Studies into factors responsible for the acceptability of pork on the Singaporean market

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ABSTRACT

The thesis reports the results of a series of studies looking into the acceptability of pork on the Singapore market. Anecdotal comments have indicated that pork from some countries had a less acceptable flavour than that produced locally, so a survey was conducted to clarify the situation. This indicated that imported pork, including that from New Zealand, had an undesirable mutton-like flavour. Using pork from female pigs fed either a plant only diet (NZP) or one that included some animal products (NZA) it was shown that Singapore consumers favoured the former due to a lower mutton note. The use of garlic essential oil (GEO) to improve the acceptability of NZ pork either by adding it directly to pork or feeding it to pigs was demonstrated. With increasing GEO, garlic flavour strength increased and mutton flavour strength decreased even when diets of the pigs included animal products.

Concentrations of indolic compounds (indole and skatole) in backfat increased with increasing dietary garlic concentration ($P<0.001$), and were higher in backfat from the NZA group ($P<0.05$), but were unaffected by different dietary lipid sources (fish oil, tallow, and a mix of linseed oil and soya oil).

A highly acceptable low-fat (<10%) and low-salt (<450 mg/100 g) pork ball with an n-6/n-3 ratio of <4 was developed as a premium product, and effects on its acceptability were assessed using pork from pigs on different diets. A supplement containing selenium, vitamin E, vitamin C and CLA fed to pigs led to pork and pork balls with increased levels of these items. Inclusion of fish oil in the diet (4.4%) increased the levels of the long chain n-3 fatty acids (LCN3FA) in the pork and pork balls, but also increased measures of oxidation (TBARs), especially after a period of storage, and decreased the acceptability of the product due to increased off-flavours (rancid and aftertaste). This occurred when fish oil was removed from the diet either 28 days or 49 days (early and late feeding stage) before slaughter. Further research into ways of improving the flavour aspects of these products is required.

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Chapter 1

Introduction

World meat consumption increased by almost 30% in the last decade of the 20th century (FAO, 2002). Pork accounted for more than half of this growth. Pork is consumed in all continents and world pork consumption was 15.9 kg per person per year for 2004 (FAO, 2004). This has risen from 9.2kg per person per year in 1970 and is predicted to reach 17.9kg per person per year in 2015 (Roppa, 2005). Consumption of pork varies widely from an annual per capita consumption of less than 3 kg in South Africa to more than 60 kg in Austria, Denmark and Spain (FAO, 2002). China, the EU and the USA are the largest producers of pigs. Asia supplies about half of the world's pork production of more than 90 million tonnes of meat.

With the increased consumption of pork around the world, knowledge of consumer preferences for pork is of great importance if the meat industry is to produce pork products that satisfy consumer demand. Sensory experience is an essential aspect of food quality to consumers (Agerhem & Tornberg, 1993). Meat quality of pork encompasses attributes such as colour, flavour and texture (Bredahl, Grunert, & Fertin, 1998). Flavour is considered to be one of the most important sensory traits of pork by consumers (Bryhni, Byrne, Rødbotten, Claudi-Magnussen, Agerhem, Johansson, Lea,, & Martens, 2002; O'Mahony, 1991), and the absence of off-flavours is expected to be critical for acceptance (Risvik, 1994).

In Singapore, pork is a popular meat consumed by non-Muslim Singaporeans with about 87,000 tonnes being consumed per year (Kanagalingam, 2005). Currently, Singapore imports its pork largely from Indonesia and Australia. Fresh pork is obtained from pigs raised in Indonesia but slaughtered at Singapore abattoirs, while chilled pork is mainly imported from Australia and is widely known as "Air Pork". Frozen pork is mainly imported from Brazil, Finland, China and France. Singaporean consumers are aware of the origin of pork from packaging labels that are readily available at supermarkets and wet markets.

The New Zealand pork industry is currently investigating the feasibility of exporting pork from New Zealand to Asian markets such as Singapore. Due to the relatively high cost of grain in New Zealand and the greater distance from the market, it is difficult for pork from New Zealand to compete on price with pork from countries

such as Indonesia, China and Australia. Consequently it will be necessary to produce a product that can attract a premium price as a result of one or more superior quality attributes such as those associated with sensory and nutritional characteristics.

The broad aim of the research reported herein was to impose treatments on pigs or pork that were expected to enhance pork quality in some way and then to quantify the effects of the treatments in terms of consumer acceptability in the market place, sensory analysis by trained panels, and selected aspects of product composition. Novel methods were investigated which involved manipulating components of pork quality with the long-term goal of devising and characterising production and processing systems that lead to pork products that satisfy consumer demand in Singapore while being profitable for the New Zealand pork industry.

The objectives of the project were as follows with the relevant chapter numbers shown in brackets:

1. To characterise the perception of Singaporean consumers towards pork from different countries by means of a survey (Chapter 3).

2. To determine the effects of dietary treatments (animal-plant vs. plant only diets) on the sensory quality of New Zealand pork using a Singaporean panel (Chapter 4), and also to compare New Zealand pork with a local reference pork sample from Indonesia.
3. To determine the usage of herbs, spices and other plant materials in the cooking of pork by Singaporean consumers by means of a survey, with a view to using an aromatic plant material to improve the flavour of New Zealand pork (Chapter 5).

4. To determine the threshold levels of garlic (as determined from objective 3) in rice bran oil and pork mince by means of both New Zealand and Singaporean panels of consumers. (Chapter 6).

5. To determine the effects of different levels of garlic essential oil in the diets of pigs on the sensory quality and acceptance of pork (Chapter 6).

6. To evaluate the effects of garlic or n-3 PUFA in the diets of New Zealand pigs on the concentrations of indole and skatole in the pork (Chapter 7).

7. To determine the effects of including n-3 polyunsaturated fatty acids (PUFA) and Sanovite™ in the diet of pigs on the sensory and nutritional quality of pork, and to evaluate the effects of storage period on these characteristics (Chapter 8).

8. To develop a healthy low-fat and low-salt pork ball product for Singaporean consumers (Chapter 9)

9. To evaluate the effects of storage time on physical, nutritional and sensory characteristics of low-fat, low-salt pork balls produced for Singaporean consumers using pork from New Zealand pigs fed either (a) GEO (Chapter 9), or (b) n-3 PUFA and Sanovite™ (Chapter 10).

Chapter 2

Literature Review

2.1 Introduction

Meat flavour is influenced by compounds contributing to the sense of taste and smell. Other sensations like mouth-feel and juiciness will also affect the overall flavour sensation, but the volatile compounds of cooked meat mainly determine its aroma attributes and flavour characteristics. There have been over 1000 volatile compounds isolated from meat (Mottram, 1991).

Meat flavour is thermally derived as raw meat possesses little odour and a mild serum-like taste, which is described as salty, metallic and bloody, with a sweet aroma (Wasserman, 1972; Hornstein and Wasserman, 1987). Much research has been aimed at understanding the chemistry of meat aroma and the nature of the reacting compounds. The major precursors of meat flavour can be divided into the two categories of water-soluble components (amino acids, peptides, carbohydrates, nucleotides, thiamine, etc.) and lipids. The Maillard reactions between amino acids and reducing sugars, and the thermal degradation of lipids result in many aroma volatiles forming during the cooking process (deRoos, 1992; Kramlich & Pearson, 1960; MacLeod & Seyyedain-Ardebili, 1981). Also lipid-derived volatiles have an important part to play in desirable meat aromas both directly as aroma compounds and as intermediates to other aroma compounds (Nawar, 1969; Mottram, 1991). In this literature review the chemistry of pork flavour is covered first, followed by a consideration of factors affecting pork flavour with a particular emphasis on pre-slaughter factors.

2.2 Chemistry of pork flavour

Raw pork meat tissue consists of water, proteins, amino acids, nucleotides, sugars, lipid, vitamins, and other compounds. All these components act as potentially important flavour compounds, flavour enhancers and aroma precursors (Mottram, 1991).

During the cooking process, various non-volatile compounds in pork undergo degradation or reaction with each other to produce numerous volatile compounds to form multiple volatiles and non-volatiles that give the characteristic pork flavour (Chen

& Ho, 1998). Flavour formation is thus closely related to the amount and nature of precursors present in the raw pork at the time of cooking (Mottram, 1991). A number of factors may influence the presence of flavour precursors, such as feed (Koutsidis et al., 2007a; Mottram, Koutsidis, Oruna-Concha, Ntova, & Elmore, 2004; Rosenvold et al., 2001), pre-slaughter stress (D´ Souza, Dunshea, Warner, & Leury, 1998; Wiklund, Andersson, Malmfors, & Lundström, 1996) and ageing (Koutsidis, Mottram, Elmore, & Oruna-Concha, 2003; Koutsidis et al., 2007b; Parrish et al., 1969; Tikk et al., 2006).

The types of volatile compounds found in pork flavour have been identified as hydrocarbons, alcohols, carbonyls, carboxylic acids, esters, lactones, ethers, sulphur-containing compounds as well as different classes of heterocyclic compounds, including furans, pyridines, pyrazines, oxazoles, thiazoles and thiophens (Table 2.1). Shahidi, Rubin & D'Souza (1986) noted that the number of volatiles present in cured pork is about one third of those in uncured pork.

Table 2.1
Classification of volatile compounds found in uncured and cured pork (modified from Shahidi et al. 1986)

Class of compound	Number of compounds	
	Pork (uncured)	Pork (cured)
Hydrocarbons	45	4
Aldehydes	35	29
Ketones	38	12
Alcohols	24	9
Phenols	9	1
Carboxylic acids	5	20
Esters	20	9
Lactones	2	-
Furans	29	5
Pyridines	5	-
Pyrazines	36	-
Other nitrogen compounds	24	3
Sulphur compounds	31	31
Halogenated compounds	4	1
Miscellaneous compounds	7	11
Total	314	135

Generally the characteristic species-associated flavours and aromas that occur in pork, beef, lamb and poultry are located in the lipid fraction (Hornstein and Wasserman, 1987; Macy et al., 1964), while the water-soluble fraction contains components contributing to the development of 'meaty' flavour (Macy et al., 1964). Differences in the fatty acid composition in animals may contribute to characteristic species flavour differences. It is also known that lipids alone are not responsible for these characteristic aromas (Mottram, Edwards & MacFie, 1982). A study has shown that regardless of the source (beef or pork) of the fat added to lean meat, the inclusion of 10% subcutaneous fat allowed panellists to more easily correctly differentiate minced pork and beef patties (Mottram, 1979).

During cooking, lipids decompose to create a myriad of volatiles. The main reactions involved are oxidation and degradation of both unsaturated and saturated fatty acids. The primary oxidation products, known as the monohydroperoxides, decompose via an intermediate alkoxy radical, thus forming a range of aroma volatiles. Such decompositions include many aliphatic hydrocarbons, alcohols, aldehydes, ketones, acids, lactones, esters and long chain alkyl-substituted heterocyclic compounds (Macleod and Ames, 1986). Lipid derived volatile compounds dominate the flavour profile of pork cooked at temperatures below 100^{o}C. Table 2.2 lists some of the volatile compounds generated from lipid degradation in cooked pork volatiles.

Aldehydes are the major components identified in the volatiles of cooked pork. Octanal, nonanal and 2-undecenal are oxidation products of oleic acid, hexanal, 2-nonenal and 2,4-decadienal are major volatiles products of linoleic acid. Oleic and linoleic acids are the two most abundant unsaturated fatty acids in pork (Schliemann, Wolm, Schrodter & Ruttloff, 1987). 1-octen-3-ol may be derived from the 12-hydroperoxide of arachidonic acid in cooked pork (Mottram, 1985; Chou and Wu, 1983). 2-pentyfuran, which has been identified in cooked pork, is an autooxidation product of linoleic acid (Ho, Smagula & Chang 1978).

Table 2.2

Examples of volatile lipid oxidation products identified in cooked pork (Ramarathnam , Rubin & Diosady, 1993; Chou & Wu, 1983; Mottram, 1985)

Compounds			
Aldehydes			
Hexanal	Heptanal	3-methylhexanal	Octanal
Nonanal	Dodecanal	Tridecanal	Tetradecanal
Hexadecanal	Octadecanal	2-hexenal	2-heptanal
2-octenal	2-nonenal	4-decenal	2-undecenal
2-dodecenal	2-tridecenal	2-tetradecenal	17-octadecenal
16-octadecenal	15-octadecenal	9-octadecenal	2,4-nonadienal
2,4-decadienal	2,4-undecadienal		
Ketones			
2-heptanone	2-tetradecanone	2-hexadecanone	2,3-octanedione
Alcohols			
1-pentanol	1-hexanol	1-heptanol	1-octanol
2-heptenol	1-octen-3-ol	1-nonanol	1-nonen-3-ol
Alkyfurans			
2-pentylfuran			

2.3 Factors affecting pork flavour

The flavour development of pork following standard cooking procedures depends on the amount and nature of precursors present in meat, which, in turn, depends on several factors including feed (Mottram, Koutsidis, Oruna-Concha, Ntova, & Elmore, 2004), post mortem treatments such as aging (Koutsidis, Mottram, Elmore, & Oruna-Concha, 2003; Parrish et al., 1969), and genetic variations (Lawrie, 1991). Some of the factors that may influence the flavour of pork at different stages of animal production and meat processing were outlined as follows (adapted from Ngapo and Gariépy (2008)):

Pre-slaughter factors

Genetic factors

1. Breed and genetic lines

Gender effects and Nutrition effects

1. Direct transfer of aroma active components
2. Changes in the fatty acid composition
3. Diet effects on hind gut digestion
4. Diet effects on liver metabolism

Post-slaughter factors

Post-mortem and pre-cooking factors

1. Patterns of temperature change
2. Conditioning or ageing
3. Storage before cooking

Preparation before consumption

Cooking - Cooking temperature and cooking time

In this review, only the preslaughter factors as set out in Figure 2.1 will be reviewed in detail as they are of most relevance for the project reported in this thesis.
They are:

1. Genetic effects
2. Gender effects
3. Nutrition effects

2.3.1 Genetic effects on pork flavour

2.3.1.1 Breeds

The genetic background of pigs plays an important role in the eating quality of pork (Casteels, Van Oeckel, Bosschaerts, Spincemaille & Boucqué, 1995; de Vries, Van der wal, Eikelenboom, Merks, 1992) due at least in part to genetic effects on the level of intramuscular fat (IMF). Casteels et al. (1995) compared three genotypes for eating quality in a preference test and showed lower preference scores for taste intensity, tenderness and juiciness for pork from Belgian Landrace pigs relative to pork from Large Whites and hybrids which had the highest IMF levels and also scored best for intrinsic and sensory meat quality parameters.

