food systems and concluded on it usefulness as a future common method of analysis.

1.3.3.6. Concluding remarks on chemical measurement of lipid oxidation

While measurement of TBA-reactive substances has been, and largely still is, the most convenient way of assessing lipid oxidation in cooked and chill-stored meats, reliance on methods based on gas chromatography and HPLC will increase due to the superior chemical specificity of these techniques. Moreover, the use of spectroscopic techniques in novel ways for assessing lipid oxidation may come more to the fore, especially if they can also be used to further elucidate the lipid oxidation mechanism.

1.4. Evaluation of WOF from a sensory perspective

1.4.1. General introduction

Sensory scientists, food technologists and lipid chemists have for many years been interested in terms to describe the sensory characteristics of lipid based off-flavours in meats. The quality, meaning and implications of the terms selected and used may be viewed differently by the different disciplines. Food technologists and lipid chemists seem content with words that describe the apparent cause of the flavours. Sensory analysts, however, are often more interested in describing the perceived characteristics. To the food technologist or research chemist, terms such as 'rancid', 'oxidised' and 'warmed-over' describe reasons for the off-flavours. For the sensory analyst these terms also suggest the occurrence of specific chemical reactions or probable causes, however, they do not provide a description of the specific flavour characteristics perceived. Thus, sensory analysts have endeavoured to develop sensory methodologies to describe the specific off-flavour notes caused by oxidation. This has resulted in descriptive lexicons or vocabularies that describe the perceived sensory characteristics rather than the causes or reasons for the off-flavours (Civille & Dus, 1992; Muñoz & Civille, 1998).

There are two possibilities for assessing the perceived characteristics of muscle foods, consumer studies or using trained sensory panels. Consumer investigations and the methods and principals for performing trained sensory evaluation of cooked meat products have been discussed by Miller (1994). The use of trained sensory panels in cooked muscle food evaluation only will be presented here.

1.4.2. Evaluation of the sensory characteristics of muscle foods using trained sensory panels

To conduct sensory evaluations involving trained sensory panels, three areas have to be considered: (1) the environmental, product and panel conditions that will be used for sensory evaluations, (2) the selection and training of sensory panellists, and (3) the type and structure of sensory methodologies that are used to assess the sensory properties of muscle foods.

1.4.2.1. Environmental, product and panel conditions

The environment where sensory analysis is carried out is of major importance to the success of sensory evaluation. Removal of as many negative environmental factors as possible or minimising and standardising those factors that cannot be removed across sample treatments is critical. Overall, the environment must not interfere with the sensory judgements made. This ensures that the sensory responses of the panellists are the result of the product characteristics and not a response confounded with an effect of the environment where sensory evaluation was carried out. There are a multitude of points involved in ensuring that the sensory evaluation environment does not affect the sensory responses of panellists. These are generally minimised if the design of the test room and sample preparation areas comply with ASTM (1986) and ISO (1988), which detail general guidelines for the design of test rooms. In general, the test room and preparation areas should be separated to reduce odour transfer. Moreover, the test room must be divided into separate panel

booths and have a controlled atmosphere and adequate lighting (ISO, 1988).

In terms of panellists, generally they should be in optimal physiological condition as is required at the time of evaluation. In addition, panellists should not have consumed food at least 1 hour before conducting oral evaluations to minimise the effect of carry over and taste adaptation on their sensory judgements. General guidelines for instructions to panellists prior to sensory evaluations are detailed in ISO (1985b).

In presentation and preparation of the samples for sensory tests there are also many points to note, which reduce sources of error ensuring that the data generated reflects the true response. Of most importance, sample presentation must be in an unbiased randomised order to eliminate confounding effects on panellists responses. All methods of sample preparation and presentation must be standardised to ensure minimal influence on the sensory properties of the samples being evaluated. This ensures that only the responses to the sensory characteristics of the samples are measured. Authors such as Meilgaard et al. (1999) have produced an excellent review which discusses the evaluation environment, panellists and product requirements for sensory evaluation.

1.4.2.2. Selection and training of sensory panel members

Selection of sensory panellists is critical in obtaining reliable sensory data. Panellists can be selected internally or externally to a company, institute or program. Each has advantages and disadvantages, internal panellists are on site and may not require a monetary reward. However, they may not be totally unbiased and work responsibilities may interfere with their sensory panel duties. In contrast external panellists are less biased and available at all times when required. The negative aspect of external panellists is they are expensive in that they require payment for their time.

In selecting panellists a pre-screening interview should be carried out to address the following issues: (1) inform the potential panellist in detail about what the job of sensory evaluation involves, (2) determine the persons availability, when can they perform the evaluations, (3) obtain an impression of the level of interest of the individual in participating in sensory evaluations, (4) evaluate their general state of health, and (5) stress the importance of the sensory work in which they will be involved (Meilgaard et al., 1999).

Once panellists are selected they need to be screened to determine their sensory acuity and ability to perform the sensory tasks required of them. Screening of panellists may be conducted in four phases: (1) undertake scaling tests to determine if individuals can follow directions, make sensory judgements and express these perceptions verbally, (2) evaluate sensory acuity, the individuals ability to discriminate using, e.g. triangle tests, (3) conduct tests to investigate panellists ability to rank or rate sensory differences, and (4) conduct a personal interview to determine persons continued interest. At the end of each phase panellists should be accepted for each subsequent phase, rejected or testing should continue with the individual. For overall acceptance a panellist should have passed all four phases of screening (Miller, 1994).

After panel selection, panel training for specific sensory evaluations begins. The goal of training is to familiarise the individuals with the test procedures and to improve the panellists ability to

recognise, identify and quantify sensory attributes. In the initial sessions panellists are generally informed about the nature of the testing in which they will participate. They are also given details about the product, and in the nature of the sensory attributes they will be assessing in that product. The subsequent sessions are used to familiarise the panellist with the test procedures (Meilgaard et al., 1999).

The degree and length of training varies widely in sensory evaluations, and depends on the type of panellists, the number of descriptive attributes and previous panel experience. Miller (1994) has indicated that in the sensory evaluation of an off-flavour such as WOF in cooked meat panel training may take up to one year if a large number of attributes are used and the panellists have had no prior meat evaluation experience. If however, the panel have been involved in evaluations it may take only a number of weeks to train the panel for different meat products. Overall, it can be said that training never ends, and the panel leader must use verification and performance evaluation procedures to determine when retraining or removal of a panellist is required.

A number of publications are available which give detailed guidance in the procedures mentioned for the selection and training of sensory panellists. These include ISO (1991) methodologies for investigating taste, ISO (1992a) methodologies for initiation and training assessors in the detection and recognition of odours and ISO (1993) and ISO (1994a), which detail guidelines for the selection, training and monitoring of selected assessors and experts, respectively. Overall, these publications, from a standards organisation, in reality only offer practical guidelines and suggestions as to how the various aspects of panel training should be carried out. On a day to day basis previous experience in the field of training sensory panels and basic 'scientific' common sense, are also required to ensure a panel are sufficiently trained for the sensory evaluation task at hand, i.e. the standards alone cannot train the panellists.

1.4.2.3. Sensory methodologies used to assess the sensory properties of muscle foods

The first step in evaluating the sensory properties of meats is to decide on the type of trained panel required to meet the objectives of the study. Two types of trained sensory panels are generally employed in the evaluation of muscle foods, i.e. 'difference' testing panels and 'descriptive attribute testing' panels.

Difference testing can be categorised into overall difference tests and attribute difference tests. Overall, difference tests provide a method to determine if a sensory difference exists between the samples, whereas attribute difference testing asks if a specified attribute differs between samples. Commonly used difference tests are the triangle test, two-out-of five test, duo-trio test, simple difference test, "A"–"not A" test, difference-from-control test, sequential test, and similarity tests. Attribute difference tests include directional difference test, pairwise ranking test and simple ranking test. Meilgaard et al. (1999) have described in detail the methodologies of these tests and their use in sensory evaluation.

Descriptive attribute evaluation, also referred to as quantitative sensory profiling, is a method where specific sensory attributes of, e.g. muscle foods, are quantified by trained panellists. A list of

descriptive words, referred to as a lexicon, describe the specific sensory attributes in a muscle food and can be used to evaluate the development or change in these attributes, e.g. the change in flavour characteristics of cooked, chill-stored and reheated meats. A descriptive vocabulary of attributes can be developed for any meat or meat product to describe any particular sensory changes or implications that are of interest. A number of lexicons have been developed for WOF, e.g. Johnson and Civille (1986) in beef and Lyon (1987) in chicken (see section 1.5.3.).

Overall, the descriptive attributes of muscle foods can be defined by a trained sensory panel through: (1) ballot development sessions where a trained panel comes to a consensus as to the descriptors for a muscle food, (2) established lexicons, e.g. for WOF description those of Johnson and Civille (1986) and Lyon (1987), (3) free profiling where individually trained panellists derive their own vocabularies for a meat or meat product.

Detailed descriptions of sensory terminology and procedural guidelines for the identification and selection of descriptors for establishing a sensory profile by a multidimensional approach have been described in ISO (1992b) and ISO (1994b), respectively. Moreover, a number of defined methodologies have been proposed for sensory descriptive analysis of food products e.g. the Spectrum method and Quantitative Descriptive Analysis (QDA). Authors such as Muñoz and Civille (1992) and Meilgaard et al. (1999) have extensively reviewed the Spectrum method. Whilst the QDA method first proposed by Stone et al. (1974), has been reviewed recently by Lawless and Heymann (1998) and Meilgaard et al. (1999). Moreover, in relation to muscle food evaluation, QDA has been extensively discussed by Miller (1994). The methodologies of Free choice profiling have also been discussed in detail by Lawless and Heymann (1998) and Meilgaard et al. (1999).

1.5. Sensory studies on WOF development in cooked, chill-stored and reheated meats

1.5.1. Initial WOF research: late 1950s to early 1980s.

An appropriate point at which to begin a discussion of WOF research as a sensory phenomenon is to examine the descriptors that have been commonly used in the earlier lipid oxidation investigations to define off-flavours in cooked and chill-stored meat. Tims and Watts (1958), the first authors to report that the flavour of cooked meats changed rapidly during storage, indicated that terms such as ‘stale’, ‘rancid’ or ‘warmed-over’ appeared to describe the characteristic off-flavour that developed. Hence, the phenomenon has been referred to as warmed-over flavour or WOF. In studies that were subsequently undertaken many included a sensory aspect and panellists were generally asked to evaluate the intensity of single attributes in order to describe WOF. Overall, descriptors such as ‘warmed-over’, itself (Tarladgis et al., 1959; Ingene et al., 1979; Younathan & Watts, 1960), ‘rancid’ (Younathan & Watts, 1958) and ‘oxidised’ (Greene, 1969; Greene et al., 1971) have often been employed. All of these studies involved the measurement of the sensory term using a hedonic scale. Another approach in the earlier WOF investigations was to use a horizontal line scale to estimate the intensity of the oxidised flavours (Winger & Pope, 1981). This type of scale was first used in the QDA method (Stone et al., 1974).

Overall, the terms used generally described the causes of the off-flavour and were not specific to

characterising the flavour notes involved. However, these studies must be commended as they at least monitored WOF as a sensory phenomenon using trained sensory panellists. Authors such as Sato et al. (1973) in a study involving WOF development in stored, cooked ground beef and pork, suggested that as the differences in WOF developed in the samples were "so obvious", formal taste panel evaluations were not required. The authors used what they referred to as "subjective flavour and odour evaluations" to evaluate WOF in their meat samples. The terms they used to describe WOF development included 'warmed-over' itself, in association with 'bitter', 'bland' versus 'meaty' and 'good' to describe a lack of WOF development in samples where WOF inhibition was investigated.

Thus, researchers in many initial investigations recognised that WOF could be monitored using sensory terms. In general the single terms used were assessed hedonically and described the cause of the off-flavour and not the specific sensory notes involved.

1.5.2. Earlier WOF description

Another objective of sensory evaluation in the early WOF research was to describe the off-flavour with a number of terms as opposed to monitoring its development with a single term. Early research reporting sensory analysis of WOF where sensory description was used however, were not particularly verbose (Melton et al., 1987).

Jacobsen and Koehler (1970), Harris and Lindsay (1972) and Joseph et al. (1980) for example were some of the studies that employed more than one term to describe WOF. In these investigations the terms most often used by panellists to describe WOF or those which were scored highest in intensity were musty, stale or 'old', rancid, warmed-over, and other off flavour (Table 2, Appendix B). It should be noted that these earlier meat descriptor lists also included some terms to describe the flavour of freshly cooked meat, what could be described as 'on-notes'. Jacobsen and Koehler (1970) were also one of the first studies to indicate that as the usage of terms such as musty, stale or 'old', and rancid increased in cooked poultry with storage, effective usage of words such as sweet, meaty and rich decreased. Thus, an early indication that WOF development was not perceived from a sensory perspective solely as the result of lipid oxidation derived off-notes, but that it also involved the loss of a fresh 'meaty' notes. In general, of the off-notes these studies used to describe WOF, only musty could be considered to describe a sensory characteristic. Stale, rancid and warmed-over once again described the process suspected of producing the off-notes.

One of the earliest publications that used sensory descriptors to describe more specific characteristics was by Cipra and Bowers (1970). These authors used a trained panel to study the flavour changes caused by chill-storage and reheating of turkey breast muscle (*pectoralis major*). The samples evaluated were freshly braised turkey and braised turkey held for 24 hours at 6^{o}C then reheated to 60^{o}C prior to sensory evaluation. The freshly braised meat had a more intense meaty/brothy flavour and aroma than the reheated meat. Stored samples had increased intensities of stale, acid, bitter and salty flavour components. In addition, significant increases for aromas but not flavours were observed for rancid and sulfur components with WOF development. Staleness was

described as aldehyde-like, whereas, rancidity was characterised as similar to 'old oil' or fat. Thus, these authors, like Jacobsen and Koehler (1970), noted a decreasing 'meaty' aspect as WOF increased.

From studies such as these, cooked, chill-stored and reheated meats were shown to undergo a series of characteristic changes over time of storage and it was clear that different descriptors or groups of descriptors appeared to generally describe these changes, i.e. fresh meatiness decreased as warmed-over, stale and off-aroma increased. However, many of the descriptors used were not clearly defined, and the definitions of terms such as, e.g. 'staleness' and 'off-aroma' were not particularly clear.

By the mid 1980s investigations using single or few terms, many cause related in nature and ill-defined, were superseded by studies that provided a more complete description of the complex meat flavour changes that occur as a result of chill-storage of cooked meat. Descriptive sensory analyses, involving the discrimination and description of a product by a trained panel of judges has been employed in such studies (see section 1.4.2.3.). Examination of the published research of stored-meat flavour using such descriptive analysis may aid in the clarification of the sensory phenomenon called WOF.

1.5.3. Standardised WOF vocabularies

One of the first studies, involving the development of a standardised vocabulary for the description of the flavour of stored ground beef was published by Lynch et al. (1986). Panellists evaluated different meat samples to develop the ballot shown in Table 3 (Appendix B). Using this ballot, the flavour profile panel trained by a method described by Caul (1957), found that cooked patties prepared from meat immediately after grinding were characterised as beefy, fresh and bloody/serumy. After 1 day of storage in polyvinyl chloride, these notes decreased in intensity. At 3 days, beefy, fresh, and bloody/serumy notes declined further and a stale characteristic was detected. After 5 days, beefiness was very low, bloody/serumy was not detected, and a stale note lingered into the aftertaste. The loss of beefiness and an increase in staleness were attributed to lipid oxidation or WOF with storage up to 5 days.

In the same study, Lynch et al. (1986) reported vacuum packed beef to develop a threshold stale note at 10 days of chill-storage. The bloody/serumy note in the fresh samples decreased up to 10 days, then changed to a metallic or sharp note. Sourness in vacuum-packaged meat patties peaked at 10 days. Overall, the authors reported that with chill-storage the meat patties first became bland, then stale, and later took on, what they referred to as an 'unpleasant' stale flavour. The nature of the 'unpleasant' stale flavour was not further defined. Of particular note in this study was the fact that the authors had developed one of the first lists of descriptive terms for WOF that were generally well defined and related to specific sensory characteristics. Overall, the study also indicated a loss of meatiness with WOF development similarly to the earlier reports of Jacobsen and Koehler (1970) and Cipra and Bowers (1970).

A further study that involved a concerted attempt to describe the perceived characteristics in

warmed-over meat in detail, was undertaken by Johnson and Civille (1986). These authors published what they called a "standardised lexicon" of meat descriptors for WOF. The protocol used to develop this lexicon involved the meeting of an expert group of WOF researchers whom derived a general list of WOF terms that were to be used. Following this a draft of the flavour lexicon was presented to participants from the American food industry and US Department of Agriculture laboratories, and the final lexicon reflected their comments and suggestions.

Following a review of the literature on WOF in meat, a variety of meats, cooking procedures, storage times, and reheating procedures which manifested the flavour and combination of flavours representing WOF were selected for sensory evaluation using the developed lexicon. The samples selected were meat patties of chicken, turkey, pork, or beef as well as slices of pre-formed roasts of beef. For initial cooking the meats were either grilled or steam cooked. Following cooking, samples were chill-stored at 4^{o}C for up to 7 days. Prior to the sensory evaluation sessions three different methods were used to reheat the cooked meats: baking, broiling and boiling.

As a result of four sessions the panellists found that WOF was equally identifiable in meat from different species (beef, pork, turkey and, chicken) or when using different cooking (grilling, steaming) and reheating (baking, broiling and boiling) treatments within a species, although WOF in the samples varied in intensity. This was also true whether the meats were in sliced or pattie form. Based on these results the authors proceeded with sensory evaluation of the beef samples alone. However, they indicated that, although the fresh meat descriptors did not apply to the different species, the descriptors characterising WOF should apply to other meats. As such the authors concluded the terms developed should help researchers elucidate the causes of the phenomena of off-flavour in meats per se. Moreover, the meat industry should be able to apply these terms in new product development, quality control and shelf-life stability testing. The list of terms and their descriptions developed for beef by Johnson and Civille (1986) is presented in Table 4 (Appendix B).