Positive relationships between IMF levels and eating quality of pork were reported by Kirkegaard, Møller, & Wismer-Pedersen (1979); Bejerholm & Barton-Gade (1986); Gandemer et al. (1990); Jakobsen (1992) and Hovenier, Kanis, and Verheven (1993), with recommended IMF levels being from 1 to more than 4%. It should be possible to modify the IMF content by selection as the heritability of IMF is in the range of 0.4 – 0.6 (Cameron, 1990; de Vries, Hovenier, Brascamp, & Westerink, 1992; Lo et al., 1992; Schwörer, Blum, & Rebsamen, 1986; Sellier, 1988; Touraille & Monin, 1982; Touraille & Monin, 1984). The pork from 100% Duroc pigs which was juicier, had a higher IMF content compared with 0 and 50% Duroc pigs (Channon et al., 2004). The intramuscular neutral lipid was higher in saturated fatty acids 14:0 and 16:0 and polyunsaturated fatty acids (PUFA) in the meat of Duroc and Large White pigs compared to that from Berkshire and Tamworth pigs (Wood et al., 2004).

Other examples of where increased IMF level has resulted in better sensory qualities in pork have been from pig crosses (Touraille, Monin & Legault (1989) for Large White (LW) and MeiShan x Large White (MS x LW) pork. The half-Chinese crossbred pigs gave a meat of higher palatability than the corresponding purebred European pigs. MS x LW meat was evaluated as being tastier, more tender and more juicy as compared to the LW.

Sellier (1988) and Warriss, Brown, Franklin, & Kestin (1990) suggested that increased IMF levels can be achieved without having carcasses with an undesirable amount of subcutaneous fat that has to be trimmed, thereby decreasing the yield of saleable meat. Correlations reported by Casteels, Van Oeckel, Bosschaerts, Spincemaille & Boucqué (1995) between the IMF level and daily gain and back fat thickness indicated that it was possible to increase the IMF level by selection programs, without negative consequences for the growth and back fat thickness of the animals.

2.3.1.2 Individual gene effects

It has been shown that cooked pork from carriers of the RN^- allele has significantly higher levels of both glucose and glucose 6-phosphate, and that the fatty acid composition in this pork includes more unsaturated fatty acids compared with that from non-carriers (Meinert, Andersen, Bredie, Bjergegaard, & Aaslyng, 2006). The lipid of pork from RN^- carrier pigs had more polyunsaturated phospholipids and more monounsaturated triglycerides than non-carriers in the study of Enser, Hallett, Hewett, Fursey, and Wood (1996). In the studies by Johansson et al. (1999) and Lundström et al (1996, 1998), the RN- allele in Hampshire crosses had significant effects on the sensory properties of pork loin, with RN- meat more acidulous in taste (due to low pH) than meat from non-carriers, and having a more intense meat taste (Johansson, et al., 1999; Jonsall et al., 2001, 2002; Lundström et al., 1996). Le Roy et al. (1996) obtained better sensory scores for flavour, but worse scores for tenderness, juiciness and mellowness for RN^-carriers than for non-carriers. RN^- carriers have also been found to have lower protein content (Estrade et al., 1993; Enfält et al., 1997), higher drip and cooking losses, and higher internal reflectance values (Lundstrom et al., 1996).

The halothane gene can affect the quality of pork. The most obvious quality defect associated with the halothane gene is the high incidence of pale, soft exudative (PSE) meat (Briskey, 1964; Sellier and Monin, 1994). A study by Shen, Underwood, Means, McCormick & Du, 2007 showed that the presence of the halothane gene induced early energy depletion, which could be the main change causing the adenosine monophosphate-activated protein kinase (AMPK) activation that leads to accelerated glycolysis and an increased incidence of PSE meat.

In conclusion, the genetic influence on pork quality comprises differences among breeds as well as differences among animals within the same breed. These differences can be caused by a large number of genes with small effects, known as polygenic effects, and in principle most traits of interest for meat quality like flavour and texture have a multifactorial background (Andersson, 2001). A comprehensive list of genetic markers for carcass and meat quality in pigs has been published by Garnier, Klont, and Plastow (2003).

2.3.2 Gender effects on pork flavour

Growing entire male pigs for meat production offers economical advantages in production costs as well as the advantage of increased carcass leaness (Malmfors & Lundstrom, 1983; Cliplef, Grinwich & Castell, 1984; Diestre, Oliver, Gispert, Arpa & Arnau, 1990; Bekaert, Casteels, Eexkhout, & Buyssee, 1974; Van Oeckel, Casteels, Warnants, De Boever, et al., 1996).

However, boar meat sometimes has a boar taint, which can reduce consumer acceptability (Bañón, Andreu, Laencina & Garrido, 2004; De Kock, Heinze, Potgieter, Dijksterhuis & Minnaar, 2001). A recent international study involving seven EU countries (Bonneau et. al., 2000) showed that a higher proportion of consumers were not satisfied with pork from entire males when compared with gilt pork (31.9 vs 26.0% for odour; 21.5 vs 18.5% for flavour). The major causes of the taint have been recognised as androstenone (Patterson, 1968) together with other 16-androstene steroids (García-Regueiro & Diaz, 1989) produced by testes, and skatole (Vold, 1970),

which is produced by bacteria in the gut. These compounds accumulate in fats and generate offensive off-odours and off-flavours of meat when it is heated (Babol, Squires & Gullett, 1999).

In the U.K., pigs are slaughtered at relatively light weights and young ages compared to most other countries, which reduces the likelihood of boars developing high levels of androstenone or skatole (Malmfors & Lundstrom, 1983). The main limiting factor with regard to the use of heavier entire males is boar taint in unprocessed meat, but this defect is better tolerated in processed meat products, so pork from heavier males may be best used for processed products (Banón, Costa, Gil, & Garrido, 2003; Bonneau, Denmat, Vaudelet, Veloso-Nunes, Mortensen & Mortensen 1992; Diestre, Oliver, Gispert, Arpa, & Arnau, 1990).

Another study by Gullett, Partlow, Fisher, Halina & Squires (1993) compared the consumer acceptability of fresh and cured pork from gilt, intersex, cryptorchid, and intact male pigs. The intersex pigs used in the study were pseudo-hermaphrodites possessing a female external morphology and an internal morphology of male, male/female or female. Cryptorchidism is either a developmental defect where the testes fail to descend into the scrotum, or it can be induced. Consumer rating of pork chops from intact males received the lowest score, followed by the cryptorchid while the gilts and the intersex pigs received the highest. Castration reduces androstenone and skatole in meat (Bañón, Andreu, Laencina, & Garrido, 2004), but it also slows down the growth of pigs and makes them fatter, thereby increasing production costs (Bonneau, 1998). Castration of male pigs has been the traditional method of preventing boar odors since boars are the main source of the problem. Skatole concentrations in adipose tissues of boars can vary between 0.0 and 1.5 ppm, whereas for gilts and barrows the concentration seldom reaches 0.3 ppm. The acceptable limit for skatole was 0.25 ppm (Mortensen et al., 1986; Bejerholm & Barton-Gade,1993).

Not everyone is necessarily sensitive to boar taint and some people are more affected by it than others (Claus et al., 1994), as the ability to smell androstenone is genetically determined (Wysocki & Beauchamp, 1984) and is predominant in women. Only 56% of men can detect androstenone, compared with 92% of women. The odour

was shown to be more unpleasant to women than to men by Claus et al. (1994). Skatole, however, is highly offensive to most people (Babol et al., 1996). While it is interesting to note the eating quality differences between animals of differing gender, there will always be both male and female animals producing pork for the market. The ratios of different sexual groups very much depends on the demand in the market and may be influenced by criteria other than production efficiencies or eating quality, such as, the ethical debate of castration of boars.

The findings on effects of boar taint on pork and its products are summarised in Table 2.3. Based on the six studies summarised in Table 2.3, it can be concluded that:

1. Boar taint is associated with androstenone, skatole and indole.
2. The presence of boar taint in pork is undesirable among a variable proportion of consumers.
3. Pork acceptance has been shown to be better for pork from castrated males than entire males.
4. Curing and smoking of ham can suppress boar taint odour.

Table 2.3
A summary of the results from the studies showing the effects of boar taint on chemical and sensory properties of fat, pork meat and pork products with emphasis on effects on flavour

Animal and Experimental design	Measurements made and methods used	Results and Conclusions	References
40 entire males (EM) and 40 castrated males (CM) received feed made from cereals (corn, barley and wheat), molasses and soya ad libitum.	Androstenone and skatole content of the fat was determined using HPLC. 8 trained judges evaluated five attributes in cooked loin using 5-point scales from 1 (minimum) to 5 (maximum). 68 untrained consumers assessed preference and acceptability of cooked loin samples on a scale of 1 (very poor) to 5 (very good).	• Castration caused a considerable reduction in androstenone, skatole and indole levels in subcutaneous fat. • Androstenone was reduced by more than 70% by castration, while the fall in skatole and indole was less pronounced. • Increased meat fatness and reduced boar taint, aroma and taste for pork from castrated pigs. • Pork acceptance was better in castrated males than in entire ones. Castrated ones were preferred by 75% of consumers.	Bañón et al., 2004
Back fat samples and samples of the corresponding L. dorsi and fat covering L. dorsi were obtained from 12 boars and three gilts.	Indolic compounds were determined using HPLC.	• A correlation was observed between the concentration of indole and skatole in the back fat and fat covering the L. dorsi samples ($p<0.001$, $r=0.99$). • No significant correlation was obtained in L. dorsi samples, between skatole and indole levels. • The mean concentrations of these indolic compounds were significantly higher ($P<0.05$) in the back fat samples than fat covering the L.dorsi samples.	Rius et al., 2001
Pork fat samples from loins from boars with low, medium or high skatole and androstenone concentrations were obtained from a commercial abattoir.	300 pork consumers evaluated the pork using a nine-point scale with word anchors (dislike extremely and like extremely) at the extreme ends.	• Majority of consumers were less willing to consume pork meat exhibiting detectable levels of boar odour. • Most of them were dissatisfied with pork meat with detectable levels of skatole. • More females compared with males responded more negatively towards samples with detectable levels of androstenone.	De Kock et al., 2001

Table 2.3 (continued)

718 meat samples with 646 from entire male pigs and 72 from gilts.	Trained analytical sensory panels in seven European countries assessed pig meat with known levels of androstenone and skatole.	• Sensory panels in general were able to differentiate between the two compounds and between different levels of the compound. • Androstenone was found to relate mostly to the urine attribute, while skatole related mostly to manure and, to a lesser extent, to naphthalene	Bonneau et al., 1996
48 cooked loins and 48 dry-cured hams from entire males and castrates were studied.	8 trained panellists evaluated the sensory attributes of dry-cured ham. The scale ranged from 1 (minimum) to 5 (maximum) for intensity of boar odour , boar flavour, saltiness, graininess, toughness , juiciness and marbling . 268 randomly chosen consumers carried out a preference and acceptability test using a scale of 1 (very poor) to 5 (very good).	• The dry cured ham from castrates was scored as more flavoured, more marbled and softer. It has less grainy, less salty and had less boar odour and flavour. • Dry-cured ham from castrated males was more accepted by consumers, especially women and habitual consumers. • Castration of male pigs improved the quality of dry-cured ham.	Bañón et al., 2003
Model and commercial type Swedish fermented sausage products based on low or high levels of boar tainted fat, three different starter cultures and two different levels of smoking were studied.	The sensory panel consisted solely of women (21–56 years of age) using a 15-cm unstructured line scale anchored from “none” to “extreme” for odour, flavour and texture of pork.	• The perception of boar taint was significantly negatively correlated to the overall positive impression of the commercial sausages. • Liquid smoke masked the perception of boar taint.	Stolzenbach et al., 2009

2.3.3 Nutrition effects on pork flavour

Many reports have shown that the diet fed to pigs can influence the flavour quality of pork. The reasons for modifying the diets are mainly to improve pig performance, but this can also influence the sensory quality of pork (Warnants, Van Oeckel & Boucqué, 1996a, b; Ahn, Lutz & Sim, 1996; Cameron & Enser, 1991; Rhee, Ziprin, Ordonez & Bohac, 1988).

According to Hansen, Agerhem, Rosenvold, & Jensen (2001), the sensory quality of pork can be influenced by the following feeding factors:

1. Direct transfer of aroma active components from the diet to meat
2. Changes in the fatty acid composition
3. Diet effects on hind gut digestion
4. Diet effects on liver metabolism

2.3.3.1 Direct transfer of diet components to meat and/or fat

The most significant example of transfer of aroma-active components from feed to meat is the fishy taint and other off-flavours obtained by feeding fish meal or oil to growing/finishing pigs (Table 2.4). This is due to fish-smelling components being absorbed from the intestine and deposited in the meat (Hertzman, Göransson, & Ruderus, 1988; Jaturasitha, Wudthithumkanaporn, Rorksasen, & Kreuzer, 2002, Kjos, Skrede, & Óverland, 1999; Lauridsen et al., 1999; Maw, Fowler, Hamilton, & Petchey, 2001).

a. Polyunsaturated fats – Fish oils & plant oils

In a study by Valaja et al., 1992, fish meal addition to the porcine diet did not significantly affect the eating quality of fresh pork, but in pork that had been frozen for 6-8 months the off-flavour increased with increasing fish meal concentration. It was observed that the PUFA content in the pork increased with fish meal concentration, and it is known that these fatty acids are more susceptible to oxidation than saturated fatty acids (Whittington et al., 1986; Rhee et al., 1988). In earlier studies, the addition of fish

meals (Lyso, 1979; Van Wyk et al., 1977) and krill meals (Grajewska et al., 1981) to the porcine diet led to decreased acceptability scores for flavour, taste and overall quality of the pork. In a later study, the use of fish silage in the porcine diet was compared to fish meal and no flavour differences were observed (Van Wyk et al., 1977). Off-flavour for pork from pigs fed 2.5 and 9.5 g kg^{-1} fish fat diets were detected (Kjos et al., 1999). Based on the study by Kjos et al. (1999), the recommendations for maximum levels of fish fat in diets for growing-finishing pigs is 3 g kg^{-1} diet when used up to slaughter, and 5 g kg^{-1} when used up to 60 kg live weight with a diet without fish fat for the rest of the finishing period.

By feeding pigs fish oils such as tuna oil, the concentrations of unsaturated fatty acids are increased in meat. This results in a product that is more prone to oxidation (Arnkværn & Bronken Lien, 1997; Øverland, Tangbol, Haug, & Sundstol, 1996; Kjos, Skrede, & Øverland, 1999; Bryhni, Kjos, Ofstad, & Hunt, 2001). Incorporating fish oils at relatively low levels (10-30 mg/kg diet) can lead to off-flavours and odours. Leskansich et al. (1997) reported lower oxidative stability in tissues and sausages from pigs fed a diet of 20g/kg rapeseed and 10 g/kg fish oil.

Table 2.4 summarises the results of studies showing the effects of fish meals or fish oil in the diets of pigs on the chemical and sensory quality of pork. From the results of the 16 studies summarised in Table 2.4 it is concluded that:

1. The majority of the studies showed that feeding > 3% fish meal in the diet of pigs caused off flavour in pork. One study showed the adversity of taste when 2% fish meal was added into the diet (Lyso, 1979).
2. Feeding fish oil at a level of up to 0.5% of the diet produced pork without adverse sensory effects..
3. Increasing the period over which fish oil or fish meal was fed increased the off-flavour in pork.
4. Fish oil supplementation increased concentrations of the long-chain n-3 fatty acids, DHA, EPA and DPA in the fat.
5. Concentrations of unsaturated fatty acids increased in the meat with increasing amounts of fish oil fed.
6. Pork from pigs fed diets containing fish oil has lower oxidative stability as shown by the TBARs assay.

Table 2.4

A summary of the results from the studies that assessed the effects of including fishmeal or fish oil in the diet of pigs on the chemical and sensory characteristics of pork

Animal and Experimental design	Measurements made and methods used	Results and conclusions	References
110 pigs were fed test diets containing 1·1%, 3·3% or 5·5% fishmeal, 10% rape-seed or 15% rape-seed meal. The control diet contained soya-bean meal. 30 IU of vitamin E/day were added to diets. Two of the diets contained extra dose of vitamin E of 200IU/day	Sensory analysis was performed by a trained panel on cutlets using a 10-point scale and intramuscular fats (IMF) Peroxide values were determined	Relative to pork from the control group, that from the treated groups had: • Slightly increased levels of off-flavours with 3% fishmeal or more in the diet after 6 mo freezer storage. • No increase in storage stability of pork fat with extra vitamin E in diets. • Lower storage stability in fats from pigs fed a combination of rape-seed and fishmeal.	Hertzman et al.,1988
20 pigs were fed diets containing 35%corn, 25%peas, 19%barley, 17%canola, and vitamin premix including 100 IU/kg vitamin E and 0.5 mg/kg selenium, mixed with DHA, added in the form of alga biomass at 0.06, 0.6 and 1.6%	Fatty acids by GC. Sensory analysis was performed on bacon by a panel using a 5-point scale where 1= very unpleasant and 5 =very pleasant. TBA test was conducted using a spectrophotometric method	• Bacon content of DHA was increased by almost 3 times when 1 g of DHA was added to a kilogram of feed. • The pigs fed the highest diet level of alga biomass, containing 0.29% DHA, produced bacon with 3.4 mg of DHA/g and 1.2% of the fat as omega-3fatty acids. • Off-odours and off-flavours were reported in the bacon by the taste panel. • Polyunsaturated fat oxidation as indicated by malonaldehyde levels was significantly higher in the pigs fed the higher concentrations of DHA.	Meadus et al., 2010

Table 2.4 (continued)

72 pigs were fed two low-fat diets with or without 0.5% fish oil added, and four medium-fat diets with palm kernel oil to fish oil in ratios given as % inclusion: 4.1:0.0, 3.9:0.3, 3.6:0.5 and 3.4:0.7.	Panellists evaluated belly and neck samples on the intensity of 21 sensory attributes using a scale where 1 was the lowest and 9 the highest intensity. Fatty acids by GC.	• No significant difference in sensory attributes for short-term stored neck and belly. • For pigs fed the highest level of fish oil (0.7%) long term stored product showed a slight increase in fish oil flavour. • Fish oil up to 0.5% produce a healthier fatty acid composition, without adverse effects on sensory profile. • Feeding fish oil increased the level of very long chain n−3 fatty acids, especially the C22:5n−3 (DPA). • Female pigs had a significant higher percentage of monounsaturated fatty than males suggesting a gender related difference in the delta-9-desaturase activity.	Hallenstvedt et al., 2010
Tuna oil (0, 1, 2 and 3%) was added to pig diets.	6 trained persons were used for grading tenderness, juiciness, flavour, on a scale from 1 to 5. Fatty acids by GC. TBA test was conducted using a spectrophotometric method	• Significant adverse effects on flavour and overall acceptance were observed. • Tuna oil + α-tocopherol decreased ω-6:ω-3 ratio • Total PUFA increased as tuna oil concentration increased • Tuna oil supplementation decreased C18:1 and C18:2 • Shelf life of the products, determined as TBA value after different storage periods at 4°C in M. Longissiumus dorsi, back fat and bacon, was significantly reduced.	Jaturasitha et al., 2002

Table 2.4 (continued)

56 pigs were assigned to one of these diets: 0% tuna oil in diet (T0), 1% of unrefined tuna oil in diet (T1), 3% of unrefined tuna oil fed in growing period (T3-E) and late stage of fattening (T3-L).	6 trained persons were used for grading tenderness, juiciness, flavour, on a scale from 1 to 9. Fatty acids by GC.	• Samples from pigs that received tuna oil in early fattening were superior to the others in scores given for sensory flavour and overall acceptability. • Feeding tuna oil at T3-L or T1 had similar efficiency in increasing n-3 fatty acid content of lean and adipose tissue (to about 1.6-fold of T0). • Two-thirds of this increase was found when the same amount of tuna oil had been fed at T3-E. • T3-E has better sensory flavor, overall acceptability grading, and oxidative status compared to the T3-L and T1.	Jaturasitha et al., 2008
The diets were barley-wheat-soya bean meal based and contained either 6% animal fat; 3% animal fat + 3% fish oil; 6% fish oil; or 6% of a mixture of fish oil and coconut oil. All pigs were switched to the finisher-diet added 2% tallow.	Fatty acids by GC.	• 3-6% unrefined fish oil caused off-flavours in the pork. • Fish oil supplementation increased the concentration of C22:6n-3 (DHA), C20:5n-3 (EPA), and C22:5n-3 (DPA), • Coconut oil increased the concentration of the fatty acids C12:0 and C14:0 in the subcutaneous fat and muscles.	Lauridsen et al., 1999
150 pigs were assigned either: Diet A-3% of a 4:1 tallow-soybean oil mixture with 100 mg of α-tocopheryl acetate/kg of diet, Diets B and C- 2% rapeseed oil plus 1% fish oil with supplementation with 100, and 250 mg of α-tocopheryl acetate/kg of diet, respectively.	A trained panel assessed sensory characteristics including both texture and flavour ratings and were rated for their overall acceptability on a scale of 1 (low intensity) to 24 (high intensity). Fatty acids by GC.	• Organoleptic properties of meat, fat, and sausages of the pigs were not adversely affected • The feeding of diets containing 2% rapeseed oil and 1% fish oil resulted in significant alterations in the fatty acid composition. • Ratio n-6: n-3 reduced from 8.5 to 4.9 and 4.6 in diets A, B, & C, respectively.	Leskanich et al., 1997

Table 2.4 (continued)

Bacon samples from four animals (two male, two female) from each farm (total of 23 farms) were subjected to sensory analysis. The pigs were given fishmeal in their diet (either < 2.5% or >2.5%)	7 trained panellists assessed bacon based on a 100 mm line scale.	Fish taints persisted in the bacon. Increased used of fish meal in the diet was associated with increased levels of fishy flavour in the bacon.	Maw et al., 2001)
30 pigs were assigned diets which were barley-soybean meal based and contained either 3% soy oil; 1% fish oil + 2% soy oil; or 3% fish oil. Fish oil was eliminated five weeks prior to slaughter.	Fatty acids by GC.	• 1–3% fish oil had an adverse effect on the sensory profile of pork, especially when used up to the day of slaughter. • Concentration of unsaturated fatty acids increased in the meat with increasing fish oil. • Fish oil increased n-3 fatty acids in muscle & fat tissue, decreased n-6/n-3 ratio & 36 d withdrawal reduced concentration of n-3 fatty acids	Øverland et al., 1996
36 pigs were fed either a control diet, or one of three diets containing 50 g kg^{-1} fish silage and different levels of fish fat (2.5, 5.5 or 9.5 g kg^{-1}).	8 trained panellists evaluated the samples, using a scale from 1 to 9, where 1 was the lowest and 9 the highest intensity. Fatty acids by GC.	• The diets containing 2.5 and 9.5 g kg^{-1} fish fat until slaughter caused off-flavour of bacon after both 1 and 6 months of frozen storage, and of loin muscle after 6 months frozen storage. • The contents of the fatty acids C20:1 and C22:6 in the subcutaneous fat were increased by the dietary inclusion of fish silage. • The total levels of omega-3 fatty acids were highest for the 9.5 and the 5.5 g kg^{-1} fish fat diets when they were fed until slaughter.	Kjos et al., 1999
48 pigs were assigned to six diets which included two levels of PUFA (low 31% and high 50% of total fat content) and three levels of fish oil (0, 0.2, 0.4% of concentrated commercial capelin oil).	10 trained panellists evaluated the samples on a continuous scale where 1=no intensity and 9=high intensity. Fatty acids by GC.	• The diet with high PUFA resulted in higher levels of PUFA in back fat and more rancid loin and sausage products after one and eight months of freezer storage. • Less than 50 g PUFA/kg feed and 23% PUFA in back fat is recommended for finishing pigs to help reduce oxidative problems.	Bryhni et al., 2002

Table 2.4 (continued)

24 pigs were fed either a standard finisher diet (control) or an identical diet supplemented with 15% tuna fishmeal.	A total of ten cuts of pork were assessed for sensory characteristics on an unmarked 100-point visual analogue scale with a higher score indicating a more favorable attribute. Fatty acids by GC. TBARs test was conducted using a spectrophotometric method	• *n*-3 PUFA contents of pork products from pigs fed fishmeal were higher than in controls (steak 300%, stirfry 250%, diced 520%, mince 480%, sausage 360%. • There were no differences between n-3-enriched and regular pork in either TBARS content of stored raw products or sensory characteristics after cooking.	Sioutis et al., 2008
48 pigs were fed diets with variable combinations of corn, linseed and fish oil.	Fatty acids by GC.	Strong correlations between dietary and fat tissue polyunsaturated fatty acids were observed, indicating that the fatty acid composition of the diet may be used as an index of the fatty acid composition of fat tissue.	Nguyen et al., 2003
192 pigs were fed either a control diet of barley and soybean meal with fish meal at 2.7, 6.5 & 13%, or fish meal at 2.5 & 10% withdrawn at 7, 5, 3, & 0 weeks pre-slaughter.	Fatty acids by GC.	• No effects on organoleptic score of fresh meat. • Increasing fish meal increased off-flavour in frozen meat. • Increasing fish meal & feeding period increased long chain PUFA.	Valaja et al. (1992)
64 pigs were fed on Herring meal (2%) ± α-tocopherol (2 g day-1).		Herring meal decreased taste, flavour & overall quality α-tocopherol had no effect	Lyso (1979)
72 pigs were fed on either 5 or 10% krill.		10% krill adversely affected taste	Grajewska et al. (1981)

The amount and composition of dietary fatty acids influences the levels of fatty acids of fat tissues in pigs (St John et al., 1987). Effects of long chain polyunsaturated fatty acids (PUFA) in the diet on fatty acid profile and flavour in pork have been reported for fish oil (see Table 2.4) and other feed ingredients like oilseeds (see Table 2.5). The level of saturated fat in pork can be readily reduced by the inclusion of unsaturated fat in the pig's diet. However, the level of PUFA incorporation is restricted by the associated increases in lipid oxidation which causes the sensory quality of meat to decline (Gray, Gomaa, & Buckley, 1996, Enser, Hallet, Hewitt, Fursey & Wood, 1996). It has been shown that saturated and monounsaturated fatty acids generally are associated positively with eating quality traits (Cameron and Enser, 1991). Oxidation of PUFAs leads to off-flavours and odours which can be detected by sensory panels, or by increased peroxide values (PV) or TBARs values.

Table 2.5 summarises the results of studies showing the effects of dietary PUFA other than fish oil in the diets of pigs on the chemical and sensory quality of pork. From the results of the 22 studies summarised in Table 2.5, it can be concluded that:

1. Plant oils commonly used in the diets of pigs included mainly those derived from linseed, corn, soy bean, sunflower seed and canola.
2. Increases in duration and levels of plant oil feeding (up to 15% of the diet) increased the MUFA and PUFA levels in pork fat.
3. Incorporation of plant oils into diets for pigs have led to increases in lipid oxidation which have been shown to cause rancidity in pork and pork products.
4. The use of vitamin E at concentrations of more than 200 mg/kg feed will result in improved oxidative stability of pork from pigs fed diets supplemented with PUFA.
5. Pork and its products had elevated levels of MUFA and PUFA when the diet of the pigs it came from was supplemented with plant oils.

b. Conjugated linoleic acids

Conjugated linoleic acid (CLA) is a collective term for a group of octadecadienoic acids that are geometric (cis, cis; cis, trans; trans, cis; or trans, trans) and positional (c8, c10; c9, c11; c10, c12, and c11, c13) isomers of linoleic acid (C18:2) (Pariza, Park, & Cook, 2001). CLA has been shown to have health benefits that may include anti-oxidation, anti-atherosclerosis, anticarcinogenic and improvements in immune-responses (Belury, Nickel, Bird, & Wu, 1996; Lee, Kritchevsky, & Pariza, 1994; Miller, Stanton, & Devery, 2001; Pariza & Hargraves, 1985; Park et al., 1999). Almost all of the research on CLA to date has been conducted in in vitro and experimental animal studies. Only recently has human clinical trials been initiated. New evidence is beginning to emerge that CLA found in foods such as dairy foods may be beneficial to health and that different forms of CLA exert unique health effects, including tumor shrinkage (Ochoa et al., 2004, Chajes et al., 2003; McCann et al., 2004), reducing the plasma concentrations of total and low density lipoprotein (LDL)-cholesterol (Ramakers et al., 2005), an anti-inflammatory effect (Turpeinen et al., 2008), the lowering of body fat (Sneddon et al., 2008, Kim et al., 2008) and the lessening of arteriosclerosis (Nakamura et al., 2008). It is unknown how CLA accomplishes all that it does. The mechanisms of CLA's actions may involve nothing less than the regulation of gene expression. The unique mechanisms by which CLA can enhance immune function and help protect us from free radicals and age-related degeneration make it a valuable addition to any life extension program (Nagpal et al., 2007). However, there were some negative impacts of CLA that cannot be ruled out. Some human CLA supplementation studies have often shown conflicting and less convincing health benefits, like the fact that CLA has no effect on glucose metabolism or insulin sensitivity on obese population (Syvertsen et al., 2007); that CLA can act as a cancer promoter in colon carcinogenesis (Rajakangas et al., 2003), and that CLA-mediated induction of hepatic lipogenesis aggravates glucose intolerance and hyperinsulinemia, despite being potentially effective in preventing fatty liver (Ide, 2005).