Jonhson and Civille (1986) also described a general pattern in WOF development: fresh flavours (cooked beef lean, cooked beef fat, serum/bloody, grainy/cowy) disappeared after the first 1-3 days, cardboard flavour appeared after 2 days and disappeared again as oxidised and rancid notes began to dominate over all other notes up to 7 days. This pattern was somewhat similar to the loss of meatiness and increase in staleness with WOF development noted by Lynch et al. (1986). Overall, the list developed and used by Johnson and Civille (1986) to describe WOF development in cooked beef samples, contained largely unambiguous well defined sensory terms which described specific sensory on and off-notes. The sensory perception of these notes was found to change dynamically with increasing chill-storage and WOF development.

Another major study that involved the development of a descriptor list specifically for WOF, was by Lyon (1987) for cooked and stored chicken meat patties. This study was modelled on the protocol presented by Jonhson and Civille (1986). Initially the author developed a 45 word 'starting' list, based on previous WOF literature to describe flavour changes in cooked, refrigerator stored and reheated chicken patties (Table 5, Appendix B). This list was subsequently reduced over

a 14 week period (3 hours/week), using a trained panel of 10 assessors, to 12 terms with associated reference descriptions (Table 6, Appendix, B). Prior to the list reduction process panellists were screened for ability to discriminate odours and tastes and for their proficiency in verbal expression. These initial 'training sessions' focused on basic taste and odour recognition and on threshold sensitivity. The authors described such sessions prior to list reduction as designed to "gain an appreciation for the complexities of human beings as sensitive research tools". Subsequently panel input and multivariate statistical procedures such as Factor analysis, Principal Component Analysis and Discriminant Analysis were utilised in the term list reduction process.

The final list of 12 terms contained a number of freshly cooked meat descriptors (chickeny, meaty and brothy) and WOF related descriptors (cardboard/musty and rancid/painty) (Table 6, Appendix B). These reflected the descriptor lists of Lynch et al. (1986) and Johnson and Civille (1986). However, the term 'warmed-over' was included as descriptor, as in the earliest WOF studies, e.g. Tarladgis et al. (1959). The authors indicated that even though the term did not fit the criteria of specificity, association or cognition, as did all the other terms chosen, the panel had insisted on its inclusion. Lyon (1987) indicated that the inclusion of the term was a compromise, and its use would be closely monitored in subsequent WOF studies that utilised their descriptor list.

In conclusion, Lyon (1987) noted that the flavour change due to short term refrigerated storage after cooking and reheating was detectable within 24 hours, and that description of this change was decidedly more complex than simply being a unidimensional WOF or rancid off-flavour. Moreover, the authors indicated that the 12 descriptors derived through the panel process were appropriate for methodical assessment of the sensory changes that occurred with WOF in cooked chicken meat.

The final published study that involved the development of a descriptor list for WOF was reported as part of a review of sensory analysis of WOF in meat by Love (1988). This author used a sensory panel trained in general sensory descriptive techniques according to a method described by Civille (1979). The descriptors were developed by the presentation of a variety of meat samples similar to those used in the protocol of Johnson and Civille (1986). Panellists individually listed terms that described the notes they perceived in the samples and then reported their perceptions verbally. Through guided discussions, redundant terms were eliminated and agreement was reached on terms for a ballot. Descriptors were derived for beef, chicken and turkey. Love (1988) indicated although the terms used to describe the meaty notes in freshly cooked samples varied, the same terms (cardboard and painty) appeared in stored meats from the different species. This finding was in agreement with the conclusions of Johnson and Civille (1986). Moreover, as in Johnson and Civille (1986), Love (1988) published only the descriptor list developed for WOF in beef (Table 7, Appendix B).

In the Love (1988) beef evaluations panel selection and training took place over a period of 16 weeks, thus, a similar time period to the 14 weeks used for descriptor development study by Lyon (1987). During training sessions panellists individually evaluated beef patties or roasts prepared by a variety of cooking and reheating methods that were refrigerator stored for 0 to 7 days. Following each session, group discussions served to clarify the meaning of descriptors and the way that the

characteristics were perceived in the various samples.

Overall, the descriptor list published by Love (1988), was very similar to that presented by Johnson and Civille (1986) for WOF in beef (Table 4, Appendix B). However, Love (1988) found a cooked liver note not reported by Johnson and Civille (1986), and they did not identify any fishy flavours in any beef samples. Metallic and astringent qualities were also found in some of the meat samples. Panellists defined 'cooked liver' as the aromatic associated with cooked beef liver. Lynch et al. (1986) also reported that 'livery' flavours were sometimes noted in cooked beef patties. The remaining descriptors were defined in a similar fashion to those proposed by Johnson and Civille (1986). In addition, the general pattern of WOF described by the panel agreed with that described by Johnson and Civille (1986): reduction in intensity of cooked beef aromatics and appearance of cardboard and painty notes with WOF development.

When the panel data from replicate sessions were tested to determine which descriptors significantly differed in stored samples, all aromatics were significantly different except green grain/vegetable, cooked liver and burned/scorched. Thus, Love (1988) concluded these terms may be present in cooked and chill-stored beef patties but were not related to describing WOF.

Overall, the descriptors evaluated by Johnson and Civille (1986) were developed by meat flavour experts. The similarity of terms developed independently by Love (1988) using a descriptive analysis panel supported the Jonhson and Civille (1986) contention that the terms they developed should be useful in research to determine causes of WOF in product development, quality control, and shelf-life stability studies. Love (1988) further indicated that if only a few attributes are evaluated, as in the earliest WOF studies, subtle but important sensory nuance differences would most likely be overlooked.

1.5.4. Sensory WOF studies that have employed standardised vocabularies

Since the studies of Johnson and Civille (1986), Lyon (1987) and Love (1988) no further studies related to developing lists of descriptive terms for WOF in meats. Thus, it appeared that researchers may have considered the conclusion of Johnson and Civille (1986), that the WOF terms developed could be employed in any meat that had been cooked, and refrigerator stored, to somehow bring to a close research into sensory WOF descriptors. There have however, been a number of studies that have utilised the descriptor lists developed by Johnson and Civille (1986), Lyon (1987) and Love (1988).

For example St. Angelo et al. (1987) used the beef descriptor list of Johnson and Civille (1986) to investigate WOF in roasted beef by chemical (TBA number) and instrumental analysis (GC). Overall, the authors found many volatile secondary reaction compounds of lipid oxidation to increase with WOF development. A number of these compounds, including hexanal, pentanal and 2,3 octanedione were suggested as marker compounds to monitor the increase in WOF with storage. Their increase also paralleled TBA number increase. Moreover, these volatile compounds were highly correlated with increasing sensory off-flavour notes, e.g. cardboard and oxidised/rancid/painty and negatively correlated to decreasing fresh meaty descriptors with WOF

development (see Table 4, Appendix B). Overall, WOF in stored roast beef was attributed to result from lipid oxidative degradations with concomitant production of compounds which mask 'normal' desirable beef flavours. Moreover, St. Angelo et al. (1988) in a similar study, involving the Johnson and Civille (1986) vocabulary, found a number of sulfur containing heteroatomic meaty type compounds to decrease with WOF development as measured by GC-MS. Thus, the authors concluded the meaty decrease not only involved WOF masking the meaty notes, but also a reduction occurred in the level of compounds perceived as meaty from a sensory perspective as WOF developed.

A further study by St. Angelo et al. (1990) utilised the descriptor list by Love (1988) in a study involving chemical and sensory studies of WOF development in cooked and refrigerator stored antioxidant treated beef. Results indicated chelators (e.g. ethylene diamine tetra acetate sodium salt or EDTA) and free-radical scavenger (e.g. Propyl gallate) type antioxidant compounds could inhibit WOF development in the meat samples. However, intensity of desirable cooked beef flavour note, was reduced after 2 days of storage in patties treated with some of the compounds. Inhibitors that showed the smallest loss of the beefy note were the free-radical scavengers. Thus, indicating that free-radical chemistry plays a very important role in WOF development. Overall, the study concluded that a comprehensive sensory evaluation of experimental samples should be included with chemical and instrumental analysis to evaluate compounds as potential WOF inhibitors.

A study using the vocabulary developed by Lyon (1987) was published by Lyon et al. (1988). In this study the list was used to examine the relationships among the individual sensory character notes and TBA values of chicken patties made from two different formulations (with and without 20% chicken skin) stored up to 3 days and reheated by conventional or microwave ovens. With increasing storage days chickeny, meaty, brothy, liver/organy and sweet character notes decreased, whereas browned, burned, cardboard, warmed-over, rancid/painty, bitter, and metallic character increased. Rancid/painty was found to have much higher sensory scores after 2 days for the patties that included chicken skin. This was attributed to greater lipid oxidation as the result of a higher lipid content in these samples, resulting from the addition of chicken skin. TBA values in the study were found to be higher in microwave-reheated samples, and to increase with refrigerator storage time. In addition the TBA values were negatively correlated with the increasing chickeny, meaty, brothy, liver/organy and sweet on-flavour notes and were positively correlated with all other attributes, i.e. the WOF related attributes. Storage time was found to be the primary source of variation in the sensory data and method of reheating was not reported as differentiated by any of the sensory variables.

A further study that used the Lyon (1987) vocabulary to evaluate WOF using oven cooking in intact broiler breast, thigh and skin tissues by chemical and instrumental means was published by Ang and Lyon (1990). In all tissues up to 5 days chill-storage, TBA values and levels of the headspace volatiles, pentanal, hexanal and heptanal were found to increase with WOF development. Specifically, fresh day 0 breast muscle had the lowest TBA values among the three tissues. However, after 2 days storage and thigh meat TBA values did not differ. After 2 days both thigh

and breast meat had higher TBA values than chicken skin. Off-flavour notes cardboard, warmed-over, rancid/painty and a composite term introduced to register off-flavours called 'overall intensity' increased with chill-storage of 1 day or more. TBA value and headspace volatiles were highly correlated to off-flavour development. Moreover, TBA values were highly correlated with a number of volatiles, predominantly hexanal. Overall, the authors suggested hexanal could be used as a substitute for TBA in the evaluation of off-flavour development in chicken tissues based on their findings.

1.5.5. Concluding remarks on the sensory evaluation of WOF

The development of standardised vocabularies in the later WOF studies has resulted in a 'general' elucidation of WOF in a number of meats. These vocabularies appear to contain defined sensory notes for 'on' and 'off-flavours' that change with WOF development. In deriving these lexicons a general pattern for WOF development in meat from a sensory perspective emerges: involving a decrease in fresh meatiness and the development of cardboard and finally rancid/painty notes with more prolonged refrigerator storage. The WOF terms have been proposed to apply more or less to all cooked meats. However, the on-notes describing the loss of fresh meatiness appear to be species specific.

Studies utilising the developed sensory lexicons have come closest to investigating WOF in a scientifically productive manner, e.g. St. Angelo et al. (1990) who used the Love (1988) beef lexicon to investigate the effects of antioxidants on the sensory development of WOF. Since these studies there has been little research into WOF from a largely sensory perspective. If anything it can be said that the 'research pendulum' has swung back to WOF investigation from a largely chemical perspective with minimal use of sensory descriptors.

An example would be a recent study by Lai et al. (1995) where the development of WOF in restructured chicken nuggets treated with antioxidants was investigated. In this study the authors measured TBARS and hexanal to monitor the development of lipid oxidation, at various levels and combinations of sodium tripolyphosphate, tertiary butylhydroquinone and oleoresin rosmary. The sensory aspect of the study involved relating the chemical measurements to a single WOF descriptor 'warmed-over' assessed in the chicken nuggets by a 'trained' panel using a 6 point hedonic scale, similar to that of Taralagis et al. (1959). The authors indicated that the panel were unable to differentiate many of the WOF samples and attributed this to a lack of training. Overall, high correlations were obtained between the sensory term warmed-over and the chemical measurements and the WOF term decreased with use of antioxidants. However, the authors were unable to formulate any conclusions on the sensory impact of the reduction in WOF with antioxidant treatment. They indicated that descriptive analysis was needed before this was possible and quoted earlier studies that had already made such a recommendation, e.g. Love (1988).

Thus, the story of sensory WOF investigations appears to have come full circle. Have we learned anything from the experiences of the authors of the late 1980s who utilised developed sensory lexicons for WOF in association with chemical an instrumental analysis, and from such

studies were able to formulate very convincing arguments for why certain factors had different effects on WOF development? It appears not, particularly when subsequently authors realise the shortcomings of their studies from a sensory perspective after reports already exist that have noted the problems involved in using badly trained panels and single non specific terms to monitor WOF.

In conclusion, in studying WOF and off-flavour problems in general, comprehensive descriptive vocabularies *are* required and *must* be developed for *each* individual problem, then and only then can the sensory implications be discussed to any useful degree in association with chemical/instrumental measurements. As the cost in time and money, of training a sensory panel and developing such descriptive vocabularies has been considered prohibitive, faster and cheaper methodologies need to be developed. Such methodologies must incorporate all the critical aspects involved in the prolonged comprehensive methods described for training and vocabulary development, in a more concise way, to produce effective sensory evaluations in shorter time frames.

1.6. WOF, its importance to the meat industry and impact on consumer acceptability

1.6.1. A meat industry survey

A single report by Cross et al. (1987) has investigated the importance of WOF to the meat industry and its impact on consumer product acceptability. The authors reported that an American meat industry survey prior to their study concluded that WOF in meat was a major "stumbling block" to the introduction of new pre-cooked meat products into the market place. Therefore, WOF was considered to be one of the most important problems facing the meat and food industries, in terms of trying to gain consumer acceptance of such products.

In addition, the survey indicated that the meat industry considered the development of WOF to involve the loss of fresh meat character in association with an increase in stale or oxidised off-notes. This was in accordance with the body of published sensory studies on WOF development that existed at the time. One of these studies, by Johnson and Civille (1986) enlisted the input of the American food industry in developing a descriptor list for WOF thus, indicating the level of awareness industry had for the problem in cooked and chill-stored meat and meat products.

1.6.2. Surveys of WOF in relation to the food service sector and consuming public

Based on the meat industry findings, Cross et al. (1987) initiated two independent surveys to investigate the position of the food service sector and consumer on WOF. The first, a U.S. nation-wide survey, investigated the impact of WOF in the "public feeding system", i.e. food service sector. This involved 72 university food service managers. The second, was initiated to gather information concerning the impact of WOF from the consuming publics perspective. This study involved 91 staff of Texas A&M University.

From the questions asked in the first independent survey it emerged that the food service industry felt that very little if any "significant" WOF problem existed, even though they admitted to encountering it in 40% of reheated meats, particularly beef and poultry dishes, served over a one

month period. Consumers of the pre-cooked products surveyed in the second study appeared to substantiate the claim that a prominent WOF problem existed, in that 72% of people had encountered WOF in reheated meats served to them over a one month period. It was noted however, that only 23% of the consumers had actually complained. Thus, a situation appeared to exist where WOF was a major problem, as determined from both surveys, but consumers did nothing to promote the food service sector and thus the food industry into trying to reduce the prevalence of WOF. Therefore, Cross et al. (1987) concluded that the climate in the food processing segment of the meat industry was to ignore WOF as a problem as complaints were minimal and therefore, maybe "WOF could be considered a minor problem of little concern". This stance by food processors was felt by Cross et al. (1987) to be a risky one as they reported that one consumer complaint may be equal to as many as 800 dissatisfied customers.

1.6.3. WOF in relation to the food service sector and consumers today

In terms of the position of the meat industry and food catering sector today on WOF, one may assume it is still understood to be a problem and that research is being carried out in private to alleviate its presence in products containing pre-cooked meat. However, as no reports have been published by the food industry recently, one must assume that it may be 'bad for business' to advertise the fact that WOF exists in pre-cooked meat products and that it reduces their palatability in a very short space of time. Overall, the food industry in the long term may all but eradicate WOF from pre-cooked meat products if the published research on practical preventive measures is investigated as part of the development of new ready-to-eat meat products. However, to ensure that the preventative measures in reality have been effective, sensory analysis must be utilised as part of the product development programme. In the long run this will ensure a much higher level of new product acceptability as opposed to the high level of unacceptability which prevails if a meat product is perceived 'consciously' or 'subconsciously' by the consumer to contain the off-flavour that is WOF.

1.7. Multivariate data analysis of sensory data from meat and meat products

1.7.1. General introduction

Meat and meat products are composed of mixtures of chemical components combined in complex physical structures. The sensory characteristics perceived in meat products that have been cooked and chill-stored and their interrelationships may be many and multidimensional in nature. Thus, in order to facilitate interpretation of the large data matrices that result from the sensory analysis of such meat products suitable data analysis techniques are required. Techniques that can be applied to such matrices to allow presentation of the results in an understandable way are usually multivariate statistical methodologies, e.g. Principal Component Analysis (PCA), Partial Least Squares Regression (PLSR) and Generalised Procrustes Analysis (GPA). There are also many other forms of multivariate data analysis and these have been reviewed extensively by, e.g. Piggott and Sharman (1986) and Martens and Næs (1989). Multivariate methods that have been used

specifically to aid in the interpretation of data from sensory and chemical analysis of meat quality and to determine their relationships have been discussed by Næs et al. (1996).

1.7.2. Principal Component Analysis (PCA)

One of the most basic multivariate methods that is used to aid in the interpretation of large data matrices, such as those that result from descriptive sensory analysis, is the bi-linear modelling method termed Principal Component Analysis or PCA (see Piggott & Sharman, 1986; Wold et al., 1987). In simple terms PCA involves the decomposition of a single data matrix X into an interpretable "structure part" and a "noise or error part" which is the fraction of the variance that should not be interpreted. Overall, the PCA algorithm identifies directions of main variability in the multivariate data space, such that the most important source of systematic variance is set as the first Principal Component or PC 1 and the second largest orthogonal source of variation is set as PC 2. PCA continues to find further orthogonal PCs (PC 3, 4, 5 etc.) in decreasing order or importance until the desired amount of variation is extracted from the data matrix. In practice in descriptive sensory analysis the first 2 or 3 PCs may encompass a high percentage of the main structural variation and additional PCs may primarily constitute noise (Næs et al., 1996). Graphical inspection of bi-variate plots of these first few PCs then gives a good overview of the main information in the input data. Being a simple bi-linear method to describe most of the systematic variation by few PCs, PCA reveals relationships between the variables (loadings) and samples (scores) (see Esbensen et al., 1996).