CLA have been found in the meat and milk of ruminants, but pork contains only small amounts of CLA as the pig is a mono-gastric animal (Chin, Liu, Storkson, Pariza,

& Ha, 1992). Interest in dietary supplementation with CLA for pigs has increased during the last decade from an animal production perspective, due to its potential to improve productive carcass and meat quality traits and to obtain meat and meat products enriched in CLA (Gatlin, See, Larick, Lin, & Odle, 2002; Martín, Antequera, Muriel, Andrés, & Ruiz, 2008; Raes, DeSmet, & Demeyer, 2004; Schmid, Collomb, Sieber, & Bee, 2006; Wiegand, Sparks, Parrish, & Zimmerman, 2002). Some studies have focused on the influence of processing and cooking on CLA content in meat products (Badiani et al., 2004; Ma, Wierzbicki, Field, & Clandinin, 1999; Shantha, Crum, & Decker, 1994), but there has been little focus on pork products.

CLA can affect the oxidative stability of pork and pork products positively and negatively. In some studies, increases in TBARS in pork products with increased CLA content have been reported (Hur et al., 2007; Martín et al., 2008). Conversely, Corino et al. (2003) found that CLA increased the oxidative stability of their product. Joo, Lee, Ha, and Park (2002) also observed how TBARS of loins from CLA fed pigs were more stable than control loins which displayed a rapid increase with storage. The TBARS did not rapidly increase in the presence of CLA. The lack of changes in CLA content during storage could be due to the greater stability of CLA compared with polyunsaturated fatty acid. The antioxidative effect of CLA in animal products is still controversial. Thus, more research is needed to determine the effects of CLA on antioxidative activity in animal products and on the mechanisms involved.

Table 2.6 provides a summary of results from seven studies that have investigated the effects of dietary CLA on quality characteristics of pork. Results from the 7 studies summarised in Table 2.6 lead to the following conclusions:

1. The oxidative stability of pork and pork products increased if the diet of pigs was supplemented with up to 2% CLA, except for one study where rancid odour was encountered when 0.31% of CLA was used.
2. The majority of the studies showed that supplementation of diets with CLA did not have any detrimental effects on sensory quality.
3. CLA supplementation resulted in an increased saturated fatty acid content.
4. CLA concentrations increased in pork lipid with increased CLA supplementation of the diets.

Table 2.5

A summary of the results of studies that have investigated the effects of dietary PUFA other than fish oil on the chemical and sensory characteristics of pork

Animals and Experimental design	Measurements and methods of analysis	Results and Conclusions	References
80 pigs were fed 3 levels of extruded flaxseed (5%, 10% and 15%) and 3 durations of feeding (4, 8 and 12 weeks)	Fatty acids by GC.	• Duration and level of flaxseed feeding affected ($P < 0.05$) most fatty acids except for 22:6n-3 ($P > 0.05$). • Increasing the duration of flax feeding led to significant quadratic increases in back fat 18:3n-3 ($P < 0.001$) and total n-3 fatty acids ($P = 0.002$) when feeding 5% co-extruded flaxseed.	Juárez et. al., 2010
70 pigs were fed either diets including 10% tallow (T), high-oleic sunflower oil (HOSF),sunflower oil (SFO), linseed oil (LO), a fat blend (FB), or an oil blend (OB) in finishing diets vs. feeding a semi-synthetic diet with no added fat (NF).	Fatty acids by GC.	• Feeding HOSF, SFO and LO enriched diets elevated the percentages of MUFA (56.7%), n-6 (30.0%) and n-3(16.6%) PUFA, respectively. • Carcasses from gilts fed OB had greater percentages of n-3 FA (14.8% n-3, 0.9% EPA, 1.0% DPA, 3.1% DHA) than gilts fed FB (6.72% n-3, 0.1% EPA, 0.4% DPA, 0.1% DHA).	Realini et al., 2010
60 Iberian pigs were either (i) free-range reared on acorn and pasture or (ii) in confinement with and without α–tocopherol acetate added to mixed feeds (barley, wheat bran and soybean).	Fatty acids by GC.	Both α-tocopherol acetate supplementation and feeding on acorn and pasture significantly decreased the total and individual aldehydes (products of lipid oxidation) at days 0, 210 and 700 in comparison with pork from pigs fed the non-supplemented diet.	Cava et al., 1999
48 piglets were randomly allotted to four experimental diets containing four levels of α–linolenic acid (0, 1.5, 2.5 and 3.5%). The diets were wheat-barley-soybean meal based.	52 untrained panellists with a 15 cm linear hedonic scale. TBARS values determined by the extraction procedure	• The increase in dietary α-linolenic acid had a detrimental effect on the acceptability of cooked pork loins held for 2 days in loose packaging. • The TBARS of loins after α-linolenic acid -enrichment were significantly higher than those of the control in both vacuum and loose packaging.	Ahn et al., 1996

Table 2.5 (continued)

Pork chops, liver, bacon and sausages from 80 pigs fed on a control or a linseed-rich diet were assessed for sensory quality and oxidative stability.	10 trained panellists evaluated odour, flavour, & texture as well as the acceptability of these attributes. TBA test by a spectrophotometric method	• Grilled loin chops, bacon and sausages from male pigs received significantly higher abnormal flavour scores than those from females. • No significant effects of diet on lipid oxidation for pork, liver or sausages.	Sheard et al., 1999
40 pigs on a control (CO) diet or a 15% flaxseed (FS) diet fed for 7, 14, 21, or 28 d prior to slaughter.	6 trained panellists evaluated bacon for visual appearance, texture, mouthfeel, flavour intensity, and flavour defects. 105 untrained consumer panellists evaluated the bacon for overall desirability. TBA by a spectrophotometric method.	• Trained panellists rated FS bacon more flavour-intense than CO bacon and recorded more flavour defects for FS bacon. • Consumer group showed a higher frequency of "dislikes" for FS bacon than for CO bacon. • Diet did not affect TBA development in bacon; however, 15% dietary FS did cause TBA levels to rise in lard ($p = 0.03$).	Romans et al., 1995
60 pigs were assigned to diets of high or low protein(HP or LP) and either Palm kernel oil (PKO), Palm oil (PO), or Soybean oil (SO)	10 trained panellists evaluated odour, flavour and texture on a scale of 1 (none) to 8 (extremely) Fatty acids by GC.	• PKO meat was more tender than SO. • LP meat was more tender & juicy than HP. • No differences in flavour intensity, flavour liking or overall liking for oils or protein levels. • Total fatty acid (FA) content unaffected by oil. • Total saturated FA lower in PO than PKO or SO pork. • PUFA lower in PKO than SO pork. • LP increased IMF over HP. • Total saturated FA & MUFA lower but PUFA higher in HP than LP pork.	Teye et al., 2006
48 pigs were fed on diets with high oleic acid peanuts (HOP), commercial peanuts (CP), canola oil (CO) with corn/soybean meal as the base.	Fatty acids by GC.	• No difference in flavour, juiciness & tenderness • HOP increased MUFA, CP & CO increased PUFA • HOP, CP & CO decreased saturated FA & increased unsaturated:saturated FA ratio	Myer et al., 1992

Table 2.5 (continued)

48 pigs were fed barley/wheat/ soybean meal with substitution of **soybean meal** with 50, 100 or 150 g bacterial protein meal (BPM) kg−1 feed.	10 trained panellists evaluated odour, flavour and texture on a scale of 1 (no intensity) to 9 (high intensity)	• Rancid odour and taste was reduced with BPM substitution after 1 month frozen storage, but no differences were observed after 3 months • No other differences in taste, odour, tenderness or juiciness were observed	Øverland et al. (2005)
48 pigs were fed on diets with extruded chickpeas (100, 200 & 300 kg t^{-1} feed) substituting for soybean meal.	10 trained panellists evaluated odour, flavour and texture on a scale of 1 (no intensity) to 10 (high intensity)	• No differences odour & taste were found • No consistent differences in tenderness & juiciness were observed • Chickpea increased cooking loss • No differences in fatty acid profiles were observed	Christodoulou et al. (2006)
28 pigs were fed a barley-based control diet and 4 groups of five pigs received the control diet plus essential oil or oleoresin of either rosemary, garlic, oregano, or ginger.	18 trained panellists used triangle tests to compare one oil/oleoresin treatment with the control. TBARs by a spectrophotometric method.	• Sensory panellists were unable to detect a flavour/aroma difference between treated and control pork. • Reduction of lipid oxidation was noted in oregano-fed pork.	Janz et al., 2006
Effects of feeding elevated levels of oleic acid (0%, 10% or 20% canola oil) on the quality of a normal or reduced fat frankfurter.	Fatty acids by GC.	The 20% canola frankfurter had a higher concentration of unsaturated fatty acids (including monounsaturated fatty acids) (82.5% vs 63.9%) and a lower concentration of saturated fatty acids (16.6% vs 34.8%).	St John et al., 1986
10 pigs were fed on diets containing sorghum/ soybean meal or supplementation with high oleic sunflower oil (HOSO; 12%).	8 trained panellists evaluated odour, flavour and texture on a scale from 1 (none) to 8 (extreme). Fatty acids by GC.	• HOSO increased juiciness & overall tenderness. • No effect on off-flavour intensity, overall flavour & oiliness scores .. • HOSO had higher ratio of unsaturated to saturated fatty acids.	Rhee et al., 1990
60 pigs were fed on diets containing animal fat, safflower oil, sunflower oil, or canola oil in a corn/soybean meal as base	8 trained panellists evaluated odour, flavour and texture of the samples on a scale from 1 (low intensity) to 8 (high intensity). Fatty acids by GC.	• No effect on juiciness, tenderness & flavour intensity. • Canola increased off-flavours, decreased flavour quality & overall palatability. • The % of total saturated fatty acids in subcutaneous and intermuscular fat decreased from ~40% in the control to ~31%, 25%, 24% and 24% for the animal fat, safflower oil, sunflower oil and canola oil treatments, respectively.	Miller et al., 1990

Table 2.5 (continued)

18 pigs were fed on control diet supplemented with (i) **Garlic** (0.25%), or (ii) Zinc bacitracin (0.025%) Both ± 22 days withdrawal	Sensory evaluation details not available (Abstract only)	• No differences in flavour, colour &tenderness	Piccolo et al., 1979
30 pigs were fed on a control diet supplemented with bamboo vinegar (2 & 4%)	Sensory evaluation details not available (Abstract only)	• Increased aroma, appearance & flavour scores • Vinegar decreased pH & TBA	Kook & Kim, 2003
72 pigs were fed with either dietary treatments were control diet with 0.003% chlortetracycline added, and diets containing 0.5% green tea by-product or 0.5% green tea probiotic supplementation.	Sensory evaluation details not available (Abstract only)	Pigs fed diets containing 0.5% green tea probiotic supplementation had lowered meat TBA values compared to those fed 0.5% green tea by-product ($p<0.05$).	Ko et al., 2008
50 pigs fed sunflower oil, linseed oil and a 1:1 (w/w) mixture of linseed oil and olive oil. Also either 20 or 220 mg α-tocopheryl acetate/kg diet	TBA test by a spectrophotometric method	Hams from animals fed on diets with added linseed and α-tocopheryl acetate (20 mg/kg diet) (batch L) have higher TBARs.	Santos et al., 2008
60 pigs fed low, medium and high sunflower oil (SFO) diets (0.9%, 1.8% and 3.6% (SFO) and low, medium and high CLA diets (0.9%, 1.8% and 3.6% CLA).	TBA test by a spectrophotometric method	Improved oxidative stability was observed in sausages from CLA diets.	Marco et al., 2009
36 pigs were fed with either 0%, 0.25% or 0.5% of CLA in their base diet.	TBA test by a spectrophotometric method	CLA increased the oxidative stability of their product.	Corino et al., 2003
20 pigs were randomly assigned four diets containing 0 (control), 1, 2.5, or 5% CLA in their base diet.	TBA test by a spectrophotometric method	CLA increased the oxidative stability of their product.	Joo et al., 2002
288 pigs were fed 3 CLA levels (0%, 1% and 2%) and 2 MUFA levels (19% and 39% average)	TBA test by a spectrophotometric method	Increased TBARS levels in pork products with increased CLA content	Martin et al., 2008

Table 2.6

A summary of the results of studies that have investigated the effects of dietary CLA on the chemical and sensory characteristics of pork and pork products

Animal and Experimental design	Measurements and their methods	Results and Conclusions	References
144 pigs were randomly assigned to a 3 × 2 factorial design, consisting of supplemented fat (3 sources of supplemental fat (SF) and 2 sources of linoleic acid (LA): 1) 0% SF + 1% LA; 2) 0% SF + 1% CLA; 3) 4% YG + 1% LA; 4) 4% YG + 1% CLA; 5) 4% tallow + 1% LA; 6) 4% tallow + 1% CLA.	6 trained panellists evaluated bacon samples and pork loin chops using a 14-point universal intensity scale.	The trained taste panel identified minor differences in the flavour of bacon and pork from pigs fed CLA that would be unlikely to be detectable by consumers.	Gatlin et al., 2006
60 pigs were fed medium and high sunflower oil (SFO) diets (0.9%, 1.8% and 3.6% SFO) and low, medium and high CLA diets (0.9%, 1.8% and 3.6% CLA) in a 3 x 3 factorial.	FAMES were analysed by GC.	• Fatty acid profiles were altered in the sausages following all treatments. • SFO in the diet also resulted in increases in CLA in the sausages. • CLA supplementation resulted in increased saturated fatty acid content.	Marco et al., 2009
40 pigs were fed wheat/barley/ corn/soybean meal as control or supplementation with CLA (2%).	8 trained panellists assessed tenderness, juiciness, and flavour (intensity and quality) and palatability (intensity and quality).	• No effect on juiciness, tenderness & palatability. • Increased flavour intensity & quality with CLA. • The CLA group had lower ultimate pH,PUFA content, n-6/n-3 PUFA ratio, higher CLA content.	Migdal et al., 2004
60 pigs were fed corn/soybean meal as control or supplementation with CLA (0.75%).	8 trained panellists evaluated samples for tenderness, juiciness, and flavour intensity based on an 8-point descriptive scale	No effect on tenderness, colour, juiciness and flavour intensity	Wiegand et al., 2001
54 pigs were fed wheat/barley/soybean meal as control or supplemented with CLA (2%) or sunflower oil (2%).	6 semi-trained panellists evaluated the samples for flavour, texture, desirability and palatability on a scale from 1 (none) to 9 (extreme).	No effect on tenderness, juiciness, flavour desirability or intensity & overall palatability	Dugan et al., 1999