An important question in PCA is how does one know how many PCs to interpret? In order to know this a validation technique must be employed. Cross-validation for example can be a useful tool to determine the number of 'significant' components. This method is based on a strategy that repeatedly leaves one new sample or a new sub-set of samples out of the data and tests the estimated model on the left out samples. In this way, the estimated model is tested on samples that have not been present in the estimation. This is repeated until all samples have been validated once, and the results are summarised in terms of explained variance (Martens & Næs, 1989). Other methods available for validation in PCA are test set validation and leverage correction (see Esbensen et al., 1996).

Studies where PCA has been used to investigate sensory characteristics of WOF are few. In one example Lyon (1987) utilised PCA to aid in the removal of redundant sensory terms in developing a sensory vocabulary to describe WOF in chicken. More recently Spanier et al. (1998) have used PCA to determine sensory attribute (e.g. cardboardy, painty and meaty) and chemical (TBA and hexanal) relationships in data as a single matrix, from an earlier study on WOF in lamb meat by St. Angelo et al. (1991). The authors found TBA, hexanal and cardboard/painty to be directly related, but inversely related to meatiness. Thus, indicating that the chemical measurements were probably good predictors of the off flavour characteristics of WOF in the experiment in question.

1.7.3. Partial Least Squared Regression (PLSR)

A further bi-linear method in the multivariate data analytical arsenal which can be used to determine the relationships between sensory and chemical variables is Partial Least Squares Regression (PLSR) (Wold et al., 1983). PLSR as opposed to PCA, involves two data matrices where a one set of data is set as X and the other as the Y matrix. The technique predicts a block of Y variables from a block of X variables by extracting a combination of X variables that are relevant for the prediction of Y (Martens & Martens, 1986; Martens & Martens 2000a). Similar to PCA, the associations between X and Y are interpreted by numbers of PCs which explain the highest percentage of the total co-variation. A high level of explained variance is an indication that relevant systematic information has been modelled whereas, low explained variance may be indicative of random measurement noise and/or error in the model. Validation techniques such as cross-validation, as used PCA, is utilised to determine the number of PCs that are reliably interpretable, and to estimate the explained variance and stability of the obtained model parameters. For visual summaries, the correlation coefficients between the first few PCs and the X and Y variables (the 'correlation loadings') are displayed (Martens & Martens, 2000a). Overall, the explained variance and correlation coefficients are used to determine the relationships between the X and Y variables.

A special use of 'two block' PLSR is APLSR (the 'ANOVA like use of PLSR'). In APLSR the X variables are 0/1 experimental design variables (dummy variables (0/1) for products, assessors, replicates etc.) and the Y matrix are set as the response data matrix, be it sensory and/or chemical data. This form of PLSR projects the response variables onto the design variables in order to determine to which degree each of the design variables in X contribute to the variation in the response variables Y (Martens & Martens, 2000a).

When the 0/1 design variables are placed in Y the application may be referred to as DPLSR ('Discriminant PLSR'). The question to be answered by this type of PLSR is: given the experimental design indicators, what patterns of sensory and/or chemical variation are relevant for discriminating between the different design conditions? (Martens & Martens, 2000a).

To derive significance indications for the relationships determined in APLSR, DPLSR and predictive PLSR, the regression coefficients can be analysed by 'jack-knifing' which is part of the cross-validation. It may be used for assessing different types of validity, e.g. between-sensory-replicates, reproducibility or between-products extrapolation ability (Martens & Martens 2000b; Martens et al., 2000c). This jack-knifing allows reliability assessment of the PLSR estimated regression coefficients, e.g. by checking uncertainty limits of ± 2 standard uncertainties estimated by leave-one-replicate-out jack-knifing. From these, the statistical significance of the variable relationships in the X- and Y-matrices can be determined.

Overall, PLSR has recently been utilised in the investigation of instrumental measurements and their relationships to sensory meat quality data by a number of authors (Næs et al., 1996; Toscas et al., 1999). However, no reports have been published where PLSR is utilised to investigate WOF in relation to chemical and instrumental measurements.

1.7.4. Generalised Procrustes Analysis (GPA)

Procrustes Analysis was originally developed to match two different solutions from Factor Analysis (Hurley & Cattell, 1962). Generalised Procrustes Analysis (GPA), which matches more than 2 data sets is a modification of this technique and is commonly applied to the data from different assessors in a sensory panel (Gower, 1975). In GPA the data from each panellist is analysed separately, correcting through transformations, termed shifting, rotating, reflecting and stretching, for individual differences in use of the line-scale and in the interpretation of the sensory attributes (Arnold & Williams 1986; Dijksterhuis, 1996). Since data from sensory analysis almost invariably displays significant assessor effects, analysis such as GPA which takes into account such effects is apt for the field (Arnold & Williams 1986, Dijksterhuis, 1996).

Overall, GPA has been used extensively in the analysis of sensory and consumer science data (e.g. Dijksterhuis, 1995; Oreskovich et al., 1991). The technique has been determined useful as it provides information on all three modes present in such data: products, terms and panellists. GPA has also shown much potential for the investigation of sensory and instrumental relationships, however it has not often been used for this purpose (Dijksterhuis, 1994).

2. Materials and methods

In the following section background information about the source animals and birds used in the different studies (I, II, III, IV, V and VI) comprising the present thesis are detailed. Methods for meat sample preparation and heat treatment, physical/chemical and instrumental measurements and the sensory methodologies utilised are also presented. Furthermore, details of the multivariate statistical methodologies employed in data analyses are included.

2.1. Pigs

2.1.1. Background

The pork meat used in studies I, III, IV and VI was from animals that were part of ongoing investigations at the Department of Animal Quality, Danish Institute of Agricultural Sciences (DIAS), Research Centre Foulum. Animals in studies I and IV were from an experiment that investigated the control of post-mortem pH decrease in pig muscles (Henckel et al., 2000). While pigs in studies III and VI were from a study to investigate the effects of the RN^- allele on pork meat quality traits.

2.1.2. Breed and Sex

The animals in studies I and IV were crossbred pigs, from pure-bred Duroc (sires) mated with dams of a Danish Large White × Danish Landrace cross, while those in studies III and VI were crossbred pigs from Danish Large White × Danish Landrace cross dams sired with heterozygous (RN^-/rn^+) pure-bred Danish Hampshire boars. As the halothane gene is known to influence muscle traits, post mortem energy metabolism and meat quality (e.g. Jensen & Barton-Gade, 1985; Lundström et al., 1989), all parents in studies I, III, IV and VI were halothane tested to exclude this gene from the populations. All pigs used in studies I, III, IV and VI were gilts.

2.1.3. Rearing

Animals in studies I and IV were produced and reared at DIAS. Those in studies III and IV were produced and reared on a commercial pig farm in Denmark. The pigs in studies I, III, IV and VI were given similar standard growing/finishing diets ad libitum, consisting mainly of barley, soya and wheat. All diet compositions and calculated nutrient contents were based on the NRC (1998) specifications for swine rations. Water was also available ad libitum throughout the growing period.

2.1.4. Pre-slaughter stress treatment

The day before slaughter in studies I and IV, littermates were assigned randomly to four different groups or models. Model A served as the control and animals were given no treatment. In model B, animals were exercised on a treadmill at a speed of 3.8 km/h for 10 min immediately prior to stunning. In model C, animals were administered 0.2 mg adrenaline/kg live weight 15 h pre-slaughter, and in model D, 0.3 mg adrenaline/kg live weight was administered 15 h pre-slaughter and the animals then exercised on a treadmill at a rate of 3.8 km/h for 5 min, immediately prior to

stunning. Animals in model A and B were given 13 ml of physiological saline solution as a placebo treatment at the same time as models C and D were administered adrenaline.

2.1.5. Slaughter

Pigs in studies I, III, IV and VI weighed approximately 23 kg when the experiments started, and all were slaughtered at DIAS in the week their live weight reached at least 80 kg in studies III and VI and 90 kg in studies I and IV. The pigs were weighted every two weeks during the fattening period.

Animals in all the pig studies (I, III, IV, VI) were stunned with 80 % CO_2 for 3 min. Thereafter, the animals were exsanguinated and scalded at 62^{o}C for a further 3 min. Cleaning and evisceration of the carcasses was completed within 30 min post-mortem. Thereafter, the carcasses were divided and weighted. At 45 min post-mortem they were transferred to a cold room at 12^{o}C and after 60 min were moved to and stored subsequently in a chill room at 4^{o}C for 24 h. The muscles for studies I, III, IV and VI were then removed, individually vacuum packed and stored at –20^{o}C prior to experimental investigations.

2.2. Birds

2.2.1. Background

Chickens were obtained from an on going study at the Department of Breeding and Genetics, DIAS, with the objective of finding the elements in the living environment of broilers, which could provide better leg constitution and thus, a generally better health condition.

2.2.2. Breed and Sex

Birds in studies II and V were obtained as 1-day-old Ross 208 chicks from a commercial hatchery in Denmark. Birds were sexed and placed in growing pens (50 % male/female in each pen).

2.2.3. Growing

Chickens in studies II and V were housed in wire pens (17m^2 with 150 birds/cage) in an environmentally controlled facility (33^{o}C initially decreasing gradually to 21^{o}C after 6 weeks), employing a typical light cycle programme of 21 h light, 3 h dark. The dark period was preceded by a 30 min dusk period. For the first 14 days birds were fed a standard starter-feed and thereafter were given a standard grower-feed. The composition and calculated nutrient content of the diet conformed to NRC (1994) specifications for poultry feeds. Feed and water were available ad libitum throughout. After 6 weeks, birds were fasted overnight and slaughtered by conventional methods. The muscles for studies II and V were removed, vacuum packed separately and frozen at –20^{o}C prior to subsequent experimental work.

2.3. Meat samples

2.3.1. Pork meat

In study I, four different sets (left and right side) of *Musculus semimembranosus* were obtained, each from a pig (4 gilt littermates) subjected to a different stress level prior to slaughter, i.e. models A, B, C and D. For study IV two batches of four sets of *Musculus semimembranosus* (left and right side), each batch from a different animal litter (4 gilt littermates in each) were utilised. Each batch was from animals also subjected to the four different pre-slaughter stress treatments A, B, C and D.

Two sets of pork loin muscles (*longissimus dorsi*) from animals of different RN^- genotypes were utilised in studies III and VI, one from Hampshire cross heterozygous carriers (RN^-/rn^+) and the other from homozygous non-carriers (rn^+/rn^+) of the RN^- allele. The muscles of each genotype were from gilt animals.

2.3.2. Chicken meat

Chicken breast muscle (*Pectoralis major*) was obtained from 6-week-old birds for studies II and V.

2.3.3. Meat pattie preparation

Prior to all studies (I-VI) muscles were stored vacuum packed in darkness at –20°C for a maximum of 4 months before analysis. Muscles were held at 4°C for 12 h in the pork studies and 2 h in the chicken studies prior to handling to allow ease of cutting and grinding. For all studies (I-VI) visible fat and connective tissues were removed and muscles were cut into cubes (3 cm^3) and mixed thoroughly. Left and right muscles (50%/50%) for a specific treatment were utilised, and mixed together thoroughly once cubed.

Each batch of muscle cubes was ground in a rotary screw mincer (Scharfen GmbH & Co. Maschinenfabrik KG, Germany) through a 4.5 mm plate. The minced samples were shaped into patties of 70 g and approximately 1 cm thickness using a commercial burger maker (i.d. 9 cm). Patties were vacuum packed in oxygen impermeable plastic laminate bags (polyamide/polyethylene 20/70, oxygen transmission rate of 32 cm^3/m^2 atm day at 23°C, RH 75 %). The vacuum-packed patties were then frozen at –20°C and stored for up to 3 weeks prior to all studies (I-VI).

2.3.4. Heat treatment and storage

For the pig studies I and IV frozen vacuum-packed patties were placed in a 25°C water bath until the sample core temperature reached 24.5°C. The patties were then held for 5 min to attain an equal starting point temperature prior to the heating/cooking stage. Heat treatment involved placing the patties in a water bath at 80°C for 25 min after which the patties reached a core temperature of 78°C. Water bath temperature and control patties core temperatures were measured continuously throughout the heat treatment by thermocouples and a data logger (Grant Instruments Ltd., United Kingdom). After heat treatment, patties were cooled on ice to, and held at, 5°C or lower for 5 min. Finally, the samples were taken from their vacuum bags, removed from the exudate, repackaged in

oxygen permeable polyethylene bags (oxygen transmission rate 2800 cm^3/m^2 atm day at 23°C) and stored at 4°C for up to 5 days to facilitate WOF development.

Prior to heat treatment in studies II, III, V, VI, patties were placed in a 25°C water bath until a core temperature of between 18 and 20°C had been reached. Subsequently patties were removed from their plastic vacuum bags and batch cooked in convection ovens set to 160, 170, 180, 190°C in chicken studies II and V, and 150 and 170°C in the pork studies III and VI. The convection ovens utilised were determined to have comparable heating cycles. During cooking, samples were heated initially for 5 min on each side followed by a further 10 minutes per side. In each oven, a control patties core temperature was monitored throughout the heat treatment by a thermocouple and data logger (Grant Instruments Ltd., United Kingdom). The final internal temperature reached over all pattie batches was found to reach a minimum of 78°C. After cooking and cooling to 5°C, the pattie batches were placed in oxygen permeable polyethylene bags and stored at 4°C for up to 4 days in studies II and V and 5 days in studies I, III, IV and VI, to allow the development of WOF.

2.3.5. Reheating treatment

To prepare the samples for sensory assessment, in all studies (I–VI) patties were divided into equal triangular pieces and individually vacuum packaged in plastic laminate bags. For reheating samples were placed in steel trays filled with water and heated in a convection oven at 140°C for 19 min. The mean serving temperature to the sensory panellists was 65°C.

2.4. Sensory evaluations

2.4.1. Assessors

In the vocabulary development studies I-III, study I utilised a 10-member panel of 8 females and 2 males, aged from 21 to 56 years, study II an 8-member panel of 3 females and 5 males, aged from 25 to 64 years and in study III the sensory panel consisted of 8 persons, 6 females and 2 males, aged from 26 to 56 years. All of panels in these studies (I-III) were recruited from the public and students of the Royal Veterinary and Agricultural University, Frederiksberg, Denmark. The individuals used in studies I-III had between 1 and 6 years previous experience as sensory judges.

Panellists in studies I-III, prior to vocabulary development were pre-screened for ability to discriminate odours and tastes (ISO, 1991; ISO 1992a). The basic requirements for assessor selection was their availability and motivation to participate on every day required. The sensory laboratory at the University which conformed to ASTM (1986) and ISO (1988) standards was utilised for all vocabulary development work.

2.4.2. Vocabulary development

Prior to vocabulary development sessions in studies I-III, expert sensory assessments of patties were made to generate preliminary attributes that spanned the sensory variation in the sample material. Literature sources were utilised to aid in this process (Civille & Lyon, 1996; ISO, 1992b; Jonhson & Civille, 1986; Lyon, 1987). In addition, sample sets for vocabulary development were

also determined from this preliminary phase in each study (I-III). These samples, spanned the experimental sensory variables in larger sample sets which were used in the future descriptive profiling studies IV, V and VI. References for each of the terms in the initial lists were also determined. Expert knowledge and literature sources were used to determine the specific materials chosen as representative of each term (Civille & Lyon, 1996; ISO, 1992b).

Prior to vocabulary development sessions each panels discriminative abilities for WOF, and the associated sensory variation in each of studies I-III were investigated using triangle tests (ISO, 1983). In each of studies I-III a descriptive vocabulary was subsequently developed over a series of daily sessions of 2 h each. Representative sample sets were presented for term intensity scoring (on paper) on unstructured line scales (150 mm) at all sessions in each of studies (I to III). Line scales were anchored on the left by 'none' and on the right by 'extreme' (Meilgaard et al., 1999). In the initial sessions panellists scored the samples based on panel leader explanations and their inherent perceptions of the sensory terms. Reference materials were then introduced to aid in the assessment of the sensory terms and were also used thereafter in the final sessions of studies I-III. Panellists were instructed to familiarise themselves with each reference material according to its associated sensory terms mode of assessment (i.e. odour, flavour, taste or aftertaste) prior to line scale scoring of samples in the later sessions. In all sessions, panellists were asked to indicate term redundancy on sheets called 'term grouping sheets', and to suggest new terms not present in the initial list of terms. All vocabulary development sessions were conducted in the English language, however, the descriptive terms were presented with their Danish translation. Descriptive sensory terms were removed or merged after each session based on selection criteria, representative sample and reference assessment, panel discussions, panel term grouping suggestions and interpretation of Principal Component Analysis (PCA) (see section 2.5.3.). Selection criteria in each session were that terms should; have relevance to the product, discriminate clearly between samples, be non-redundant, and have cognitive clarity to the assessors (Claassen & Lawless, 1992; ISO, 1994b; Wolters & Allchurch, 1994; Thybo & Martens, 1998).

Overall, comprehensive descriptive vocabularies of 16, 18 and 20 sensory terms were developed in studies I, II and III respectively. These terminologies were deemed to describe the odour, flavour, taste and aftertaste notes of the sample sets sensory dimensions. Each of the terms in the lists produced was assigned a corresponding reference material.

In general, the basic strategies utilised in the vocabulary development studies I-III were based on the International Standardisation Organisation or ISO guidelines in sensory analysis for training and selection of assessors (ISO, 1993; ISO, 1994a), identification and selection of descriptors for establishing a sensory profile (ISO, 1994b) and on a vocabulary development protocol utilised by Lyon (1987) for WOF in chicken meat.

2.4.3. Descriptive sensory profiling

All descriptive sensory profiling, studies IV, V and VI were carried out at the sensory laboratory at the University (ASTM, 1986; ISO, 1988). The sensory panels used to develop the vocabularies in

studies I, II and III were also used in the profiling studies IV, V and VI, respectively. Panellists in study I were used in the corresponding profiling study IV, panellists in study II in study IV and those in study III were used in study VI.

For study IV, two independent identical sensory profiling experiments were carried out. The sample sets presented in each profiling experiment consisted of four pre-slaughter stress treatments (A, B, C and D) each at five storage times 0, 1, 2, 3 and 5 days. Each sample set ($4 \times 5 = 20$) was assessed by the 10 panel members 4 times, as replicates in each profile. Thus, $20 \times 10 \times 4 = 800$ 'objects' were obtained for each of 16 sensory terms in both profiles.

In study V, the sample set presented for descriptive profiling contained four oven cooking temperatures, 160, 170, 180 and 190^{o}C referred to as T160, T170, T180 and T190^{o}C, respectively, each at four storage times 0, 1, 2, and 4 days. This sample set ($4 \times 4 = 16$) was assessed by each of 8 panel assessors in 4 replications. Thus, there were $16 \times 8 \times 4 = 448$ 'objects' in the profile data set for each of 18 sensory terms.

In study VI, the sample set presented for profiling consisted of two RN^{-} genotypes (carriers RN^{-}/rn^{+} and non-carriers rn^{+}/rn^{+}) both cooked at 150 and 170^{o}C and stored for 0, 1, 3 and 5 days. This sample set ($2 \times 2 \times 4 = 16$) was assessed by each of the 8 panel assessors 4 times, as replicates. This produced a total of $16 \times 8 \times 4 = 512$ 'objects' for each of the 20 sensory terms.

In all of the profiling studies (IV-VI) each of the 4 replicates (a full sample set) was presented over two days to each panellist, half the sample set on each day. In all 8 days of profiling sessions of 1.5 hr each were carried out to facilitate the assessment of the 4 replicates in each of the studies. In all of the profiling studies (IV-VI) sample presentation to the individual panellists was in a randomised order. Quantitative sensory data in each profiling study (IV-VI) was collected using the FIZZ Network data acquisition software (Biosystems, France). Overall, the methodologies used in descriptive profiling (studies IV-VI) followed ISO (1985a), guidelines for sensory methodologies in flavour profiling.

2.5. Data analyses

2.5.1. Monte Carlo testing of experimental designs

The statistical power of the experimental designs in studies IV and VI were tested by Monte Carlo simulation as described in Martens et al. (2000d). This method involved testing several alternative experimental designs with respect to balancing the risk of committing Type I errors, i.e. being fooled into believing that random errors in the data represent real effects and Type II errors, overlooking interesting effects as if they were just random errors. Overall, Monte Carlo power estimation of a design involves: initial generation of artificial data for a number of (say 5000) hypothetical experiments; then based on the experimental design and on certain assumptions each artificial data set in analysed in the same way that the future, real data is intended to be analysed, and from the distributions of the obtained parameter estimates the risks associated with a given experimental design can be studied (Martens & Martens, 2000a).

2.5.2. Descriptive statistical analysis

To gain an overview of the sensory terms and chemical measurements in studies IV, V and VI, descriptive statistical analysis providing, means, standard deviations, ranges, medians and percentile distributions was performed.

2.5.3. Principal Component Analysis

From the vocabulary development sessions where line scale scoring data was collected in studies (I-III), panel mean term scores for the samples were analysed by Principal Component Analysis (PCA). Moreover, in studies II and III the mean term data for the final lists of terms from each of sessions were data was collected were analysed in a single matrix by PCA. In both cases the sensory data were analysed, centred, unweighted and with full cross validation

2.5.4. ANOVA-Partial Least Squares Regression

In study II, ANOVA Partial Least Squares Regression (APLSR) was performed on the raw data for the final list of 18 terms from each of sessions 1 to 4, with the X-matrix set as 0/1 design variables for WOF and cooking temperature and the Y-matrix as sensory variables mean centred for assessor and session level effects, to investigate the remaining systematic structure. From this analysis the total variance (signal) and the residual variance (noise) after modelling optimal factors (Aopt), were plotted as a signal to noise (S/N) ratio against each of the final 18 terms over sessions 1 to 4. To determine the optimal number of factors for the S/N the validated explained variance in X and Y was investigated. From this 2 PCs were determined to be the optimal number of factors.

In study IV, for initial exploration of the sensory data, APLSR was performed for each profile. The X-matrix was set as design main effect variables A, B, C, D and WOF storage days and the Y-matrix as the sensory data (averaged over assessor and replicate) and selected chemistry measurements TBARS, water and pH. 0/1 indicator variables for the sample combinations of pre-slaughter stress and WOF were also included in the X-matrix to allow the individual samples to appear in the resultant correlation loadings plot. For contextual validation in the regression analysis, the conventional loadings plot was replaced by a plot of correlation loadings in this study (IV). This allowed easier interpretation since it revealed the structures in the data and their degree of fit at the same time. Full cross-validation was used in model validation.

To gain more quantitative information from the two profiles in study IV, further APLSR was carried out with the X-matrix comprising three qualitative pre-slaughter stress level indicators B, C, D (representing stress increase relative to the control level A), a single quantitative variable for WOF called Days, interactions (B×Days, C×Days, D×Days) and a squared term ($Days^2$). Sensory data (averaged over assessors and mean centred within each replicate) and the selected chemistry measurements TBARS, water and pH were again set as the Y-matrix. Sensory replicates were used in model cross-validation.

In study V, APLSR was performed similarly to study IV, with X as main design indicators for WOF and cooking temperature and individual sample indicators and Y as the sensory data

(averaged over assessors and mean centred for replicates). Also as in study IV, further APLSR was performed for more quantitative modelling of the data. In this analysis the X-matrix was comprised quantitative variables for WOF and cooking temperature called Days and Temp, respectively, their interaction (Days×Temp) and squared terms ($Days^2$, $Temp^2$). Sensory data (averaged over assessors and mean centred within each replicate) was set as the Y-matrix. Sensory replicates were used in both APLSR analyses for model cross-validation.

In study VI, an initial APLSR performed as in studies IV and V with the X-matrix as main and sample design indicators for WOF, cooking temperature and genotype and Y as the sensory data (averaged over assessors and mean centred for replicates) and chemistry/physical measurements (TBARS, pH, thawing losses and cooking losses).

In addition, more quantitative APLSR was also carried out in study VI, similar to study V, with the X-matrix comprising, a single quantitative variable for WOF, called Days (with values 0, 1, 2, 3, 5) and two qualitative indicators RN and T170 representing genotype and cooking temperature relative to the control level rn and T150, respectively, interactions (T170×RN, T170×Days, RN×Days) and a square term ($Days^2$). Sensory data (average over assessor only and mean centred for replicates) and the chemistry measurements (thawing loss, cooking loss, pH, TBARS) were again set as the Y-matrix. Sensory replicates were used in both APLSR analyses for model cross-validation.

2.5.5. Partial Least Squares Regression

In study IV, Partial Least Squares Regression (PLSR) was performed to investigate the predictive ability of the sensory data from the chemical data (as used in APLSR). The X-matrix was set as the sensory data (averaged over assessor and mean centred for replicates) and Y as the chemistry data, with replicates utilised for model cross-validation

2.5.6. Jack-knife uncertainty testing of significances in APLSR and PLSR

Estimated regression coefficients of the quantitative APLSR in studies IV, V and VI and predictive PLSR in study IV were analysed by jack-knife uncertainty testing which is based on cross-validation and stability plots (Martens & Martens 1999; Martens & Martens 2000b). This allowed determination of the estimated regression coefficients ($\hat{b}$) with uncertainty limits that correspond to ± 2 standard uncertainties, i.e. $\hat{b} \pm 2\ \hat{s}(\hat{b})$ under ideal conditions. From these, the significance ($P<0.05$) of the variable relationships in the X and Y matrices were determined.

All PCA, APLSR and PLSR analysis was performed using the Unscrambler Software, Version 7.5 (CAMO ASA, Norway). In all regression analysis data were analysed, centred and with both the X and Y matrices standardised in all cases.

2.5.7. Generalised Procrustes Analysis

Separate Generalised Procrustes Analysis (GPA) was performed on the raw data from each of the 7 vocabulary development sessions in study III. Changes in overall panellist agreement on the

sensory term meanings over the sessions was investigated using the total percentage explained variance over all dimensions (5) of the GPA solutions from the vocabulary development sessions. Subsequently, the breakdown of this total percentage explained variance in each dimension of the GPA solution from each session, referred to as percent variance accounted for (%VAF), was also investigated. Differences in panellists use of the line scale over the session was investigated via their individual scaling weights from GPA of each session.

To investigate changes in the level of panellist agreement on the meaning of each of the final list of sensory terms over the vocabulary development sessions, assessor mean term correlation vector lengths for each term was extracted from the GPAs performed on each session. The derivation and interpretation of these correlation vector lengths is addressed in detail in study III. To investigate agreement of the individual assessors over the sessions, their individual vectors in each session for specific terms were investigated.

Finally, progression of sample discrimination over the sessions was investigated by the ratio of total explained variance to total residual variance per sample using all dimensions of the GPA solution from each of the development sessions. These ratios are essentially GPA derived Signal to Noise (S/N) ratios.

All GPA investigations were carried out using the Senstools Software, Version 2.2 (OP&P, The Netherlands).

2.5.8. Analysis of variance

In study III, comparison of GPA derived S/N ratios was performed by analysis of variance (ANOVA) using the Tukey honestly significance difference (*HSD*)-test at the 5% level (Statistical Package for the Social Sciences, SPSS, Chicago, USA). In study VI, Analysis of Variance (ANOVA) using Generalised Linear Model (GLM) procedure from SPSS (Chicago, USA), was performed for analysis of the physical/chemical data, thawing loss, cooking loss, pH, TBARS and means were separated by Least Significant Difference (LSD) testing at the 5% level ($P < 0.05$).

2.6. Chemical/instrumental/physical measurements

2.6.1. Background

All chemical/instrumental analyses in study IV were carried out at The Royal Veterinary and Agricultural University, Department of Dairy and Food Science, Denmark. Gas Chromatography/Mass spectroscopy in study V was carried out at the University of Reading, Department of Food Science, United Kingdom. Chemical analyses in study VI was carried out at DIAS, Department of Animal Quality, Denmark.

2.6.2. Chemicals

Reagents in studies V-VI were of analytical grade (‘AnalR’) and the highest purity.

2.6.3. Lipid analyses

2.6.3.1. Extraction of lipids

In study IV, lipid extraction was performed by a method described by Brøndum et al. (2000). Analysis was performed in duplicate on all WOF samples at each pre-slaughter stress level in study IV and total lipids were expressed as g/100g meat.

2.6.3.2. Conjugated dienes

In study IV, the extracted lipids were analysed for conjugated diene as described by Brøndum et al. (2000). Spectra were transformed to the secondary derivative as described by Corongiu and Milia (1983) and the relative difference of absorbance at 254 nm and 238 nm was used to express the concentration of components with *trans, trans* conjugated bonds. Duplicate analysis was performed on day 0 and 5 WOF samples only, at each pre-slaughter stress level. Results were expressed as 2nd derivative (254–238 nm)/g total lipid.

2.6.3.3. Separation of phospholipids

Phospholipid separation from the extracted lipids in study IV, was performed as described by Brøndum et al. (2000).

2.6.3.4. Fatty acid composition of total lipids and phospholipid fractions

The fatty acid composition in the total lipids and the isolated PC and PE fractions of study IV were analysed as lipid methyl esters according to the method of Jart (1997). The different fatty acid content of the total lipids were expressed as percentage of total fatty acids. Fatty acid content of the phospholipid fractions were expressed as percentage fatty acids with less than 2 double bonds (< 2db), with two double bonds (2db) and with greater than two double bonds (> 2db). Analysis was performed in duplicate on day 0 and 5 WOF samples only, at each pre-slaughter stress level.

2.6.4. Thiobarbituric acid reactive substances

Lipid oxidation was measured in studies IV and VI as thiobarbituric acid reactive substances (TBARS) by the extraction method described by Vyncke (1975) with modifications described by Brøndum et al. (2000). Analyses were performed in duplicate on two individual meat patties for all samples in profile 1 and 2 of study IV and in triplicate on two individual meat patties for all samples in study VI. In study IV and VI, TBARS were expressed as μmol malondialdehyde (MDA)/kg meat.

2.6.5. pH

For pH determination in the cooked meat patties of studies IV and VI, 10 g of frozen meat pattie was mixed in an Ultra Turrax with 10 ml of water for 45 s at 13,500 rpm. The pH of the meat slurry was measured after 30 min at ambient temperature using a pH-meter (PHM 82 Standard, Radiometer, Copenhagen, Denmark) with an Ingold pH Electrode (Ingold, Urdorf, Switzerland). pH

analyses were performed in duplicate on two individual meat patties for all samples in study IV and VI.

2.6.6. Water Content

In study IV water content was derived from cooked pattie dry matter content. Determinations of dry matter were conducted by drying 2 g of pattie meat in an oven at 104°C for 4 hours and weighing the sample after cooling for 15 min. Water content was determined from 100 less the dry matter content of 100 g meat, and expressed as g/100g meat (see Bendall & Swatland, 1988). Duplicate analysis was performed in on all WOF samples at each pre-slaughter stress level.

2.6.7. Thawing and cooking losses

In study VI, thawing loss was measured directly by allowing patties to thaw from frozen in their oxygen impermeable plastic laminate bags at 4^{o}C for 3 h. Thawed patties were then taken from the storage bags and excess exudate was removed with an absorbant paper prior to weighing. Thawing loss was determined as the weight of a meat pattie before and after thawing, expressed as a percentage of the initial weight of the sample. In total, thawing loss was measured for 32 meat patties from each genotype. Cooking losses were derived directly from the weight of the thawed pattie before, and pattie weight after cooking, expressed as a percentage of the initial pattie weight (see Bendall & Swatland, 1988). For cooking losses weights were taken for a total of 4 patties for each sample in the sensory profiling design.

2.6.8. WOF volatiles analysis

2.6.8.1. Volatile extraction

A subset of meat samples (cooking temperatures 160, 180 and 190^{o}C and storage days 0, 1 and 4) were analysed in triplicate using the dynamic headspace method of Madruga and Mottram (1995). Samples (40 g) were held at 25 °C for 1 h while nitrogen (40 ml/min) swept the volatiles onto a glass-lined, stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg Tenax TA (Scientific Glass Engineering Ltd., UK). A 100 ng of 1,2-dichlorobenzene in hexane solution (1µl) was added to the trap at the end of the collection and excess solvent and any water retained on the trap were removed by purging the trap with nitrogen at 40 ml/min for 5 min.

2.6.8.2. Gas chromatography-mass spectrometry

The analyses were performed on a HP 5972 mass spectrometer fitted with a HP 5890 Series II gas chromatograph (Hewlett-Packard, USA). A CHIS injection port (Scientific Glass Engineering Ltd., UK) was used to thermally desorb the volatiles from the Tenax trap onto the front of a BPX5 capillary column (50 m × 0.25 mm i.d., 0.25 µm film thickness). During the desorption period of 5 min, the oven was held at 0 °C. After desorption, the oven was heated at 40°C/min to 40°C and held for 2 min before heating at 4°C/min to 280°C. Helium at 1 ml/min was used as the carrier gas. The

mass spectrometer was operated in electron impact mode with an electron voltage of 70 eV and an emission current of 50 μA. A scan range of *m/z* 29 to 400 at a rate of 1.9 scans/s was employed.

2.6.8.3. Identification of volatiles

The volatile compounds in the samples were identified by comparison of their mass spectra with those in reference collections and where available with those of authentic compounds. Linear retention indices (LRI) were calculated for each compound using the retention times of a homologous series of C_6–C_{22} *n*-alkanes according to Van den Dool and Kratz (1963). Mass-spectral identification was confirmed by comparison of the LRI with those from authentic compounds run under similar conditions.

2.6.9. Genotype analysis

2.6.9.1. Glycogen

Of the muscles used in studies III and IV, all from gilt animals (female), half were from Hampshire cross heterozygous carriers (RN^-/rn^+) and half from homozygous non-carriers (rn^+/rn^+) of the RN^- allele. The animals were confirmed as RN^- carriers and non carriers by determination of glycogen levels (including glucose and not glucose-6-phosphate) in muscle biopsy samples taken from the *Musculus longissimus dorsi* of the live animal. Glycogen level was determined in 50 mg muscle samples, heated in a test tube with 5 ml of 1 M HCL at 100°C for 2 h. The samples were then analysed for glucose residues by the method of Passonneau and Lowry (1993) and expressed as μmol of glucose residues (glycogen + glucose) per gram of wet muscle. Animals with >100 μmol (glycogen + glucose)/g wet weight were regarded as RN^- carriers.

2.6.9.2. Direct genotyping

In addition, direct genotyping by DNA sequencing, carried out according to the method of De Vries et al. (1997) was utilised to confirm RN^- carrier or non-carrier status of the pigs used in study III and VI.

3. Summaries of investigations

Paper I. Development of a sensory vocabulary for warmed-over flavour: part I in porcine meat

Pork patties from *Musculus semimembranosus*, were utilised by a sensory panel to develop a descriptive vocabulary for the sensory profiling of WOF. Patties were derived from the meat of non-stressed and stressed animals and were stored at 4^{o}C for up to 5 days. An initial list containing 45 descriptive terms developed from the literature and a preliminary sample evaluation was presented to the panel. This list was modified over a 7 session period to 16 terms each with a corresponding reference material. Selection criteria were, that terms should; have relevance to the product, discriminate clearly between samples, be non-redundant, and have cognitive clarity to the assessors. Criteria fulfilment was determined via representative sample and reference assessment, panel discussions and interpretation of Principal Component Analysis. During vocabulary development the panel showed dynamic changes in their use of the sensory vocabulary. Discriminative abilities were found to increase over the early sessions and appeared to stabilise in the final two sessions.

Paper II. Development of a sensory vocabulary for warmed-over flavour: part II in chicken meat

A sensory panel utilised chicken patties from *Musculus pectoralis major* to develop a descriptive vocabulary for WOF. Patties were subjected to 4 different cooking temperatures and stored at 4^{o}C for up to 4 days. A list of 33 descriptive terms, developed from the literature and a preliminary sample evaluation was modified over 5 daily sessions to 18 terms with corresponding references. In the term selection process the criteria used were, that terms should; have relevance to the product, discriminate clearly between samples, be non-redundant, and have cognitive clarity to the assessors. A comprehensive vocabulary was developed that described the sensory dimensions present in the samples. Over sessions the panel was found to display dynamic changes in their use of the sensory vocabulary. Overall, panel discriminative abilities were enhanced and a process of sensory learning was observed.