Table 2.6 (continued)

180 pigs in 6 treatments with 3 levels of CLA (0, 0.25, and 0.5%) and two levels of total oil (2 and 5% made up with canola oil).	6 trained panellists evaluated samples for tenderness, juiciness, and flavour intensity based on a 9-point descriptive scale	Supplementing diets with CLA in combination with canola oil did not have any detrimental effect on pork quality, composition or palatability.	Dugan et al., 2004
59 pigs were fed on diets containing either animal plus plant products (the animal group) or plant products only, with or without a supplement (0.31% of the diet) containing extra CLA, selenium, and vitamin E.	13 trained panellists evaluated the samples using a 10-point scales (0 = "none", 10 = "strong")	A rancid odour that was greater for the supplemented plant group compared with the control plant group (25 vs. 12%).	Janz et al., 2008

c. Reduction of lipid oxidation in pork from pigs fed supplemental PUFA

There are several ways of minimising lipid oxidation in pork by including an antioxidant into the animal feed (Decker & Xu, 1998; Morrissey et al., 1998). These antioxidants, which can range from commercial phenolic antioxidants to more exotic compounds isolated from natural food products, have been described with respect to their use in meat systems in reviews by Gary & Crackel (1994), Gray & Pearson (1994) and Mielche & Bertelsen (1994). Vitamin E and selenium are examples of antioxidants frequently used as supplements in animals' diet.

Vitamin E

Vitamin E-supplemented diets are often used with pigs to increase oxidative stability and thus improve the flavour and enhance shelf-life of pork products (Asghar, Gray, Booren, Gomaa, Abouzied, & Miller, 1991; Jensen, Flesnted-Jensen, Skibsted & Bertelsen, 1998).

Dietary vitamin E can stabilise the membrane lipids and consequently enhance the quality of meat during storage. Elevated concentrations of α-tocopherols in the cell membranes, especially in the mitochondria and the microsomes, result in a significantly lower susceptibility to lipid oxidation (Monohan et al., 1990a,b; Ashgar et al., 1991a). The antioxidant potential of tea catechins has been proven in raw and cooked pork patties, and dietary tea catechins have also been shown to be an effective alternative to vitamin E in chickens as well as pork (McCarthy et al., 2001a, McCarthy et al., 2001b, Tang et al., 2001a , Tang et al., 2001b).

Table 2.7 summarises the results of selected studies that assessed the effects of vitamin E-supplemented diets on pork flavour. From the results of the 14 studies summarised in Table 2.7 it is concluded that:

1. α-tocopherol acetate has frequently been used as a dietary vitamin E derivative.
2. Supplementation of pig diets with vitamin E at a concentration of 200 mg/kg feed can reduce lipid oxidation in pork and pork products.
3. The susceptibility of fatty acids in pork to oxidation is lower in pork from pigs fed diets supplemented with vitamin E.
4. Supplementation with vitamin E at a level of at least 200 mg/kg feed can reduce rancidity and improve the colour of the pork. One study show that 100 mg of vitamin E per kilogram improved eating quality with better oxidative stability (Sheard et al., 2000).

Selenium

The most important metabolic role of selenium in mammalian species is its function in the active site of selenoenzyme glutathione peroxidase (GSHPx). This enzyme, together with superoxide dismutase and catalase, protects cells against damage caused by free radicals and hydroorlipoperoxides (Flohe, 1971). GSHPx like most other enzymes, lose their activity by heating (Lindmark-Månsson et al., 2001 and Mei et al., 1994). To date there is limited study on the role of different GSHPx in modulating lipid oxidation in meat. Lee, Mei, and Decker (1996) reported that added GSHPx might diminish lipid oxidation in cooked turkey muscle. Antioxidant potential of selenium has also been shown in the study in chicken and duck by Hoac, Daun, Trafikowska, Zackrisson & Åkesson (2006) who had shown that there was a reciprocal relationship between TBARS formation and GSHPx activity and that addition of GSHPx could decrease lipid oxidation.

Vitamin C

Vitamin C, also known as ascorbic acid (AA), is a well-known antioxidant. It has been suggested to act synergistically with tocopherol to regenerate the tocopheryl radicals. Vitamin C may scavenge the peroxyl radical and inhibit cytotoxicity induced by oxidants. In addition, it can reduce or prevent H_2O_2-induced lipid peroxidation and the formation of 8OH-deoxyguanosine (Retsky & Frei, 1995; Tsou, Chen, Liu & Yang, 1996). AA can cause strand breakage in DNA in the presence of oxygen and can initiate cell death in tissue culture, possibly through the generation of H_2O_2. Reactions of AA with metals such as $Cu^{2+}$are thought to lead to the production of H_2O_2 (Zhao and Jung, 1995).

However, depending on its concentration, vitamin C either promotes or inhibits lipid oxidation in muscle foods. It has been reported that neither short-term (Ohene-Adjei, Bertol, Hyun, Ellis, Brewe & McKeith, 2001) nor long-term vitamin C supplementation (Gebert, Eichenberger, Pfirter & Wenk, 2006) to pigs affects the colour and water holding capacity of pork. In addition, there is no evidence to suggest that supplementing swine diets with vitamin C improves the oxidative stability of lipids of pork during storage or retail display (Gebert et al., 2006). Ohene-Adjei et al (2001) reported that feeding pigs diets supplemented with increased levels of vitamin C elevated TBARS values of pork chops during refrigerated storage."

Table 2.7
A summary of the results of studies that have investigated the effects of dietary vitamin E for pigs on the oxidative stability of pork

Animal and Experimental design	Results and Conclusions	References
24 pigs (12 male, 12 female), weighing about 70 kg were fed a control diet containing 30 mg α–tocopherol acetate (α–TAC) /kg feed or a diet with 200 mg α–TAC /kg feed for 2 weeks.	Oxidative stability of raw and cooked meat and membrane-bound lipids increased for the 200-mg group.	Monahan et al., 1990
64 male pigs (3 weeks old) were fed diets containing 3% beef tallow or 3% soya oil with either a basal (10-50 mg/kg diet) or supplemented (200 mg/kg diet) level of α–TAC.	▪ α–TAC supplementation significantly increased oxidative stability; ▪ higher C18:2/C18:1 ratios led to increased oxidation rates	Monahan et al., 1992
Muscle microsomes from 18 pigs (4 months old) of about 30 kg in weight and fed an α-TAC-supplemented diet (200 mg /kg diet) were compared with those from pigs fed a control diet (10 mg α-TAC-supplemented diet).	Susceptibility of pork lipids to oxidation during refrigerated storage was significantly lower in chops from pigs fed the supplemented diet.	Monahan et al., 1993
1220 pigs were fed diets containing either a basal (40 mg/kg of feed) or supplemented (200 mg/kg of feed) level of α-TAC.	▪ α-TAC was higher in muscle and adipose tissue from the supplemented group and TBARS in muscles were lower. ▪ α–T decreased with lipid oxidation in adipose tissues.	Pfalzgraf et al., 1995
Two dietary treatments were compared: (1) 60 mg of α-TAC/kg of feed from 20 to 100 kg live weight; and (2) 60 mg of α-TAC/kg of feed from 20 to 45 kg live weight and 200 mg of α-TAC/kg of feed from 45 to 100 kg live weight (10 and 11 weeks preslaughter).	α–TAC -supplemented samples were lighter and more red, tasted fresher, and extra α̃TAC supplementation increased oxidative stability.	Dirinck et al., 1996
72 pigs at a mean weight of 44 kg were assigned to a tapioca based diet, which contained 8 mg α–TAC/ kg feed, or to the same diet supplemented with 200 mg α-TAC/kg feed.	Vitamin E levels were 5 times higher in muscles of supplemented group, with reduced TBA values after frozen storage.	Hoving-Bolink et al., 1998
48 female pigs were fed with diets with 6% sunflower oil (78% oleic acid) supplemented with 100 and 200 mg of α-TAC/kg of feed	α–T levels were higher with vitamin supplementation; oxidative stability was not affected by increasing α–T, but colour stability increased.	Zanardi et al., 1999

Table 2.7 (continued)

82 female pigs were reared on ten different diets, including either a control diet (no supplementation of rapeseed oil, $CuSO_4$ or vitamin E) or 6% rapeseed oil diets supplemented with $CuSO_4$ (0, 35 or 175 mg/kg) and vitamin E (0, 100 or 200 mg α–TAC/kg).	Rapeseed oil in diets increased amount of MUFA and PUFA. Vitamin E significantly increased oxidative stability of pork chops.	Jensen et al., 1998
12castrated pigs were fed diets containing either a basal (10 mg/kg of feed) or supplemented (400 mg/kg of feed) level of α-TAC.	α–T concentrations in muscles and hams were higher in pigs fed the supplemented diet than in those without.	Harms et al., 2003
12 female pigs in a 2x2 factorial design with dietary α-TAC (10 or 200 mg/kg feed) and dietary fishmeal (0 or 5%).	200 mg of α–TAC /kg reduced lipid oxidation. Wood smoke also reduced oxidation.	Coronado et al., 2002
80 male pigs were fed *n*3 fatty acid-enriched diets (in the form of 0.5% linseed oil with either 1.5% sunflower oil or 1.5% olive oil) and *α*-TAC Supplementation (200 mg/kg feed).	α–TAC supplementation reduced lipid and cholesterol oxidation in cooked pork.	Rey et al., 2001
30 female pigs were fed six experimental diets containing 3 levels of poly and monounsaturated fatty acids. Within each dietary fat treatment, one group was fed a basal level of vitamin E (20 mg α-TAC/kg diet) and the other group received a supplemented level (200 mg α–TAC/kg diet).	α–TAC supplementation increased tissue α–T level and decreased oxidation in dry-cured hams from animals fed elevated levels of MUFA and PUFA.	Isabel et al., 2003
240 male and female pigs were fed either 12, 55, 99, 174, and 351 IU/kg of α-TAC for 6 weeks prior to slaughter.	α–TAC supplementation improved oxidative stability in vacuum-packaged chops displayed over a period of 8 days.	van Heugten et al., 2003
Pork chops, liver, bacon and sausages were made from 80 male and female pigs fed on a control or a linseed-rich test diet with 0 or 100 mg α–TAC/kg diet.	Improved oxidative stability in pork and pork products. No detection of adverse eating quality parameters.	Sheard et al., 2000

2.3.3.2 Diet effects on pork flavour through effects on hind gut digestion

Indolic compounds such as indole and skatole are produced in the gastrointestinal tract by the degradation of the amino acid, tryptophan followed by absorption and deposition in the meat. Some studies suggest that indole is the predominant metabolite resulting from tryptophan degradation (Yokoyama & Carlson, 1979). A wide range of bacterial species are capable of producing it, including *Escherichia coli, Proteus vulgaris, Paracolobactrum coliforme, Achromobacter liquefaciens, Micrococcus aerogenes* and certain rumen protozoa.

Skatole goes through several stages before it accumulates in pork fat. While many bacteria produce indole, very few produce skatole. In fact, six known species produce skatole: *Clostridium scatologenes, Clostridium nauseum, Pseudomonas sp., Rhizobium sp., Lactobacillus helveticus and Lactobacillus sp. strain 11201* (Honeyfield & Carlson, 1990). In fact, Lactobacillus sp. strain 11201 present in the normal intestinal flora of pigs (Yokoyama et al., 1977) is generally recognised as the agent responsible for producting skatole causing boar taint in pigs. Skatole-producing bacteria comprise less than 0.01% of total intestinal flora (Borg, Jensen and Jensen, 1993).

The first stage in skatole production in pigs is the ingestion of proteins containing L-tryptophan. Some sources say that this stage plays an important role in the rate of skatole deposition in fat (Babol & Squires, 1995; Claus, Weiler & Herzog, 1994), while others see no direct link (Moss, Hawe & Walker, 1992; Hawe, Walker & Moss, 1991, 1992). L-Tryptophan originates mainly from the diet, but may also originate from degradation of the intestinal mucosa. This mucosa is known to have a very high turnover rate and the resulting cell debris, containing L-tryptophan, could very well be used by indoleacetic acid producing bacteria (Claus et al., 1994).The skatole produced can remain in the intestinal content, be excreted or be absorbed by the intestinal mucosa. The degeneration products of absorbed skatole are excreted in the urine. Skatole that is not metabolised will accumulate in adipose tissue, the liver and the kidneys (Friis, 1993). No traces of skatole have been detected in urine. Correlations have been demonstrated between concentrations of skatole produce in the gut, blood

and liver and the concentration of skatole in adipose tissue (Borg Jensen and Jensen, 1993).

Indole and skatole are known to possess sensory characteristics that are undesirable to consumers, and that have been described to be acrid, mothballs, sweaty, ammonia, musty, parsnips, nosefeel, dirty, body reaction and silage (Anne-Frempong et al., 1997). Through diet manipulation, it is possible to change the metabolism of the microorganisms in the gastrointestinal tract so as to reduce skatole production. Some researchers have used herbs and spices to mask the smell through marination (Lunde et al., 2008) or supplementation in the pig's diet (Janz et al., 2006), while others have used fibre and lactobacillus to reduce metabolite formation in the intestines, or have included items in the feed of pigs to influence the degradation of skatole and other indolic compounds in the liver (Hansen et al., 2000, 2008; Oeckel et al., 1996; Byrne, 2007; Stolzenbacha et al., 2009; Lösel et al., 2006). Other factors such as ad libitum water consumption, cleanliness, good ventilation, low-fiber diet and use of antibiotics all tend to reduce skatole concentrations in fat (Lundstrom et al., 1988; Hansen et al., 1994).