Paper III. Sensory panel consistency during development of a vocabulary for warmed-over flavour

A sensory vocabulary of 20 terms each with a corresponding reference material was developed over 7 sessions using pork patties derived from the meat of carriers and non-carriers of the RN^{-} gene. Patties were oven-cooked at 150 and 170^{o}C and chill-stored for up to 5 days to facilitate WOF development. Generalised Procrustes Analysis (GPA) was used to investigate sensory terms and their individual use by panellists over the sessions. GPA explained variance indicated the final vocabulary displayed a similar amount of information to that of the initial vocabulary of 42 terms. Individual panellists scale use was found in general to converge over the sessions. Panel agreement on many odour and flavour terms appeared to be enhanced as term synonyms were removed in vocabulary development. Sample discriminability decreased from sessions 1 to 4, where term concepts were verbally communicated to the panel. Term reference

introduction in session 5 caused a levelling in sample discriminability and a reduction in agreement, most likely related to perceptual confusion. Subsequently, references enhanced both discriminability and agreement. Thus, it may be more useful to introduce reference materials earlier if not in the first session of the vocabulary development process.

Paper IV. Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress

Two independent sensory profiles were carried out to evaluate WOF development in cooked, chill-stored and reheated pork patties. The patties were derived from the *Musculus semimembranosus* of control animals and animals subjected to increasing pre-slaughter stress treatments. All patties were stored at 4^{o}C for up to 5 days to allow WOF development. In addition, TBARS, conjugated dienes, pH, water content, total lipids and the fatty acid compositions of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and total lipids, were measured in the cooked meat patties. A data analytical strategy involving ANOVA-Partial Least Squares Regression (APLSR), to determine relationships between the design variables (WOF and pre-slaughter stress) and the sensory-chemical data, and PLSR to elucidate predictive links between the sensory and chemical data was utilised. WOF was found to involve the development of lipid oxidation derived nuance off-flavour and odour notes, e.g. rancid-like flavour and linseed oil-like odour, in association with a concurrent decrease in cooked pork meat-like flavour. The reduction in "meatiness", over the initial days, 0 to 2 of WOF development was attributed to the degradation of both, unstable sulfur containing amino acids in meat proteins and sulfur containing "meaty" aroma compounds. Whereas, at the later days, 3 to 5 of WOF development the "meaty" loss was ascribed to perceptual masking by lipid oxidation products. TBARS and conjugated dienes were found to be significant ($P < 0.05$) predictors of the sensory terms related to the lipid oxidation aspect of WOF. Whilst the polyunsaturated fatty acid (PUFAs) contents of PE, PC and the total lipids were found to decrease with WOF development, reflecting their loss in lipid oxidation reactions. The sensory variation related to pre-slaughter stress appeared to be distinct from WOF variation and was described by a sour to sweet taste continuum. However, interactions were noted that indicated increasing pre-slaughter stress resulted in the decreased perception of WOF. pH and water content were found to significantly ($P < 0.05$) predict the sensory effects resulting from pre-slaughter stress. Overall, new insight into the intricate and multidimensional nature of WOF from a sensory as well as chemical perspective was gained.

Paper V. Sensory profiling and volatile analysis of warmed-over chicken patties cooked at different temperatures

A descriptive sensory profile was carried out to evaluate warmed-over flavour (WOF) development in cooked, chill stored and reheated chicken patties. Patties, derived from the *Pectoralis major* muscle were subjected to different cooking temperatures (160, 170, 180, 190^{o}C) and stored at 4^{o}C for 0, 1, 2 and 4 days to facilitate WOF development. In addition, Gas

Chromatography-Mass Spectrometry (GC-MS) was carried out on a representative sub-set of the samples (160, 180, 190^{o}C, stored for 0, 2, 4 days) used in sensory profiling. Sensory and GC-MS identified volatiles were analysed using multivariate ANOVA-PLSR and jack-knife significance testing. The descriptive vocabulary used in profiling was found to display the sensory dimensionality of WOF in cooked chicken in relation to increasing oven cooking temperature. WOF was described as involving the increase of 'rancid' and 'sulfur/rubber' sensory notes and the decrease of chicken 'meaty' characteristics. Cooking temperature increase was found to result in meat samples with a more 'roasted', 'toasted' and 'bitter' sensory nature. The 'roasted' character of the samples was determined as related to both WOF and cooking temperature. Analysis of the volatile compounds from the chicken patties showed a rapid development of lipid oxidation with refrigerated storage. This was also apparent in the sensory profiling data. Changes in identified sulfide compounds were proposed as related to the progression of lipid oxidation. Overall, cooking temperature increased the formation of Maillard reaction derived compounds, however, these did not appear to inhibit WOF development in the chicken patties.

Paper VI. Predicting sensory properties of warmed-over flavour in carriers and non-carriers of the RN^{-} allele

Descriptive sensory profiling was carried out to evaluate warmed-over flavour (WOF) development in cooked, chill-stored and reheated pork patties derived from the meat (*Musculus longissimus dorsi)* of heterozygous carriers (RN^{-}/rn^{+}) and homozygous non-carriers (rn^{+}/rn^{+}) of the RN^{-} gene. Patties were oven-cooked at 150 and 170^{o}C and chill-stored for up to 5 days to facilitate warmed-over flavour (WOF) development. In addition, thawing losses, cooking losses, pH and TBARS were determined for the meat samples. In analysis of data, a strategy that involved Analysis of Variance (ANOVA), to investigate changes in the physical/chemical measurements due to the experimental design variables (storage days, cooking temperature and genotype) and multivariate ANOVA-Partial Least Squares Regression (APLSR), to determine relationships between the design variables and the sensory-physical/chemical data was utilised. WOF was determined to involve the development of lipid oxidation derived nuance off-flavour and odour notes, e.g. rancid-like flavour and linseed oil-like odour, in association with a concurrent decrease in 'meatiness' as described by, e.g. cooked pork meat-like flavour. Cooking temperature as described by roasted-like and caramel-like odours and samples from carriers of the RN^{-} gene were described as more 'sour' and 'metallic' in nature. Thawing and cooking losses were found to be significantly ($P < 0.05$) higher in meat from carriers of the RN^{-} gene versus non-carriers. pH, responsible for increased thawing losses, was negatively related to samples from carriers of the RN^{-} gene. However, the measured pH in RN^{-} carriers could not be significantly ascribed as lower than non-carriers in the freshly cooked meat samples. TBARS were found to be significant ($P < 0.05$) predictors of the sensory terms related to the lipid oxidation aspect of WOF. Moreover, TBARS were significantly ($P < 0.05$) higher in meat from RN^{-} gene carriers but, significantly ($P < 0.05$) lower in meat cooked at higher temperature. The former effect was postulated as related to pH and the latter as related to the antioxidant effects

of Maillard reaction products developed at higher cooking temperatures. Overall, WOF, cooking temperature and genotype were differentiated as individual dimensions through sensory profiling of the meat samples and were characterised by specific groups of sensory descriptors. In addition, the predictive nature of thawing losses, cooking losses and TBARS was established for the effects of RN^- gene, cooking temperature and WOF in the meat samples.

4. General conclusions

4.1. Study I. Vocabulary development for WOF in pre-slaughter stressed pork meat

- A list of 16 terms was developed as a vocabulary to describe the aroma/flavour/taste character notes of WOF in porcine meat patties derived from pigs subjected to different pre-slaughter stress treatments. Omission of terms from an initial list over several sessions was based on the selection criteria, that terms should; (1) have relevance to the product; (2) discriminate clearly between samples; (3) be non-redundant and (4) have cognitive clarity to assessors. Criteria fulfilment by terms was determined via representative sample assessment, reference assessment, panel discussions and the multivariate statistical method PCA. The terms were seen in general to describe the underlying phenomenon when individual variables were investigated. Those chosen were deemed not to be redundant or to show limited redundancy. The 16 terms were relevant for the product and non-systematic terms were removed as they were not relevant for the samples analysed. The hypothesis that the sensory quality deterioration in samples was a dynamic multidimensional phenomenon, which could not be adequately described by an all encompassing term such as 'warmed-over flavour', was strongly supported by the PCA indications in the various sessions.

4.2. Study II, Vocabulary development for WOF in oven cooked chicken meat

- A sensory vocabulary of 18 terms was developed that described the sensory dimensions WOF and cooking temperature, in chicken meat patties as odour, flavour, taste and aftertaste character notes. The 18 terms were deemed relevant to the product and were seen to be discriminative for the underlying phenomena when individual variables in the PCA were investigated. Terms were determined not to be redundant or to show limited redundancy and all were considered cognitively clear by the panel. Vocabulary development was shown to be a dynamic process and clear sensory learning was displayed by the panel over the sessions.

4.3. Study III, GPA analysis of a sensory vocabulary developed for, WOF in pork meat from carriers of the RN^- gene

- The methodology utilised lead to the development of a vocabulary of 20 terms, over 7 sessions that described the experimental sensory variation present in the meat samples, i.e. WOF, cooking temperature and RN^- genotype. Overall explained variance in GPAs of each session indicated the vocabulary produced gave a similar amount of information to the initial list of 42 terms, with less than half the number of sensory terms. Similarity of panellists scale use was found in general to increase with vocabulary development. Increased panel agreement was observed for some terms where other terms showed little change in agreement over the sessions. Ability to achieve agreement on a term appeared to be enhanced as the number of term synonyms were removed in terminology development. Sample discrimination decreased on average over the initial sessions where term descriptions were verbally presented. Reference introduction in mid-vocabulary development initially caused confusion in panel concepts and

reduced agreement. Subsequently term references produced increased sample agreement and discrimination, with discriminative ability in the final session appearing similar to that in the first session. Sample discrimination, however, in the first session was the result of a term list upon which the panel did not agree whereas, discrimination in the final session was from a vocabulary that panellists did perceptually agree upon. Overall, to attain a more immediate increase in sample discriminative ability references may be introduced earlier in vocabulary development.

4.4. Overall on WOF vocabularies developed

- The methodology devised for vocabulary development overall was determined to produce sensory descriptor lists which characterised the sensory variation present in the meat samples within a short period. The concise strategy developed, where a start list of generally relevant terms is presented to a sensory panel and over a period of days rather than weeks or months is reduced, first without references and subsequently with references, using selection criteria, multivariate data analysis and panel discussions to a final descriptive and discriminative vocabulary, may be considered to have applications generally in developing descriptive vocabularies for the sensory evaluation of foods.
- The similarity of terms that described the WOF phenomenon in all three vocabularies was quite clear. Sweet, fresh pork or chicken meat-like to linseed oil-like, rancid-like flavour and odour characteristics, indicating the loss of freshly cooked 'meatiness' as lipid oxidation proceeded and WOF off-notes developed was common to all vocabularies. However, the additional sensory variation, i.e. pre-slaughter stress, cooking and genotype appearing to be characterised by a number of the sensory descriptors that were not WOF related in each vocabulary.

4.5. Study IV, WOF and pre-slaughter stress

- WOF from a sensory perspective was found to involve the development of a number of lipid oxidation derived nuance off-flavours and odours, in association with a decrease in 'meaty' flavour with days of chill-storage. Loss of cooked pork meat flavour was attributed to a combination of sulfur amino acid degradation and perceptual masking by lipid oxidation products. Chemical measurements TBARS and conjugated dienes were found to be strong predictors of the sensory terms related to the lipid oxidation aspect of WOF.
- Pre-slaughter stress appeared in general to manifest itself as a separate sensory dimension to WOF in the meat samples. However, some indications of interaction were found, indicating increasing pre-slaughter stress may have reduced perceived WOF development. From a sensory viewpoint increasing pre-slaughter stress was described by a sour to sweet taste continuum. This reflected increasing measured pH which was a strong predictor of the sensory effects resulting from pre-slaughter stress in the cooked meat. Water content was also found to be a highly correlated with the sensory effects of increasing pre-slaughter stress.

- PUFAs in the total lipid and phospholipid fractions PE and PC were found to decrease with WOF development, reflecting their loss in lipid oxidation reactions. Total lipids, found to be higher in the animals of profile 2 appeared to produce an increased lipid oxidation level relative to profile 1. This was reflected by higher measured conjugated dienes and an increased perception of sensory WOF terms in profile 2.
- The conclusions from both sensory profiles for WOF and pre-slaughter stress variation were in general the same. This added validity to the interpretation of the variation noted in the results as a whole. Moreover, the sensory profiles, clearly illustrated the intricate and multidimensionality of WOF both from a sensory and chemical perspective.

4.6. Study V, WOF and cooking temperature

- This study showed the sensory dimensionality of WOF in relation to oven cooking of chicken meat. WOF was described by increased 'rancid' and 'sulfur/rubber' sensory notes associated with a concurrent decrease in chicken 'meaty' flavour. Cooking temperature was described by increased 'roasted', 'toasted' and 'bitter' sensory notes. Roasted flavour was determined to be related to both WOF and cooking temperature.
- Analysis of the volatile compounds from the chicken patties showed a rapid development of lipid oxidation during refrigerated storage. This was also apparent in the sensory profiling data. Changes in sulfide compounds were conjectured to be related to the progression of lipid oxidation. Cooking temperature increased the formation of Maillard-derived compounds, however, they did not show strong effects on the prevention of WOF in the chicken patties.

4.7. Study VI, WOF, cooking and the RN gene

- Meat patties from RN^- carriers were determined to have significantly higher thawing losses than non-carriers. Cooking losses were found to be significantly higher at 170^{o}C compared to 150^{o}C for oven cooked meat pattie samples at corresponding days of warmed-over flavour and genotype. Samples from RN^- carriers also displayed significantly higher cooking losses versus non-carriers at 150^{o}C, however, not at 170^{o}C.
- pH displayed significant increases and decreases for samples from carriers of the RN^- gene versus those from non-carriers, at corresponding temperatures and days of storage. Moreover, the freshly cooked meat samples (day 0 storage) at each oven cooking temperature displayed no significant differences in pH between the two genotypes.
- TBARS were found to significantly increase with days of storage within a genotype and cooking temperature. Thus, an indication of increased lipid oxidation and WOF with days of storage. In addition, TBARS was found to significantly increase in carriers of the RN^- gene relative to non-carriers at similar days of storage and cooking temperature. Therefore, lipid oxidation was significantly higher in meat patties from carriers of the RN^- gene. This was probably related to an effect of lower pH increasing the activity of pro-oxidants in the samples from RN^- carriers versus non-carriers. Moreover, TBARS also appeared to significantly

decrease at similar genotype and days of storage with increasing cooking temperature. This was most likely due to the antioxidant effects of Maillard reaction products produced when meat is cooked at high temperatures.

- In APLSR analysis WOF development displayed a pattern involving the increase of off-flavour notes with a concurrent loss of fresh 'meaty' descriptors. Cooking temperature increase was found to be described by the terms caramel-like odour, roasted like odour and bread-like flavour. While RN^- gene was related to a group of terms that included, egg/sulfur/rubber-like flavour, lactic/fresh sour like aftertaste and flavour, sour and salt tastes and metallic flavour and aftertaste. Thus, these samples were more 'lactic/fresh sour' and 'metallic' in sensory nature compared to non-carriers.
- Overall, it appeared that the sample variables WOF, cooking temperature and genotype were differentiated by individual PCs and described by specific groups of sensory terms. Thus, the sensory terminology differentiated and characterised the sample variation present in the sensory profiling meat sample set.
- Of the physical/chemical measurements in APLSR, TBARS were highly correlated with WOF days of storage and measured cooking loss was highly correlated with the highest cooking temperature, 170^{o}C. In addition, the pattie samples from carriers of the RN^- gene appeared to have higher thawing losses and a lower measured pH than samples from non-carriers.
- Of the terms in the initial APLSR analysis related to carriers of the RN^- gene, lactic/fresh sour flavour was determined to be a significant descriptor. Thus, the general terms 'acidulous' proposed previously to describe the change in taste of meat from the RN^- carriers versus non-carriers may be better characterised as a lactic/fresh sour-like flavour reminiscent of that noted in natural yoghurt.
- In general, the experimental design variation, WOF, cooking temperature and genotype in the meat samples presented for sensory profiling were differentiated as independent phenomena and were characterised by specific groups of sensory terms. In addition, each experimental parameter differentiated in sensory profiling, WOF, cooking temperature and genotype was 'predicted' by TBARS, cooking losses and thawing losses, respectively.
- Overall, from a sensory perspective WOF development was characterised as involving the increase of off-note sensory descriptors such as linseed oil-like odour and rancid-like flavour concurrent with a decrease in fresh 'meatiness' as described by cooked pork meat-like flavour and odour.

5. Future perspectives

- The developed WOF vocabularies of the present thesis confirm conclusions from previously presented WOF lexicons, that the sensory terms related to WOF are largely common across different cooked and chill-stored meat types. However, no definitive WOF vocabulary can be said to exist as with each new context where WOF is a problem new descriptors in addition to the more common terms will always be required. This is essential to note if one hopes to fully characterise the off-flavour in new sensory studies.
- In relation to the consumer and industry, studies have indicated that WOF is a major problem from a consumer perspective in reducing the palatability of cooked and reheated meats and meat products. However, as consumers fail to report their dissatisfaction it appears that the catering sector and food industry have chosen not to acknowledge WOF as a significant problem, 'publicly' at least, in recent years. One assumes however, as WOF is a significant barrier to launching new ready-to-eat meals, industry 'privately' is devising ways to eliminate or at least reduce the prevalence of WOF. It is afterall good for business, and let us not forget the consumer, in the long term. Moreover, for WOF to be controlled, industry and the catering sector must make a concerted effort through preventive means in combination with sensory analysis to control WOF development in pre-cooked meat and meat products. In addition, the consumer should be a little more vocal as to their impressions of such food products if they find them to be unsatisfactory. This will ensure preventive action from the people who produce and sell the products that are susceptible to WOF.
- The compounds or combination of compounds produced by lipid oxidation responsible for the different sensory notes that describe WOF still need definitive elucidation. Also the interrelationship of the lipid oxidation aspect of WOF with the degradation of sulfur-containing amino acids in meat proteins and sulfur containing "meaty" flavour compounds in the network of radical reactions requires further attention.
- The reported effects of Maillard reaction products on the prevention of WOF in cooked and chill-stored meat needs further investigation from a sensory perspective as this effect was suggested in pork but not in chicken in the present thesis studies. That the latter may have been due to sensory masking by WOF needs to be confirmed.
- From a sensory perspective in the present thesis RN^- gene variation was related to a group of terms that included, egg/sulfur/rubber-like flavour, lactic/fresh sour like aftertaste and flavour, sour and salt tastes and metallic flavour and aftertaste. Thus, these samples were more 'lactic/fresh sour' and 'metallic' in sensory nature compared to non-carriers. Therefore, it may be said that the general term 'acidulous' proposed previously to describe the change in taste of meat from the RN^- carriers versus non-carriers was characterised to a degree for the first time. Further sensory studies however, need be carried out with the specific aim of characterising the effect of the RN^- gene in freshly cooked meat. This will ensure that the sensory dimensionality of the effect of the RN^- gene meat is uncovered conclusively.

- The vocabulary development methodology devised in the present thesis, to produce a discriminative sensory terminology in a short space of time, should be employed in the sensory characterisation of other meat and food products. This methodology appears to produce highly descriptive and discriminative vocabularies at very reasonable 'costs' in time and money.
- The data analysis strategies used in the present thesis allowed clear interpretation of the variation in the sensory and chemical data and their interrelationships. Thus, such a strategy may be implemented generally in food research where sensory and chemical data are under investigation. This will ensure that the maximum information is extracted from the data one has worked long and hard to obtain.
- The experimental strategies used in the present thesis profiling studies, involving sound experimental planning, testing of experimental designs to ensure sufficient replications, standardised sample preparation, randomised sample presentation and sample evaluation by highly trained panellists, who had participated in the development of a sensory vocabulary prior to each profiling study, should be considered as essential aspects to successful sensory profiling of food products.
- Overall, the present thesis studies can be said to have lead to a general elucidation of WOF from a sensory odour, flavour, taste and aftertaste perspective. However, one must not forget the influence that WOF may have on other sensory quality parameters, e.g. texture and colour. The assessment of these quality effects may be considered for sensory investigation in relation to WOF in the future. This will ensure that the overall sensory implications of the phenomenon known as WOF are elucidated.

References

AOCS (1989). *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 4th ed. New York: American Oil Chemists' Society.

Ang, C. Y. W., & Lyon, B. G. (1990). Evaluations of warmed-over flavour during chill storage of cooked broiler breast, thigh and skin by chemical, instrumental and sensory methods. *Journal of Food Science, 55,* 644-648.

Arnold, G. M., & Williams, A. A. (1986). The use of Generalised Procrustes Analysis in sensory analysis. In J. R. Pigott, *Statistical Procedures in Food Research* (pp. 233-253). London: Elsevier.

Asghar, A., Gray, J. I., Buckley, D. J., Pearson, A. M., & Booren, A. M. (1988). Perspectives on warmed-over flavour. *Food Technology, 42,* 102-108.

ASTM (1986). Physical Requirements. *Guidelines for Sensory Evaluation Laboratories*, STP 913. Pennsylvania: American Society for Testing and Materials.

Awad, A., Powrie, W. D., & Fennema, O. (1968). Chemical deterioration of frozen bovine muscle at –4°C. *Journal of Food Science, 33*, 227-235.

Bailey, C., Cutting, C. L., Enser, M. B. & Rhodes, D. N. (1973) The influence of slaughter weight on the stability of pork sides in frozen storage. *Journal of the Science of Food and Agriculture, 24*, 1229-1304.

Bailey, M. E. (1988). Inhibition of warmed-over flavour with emphasis on Maillard reaction products. *Food Technology, 42,* 123-126.

Bailey, M. E., & Um, K. W. (1992). Maillard reaction products and lipid oxidation. In A. J. St. Angelo, *Lipid Oxidation in Food* (pp. 123-139). New York: American Chemical Society.

Bendall, J.R., & Lawrie, R.A. (1962). The effect of pre-treatment with various drugs on post-mortem glycolysis and the onset of rigor mortis in rabbit skeletal muscle. *Journal of Comparative Pathology and Therapy, 72,* 118-124.

Bendall, J.R. (1973). Cold-contracture and ATP turnover in the red and white musculature of the pig post mortem. *Journal of the Science of Food and Agriculture. 23,* 55-71.

Bendall, J. R., & Swatland, H. J. (1988). A review of the relationship of pH with physical aspects of pork quality. *Meat Science, 24*, 85-126.

Berlitz, H. –D., & Grosch, W. (1999). Lipids. In *Food Chemistry* (pp. 152-234). Berlin: Springer-Verlag.

Berry, B. W., Maga, J. A., Calkins, C. R., Wells, L. H., Carpenter, A. L., & Cross, H. R. (1980). Flavour profile analysis of cooked beef loin steaks. *Journal of Food Science, 45,* 1113-1115, 1121.

Bolton, J. C., Melton, S. L., Riemann, M. J., & Backus, W. R. (1985). An isocratic HPLC method for analysis of bovine phospholipids. Presented at the 77th Annual Meeting of the *American Society of Animal Sciences*, University of Georgia, August 13-16.

Briskey, E.J. (1964). Etiological status and associated studies of pale, soft, exudative porcine musculature. *Advances in Food Research, 13*, 89-178.

Brøndum, J., Byrne, D. V., Bak, L. S., Bertelsen, G., & Engelsen, S. B. (2000). Warmed-over flavour in porcine meat—a combined spectroscopic, sensory and chemometric study. *Meat Science, 54,* 83-95.

Buckley, D. J., Morrissey, P. A., & Gray, J. I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science, 73,* 3122-3127.

Caul, J. F. (1957). The profile method for flavour analysis. *Advances in Food Research, 7*, 1-40.

Chen, C. C., Pearson, A. M., Gray, J. I., & Merkel, R. A. (1984). Effects of salt and some antioxidants upon the TBA numbers of meat. *Food Chemistry, 14,* 167-172.

Church, N. (1994). Developments in modified atmosphere packaging and related technologies. *Trends in Food Science and Technology, 5,* 345-352.

Cipra, J. S., & Bowers, J. A. (1970). Pre-cooked turkey. Flavour and certain chemical changes caused by refrigeration and reheating. *Food Technology, 24,* 921-925.

Cipra, J. S., & Bowers, J. A. (1971). Flavour of microwave- and conventionally-reheated turkey. *Poultry Science, 50,* 703-706.

Civille, G. V. (1979). Descriptive analysis – Flavour profile, texture profile, and quantitative descriptive analysis. In M. R. Johnson, *Sensory Evaluation Methods for the Practising Food Technologist* (pp. 1-6). Pennsylvania: Institute of Food Technologists.

Civille, G. V., & Dus, C. A. (1992). Sensory evaluation of lipid oxidation in foods. In A. J. St. Angelo, *Lipid Oxidation in Food* (pp. 279-291). Washington, DC: American Chemical Society, Symposium Series 500.

Civille, G.V., & Lyon, B.G. (1996). List of terms describing each product by category. In G.V. Civille & B.G. Lyon, *Aroma and Flavor Lexicon for Sensory Evaluation: Terms, Definitions, References, and Examples* (pp. 46-49). Pennsylvania: American Society for Testing and Materials.

Claassens, M., & Lawless, H. T. (1992). Comparison of descriptive terminology systems for sensory evaluation of fluid milk. *Journal of Food Science, 57*, 596-600.

Cori, C.F., & Cori, G.T. (1928a). The mechanism of epinephrine action. I. The influence of epinephrine on the carbohydrate metabolism of fasting rats, with a note on new formation of carbohydrates. *Journal of Biological Chemistry, 79*, 309-319.

Cori, C.F., & Cori, G.T. (1928b). The mechanism of epinephrine action. II. The influence of epinephrine and insulin on the carbohydrate metabolism of rats in the postabsorptive state. *Journal of Biological Chemistry, 79*, 321-341.

Corongiu, F. P., & Milia, A. (1983). An improved and simple method for determining diene conjugation in autoxidized polyunsaturated fatty acids. *Chemico-Biological Interactions, 44*, 289-297.

Coxon, D. (1987). Measurement of lipid oxidation. *Food Science and Technology Today, 1,* 164-166.

Cross, H. R., Leu, R., & Miller, M. F. (1987). Scope of warmed-over flavour and its importance to the meat industry. In A. J. St. Angelo & M. E. Bailey, *Warmed-Over Flavour in Meat* (pp. 1-18). London: Academic Press Inc.

Davies, M. J. (1987). Application of electron spin resonance spectroscopy to the identification of radicals produced during lipid peroxidation. *Chemistry and Physics of Lipids, 44,* 149-173.

Decker, E. A., & Hultin, H. O. (1992). Lipid oxidation in muscle foods via redox iron. In A. J. St. Angelo, *Lipid Oxidation in Food* (pp. 33-54) New York: American Chemical Society.

De Vries, A. G., Timm, H. H., Wilson, E. R., Evans, G., Keller, V., & Plastow, G. S. (1997). An effective DNA. marker test for the "acid meat" problem. In *48th Annual Meeting EAAP* (pp. 340-344), Vienna: Austria.

Dijksterhuis, G. B. (1994). Procrustes analysis in studying sensory-instrumental relations. *Food Quality and Preference, 5,* 115-120.

Dijksterhuis, G. B. (1995). Multivariate data analysis in sensory and consumer science: an overview of developments. *Trends in Food Science and Technology, 6,* 206-211.

Dijksterhuis, G. B. (1996). Procrustes Analysis in sensory research. In T. Næs & E. Risvik, *Multivariate Analysis of Data in Sensory Science* (pp. 185-219). Amsterdam: Elsevier.

Drumm, T. D., & Spanier, A. M. (1991). Changes in the content of lipid autoxidation and sulfur-containing compounds in cooked beef during storage. *Journal of Agriculture and Food Chemistry, 39*, 336-343.

Enfält, A.-C., Lundström, K., Hansson, I., Johansen, S., & Nyström, P.-E. (1997a). Composition of non-carriers and heterozygous carriers of the RN^- allele for carcass composition, muscle distribution and technological meat quality. *Meat Science, 45*, 1-15.

Enfält, A.-C., Lundström, K., Hansson, I., Lundeheim, N., & Nyström, P.-E. (1997b). Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. *Meat Science, 45*, 313-319.

Einerson, M.A., & Reineccius, G.A. (1977). Inhibition of warmed-over flavor in retorted turkey by antioxidants formed during processing. *Journal of Food Processing and Preservatives, 1,* 279-291.

Enser, M. (1987). What is lipid oxidation. *Food Science and Technology Today, 1,* 151-153.

Esbensen, K., Midtgaard, T., & Schönkopf, S. (1996). In *Multivariate Analysis in Practice*. Norway: Wennbergs Trykkeri AS.

Evans, C. D., List, G. R., Dolev, A., McConnell, D. G., & Hoffman, R. L. (1967). Pentane from thermal decomposition of lipoxidase-derived products. *Lipids, 2,* 432-434.

Fernandez, X., Forsilde, A., Mågård, M., Möller, M., & Tornberg, E. (1992). Effect of time between adrenaline injection and slaughter on the rate and extent of post-mortem metabolism in porcine skeletal muscle. *Meat Science, 31,* 287-298.

Fernandez, X., & Tornberg, E. (1991). A review of the causes of variation in muscle glycogen content and ultimate pH in pigs. *Journal of Muscle Foods, 2,* 209-235.

Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry, 59*, 345-353.

Frankel, N. E., Neff, W. E., & Selke, E. (1981). Analysis of autoxidised fats by gas chromatography-mass spectrometry: VII. Volatile thermal decomposition products of pure hydroperoxides from autoxidised and photosensitised oxidised methyl oleate, linoleate and linolenate. *Lipids, 16,* 279-285.

Frankel, E. N. (1984). Recent advances in the chemistry of the rancidity of fats. In A. J. Bailey, *Recent Advances in the Chemistry of Meat* (pp. 87-118). New York: The Royal Society of Chemistry, Special Publication.

Gasser, U., & Grosch, W. (1988). Flavour deterioration of soya bean oil: identification of intense odour compounds during flavour reversion. *Fett Wissenschart Technologie, 90,* 332-336.

Gower, J. C. (1975). Generalised procrustes analysis. *Psychometrika, 40*, 33-51.

Gray, J. I. (1978). Measurement of lipid oxidation: a review. *Journal of the American Oil Chemists Society, 55,* 539-546.

Gray, J. I., & Pearson, A. M. (1987). Rancidity and warmed-over flavour. In A. M. Pearson & T. R. Dutson, *Advances in Meat Research* (pp. 221-269). New York: Van Nostrand Reinhold Co.

Gray, J. I., & Monahan, F. J. (1992). Measurement of lipid oxidation in meat and meat products. *Trends in Food Science and Technology, 3*, 315-319.

Gray, J. I., Gomaa, E. A., & Buckley, D. J. (1996). Oxidative quality and shelf life of meats. *Meat Science, 43, Supplement,* S111-S123.

Greene, B. E. (1969). Lipid oxidation and pigment changes in raw beef. *Journal of Food Science, 34,* 110-113.

Greene, B. E., Hsin, I. N., & Ziprin, M. W. (1971). Retardation of oxidative colour changes in raw ground pork. *Journal of Food Science, 36,* 940-942.

Greene, B. E., & Price, L. G. (1975). Oxidation induced colour and flavour changes in meat. *Journal of Agriculture and Food Chemistry, 23,* 164-167.

Grosch, W., Konopka, U. C., & Guth, H. (1992). Characterization of off-flavours by aroma extract dilution analysis. In A. J. St. Angelo, *Lipid Oxidation in Food* (pp. 267-278). Washington, American Chemical Society Symposium Series 500.

Gutteridge, J. M. C. & Halliwell, B. (1990). The measurement and mechanism of lipid peroxidation in biological systems. *Trends in Biochemical Sciences, 15,* 129-135.

Harris, N. D., & Lindsay, R. C. (1972). Flavour changes in reheated chicken, *Journal of Food Science, 37*, 19-22.

Hatton, M. W. C., Lawrie, R. A., Ratcliff, P. W., & Wayne, N. (1972). Effects of preslaughter adrenaline injection on muscle metabolites and meat quality of pigs. *Journal of Food Technology, 7,* 443-453.

Henckel, P., Karlsson, A., Oksbjerg, N., & Petersen, J.S. (2000). Control of post mortem pH-decrease in pig muscles: experimental design and testing of animal models. *Meat Science, 55*, 131-138.

Horvat, R. J., Lane, W. G., Ng, H., & Shepard, A. D. (1964). Saturated hydrocarbons from autoxidising methyl linoleate. *Nature, 203,* 532-524.

Hsieh, R. J., & Kinsella, J. E. (1989). Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *Advances in Food and Nutrition Research, 33,* 233-341.

Hurley, J. R., & Cattell, R. B. (1962). The procrustes program: producing direct rotation to test a hypothesised factor structure. *Behavioural Science, 7*, 258-262.

Ingene, J. O., & Pearson, A.M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *Journal of Food Science, 44*, 1285-1290.

Ingene, J. O., King, J. A., Pearson, A. M., & Gray, J. I. (1979). Influence of haem pigments, nitrite and non-haem iron on the development of warmed-over flavour in cooked meat. *Food Chemistry, 27,* 838-842.

Ingene, J. O., Pearson, A. M., Dugan, L. R., Jr, & Price, J. F. (1980). Role of triglycerides and phospholids on development of rancidity in model meat systems during frozen storage. *Food Chemistry, 5,* 263-276.

Ingold, K. U. (1962). Metal catalysis. In H. W. Schultz, E. A. Day, & R. O. Sinnhuber, *Syposium on Foods Lipdis and their Oxidation.* Conneticut: AVI Publishing.

ISO (1983). International Standard 4120. Sensory analysis—Methodology—Trianglar test. Ref. no. ISO 4120:1983 (E). International Organization for Standardization, Genève.

ISO (1985a). International Standard 6564. Sensory analysis—Methodology—Flavour profile methods. Ref. no. ISO 6564:1985 (E). International Organization for Standardization, Genève.

ISO (1985b). International Standard 6658. Sensory analysis—Methodology—General guidance. Ref. no. ISO 6658:1985 (E). International Organization for Standardization, Genève.

ISO (1988). International Standard 8589. Sensory analysis—general guidance for the design of test rooms. Ref. no. ISO 8589:1988 (E). International Organization for Standardization, Genève.

ISO (1991). International Standard. 3972. Sensory analysis—Methodology—Method of investigating sensitivity of taste. Ref. no. ISO 3972:1991 (E). International Organization for Standardization, Genève.

ISO (1992a). International Standard. 5496. Sensory analysis—Methodology—Initiation and training of assessors in the detection and recognition of odours. Ref. no. ISO 5496:1992 (E). International Organization for Standardization, Genève.

ISO (1992b). International Standard. 5492. Sensory analysis—Vocabulary. Ref. no. ISO 5492:1992 (E/F). International Organization for Standardization, Genève.

ISO (1993). International Standard 8586-1. Sensory analysis—general guidance for the selection, training and monitoring of assessors: selected assessors. Ref. no. ISO 8586-1:1993 (E). International Organization for Standardization, Genève.

ISO (1994a). International Standard 8586-1. Sensory analysis—general guidance for the selection, training and monitoring of assessors: experts. Ref. no. ISO 8586-1:1993 (E). International Organization for Standardization, Genève.

ISO (1994b). International Standard 11035. Sensory analysis—identification and selection of descriptors for establishing a sensory profile by a multidimensional approach. Ref. no. ISO 11035:1994 (E). International Organization for Standardization, Genève.

Jacobson, M., & Koehler, H. H. (1970). Development of rancidity during short-time storage of cooked poultry meat. *Journal of Agriculture and Food Chemistry, 18*, 1069-1072.

Jart, A. (1997). The magnetic field as an additional selectivity parameter in fat hydrogenation. *Journal of the American Oil Chemists' Society, 74*, 615-617.

Jensen, C., Guidera, J., Skovgaard, I. M., Staun, H., Skibsted, L. H., Jensen, S. K., Møller, A. J., Buckley, D. J., & Bertelsen, G. (1997). Effects of dietary α-tocopherol acetate supplementation on drip loss, colour stability and oxidative stability of pork meat. *Meat Science, 45,* 491-500.

Jensen, P., & Barton-Gade, P. A. (1985). Performance and carcass characteristics of pigs with known genotypes for halothane susceptibility. In J. B. Ludvigsen*, Stress Susceptibility and Meat Quality in Pigs, EAAP publication No 33* (pp 80-87), Denmark: Jelling Press A/S.

Joseph, A. L., Smith, G. C., & Cross, H. R. (1980). Physical, chemical, and palatability traits of raw and pre-cooked ground beef patties. *Journal of Food Science, 45*, 168-170.

Johansson, L., Lundström, K., Jonsäll, A., & Lundh, T. (1999). Effects of red clover silage and ageing time on sensory characteristics and cooking losses of loin (M. longissimus dorsi) from Hampshire crosses with and without the RN^- allele. *Food Quality and Preference, 10*, 299-303.