Several factors affect the rate of skatole deposition in fat. Nonboe (1990) showed that brewery by-products led to high skatole deposition rates in male pigs while distillery by-products had no impact on skatole deposition rates. A diet rich in yellow peas can increase the rate of skatole deposition (Lundström et al., 1994). Lactose may reduce the rate of skatole deposition by acidifying the contents of the intestine (Hawe et al., 1992), but these effects seem to be more indirect than direct (Moss et al., 1992). The slower the fecal excretion rate, the higher the skatole production rate becomes (Claus et al., 1994). The addition of antibiotics to the diet, especially polyether antibiotics, may be an effective way to reduce skatole synthesis from L-tryptophan (Claus et al., 1994).

Genetics can also be another factor in skatole deposition. Lundström et al. (1994) hypothesized that the *ska*[1] gene, which is probably a major, recessive gene, was responsible for skatole deposition in fat. A stressful environment with high numbers of animals per pen and high temperatures, would activate the gene's expression (Hansen et al., 1994). The expression of this gene could reduce the effectiveness of the enzymatic chain of skatole degradation in the liver (Lundström et al., 1994). This is also suggested

in the work by Squires and Lundström (1997) showing that carcasses with high skatole deposition rates have low activities of liver cytochrome P450 2E, which is responsible for skatole metabolism in the liver.

The microsystem in the gastrointestinal tract in the pigs can be changed by feeding pigs with fermented liquid, so that in some cases, the skatole production in the gastrointestinal tract is reduced and this reduces the skatole concentration in back fat (Jensen, Jensen, Agergaard, Hansen, Mikkelsen & Laue, 2000; Hansen, Mikkelsen, Agerhem, Laue, Jensen, & Jensen, 1997). Fermented liquid feed containing a large population of lactic acid bacteria and yeast cells, and having a low pH, and high concentrations of lactic acid (Geary et al., 1999; Mikkelsen and Jensen, 1997) has been shown to change the composition and activity of the gastro-intestinal microbiota compared with pigs given dry or non-fermented liquid feed (Mikkelsen and Jensen, 1997; Jensen et al., 1998).

Table 2.8 summarises the results of 10 selected studies that have assessed methods to reduce production of skatole and other indolic compounds in pork. From the results of the 10 studies summarised in Table 2.8 the following conclusions can be drawn:

1. The inclusion of plant materials such as chicory, lupines, sugar beet pulp and raw potato starch in the diet can reduce skatole concentrations in the pork.
2. Liquid smoke and marination of pork with herbs and spices like oregano, garlic and paprika can mask boar taint flavour up to a skatole concentration of 0.7 ppm.
3. Fermented liquid feed with zinc bacitracin decreased skatole concentrations in pork.
4. Liver cytochrome P450 2E1 is responsible for skatole metabolism.

Table 2.8
A summary of the results of studies that have investigated methods of reducing skatole and other indolic compounds in pork

Animals and experimental design	Measurements and methods of analysis	Results and conclusions	References
80 pigs fed either a diet containing (i) crude chicory roots, (ii) crude chicory roots, dried chicory roots, or inulin; and (iii) a dried chicory diet.	Skatole in back fat and blood plasma was measured using colorimetry and HPLC respectively.	Skatole concentrations in blood plasma and back fat at slaughter were reduced to almost zero levels by including crude or dried chicory or inulin in the diet.	Hansen et al., 2006
Pork from 16 pigs was marinated with oregano oleoresin, garlic powder, bacon flavour, paprika extract. Smoke flavour was used with one neck sample.	Skatole and androstenone were measured using colourimetry and ELISA methods respectively. 10 trained panellists evaluated the samples using intensity scores from 1 to 9; where 9 corresponded to the highest intensity score.	• Marinated chops with skatole content of approximately 0.4 ppm appeared similar to castrates for boar taint. • Chops with skatole contents above 0.7 ppm remained unmasked despite the use of strongly flavoured marinades. • Unmarinated chops served at 60^{o}C were more tainted than those served at 15^{o}C,but scored lower for boar taint when reheated. • Oregano oleoresin and liquid smoke decreased the perception of boar taint.	Lund et al., 2008
Model and commercial type Swedish fermented sausage products based on low or high levels of boar tainted fat. Three different starter cultures and two different levels of smoking were studied.	Androstenone and skatole analysis by HPLC analysis. A 15-cm unstructured line scale anchored from “none” to “extreme” was used.	• In the model sausages, liquid smoke masked the perception of boar taint. The smoking procedure of the commercial sausages was insufficient to totally mask the perception of boar taint. • In both types of sausages, aroma development from the starter cultures lowered the perception of boar taint but was insufficient for total perceptual masking.	Stolzenbacha et al., 2009

Table 2.8 (continued)

48 pigs were fed three organic diets containing different fermentable fibre-rich feedstuffs - 10-13.3% dried chicory roots or 25% blue lupines.	10 trained panellists evaluated the samples on a 150 mm unstructured line scale from 'none' to 'very much'.	• Lupines significantly reduced skatole in blood and back fat for both genders after 1 week. • Indole concentration was significantly lower in chicory- than lupine-fed pigs. • Chicory and lupine feeding reduced boar taint. • Boar taint was most effectively reduced after 14 days. by both fibre-rich feeds. • Lupine had the largest influence on "boar" taint reduction in female pigs.	Hansen et al., 2008
60 pigs were fed diets with either 15% sugar beet pulp; 30% wheat bran; or 15% soybean hulls.	Skatole content in the back fat was determined using HPLC	• Back fat skatole and indole contents and boar taint scores were not influenced by diet. • The wheat bran diet group gave higher back fat skatole content than the sugar beet pulp diet group.	Oeckel et al., 1996
64 pigs were fed either (i) crude chicory for 4 and 9 weeks or (ii) crude, dried chicory and inulin for 6 weeks prior to slaughter.	Skatole and indole were measured using HPLC while androstenone was measured using an immunochemical method.	Feeding treatments significantly reduced perceived sensory boar taint in the cooked pork meat.	Byrne et al., 2008
80 pigs were fed either a conventional diet containing grain and soy; or a diet supplemented with raw potato starch (300g/kg of body weight).	Skatole and indole were measured using HPLC. 8 trained panellists evaluated samples for odour of meat juice and meat on a 5-point scale ("very unpleasant" to "very pleasant").	• Raw potato starch reduced skatole concentrations in colon content and blood plasma. • Skatole concentrations in back fat were decreased significantly from 25 to1.40 ng/g. • Odour rating was 3.07 for low skatole concentrations and 2.66 for both medium and high skatole concentrations.	Lösel et al., 2006

Table 2.8 (continued)

108 pigs were fed barley/wheat/ soybean meal with fermented liquid feed (FLF) ± Zn bacitracin and non-pelleted dry food (NPDF).	Skatole and indole were measured using colorimetry.	• FLF demonstrated no effects on skatole concentration in caecum, colon, blood and back fat and boar odour attributes, whereas administration of FLF + ZB decreased the skatole concentrations and the typical boar odours, pig and manure odour, compared especially with the pigs on NPDF.	Hansen et al., 2000
18 pigs were assigned to either: (i) a Low-Non-starch polysaccharide(NSP) diet (87 g/kg of NSP) or (ii) a High-NSP diet (160 g/kg of NSP) with sugar-beet pulp as the NSP source.	Skatole and indole were measured using HPLC.	• Dietary NSP-inclusion reduced skatole concentration. • A dietary effect of NSP on the indole concentration was reflected in blood samples only. • The absorption of skatole and indole was significantly lower in pigs given the high-NSP diet compared with those offered the low-NSP diet.	Knarreborg et al., 2002
82 pigs (74 males and 8 females) were used to determine the relationships between levels of skatole and androstenone in fat, plasma levels of skatole metabolites, plasma testosterone and estrone sulfate, and levels of cytochrome P450IIE1 in liver.	Skatole was measured using a colourimetric assay.	• Low levels of cytochrome P450IIE1 in liver may result in high levels of skatole in back fat of uncastrated male pigs due to decreased metabolism and clearance of skatole.	Squires & Lundstrom, 1997

2.3.3.3 Diet effects on liver metabolism

Studies concerning skatole have shown that the activity of the cytochrome P450 enzyme system in the liver plays an important role in determining the skatole concentration in pork (Babol, Squires, & Lundström, 1998a, 1998b; Hak, Hansen - Møller, Friis, Cornett, & Hansen, 1997). Pigs having a metabolic deficiency with one of the P450 enzymes have a lower ability to degrade skatole, thus resulting in a higher concentration of skatole in meat and fat. It has been shown that the activity of cytochrome P450 isoenzymes can be stimulated by certain compounds (glucosinates and its hydrolysis products) found in foods such as the cruciferous vegetables like cabbage and Brussel sprouts (Verhoeven, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997). Brussels sprouts can stimulate the metabolism of the liver (Sørensen et al., 2001) and thereby possibly increase the degradation of compounds that impact on the sensory quality of meat.

In conclusion, nutritional means to manipulate pork quality are possible but in many instances more work needs to be done to ensure nutritional and sensory quality of pork is not compromised and that these are profitable in a commercial setting. Integration of industry efforts to solve problems and produce quality pork will, therefore, also need to be met by greater integration of genetics, nutrition and meat quality research.

2.4 Conclusions

To market meat of better eating quality, extensive knowledge about the effects of various factors on meat quality is required. With a holistic approach on breed and genetic selection; and manipulation of dietary feeding systems, we will then be able to understand the influence of production, pre- and post- mortem factors on pork quality, which subsequently can be used in the control of the quality of pork products in the future. Ultimately, the implementation of strategies to improve meat quality depends on the willingness of consumers to purchase and pay more for meat of better quality. Certainly, improvement of meat quality by optimizing various key factors will not only be appreciated by the consumer but will also be profitable to the meat industry.

Chapter 3

A survey of the perception of pork by Singapore consumers

3.1 Introduction

Pork is a popular meat consumed by non-muslim Singaporeans with the amount consumed every year (about 87,000 tonnes) being worth $340 million (Kanagalingam, 2005). Currently, Singapore imports its pork mainly from Indonesia and Australia. Fresh pork is obtained from Indonesia, with live pigs being imported and slaughtered at Singapore abattoirs. Chilled pork is mainly derived from imports from Australia. Other countries that supply frozen pork to Singapore include North America, Canada, France, Holland and New Zealand. Singapore is a major market for Australian pork, taking about two thirds of their total monthly exports of around 3000 tonnes (Ronan et al., 2001).

Singapore's requirement for pork and Australia's proximity to Singapore and ability to supply small volumes on a daily basis are key factors in the trade. Singaporeans have a strong preference for fresh or chilled pork rather than frozen (Agri-Food Canada, 2006). North America and Europe are too far away to land chilled pigmeat that still has an acceptable shelf-life. Chilled Airpork from Australia is sold in supermarkets and typically eaten within three or four days of slaughter. Australian chilled pork is sold in many supermarkets in Singapore, along with Indonesian pork.

The objective of this study was to investigate consumer liking and perceptions related to pork consumption in Singapore by means of a consumer survey. The main emphasis was to answer the following questions:

1. What are Singaporean consumers' main reasons for buying and consuming pork?
2. What are their pork purchasing patterns?
3. Which aspects in pork are considered as undesirable?
4. Do Singaporean consumers consider that pork products from different countries have different characteristics?

3.2 Materials and methods

3.2.1 Survey

Consumers were pork eaters older than 15 years of age and chosen at random at a range of sites, including supermarkets and workplaces. In total 202 consumers completed the survey.

Consumers had to complete a socio-demographic questionnaire (16 questions) including questions about purchasing and consuming pattern of pork, reasons for purchasing pork and undesirable aspects of pork from various countries namely Australia, Canada, New Zealand, China, Indonesia, Brazil and USA.

All statistical analyses were carried out using SPSS ver. 17 (SPSS Inc, Singapore). The frequencies were expressed as percentages of the total for the following: gender, race, household income per month, dwelling type, and number of people in the household. Chi-square test for goodness of fit was used to determine the significance: among reasons for purchasing and consuming pork as well as undesirable aspects of pork among countries. Ratings of relevance of undesirable flavour terms associated with pork from different countries of origin were analysed by ANOVA (Type I Sums of Squares) at 5% level of significance using the General Linear Model (GLM) procedures to determine differences among the country groups. Discriminant analysis was carried out to investigate the association between unfavourable-attribute scores and countries of origin of pork.

3.3 Results

3.3.1 Description of the participants

The survey was conducted in January 2007 and 202 respondents filled out the survey form completely. The results can be found in Table 3.6 (data in the SN column). There were in total 87% were females and 13% males; 91.1% were Chinese and the balance Indians. There were almost equal proportions of married and single parpticipants, mainly from the under 40s age bracket. They stayed mainly in Housing Development Board apartments (HDB) where 84% of Singaporeans live (HDB, 2007). In addition, most had four to five members in the household (>30%). Few (9%) consumers were living with grandparents and most (>35%) were living with their spouse and children. Nearly 23% of the households had other people living with them and they were usually the live-in helpers from Indonesia or Philippines. About 30% of the households (32.6%) had a monthly income of more than $5000 Singapore dollars.

3.3.2 Purchasing and consuming pattern for pork

Almost half the respondents (43.6%) did the meat shopping for their household, with the majority of the purchases being made at supermarkets (65.1%). Only 7% did the purchasing from meat speciality shops. Those who did not shop for their pork depended on other members of their household to do the shopping and cooking. It would mainly be the foreign domestic worker (FDW) doing this job as they play an important role in household chores like cooking for the family of their employers. In 2006, there were approximately 160,000 FDWs working in Singapore, i.e., about one in six households employed an FDW (MOM, 2007). Most respondents (85%) consumed pork more than once a month, whilst only 9% ate pork every day (Table 3.6).

The most popular method for cooking pork (Table 3.1) was boiling (69.1%). The Chinese population uses this method mainly in making soup with other herbs and spices. The next most popular method was pan-frying (43.2%) and stewing (45.2%). Microwaving (Other category) was the least often used method for cooking of pork (1.2%).

Table 3.1
Percentage of respondents who used different cooking methods for pork (Chinese population only)[1]

Cooking method	Percentage of respondents who used the cooking method (%)
Grill	10.6
Pan-fry	43.2
Roast	32.1
Stew	45.2
Boil (soup)	69.1
Deep fry	37.5
Others	1.2

[1] Based on Chi-square test for goodness of fit, there were statistically significant differences among the cooking methods as $p < 0.05$.

3.3.3 Reasons for buying pork

Results in Table 3.2 show that Singapore consumers in this survey liked pork for a variety of reasons with "taste" cited most often (69.0%). This was followed by "availability" (38.4%), "nutrition" (29.3%), "versatility" (28.8%) and "price" (12.5%).

Table 3.2
Reasons for purchasing and consuming pork by Singapore consumers (Chinese population only)[1]

Reason for purchasing pork	Percentage of respondents who gave this as a reason.
Availability	38.4
Nutritional quality	29.3
Versatility in cooking	28.8
Taste	69.0
Price	12.5

[1] Based on Chi-square test for goodness of fit, there were statistically significant differences among reasons for purchasing pork as $p < 0.05$.

The survey results suggested that Singapore consumers depend on information about the country of origin of the pork as an indicator of the taste of the pork, which was the main reason given for purchasing pork (Table 3.2). Results in Table 3.3 indicate that pork from China (77.3%) and Indonesia (82.7%) was considered as tastier compared to that from Western countries. Pork is easily available for purchase at all supermarkets and wet markets in Singapore. In supermarkets, chilled pork from Australia is the most common sight on the shelves. In wet markets, fresh and chilled pork from Indonesia and Australia respectively can be purchased. The available cuts were mainly loin, fillet, ribs and shoulder butt. Canada, Brazil, New Zealand, France and US pork can be obtained from selected supermarkets and meat speciality shops. More specialty cuts of pork are also available at these shops. Singapore consumers can also buy New Zealand pork from selected organic food shops.

Versatility (28.8%) and nutritional quality (29.3%) scored almost equally as reasons for purchasing and consuming pork. The versatility of pork in cooking is illustrated by results in Table 3.2. The price of pork is relatively cheaper than beef and mutton in Singapore, with pork prices ranging from $13 - $22 per kg for fresh pork (Indonesia) and $16 - $22 per kg for Australian chilled pork. The price was also dependent on the country of origin, with Canadian pork at about $18 - $25 per kg and New Zealand pork (organic) at $25 – 30 per kg.

Australian pork had a score of less than 50% for its perception as a “tasty meat” (Table 3.3). Consumers purchased Australian pork because “it is easily available” (85.4%), “safe to be consumed” (84.2%) and “reasonably cheap” (68.2%). Canadian pork, on the contrary, was not seen as readily available by the respondents (15.1%). In addition, it scored low in flavour (27.4%) and was not considered cheap (23.2%) by these Singapore consumers. Unlike New Zealand beef, New Zealand pork is only available for sale in meat speciality and organic food shops. At the point of survey, *Trim Pork* from New Zealand was available at some Cold Storage supermarket outlets. New Zealand pork scored 90.3% for being “safe to be consumed”, which was a far cry from the score by China pork. Indonesian pork had a score of 75.9% in “versatility to use in cooking”, a characteristic that was probably associated with its high score as a “tasty meat” (Table 3.3).

Table 3.3
The frequency (%) with which respondents agreed with various "reasons for purchasing and consuming pork", when the pork was from China, New Zealand, Australia, Indonesia, Canada or others countries[1] (Chinese population only).

Product features	Country-of-origin (% that responded for each feature) [1]					
	Australia	Canada	China	Indonesia	New Zealand	Others (Brazil, USA)
It is easily available	85.4	15.1	50.2	69.2	9.3	10.0
It is versatile to use in cooking	66.5	55.5	73.4	75.9	62.9	60.3
It is reasonably cheap	68.2	23.2	78.0	73.7	20.9	24.5
It is a tasty meat	46.3	27.4	77.3	82.7	33.8	35.2
It has a good texture	50.4	45.3	70.1	69.0	43.9	46.5
It is safe to be consumed	84.2	86.9	10.7	67.9	90.3	54.7

[1] Responses to the question: "What are your reasons for purchasing and consuming pork from these countries? (China, New Zealand, Australia, Indonesia, Canada and others)"

Pork from other countries like Brazil and USA was only available in selected shops, and was not common in the main Singapore supermarkets with popular household names like Fairprice and Cold Storage. Pork from these countries had a score of less than 40% as "a cheap meat" and "tasty meat". In terms of texture, the purchasing intent and consumption scores were similar for Australia, Canada, New Zealand, Brazil and USA with close to 50% of the respondents giving this as a reason.

3.3.4 Undesirable aspects of pork

Safety aspect was a concern for pork from Indonesia and China, where China was given a greater concern for this aspect (Table 3.4). Consumers were generally with the safety of pork from Australia, New Zealand and Canada.

Singapore consumers, as represented by the current respondents, considered flavour and price as the least desirable characteristics for pork from the western countries (Table 3.4).

The specific flavour and taste sensory attributes associated with undesirable aspects of pork flavour shown in Table 3.5 were scored on a scale from 1 to 5, where 1 was most relevant and 5 was the least relevant. Mutton flavour was considered as the most relevant attribute for pork from Australia (1.32), Canada (1.30) and New Zealand (1.26).

Discriminant analysis was carried out to investigate the association between unfavourable-attribute scores and countries of origin of pork. The first and second functions accounted for 95.9% of the variance. Function 1 accounted for 85.8% of the variation and related mainly to mutton-like flavour, while function 2 accounted for 10.1% of the variation and reflected on acidic and metallic flavour. Two distinct groups can be seen in Figure 3.1, the Asian (Indonesia and China) group, and the Western (Australia, Canada, New Zealand, Brazil and USA) group. Function 1 clearly separated the treatments into 2 clusters- the Asian group as one cluster and the Western group as another cluster, based on the high degree of relevance of mutton flavour in pork from the Western group. This observation is also consistent with results in Table 3.3 showing that pork from Western countries scored lower as “tasty pork” compared to that from Asian countries. In contrast to the Asian pork, flavour from the Western cluster was considered as less desirable (Table 3.4).

Table 3.4
Frequency scores for reasons why Singapore consumers dislike pork from various countries of origin[1] (Chinese population only).

Reason for not liking pork	Country-of-origin (% that responded for each feature) [1]					
	Australia	Canada	China	Indonesia	New Zealand	Others (Brazil, USA)
Flavour	14.6	52.3	12.4	20.9	70.2	45.5
Price	32.8	79.2	5.8	35.2	77.9	50.9
Safety reasons	6.7	10.8	90.7	32.1	6.3	35.3
Texture	10.5	25.6	1.5	10.6	27.9	29.6
Other reasons	0.94	1.7	3.8	2.9	1.2	1.1

[1] Responses to the question: "What is the reason for not liking pork from these countries of origin? (China, New Zealand, Australia, Indonesia, Canada and others)"

Table 3.5

Least squares means for degree of relevance of undesirable flavour terms associated with pork from different countries of origin[1] (Chinese population only).

Reason	Country					
	Australia	Canada	China	Indonesia	New Zealand	Others (Brazil, USA)
No.of respondents	38	40	20	14	27	9
Presence of:						
Acidic taste	4.21c	3.95c	2.70b	1.36a	5.04d	4.91d
Aftertaste	2.11a	1.63a	4.00b	4.64b	1.94a	2.18a
Bitter taste	4.24ab	4.55b	4.30ab	4.64b	4.67b	3.82a
Metallic flavour	4.76d	4.48d	1.25a	1.93b	3.67c	3.27c
Milky flavour	1.58a	1.85a	2.75b	4.29c	1.67a	1.18a
Mutton-liked flavour	1.32a	1.30a	4.45c	4.50c	1.26a	3.36b
Stale flavour	2.34b	2.70b	4.90d	4.30c	1.52a	2.64b

[1]All attributes were scored on a scale where 1 = very relevant and 5 = not relevant

[2] Means in the same row with no letters or the same letters after them do not differ significantly ($P < 0.05$) as determined by Fisher's least significance difference (LSD) mean separation test.

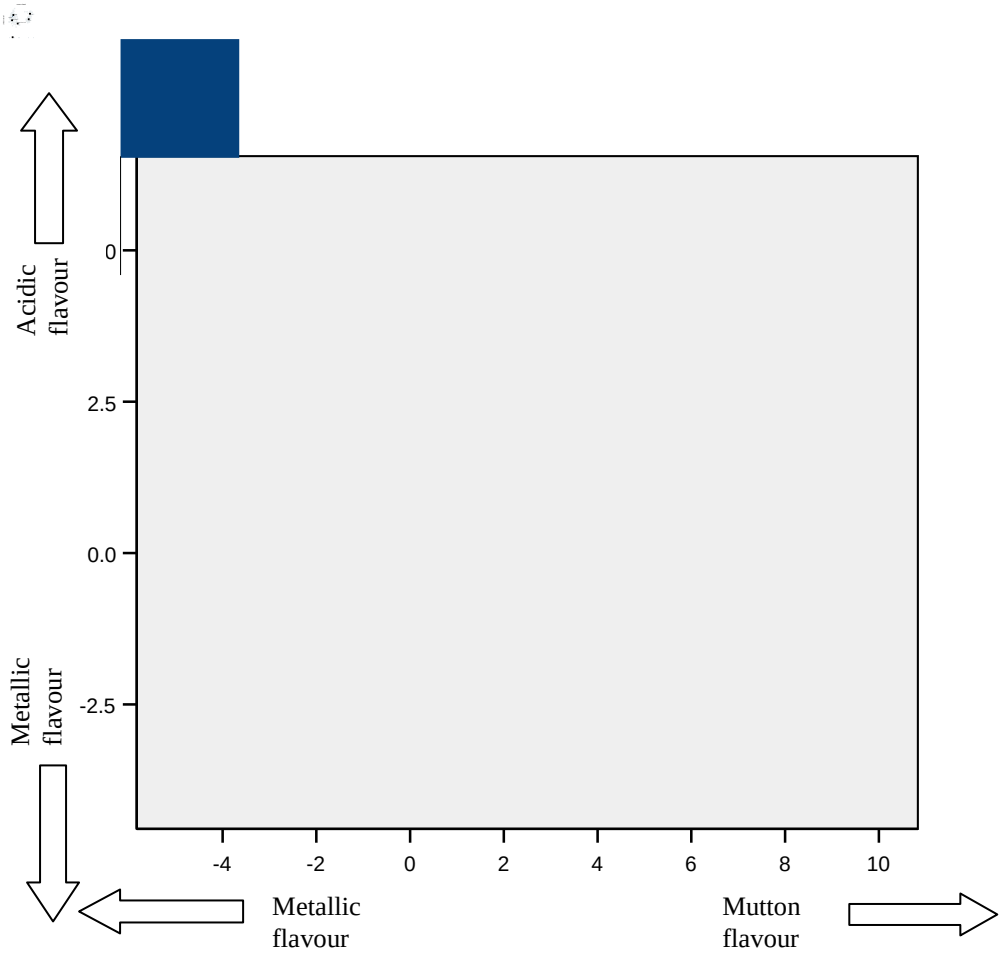

Fig. 3.1
A plot of seven countries relative to the first two discriminant functions made up of relevance scores for the seven undesirable flavour attributes (Table 3.5) as assessed by Singapore consumers. Based on Wilks' Lambda test, significance values of function 1 and function 2 were less than 0.001.

The first and second functions accounted for 95.9% of the variance. Function 1 accounted for 85.8% of the variation and related mainly to mutton-like flavour, while function 2 accounted for 10.1% of the variation and related mainly on acidic and metallic flavour.

3.4 Discussion

In Singapore, more than 40%, of the consumers in this study lived in households of 5 or more people. Few (<10%) consumers were living with grandparents. More than half of Singapore and Korean households had parents living with them. Thus the decision of purchasing pork is not just a personal issue as they need to buy and even to cook for their family members too. They may even have to consider the preferences of their family members towards the country of origin of the pork during purchasing. In Singapore, more than 60% of the consumers purchase pork at the supermarkets which offer a wide variety of pork and its parts from different countries. Thus availability of pork will not be an issue unless Singapore consumers are looking for pork from Canada and New Zealand which are mainly available from meat specialty shops or organic food stores.

Most Singaporeans consume pork at least once a week. Consumers from Asian countries like Korea (64%) and Japan (79%) also tend to eat pork at least once a week (Table 3.6), but this is not the case in Australia (19%), Mexico (34%) and New Zealand (34%). Pork is rarely eaten every day, except in China (50%). Boiling was often used in Singapore (69%). Stewing was the second most popular among consumers in Singapore. This goes in parallel with Asian countries like China. The most popular forms were grilling, frying and roasting for the western countries. Roasting was rare in China (1%), Korea (4%) and Mexico (9%).

The Singapore study also showed that 80% of the consumers like to consume pork (Table 3.6). This was similar to the respondents from China, Japan, Korea, Ireland, Mexico, Australia and New Zealand in the study of Ngapo et al. (2007) (Table 3.6). This indicates that pork is a popular meat for consumers throughout western and Asian countries. According to the USDA's Foreign Agricultural Service, nearly 100 million metric tons of pork was consumed worldwide in 2006. Increasing urbanisation and disposable income has led to a rapid rise in pork consumption in China, where 2006 consumption was 20% higher than in 2002, and a further 5% increase was projected for 2007 (USDA, 2006).

Safety aspect of pork from the Asian countries, particularly China was a big concern to Singapore consumers (Table 3.6). This could be attributed to the recent recalls of many of its products (non-food and food), so that the confidence of consumers might be affected when purchasing products from China. Availability of pork from China is not as great as that from Indonesia for Singapore consumers. Most consumers might not be aware of how pigs from Indonesia are handled before slaughtering, and hence safety becomes a concern. Pigs from Indonesia are reared on an island off Singapore and once they are mature, they are transported to Singapore for slaughtering. Safety concerns for pork from western countries were not a huge issue (Table 3.6).

Texture of pork was not an issue with Singapore consumers as they can use different cooking methods to prepare the pork to ensure a desirable texture. However, price was a main concern for pork from western countries like Canada and New Zealand. At the time of study, the prices of Canadian and New Zealand pork were 2 to 4 times higher than Australian and Indonesian pork, which were very easily available from supermarkets and wet markets in Singapore. Though widely available, Singapore consumers generally found the flavour of Australian pork less desirable compared to that from Indonesia. Therefore, the concerns about of pork flavour warranted a more thorough investigation into what makes Indonesian pork tastier than the Australian equivalent. One of the ways to approach this issue would be to investigate how components in the diets of pigs can influence the overall sensory quality of pork.