Johns, A. M., Birkinshaw, L. H., & Ledward, D. A. (1989). Catalysts of lipid oxidation in meat products. *Meat Science, 25,* 209-220.

Johnson, P. B., & Civille, G. V. (1986). A standardized lexicon of meat WOF descriptors. *Journal of Sensory Studies*, *1*, 99-104.

Kamarei, A. R., & Karel, M. (1984). Assessment of autoxidation in freeze dried meats by a fluorescence assay. *Journal of Food Science, 49,* 1517-1520, 1524.

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Science, 36,* 169-189.

Kerler, J., & Grosh, W. (1997). Character impact odorants of boiled chicken: changes during refrigerated storage and reheating. *Zeitschrift für Lebensmittel-Utersuchung und Forschung, 205*, 232-238.

Kosugi, H., Kato, T., & Kikugawa, K. (1987). Formation of yellow, orange and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Analytical Biochemistry, 165,* 45-464

Kosugi, H., Kato, T., & Kikugawa, K. (1988). Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alka-2,4-dienals. Potential products of lipid oxidation. *Lipids, 23,* 1024-1031.

Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle foods: a review. *Food Chemistry, 35,* 295-314.

Lai, S., Gray, J. I., Booren, A. M., Crackel, R. L., & Gill, J. L. (1995). Assessment of off-flavour development in restructured chicken nuggets using hexanal and TBARS measurements and sensory evaluation. *Journal of the Science of Food and Agriculture, 67,* 447-452.

Landes, D. R. (1972). The effects of polyphosphates on several organoleptic, physical, and chemical properties of stored pre-cooked frozen chickens. *Poultry Science, 51,* 258-276.

Lawless, H. T., & Heymann, H. (1998). Descriptive analysis. In H. T. Lawless & H. Heymann, *Sensory Evaluation of Food, Principles and Practices* (pp 341-378). New York, Chapman & Hall.

Ledward, D. A. (1987). Interactions between myoglobin and lipid oxidation in meat and meat products. *Food Science and Technology Today, 1,* 153-155.

Lee, Y. B., Hargus, G. L., Kirkpatrick, J. A., Berner, D. L., & Forsythe, R. H. (1975). Mechanism of lipid oxidation in mechanically deboned chicken meat. *Journal of Food Science, 40,* 964-967.

Le Roy, P., Naveau, J., Elsen, J. M., & Sellier, P. (1990). Evidence for a new major gene influencing meat quality in pigs. *Genetic Research Cambridge, 55,* 33-40.

Le Roy, P., Cartitez, J. C., Billon, Y., Elsen, J. M., Talmant, A., Vernin, P., Lagant, H., Larzul, C., Monin, G., & Sellier, P. (1995).Etude de l'effect du locus RN sur les caractères de croissance et de carcasse: premiers résultas. *Journées de la Recherche. Porcine en France, 27,* 165-170.

Le Roy, P., Juin, H., Caritez, J. C., Billon, Y., Lagant, H., Elsen, J. M., & Sellier, P. (1996). Effet du génotype RN sur les qualités sensorilles de la viande de porc. *Journées de la Recherche. Porcine en France, 28,* 53-56.

Lewis, P.K. jr., Rakes, L.Y., Brown, C.J., & Noland, P.R. (1989). Effect of exercise and pre-slaughter stress on pork muscle characteristics. *Meat Science, 26*, 121-129.

Liu, H. P., & Watts, B. M. (1970). Catalysts of lipid peroxidation in meats. 3. catalysts of oxidative rancidity in meats. *Journal of Food Science, 35,* 596-598.

Love, J. D. (1987) Mechanism of iron catalysis of lipid oxidation in warmed-over flavour of meat. In A. J. St. Angelo & M. E. Bailey, *Warmed-Over Flavour in Meat* (pp. 19-40). London: Academic Press Inc.

Love, J. D. (1988). Sensory analysis of warmed-over flavour in meat. *Food Technology*, *42*, 140-143.

Lundström, K., Essén-Gustavsson, B., Rundgren, M., Efors-Lilja, I. & Malmfors, G. (1989). Effect of halothane genotype on muscle metabolism at slaughter and its relationship with meat quality: within a litter comparison. *Meat Science, 25,* 251-263.

Lundström, K., Andersson, A., & Hansson, I. (1996). Effect of the RN^- gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Science, 42*, 145-153.

Lundström, K., Enfält, A.-C., Tornberg, E., & Agerhem, H. (1998). Sensory and technological meat quality in carriers and non-carriers of the RN^- allele in Hampshire crosses and purebred Yorkshire pigs. *Meat Science, 48,* 115-124.

Lynch, N. M., Kastner, C. L., Cavl, J. F., & Kropf, D. H. (1986). Flavor profiles of vacuum packaged and polyvinyl chloride packaged ground beef: A comparison of cooked flavor changes occurring product display. *Journal of Food Science, 51*, 258-262, 267.

Lyon, B. G. (1987). Development of chicken flavour descriptive attribute terms aided by multivariate statistical procedures. *Journal of Sensory Studies*, *2*, 55-67.

Lyon, B. G., Lyon, C. E., Ang, C: Y. W., & Young, L. L. (1988). Sensory analysis and thiobarbituric acid values of pre-cooked chicken patties stored up to three days and reheated by two methods. *Poultry Science, 67,* 736-742.

Lyon, B. G., & Ang, C. Y. W. (1990). Effects of reheating method on off-flavour development in pre-cooked, stored, chicken patties. *Poultry Science, 69,* 320-328.

MacLeod, G., & Coppock, B. M. (1978). Sensory properties of aroma of beef cooked conventionally and by microwave radiation. *Journal of Food Science, 43,* 145-161.

Madruga, M. S., & Mottram, D. S. (1995). The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture, 68,* 305-310.

Marcuse, R., & Johansson, L. (1973). Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. *Journal of the American Oil Chemists Society, 50,* 387-391.

Martens, H. & Næs, T. (1989). *Multivariate calibration.* London: J. Wiley & Sons Ltd.

Martens, H., & Martens, M. (1999) Validation of PLS Regression models in sensory science by extended cross-validation. In M. Tenenhause & A. Monineau, *Les Methodes PLS*, (pp. 149-182). France: CISIA-CERESTA.

Martens, H., & Martens, M. (2000a). In *Multivariate Analysis of Quality. An Introduction*. London: J. Wiley & Sons Ltd.

Martens, H., & Martens, M. (2000b). Modified jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality and Preference, 11*, 5-16.

Martens, H., Byrne, D. V., Westad, F. O., & Martens, M. (2000c). Cross-validated PLSR analysis of designed experiments: Reliability of the estimated effects. *Journal of Chemometrics*, in preparation.

Martens, H., Dijksterhuis, G. B., & Byrne, D. V. (2000d). Power of experimental designs, estimated by Monte Carlo simulation. *Journal of Chemometrics, 14,* 1-22.

Martens, M., & Martens, H. (1986). Partial least squares regression. In J. R. Piggott*, Statistical Procedures in Food Research* (pp. 293-359). London: Elsevier.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). Measuring responses. In *Sensory evaluation techniques* 3rd ed. (pp 43-57). Florida: CRC press.

Melton, S. L. (1983). Methodology for following lipid oxidation in muscle foods. *Food Technology, 37,* 105-111.

Melton, S. L., Davidson, P. M., & Mount, J. R. (1987) Sensory analysis of undesirable flavours in meat. In A. J. St. Angelo & M. E. Bailey, *Warmed-Over Flavour in Meat* (pp. 141-164). London: Academic Press Inc.

Mielche, M. M., & Bertelsen, G. (1994). Approaches to prevention of warmed-over flavour. *Trends in Food Science and Technology, 5,* 322-327.

Miller, R. (1994). Sensory methods to evaluate muscle foods. In D. M. Kinsman, A. W. Kotula, B. C. Breidenstein, *Muscle Foods: Meat Poultry and Seafood Technology* (pp. 333-360). New York: Chapman & Hall.

Monin, G., Mejenes-Quijano, A., Talmant, A., & Sellier, P. (1987). Influence of breed and muscle type on muscle glycolytic potential and meat pH in pigs. *Meat Science, 20,* 149-158.

Monin, G., Brad, C., Vernin, P., & Naveau, J. (1992). Effects of the RN gene on some traits of muscle and liver in pigs. *Proceedings of the 38th International Congress of Meat Science and Technology* (pp. 391-394). Clermont-Ferrand, France,.

Mottram, D. S. (1998). The chemistry of meat flavour. In F. Shahidi, *Flavour of Meat, Meat Products* and Seafoods 2nd ed. (pp. 5-26). London: Chapman & Hall.

Muñoz, A. M., & Civille, G. V. (1992). The Spectrum descriptive analysis method. In R. C. Hootman*, ASTM Manual on Descriptive Analysis*. Pennsylvania: American Society for Testing and Materials.

Muñoz, A. M., & Civille, G. V. (1998). Universal, product and attribute specific scaling and the development of common lexicons in descriptive analysis. *Journal of Sensory Studies, 13,* 57-75.

National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th Revised Edition. Washington DC: National Academy Press.

National Research Council. 1998. *Nutrient Requirements of Swine*. 10th Revised Edition. Washington DC: National Academy Press.

Naveau, J., Pommeret, P., & Lechaux, P. (1985). Proposition d'une méthode de mesure du rendement technologique: "la méthode Napole". *Techni-Porc, 8,* 7-13.

Naveau, J. (1986). Contribution à l'étude du déterminisme génétique de la qualité de viande porcine Héritabilité du Redement Technologique Napole. *Journées de la Recherche. Porcine en France, 18*, 265-276.

Næs, T., Baardseth, P., Helgesen, H., & Isaksson, T. (1996). Multivariate techniques in the analysis of meat quality, *Meat Science, 43, Supplement,* S111-S123.

Nielsen, J. H., Sørensen, B., Skibsted, L. H., & Bertelsen, G. (1997). Oxidation in pre-cooked minced pork as influences by chill storage of raw muscle. *Meat Science, 46,* 191-197.

Noble, A. C. (1976). Effect of carbondioxide and sodium chloride on oxidative stability of frozen mechanically deboned poultry meat. *Canadian Institute of Food Science and Technology Journal, 9,* 105-107.

Nolan, N. L., Bowers, J. A., & Kropf, D. H. (1989). Lipid oxidation and sensory analysis of cooked pork and turkey under modified atmosphere. *Journal of Food Science, 54,* 846-849.

O'Keefe, S. F., Proudfoot, F. G., & Ackman, R. G. (1995). Lipid oxidation in meats of omega-3 fatty acid-enriched broiler chickens. *Food Research International, 28,* 417-424.

Oreskovich, D. C., Klein, B. P., & Sutherland, J. W. (1991). Procrustes analysis and its analysis and applications to free-choice profiling and other sensory profiling. In H. T. Lawless & B. P. Klein, *Sensory Science Theory and Applications in Foods* (pp. 353-393). New York: Marcel Dekker.

Owen, J. E., Lawrie, R. A., & Hardy, B. (1975). Effect of dietary variation, with respect to energy and crude protein level, on the oxidative rancidity exhibited by frozen porcine muscles. *Journal of the Science of Food and Agriculture, 26,* 31-34.

Passonneau, J. V., & Lowery, O. H. (1993). *Enzymatic Analysis- A Practical Guide*. USA: The Humana Press.

Pearson, A. M., Love, J. D., & Shortland, F. B. (1977). Warmed-over flavor in meats poultry and fish. *Advances in Food Research, 23,* 1-74.

Pearson, A. M., & Gray, J. I. (1983). Mechanism responsible for warmed-over flavor in meat. In G. R. Waller & M. S. Feather. *The Maillard reaction in food and nutrition* (pp. 287-300). Washington, American Chemical Society Symposium Series 215.

Pegg, R. B., Shahidi, F, Gogan, N. J., & De Silva, S. J. (1996). Elucidation of the chemical structure of preformed cooked, cured-meat pigment by electron paramagnetic resonance spectroscopy. *Journal of Agriculture and Food Chemistry, 44,* 416-421.

Persson, T., Von Sydow, E., & Akesson, C. (1973). Aroma of canned beef: sensory properties. *Journal of Food Science, 38,* 386-392.

Piggott, J. R., & Sharman, K. (1986). Methods to aid interpretation of multidimensional data. In J. R. Piggott, *Statistical Procedures in Food Research* (pp. 181-232). London: Elsevier.

Poste, L. M., Willemot, C., Butler, G., & Patterson, C. (1986). Sensory aroma scores and TBA values as indices of warmed-over flavor in pork. *Journal of Food Science, 51*, 886-888.

Rhee, K. S. (1978). Factors affecting oxygen uptake in model systems used for investigating lipid peroxidation in meat. *Journal of Food Science, 43*, 6-9.

Rhee, K. S., Anderson, L. M., & Sams, A. R. (1996). Lipid oxidation potential of beef, chicken, and pork. *Journal of Food Science, 61*, 8-12.

Sato, K., & Hegarty, G. R (1971). Warmed-over flavour in cooked meats. *Journal of Food Science, 36,* 1098-1102.

Sato, K., Hegarty, G. R., & Herring, H. K. (1973). The inhibition of warmed-over flavour in cooked meats. *Journal of Food Science, 38,* 398-403.

Satyanarayan, V. T., & Honikel, K. O. (1992). Effect of different cooking methods on warmed-over flavour development in pork. *Zeitschrift für Lebensmittel-Utersuchung und Forschung, 194*, 422-425.

Schaich, K. M., & Borgi, D. C. (1980). EPR studies in autoxidation. In M. G. Simic & M. Karel. *Autoxidation in Food and Biological Systems* (pp 45-70). New York, Plenum Press.

Schieberle, P., & Grosch, W. (1987). Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Zeitschrift für Lebensmittel-Utersuchung und Forschung, 185,* 111-113.

Schmitt, A. (1977). Derivative Spectroscopy. An Introduction with practical examples. *Applied UV Spectroscopy, 1,* 1-6.

Sedman, J., Ismail, A. A., Nicodema, A., Kubow, S., & Van de Voort, F. R. (1997). Application of FTIR. ATR differential spectroscopy for monitoring oil oxidation and antioxidant efficacy. In F. Shahidi*, Natural Antioxidants: Chemistry, Health Effects and Applications* (pp. 358-378). New York: AOCS Press.

Seo, C. W., & Joel, D. L. (1980). Pentane production as an index of rancidity in freeze-dried pork. *Journal of Food Science, 45,* 26-28.

Shahidi, F., Yun, J., Rubin, L. J., & Wood, D. F. (1987). The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. *Canadian Institute of Food Science and Technology Journal, 20,* 104-106.

Shahidi, F., (1998). Assessment of lipid oxidation and off-flavour development in meat and meat products. In F. Shahidi. *Flavour of Meat, Meat Products and Seafoods* 2nd ed. (pp. 373-394). London: Chapman & Hall.

Silberstein, D. A., & Lillard, D. A. (1978). Factors affecting the autoxidation of lipids in mechanically deboned fish. *Journal of Food Science, 43,* 764-766.

Simic, M. G. (1980). Kinetic and mechanistic studies of peroxy, vitamin E and antioxidant free radicals by pulse radiolysis. In M. G. Simic & M. Karel. *Autoxidation in Food and Biological Systems* (pp 17-26). New York, Plenum Press.

Simic, M. G., Jovanovic, S. V., & Niki, E. (1992). Mechanisms of lipid oxidative processes and their inhibition. In A. J. St. Angelo, *Lipid Oxidation in Food* (pp. 14-32) NewYork: American Chemical Society.

Skibsted, L. H., Mikkelsen, A., & Bertelsen, G. (1998). Lipid-derived off-flavours in meat. In F. Shahidi. *Flavour of Meat, Meat Products and Seafoods* 2nd ed. (pp. 217-256). London: Chapman & Hall.

Sklan, D., Tenne, Z., & Budowski, P. (1983). The effects of dietary fat and tochopherol on lipolysis and oxidation of turkey meat stored at different temperatures. *Poultry Science, 62,* 2017-2021.

Slover, H. T., Thompson, R. H. jr., Davis, C. S., & Merola, G. V. (1987). The lipid composition of raw and cooked fresh pork. *Journal of Food Composition and Analysis, 1*, 38-52.

Spanier, A. M., Vincent Edwards, J., & Dupuy, H. P. (1988). The warmed-over flavor process in beef: a study of meat proteins and peptides. *Food Technology, 46*, 110-118.

Spanier, A. M., Vinyard, B. T., Bett, K. L., & St. Angelo (1998) Sensory and statistical analyses in meat flavour research. In F. Shahidi. *Flavour of Meat, Meat Products and Seafoods* 2nd ed. (pp. 395-419). London: Chapman & Hall.

St. Angelo, A. J., Vercellotti, J. R., Legendre, M. G., Vinnett, C. H., Kuan, J. W., James, C. Jr., & Dupuy, H. P. (1987). Chemical and instrumental analysis of warmed-over flavor in beef. *Journal of Food Science, 52*, 1163-1168.

St. Angelo, A., J. Vercellotti, J. R., Dupuy, H.P., & Spanier, A.M. (1988). Assessment of beef flavor quality: A multidisciplinary approach. *Food Technology, 42*, 133-138.

St. Angelo, A. J., Crippen, K. L., Dupuy, H. P., & James, C., Jr. (1990). Chemical and sensory studies of antioxidant-treated beef. *Journal of Food Science, 55*, 1501-1539.

St. Angelo, A. J., Koohmaraie, M., Crippen, K. L., & Crouse, J. (1991). Accelaration of post-mortem tenderisation/inhibition of warmed-over flavour by calcium chloride antioxidant infusion into lamb carcasses. *Journal of Food Science, 52,* 1163-1168.

Stapelfeldt, H., Bjørn, H., Skovgaard, I. M., Skibsted, L. H., & Bertelsen, G. (1992). Warmed-over flavour in cooked sliced beef. Chemical analysis in relation to sensory evaluation. *Zeitschrift für Lebensmittel-Utersuchung und Forschung, 195*, 203-208.

Stapelfeldt, H., Bjørn, H., Skovgaard, I. M., Skibsted, L. H., & Bertelsen, G. (1993). Effect of packaging and storage conditions on the development of warmed-over flavour in sliced cooked meat. *Zeitschrift für Lebensmittel-Utersuchung und Forschung, 196*, 131-136.

Stone, H., Sidel, J., Oliver, S., Woolsey, A. & Singleton, R. C. (1974). Sensory evaluation by Quantitative Descriptive Analysis. *Food Technology, 28*, 24-34.

Sutherland, E.W., & Cori, C.F. (1951). Effect of hyperglycemic-glycogenolytic factor and adrenaline on liver phosphorylase. *Journal of Biological Chemistry 188*, 531-543.

Tappel, A. L. (1962). Heme compounds and lipoxidase as biocatalysts. In H. W. Schultz, E. A. Day, & R. O. Sinnhuber, *Syposium on Foods Lipdis and their Oxidation* (pp. 122-126). Conneticut: AVI Publishing.

Tarladgis, B. G., Yonathan, M. T., & Watts, B. M. (1959). Antioxidants in irradiated cooked pork. *Food Technology, 13*, 635-639.

Tarladgis, B. G., Watts, B. M., Yonathan, M. T., & Dugan, L. R. Jr. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society, 37,* 44-48.

Tichivangana, J. Z., & Morrissey, P. A. (1985). The influence of pH on lipid oxidation in cooked meats from several species. *Irish Journal of Food Science and Technology, 9*, 99-106.

Tims, M. J., & Watts, B. M. (1958). Protection of cooked meats with phosphates. *Food Technology, 12*, 240-243.

Toscas, P. J., Shaw, F. D., & Beilken, S. L. (1999). Partial least squares (PLS) regression for the analysis of instrument measurements and sensory meat quality data. *Meat Science, 52,* 173-178.

Thybo, A. K., & Martens, M. (1998). Development of a sensory texture profile of cooked potatoes by multivariate data analysis. *Journal of Texture Studies, 29,* 453-468.

Ullrich, F. ,& Grosch, W. (1987). Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Zeitschrift für Lebensmittel-Utersuchung und Forschung, 184,* 227-282.

Van de Riet, J. J. & Hard, M. M. (1979). Flavour quality of antioxidants treated, cooked, ground beef patties. *Journal of the American Dietetics Association, 75,* 556-559.

Van den Dool, H., & Kratz, P. D. (1983). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, *11*, 463-471.

Vyncke, W. (1975). Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackeral (Scomber scombrus L.). *Fette, Seifen, Anstrichmittel, 77*, 239-240.

Warris, P.D., Bevis, E.A., & Ekins, P.J. (1989). The relationships between glycogen stores and muscle ultimate pH in commercially slaughtered pigs. *British Veterinary Journal, 145*, 378-383.

Warris, P.D., Brown, S.N., Adams, S.J.M., & Corlett, I.K. (1994). Relationships between subjective and objective assessments of stress at slaughter and meat quality in pigs. *Meat Science, 38*, 329-340.

Watanabe, A., Daly, C.C., & Devine, C.E. (1996). The effects of the ultimate pH of meat on tenderness changes during ageing. *Meat Science, 42*, 67-78.

Willemot, C., Poste, L. M., Salvador, J., & Wood, D. F. (1985). Lipid degradation in pork during warmed-over flavour development. *Canadian Institute of Food Science and Technology Journal, 18*, 316-322.

Winger, R. J., & Pope, C. G. (1981). Selection and training of panellists for sensory evaluation of meat flavours. *Journal of Food Technology, 16,* 661-669.

Wilson, B. R., Pearson, A. M., & Shorland, F. B. (1976). Effect of total lipids and phospholipids on warmed-over flavour in red and white muscle from several species as measured by thiobarbituric acid analysis. *Journal of Agriculture and Food Chemistry, 24,* 7-11.

Wold, S., Martens, H., & Wold, H. (1983). The multivariate calibration problem in chemistry solved by the PLS method. In A. Ruhe & B. Kågström, *Proceedings of the Conference on Matrix Pencils* (pp. 286-293). Heidelberg: Springer Verlag.

Wold, S., Esbensen, K., & Geldi, P. (1987). Principal Component Analysis. *Chemometrics and Intelligent Laboratory Systems, 2,* 37-52.

Wolters, C.J., & Allchurch, E. M. (1994). Effect of training procedure on the performance of descriptive panels. *Food Quality and Preference, 5*, 203-214.

Wu, T. C., & Sheldon, B. W. (1988). Flavor components and factors associated with the development of off-flavors in cooked turkey rolls. *Journal of Food Science, 53*, 49-54.

Youanthan, M. T., & Watts, B. M. (1958). Relationship of meat pigments to lipid oxidation. *Food Research, 24,* 728-731.

Youanthan, M. T., & Watts, B. M. (1960). Oxidation of tissue lipids in cooked pork. *Food Research, 25,* 538-541.

Appendix A

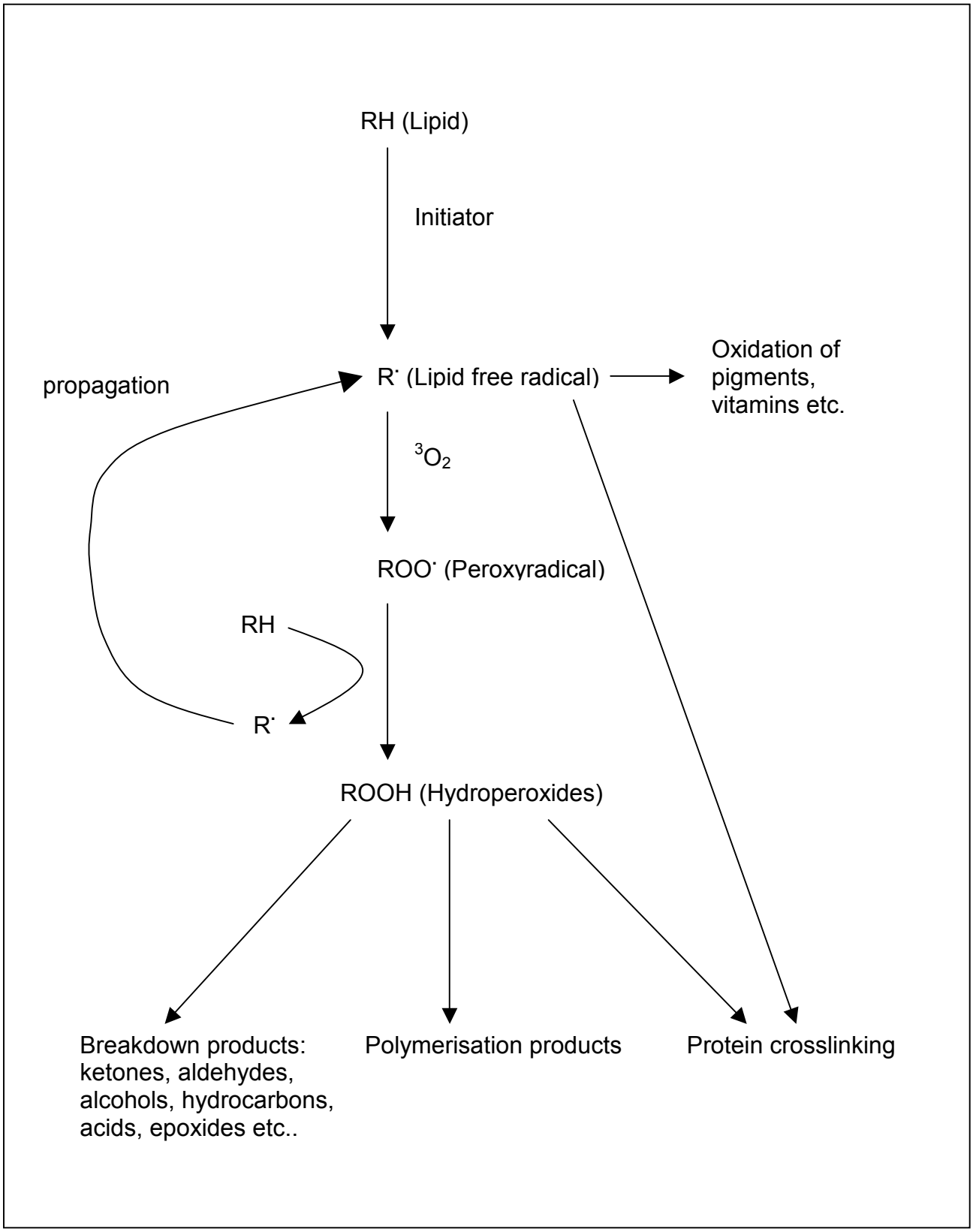


Figure 1. Lipid oxidation of meat lipids: adapted from Shahidi (1998)

Appendix B

Table 1

Overview of the methods that are commonly used to measure lipid oxidation and their potential to monitor the development of warmed-over flavour in cooked and chill-stored meat

Primary changes:	Potential for WOF measurement
Loss of polyunsaturated fatty acids (GC-MS or HPLC)	High
Increased conjugated dienes (spectroscopy)	High
Formation of hydroperoxides (peroxide value)	Low
Oxygen uptake (AOCS method, 1989)	Low
Secondary changes:	
Formation of malonaldehyde (TBA/TBARS tests)	High
Formation of hydrocarbons (e.g. hexanal by GC-MS)	High
Formation of fluorescent products (spectroscopy)	High

Table 2

Sensory descriptors used in some earlier investigations of warmed-over flavour. From Melton et al. (1987)

Jacobsen & Koehler (1970)	Harris & Lindsay (1972)	Joseph et al. (1980)
Bland	Greasy	Sour
Sweet	Sulfur	Bitter
Rich	Liver-giblet	Metallic
Meaty	Warmed-over[a]	Sweet
Sulfury	Stale[a]	Putrid
Gizzard-like	Rancid[a]	Salty
Musty[a]		Rancid[a]
Stale or old[a]		Other off-flavour
Rancid[a]		

[a] These descriptors were highest in meats having warmed-over flavour or were used most frequently by panellists describing meat containing this flavour.

Table 3

Flavour profile ballot terms and definitions for polyvinyl chloride and vacuum packaged ground beef. From Lynch et al. (1986)

Sensory term	Definition/Reference
Aromatics	
Beefiness	— 95 % lean tenderloin steak cut from carcass 24 hr post-mortem and broiled to a medium-rare degree of doneness
Fat	— Beef fat trimmed from carcass and broiled
Freshness	— Cooked ground beef (ground 2 hr before cooking) from 24 hr post mortem carcass, 0 on the freshness scale was represented by bland-tasting ground beef
Stale/off	— Cooked, 5 day display, PVC packaged ground beef
Bloody/serumy/metallic/sharp	— Drip from cooked sample from 24 hr post mortem carcass
Dairy/milky	— Fresh whole milk
Basic tastes	
Sweet	— Sucrose
Sour	— Citric acid
Bitter	— Caffeine
Salt	— Sodium chloride
Mouthfeel	
Oily	— The coating left in the mouth when vegetable oil is swallowed
Metallic	— The feeling that results when one places a penny in the mouth
Astringent/drying	— The sensation that occurs when alum is placed in the mouth
Aftertastes	
Oily/fatty	— Same as above
Beefiness	— Same as above
Stale/off	— Same as above
Astringent/drying	— Same as above

Table 4

Beef flavour descriptors by an expert panel. Johnson and Civille (1986)

Sensory term	Definition/Reference
Aromatic	The aromatic associated with:
Cooked beef lean	— cooked beef muscle meat
Cooked beef fat	— cooked beef fat
Browned	— the outside of grilled broiled beef (seared but not blackened/burnt)
Serum/bloody	— raw beef lean
Grainy/cowy	— cow meat and/or beef in which grain/feed character is detectable
Cardboard	— slightly stale beef (refrigerated for a few days only) and associated with wet cardboard and stale oils and fats
Oxidised/rancid/painty	— rancid oil and fat (distinctly like linseed oil)
Fishy	— some rancid fats and oils (similar to oil fish)
Taste	The taste on the tongue associated with:
Sweet	— sugars
Sour	— acids
Salty	— sodium ions
Bitter	— agents such as caffeine, quinine etc.

Table 5

The initial list of 45 terms describing the range of sensations perceived in a variety of test chicken products. From Lyon (1987)

1. Chickeny[d]	16. Bouillon-like[a]	31. Reheated
2. Meaty[b, c, d]	17. Serum/bloody[a, e, f, g]	32. Greasy
3. Meaty, chickeny	18. Liver/organy[a]	33. Fatty[d, e, f, g]
4. Meaty, cooked[f,g]	19. Bone-marrow	34. Oily[d, e, f, g]
5. Meaty, raw[f]	20. Earthy[f, g]	35. Oxidised
6. Gamey, fowl-like	21. Grassy[a, g]	36. Rancid[b, c, d, f, g, h]
7. Roasted[f]	22. Feedy[g]	37. Painty[f]
8. Boiled	23. Vegetable (cooked)[f, g]	38. Fishy[g]
9. Canned[g]	24. Musty[a, c, f, g]	39. Salt[e]
10. Browned[a]	25. Moldy[a, f, g]	40. Sweet[b, c, e, f, g]
11. Burned[f, g]	26. Papery	41. Sour[b, e, g]
12. Toasted[f]	27. Nutty[a]	42. Bitter[g, e]
13. Scorched	28. Cardboard	43. Metallic[a, f, g]
14. Heated protein	29. Stale[b, c, h]	44. Chemical[a, h]
15. Brothy[b,h]	30. Warmed-over[d]	45. Astringent[a, e, h]

[a-h] Reference literature citing similar use of terms:

[a] Berry et al. (1980). Beef.

[b] Cipra and Bowers (1971). Turkey.

[c] Jacobsen and Koehler (1970). Chicken.

[d] Landes (1972). Chicken.

[e] Lynch et al. (1986). Beef

[f] MacLeod and Coppock (1978). Beef.

[g] Persson et al. (1973). Beef.

[h] Van de Riet and Hard (1979). Beef.

Table 6

Twelve sensory descriptive terms with definitions developed for evaluation of chicken flavour. From Lyon (1987)

Sensory term	Definition
	Aromatic taste sensation associated with:
Chickeny	— cooked white chicken muscle
Meaty	— cooked dark chicken muscle
Brothy	— chicken stock
Liver/Organy	— liver, serum or blood vessels
Browned	— roasted, grilled or broiled chicken patties (not seared, blackened, or burned)
Burned	— excessive heating or browning (scorched, seared and charred)
Cardboard/Musty	— cardboard, paper, mould, or mildew; described as nutty, stale
Warmed-over	— reheated meat: not newly-cooked nor rancid/painty
Rancid/Painty	— oxidised fat and linseed oil
	Primary taste associated with:
Sweet	— The taste on the tongue associated with sugars
Bitter	— The taste on the tongue associated with acids
	Feeling factor on the tongue associated with:
Metallic	— The taste on the tongue associated with sodium ions

Table 7

Flavour descriptors for beef patties. From Love (1988)

Aromatics	Tastes
Cooked beef	Sweet
Beef fat	Sour
Browned	Salty
Serum/raw	Bitter
Cooked liver	Mouthfeel
Green grain/vegetable	Metallic
Cardboard	Astringent
Painty	
Burned/scorched	

Due to restrictions from the publishers of the journals in which the articles from this PhD dissertation have been published, they are not present in this PDF. The papers can be found in:

Article I:
Byrne D. V., Bak L. S., Bredie W. L. P., Bertelsen G., Martens M. (1999a). Development of a sensory vocabulary for warmed-over flavor: Part I. In porcine meat. *Journal of Sensory Studies, 14*, 47-65.
DOI: 10.1111/j.1745-459X.1999.tb00104.x

Article II:
Byrne D. V., Bredie W. L. P., Martens M. (1999b). Development of a sensory vocabulary for warmed-over flavor: Part II. In chicken meat. *Journal of Sensory Studies, 14*, 67-78.
DOI: 10.1111/j.1745-459X.1999.tb00105.x

Article III:
Byrne, D. V., O'Sullivan, M. G., Dijksterhuis, G. B., Bredie, W. L. P., & Martens, M. (2001). Sensory panel consistency during development of a vocabulary for warmed-over flavour. Food Quality and Preference, 12, 171-187.
DOI: 10.1016/S0950-3293(00)00043-4

Article IV:
Byrne, D. V., Bredie, W. L. P., Bak, L. S., Bertelsen, G., Martens, H., & Martens, M. (2001). Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. Meat Science, 59, 229-249.
DOI: 10.1016/S0309-1740(01)00072-9

Article V:
Byrne, D. V., Bredie, W. L. P., Mottram, D. S., & Martens, M. (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. Meat Science, 61, 127-139.
DOI: 10.1016/S0309-1740(01)00171-1

Article VI:
Byrne, D. V., O'Sullivan, M. G., Bredie, W. L. P., Andersen, H. J., & Martens, M. (2003). Descriptive sensory profiling and physical/chemical analyses of warmed-over flavour in pork patties from carriers and non-carriers of the RN^- allele. Meat Science, 63, 211-224.
DOI: 10.1016/S0309-1740(02)00072-4

8. Thesis articles I-VI

I. Byrne, D. V., Bak, L. S., Bredie, W. L. P., Bertelsen, G. and Martens, M. (1999a). Development of a sensory vocabulary for warmed-over flavour: Part I. in porcine meat. *Journal of Sensory Studies, 14*, 47-65.

II. Byrne, D. V., Bredie, W. L. P., and Martens, M. (1999b). Development of a sensory vocabulary for warmed-over flavour: Part II. in chicken meat. *Journal of Sensory Studies, 14*, 67-78.

III. Byrne, D. V., O'Sullivan, M. G., Dijksterhuis, G. B., Bredie, W. L. P. and Martens, M. (2001). Sensory panel consistency during development of a vocabulary for warmed-over flavour. *Food Quality and Preference, 12,* 171-187.

IV. Byrne, D. V., Bredie, W. L. P., Bak, L. S., Bertelsen, G., Martens, H and Martens, M. (2001). Sensory and chemical analysis of cooked porcine meat patties in relation to warmed-over flavour and pre-slaughter stress. *Meat Science*, *59,* 229-249.

V. Byrne, D. V., Bredie, W. L. P., and Martens, M. (2002). Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. *Meat Science, 61,* 127-139.

VI. Byrne, D. V., O'Sullivan, M. G., Bredie, W. L. P., and Martens, M. (2003). Descriptive sensory profiling and physical/chemical analyses of warmed-over flavour in meat patties from carriers and non-carriers of the RN^- allele. *Meat Science*, *63,* 211-224.

Paper I

Paper II

Paper III

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