Chapter 3

Table 3.6

A comparison of results from the present study (SN – shown in bold in the first data column) against those for seven other countries [1, 2] from Ngapo et al. (2007)

Question	Response options								
		SN	CH	JA	KO	IR	ME	AU	NZ
What is your age?	<35	**55**	50	50	50	50	50	50	-
	>=35	**45**	50	50	50	50	50	50	-
Gender?	Female	**87**	50	50	50	50	50	50	61
	Male	**13**	50	50	50	50	50	50	28
Marital status	Single	**57**	27	42	42	49	27	48	32
	Married	**43**	72	58	58	52	73	52	67
How many people in your household?	1	**0**	1	21	2	6	2	12	9
	2	**7**	13	24	6	27	8	31	35
	3	**12**	64	27	16	24	27	19	18
	4	**26**	12	19	47	32	36	17	17
	5+	**55**	12	7	31	12	28	22	12
They are:	Spouse	**35**	71	54	56	57	73	52	67
	Children	**40**	62	43	52	44	77	43	44
	Parents	**61**	31	25	53	18	15	24	7
	Grandparents	**9**	2	2	8	1	2	1	0
	Others	**23**	9	5	38	16	23	13	9
Where do you purchase pork?	Wet market/Butcher	**28**	75	9	54	30	64	49	33
	Supermarket	**65**	26	89	45	54	45	64	82
	Others like farm	**7**	0	1	1	1	1	1	1
Are you the member of your household who shops for pork?	Yes	**44**	44	53	85	59	61	58	72
	No	**56**	57	47	15	41	39	42	26
Do you like pork?	Yes	**80**	79	87	82	100	93	80	90
	No	**20**	21	9	18	0	7	20	9
For what reasons you like pork?	Availability	**38**	12	27	63	10	1	11	18
	Nutritional quality	**29**	20	37	36	10	2	24	8
	Versatility	**29**	35	46	10	18	3	14	29
	Taste	**69**	42	58	4	99	92	84	85
	Price	**13**	8	41	11	18	4	24	26
How often do you eat pork?	Everyday	**9**	50	4	1	5	0	0	0
	> once/week	**66**	41	79	64	53	34	23	34
	>once/ month	**19**	9	10	29	29	46	45	39
	<once/ month	**6**	1	8	6	11	20	33	24
How do you cook pork	Grill	**11**	0	82	75	78	33	45	53
	Pan fry	**43**	78	27	6	24	64	28	37
	Roast	**32**	1	23	4	23	9	48	32
	Stew	**45**	46	36	6	8	17	12	5
	Boil (soup)	**69**	14	20	9	3	47	1	1

[1]Based on the sub-panels of 200 consumers for all countries except New Zealand (327 consumers) and Singapore (202).
[2]Country codes are Singapore (SN), China (CH), Japan (JA), Korea (KO),Ireland (IR), Mexico (ME), Australia (AU), New Zealand (NZ)

3.5 Conclusions

A consumer survey (n=202) was used to obtain an improved understanding of the perception of pork by Singapore consumers. The consumers liked pork for a variety of reasons, but taste and availability were the main reasons for buying and consuming pork. The present results indicate that most consumers perceived differences in pork from different countries of origin. Australian and Indonesian pork scored highest for availability, while pork from China and Indonesia were considered to be tasty and cheap. This was in contrast with the tastiness of pork from the Western countries like Australia, Canada, New Zealand, Brazil and USA. Flavour attributes of pork from various countries were perceived differently, with an undesirable mutton-like flavour being the most relevant for pork from Australia, Canada and New Zealand.

Pork should therefore, be produced in ways to ensure good eating quality, as consumers clearly perceive differences in pork quality and this affects their interest in the meat. It is important to produce pork with good flavour in accordance with consumers' expectations.

Chapter 4

The effects of excluding animal products from the diet on sensory properties of pork from pigs grown in New Zealand as assessed by Singaporean panellists

This chapter has been partly published in Asian - Australasian Journal of Animal Sciences in 2010.

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4.1 Introduction

Flavour is a very important quality factor in pork which is a popular meat consumed by non-Muslim Singaporeans who make up more than 70% of the Singapore population. Currently, the imports of pork are mainly from Australia, Indonesia, Brazil, France and USA, with pork from Australia and Indonesia being consumed most widely due to its ready availability at supermarkets and wet markets. A recent survey showed that Singapore consumers frequently associate non-Indonesian pork with the presence of an unpleasant mutton-like off-flavour (Leong, Purchas, Morel & Wilkinson, 2008).

One possible cause of off- flavours in pork is by the oxidation of lipids, leading to the formation of aldehydes and short-chain fatty acids (Reindl & Stan, 1982; Devol, McKeit, Bechtel, Novakofski, Shanks & Carr, 1988). The rate and extent of lipid oxidation depends on a number of factors, the most important being the level of polyunsaturated fatty acids (PUFA) in muscle (Allen and Foegeding, 1981). Pork contains high levels of unsaturated fatty acids relative to ruminant meat (Enser, Hallett, Hewitt, Fursey & Wood, 1996) and is more susceptible to oxidative deterioration of lipids and myoglobin. Feeding of PUFAs to pigs can improve the nutritional quality of pork, but may also increase the susceptibility to oxidation (Sheard, Enser, Wood, Nute, Gill & Richardson, 2000; Kouba, Enser, Whittington, Nute, & Wood, 2003; Morel et al., 2006). There have been many reports of PUFA-rich feeds leading to increased lipid oxidation and thus off-flavour in pork (Houben & Krol, 1980; Warnants, Van Oeckel & Boucque, 1998; Romans, Wulf, Johnson, Libal & Costello 1995, Overland et al., 1996; Leskanich, Matthews, Warkup, Noble & Hazzledine, 1997; Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard & Enser, 2003). There have also been examples of off-flavours in pork arising from the direct transfer of aroma components from feed to meat, including several reports on how feeding of fish oil and high fat fish meal to finisher pigs has caused “fishy” and other off-flavours in pork products (Kjos, Skrede & Overland, 1999; Lauridsen, Anderson, Andersson, Danielsen, Engberg & Jakobsen, 1999; Maw, Fowler, Hamilton & Petchey, 2001; Jaturasitha, Wudthithumkanaporn, Rurksasen & Kreuzer, 2002).

The current chapter compares sensory assessments of the flavour of pork from the legs of pigs finished in New Zealand on three diets (Morel, Janz, Purchas, Hendriks & Wilkinson, 2008) with a local reference pork sample from Indonesia using Singaporean panellists. The objective was to determine the extent to which dietary feed treatments received by the New Zealand pigs influenced the sensory properties of pork using trained and untrained Singaporean panels. Results of sensory analyses of pork from the loins of the same New Zealand pigs using New Zealand panellists were reported by Janz, Morel, Purchas, Corrigan, Cumarasamy, Wilkinson & Hendriks (2008).

4.2 Materials and Methods

4.2.1 Samples

Pork samples comprising the semimembranosus, adductor, and semitendinosus muscles were obtained from 18 female pigs (Duroc x (Large White x Landrace)) raised in New Zealand and 6 female pigs from Indonesia (Duroc cross, SG1; n=6). The pigs from New Zealand were made up of three dietary groups, with one receiving a diet containing some animal products (NZA; n=6), and two receiving diets containing plant products only (NZP; n=6 & NZP+; n=5), with the NZP+ diet containing a supplement (Sanovite™; 0.614% of the diet) of conjugated linoleic acid (CLA), selenium, and vitamin E. Details of these diets were given by Morel *et al.* (2008) and are shown in Table 4.1. The New Zealand pigs (NZA, NZP and NZP+) were slaughtered under normal commercial conditions in a New Zealand abattoir in Wanganui (Morel *et al.*, 2008). A fourth group of Indonesian pigs (SG1) were slaughtered at an abattoir in Singapore for the Indonesian group as a local reference control. The carcass weights ranged from 65.2 to 87 kg for the New Zealand pigs and 60 to 70.5 kg for the Indonesian pigs. The experiment was conducted in accordance with the Massey University Animal Ethics Committee and the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Table 4.1

Composition of NZA, NZP and NZP+[1,] diets for pigs raised in New Zealand

Ingredient, % of diet	Treatment group	
	NZA	NZP
Barley	63.38	67.35
Broll	10	10
Soybean meal	6.5	14
Blood meal	3.00	-
Meat and bone meal	13.00	-
Tallow	3.5	-
Soybean oil	-	1.90
Linseed oil	-	0.60
Lysine	-	0.35
Methionine	0.16	0.22
Threonine	0.06	0.18
Dicalcium phosphate	-	3.20
Limestone	-	1.50
Sodium chloride	0.10	0.10
Disodium phosphate	-	0.30
Vitamin-mineral premix[2]	0.30	0.30

[1]NZA=diet with animal and plant products; NZP=diet with plant products only; NZP+=diet with plant products & Sanovite™; SG1=Indonesian pigs

[2] Vitamin-mineral premix provided the following (unit kg^{-1} diet): 10,000 IU Vitamin A, 2000 IU Vitamin D3, 50 mg Vitamin E, 2 mg Vitamin K, 1 mg Vitamin B1, 2.5 mg Vitamin B2, 2 mg Vitamin B6, 10ug Vitamin B12, 10 mg calcium pantothenate, 15 mg niacin, 10 ug biotin, 0.5 mg folic acid, 100 mg choline, 100 mg iron, 45 mg manganese, 0.5 mg cobalt, 0.3 mg selenium, 120 mg zinc, 25 mg copper, 1 mg iodine.

4.2.2 Sensory Evaluation

Sensory evaluation was conducted at the Food Quality and Sensory Evaluation laboratory of the Singapore Polytechnic. Quantitative descriptive analysis (QDA) which has gained acceptance for sensory evaluation of various food products (Stone & Sidel, 1998) was carried out by trained panellists who were screened based on their sensory acuity and their liking of pork. They were considered to like pork if they consumed it at least twice a week and described it as one of their favourite meats. Triangle tests using different concentrations of sucrose, sodium chloride, citric acid and caffeine were used

to perform the screening and ultimately the six selected panellists participated in three training sessions over a period of two days.

Training under the direction of the panel leader led to the development of a common sensory language over three 1.5 hour training sessions. This relatively short training period was considered sufficient because all panellists had considerable previous experience. During the training sessions, the panellists practised scoring sensory attributes of the pork samples that were presented at least twice per session to allow panellists to re-familiarise themselves with the typical flavour associated with each attribute. The questionnaire for the trained panel included 18 items (Table 4.2) each of which was assessed on a 150 mm unstructured scale from "None" to "Strong".

For the trained panel, samples of the semimembranosus and adductor muscles were minced (Moulinex brand, model no.HV8) through a plate with 8 mm diameter holes at a rate of 1.6 kg /minute. For evaluation of meat aroma, 10 g of minced meat were placed in a 30 mL polypropylene bottle that was covered with a cap, and cooked for 10 min in a water bath at 100^{o}C (Memmert brand; model no. W350). The bottles were capped throughout the cooking process to retain aromas and were opened by the panellists during the evaluation. For taste evaluation, 50 g of minced meat were placed into 150 mL glass jars that were capped and cooked in water (100^{o}C) for 30 mins. During the cooking process, the minced meat was stirred to prevent clumping together. By opening and closing the jars to do the stirring, much aroma would have been lost, which is why aroma was evaluated separately as described above. Each panellist was provided with an evaluation form, napkins, a covered container for expectoration, water, and plain white bread for cleansing the palate. Each sample was tasted once in each of two sessions with 11 or 12 samples per session to give a total of four sessions. Samples were presented to the panellists randomly and one at a time.

Table 4.2
Definitions of the sensory attributes of cooked pork developed by the trained panellists during training, together with the anchor points at each end of the 150 mm scale

Sensory attribute	Interpretation	Anchor points
Aroma & Odour attributes:		
Meaty aroma	Aromatics associated with cooked meat[1]	None / Strong
Brothy aroma	Aromatics associated with pork cooked in water[1]	None / Strong
Metallic aroma	Aromatics associated with presence of iron ions[1]	None / Strong
Acidic aroma	Aromatics associated with presence of citric acid[2]	None / Strong
Mutton aroma	Aromatics associated with presence of mutton[1]	None / Strong
Stale odour	A typical aroma generally associated with rancidity of meat and its fat[1]	None / Strong
Flavour & Taste attributes:		
Meaty flavour	Sensations associated with cooked meat[1]	None / Strong
Brothy flavour	Sensations associated with pork cooked in water[1]	None / Strong
Metallic flavour	Sensations associated with the presence of iron ions[1]	None / Strong
Acidic taste	Taste on the tongue associated with citric acid[2]	None / Strong
Mutton flavour	Sensations associated with cooked mutton[1]	None / Strong
Stale flavour	Atypical taste generally associated with rancidity of meat and its fat[1]	None / Strong
Bitter taste	Taste on the tongue associated with caffeine[2]	None / Strong
Aftertaste	Sensation of lingering taste on the tongue after ingestion[1]	None / Strong
Other attributes		
Brownness	Degree of brownness[1]	Grey / Brown
Lightness	Degree of darkness/lightness[1]	Light / Dark
Juiciness	Sensation of presence of moisture or liquid exudates in the mouth[1]	None / Strong
Tenderness	Ease of breaking down of meat into fine particles when chewed[1]	None / Strong

[1]Definitions as developed by the panellists
[2] Definitions of Meilgarrd, Civille, & Carr (1999)

For the untrained panel, each of the 20 untrained panellists (5 males and 15 females, aged between 18 to 45 years) assessed samples from each pig twice for aroma, flavour, juiciness, tenderness and overall acceptability on a scale from 1 to 9 where 1 was “Dislike extremely” and 9 was “Like extremely”. They also assessed the intensity of mutton aroma and mutton flavour on a scale where 1 was “None” and 5 was “Intense”. Both panels assessed the pork samples using normal white light at the sensory evaluation booths.

Samples of the semitendinosus muscle were cut into strips (approximately 50 mm x 30 mm x 5 mm) and marinated with dark soy sauce (Tai Hua Food Industries Pte Ltd, Singapore; 1 mL of sauce per 2.5 g of pork) for 10 minutes to impart a dark brownish black colour and a slight salty note to the meat, and then simmered in a covered 3.5 litre slow cooker (Cornell brand: CSJ35) for 30 min set at 90°C, 30 min at 80°C, and then held at 60°C until served. Panellists were served with a tray of four samples (in lidded plastic containers) that they tasted individually on a flour bun (commonly known as 'Mantou'; 80 x 50 x 5 mm). Three trays of samples were evaluated per session with a total of 46 samples in four sessions over two weeks, so each panellist tasted pork from each pig twice. This method of cooking and presentation was used because it is popular with Singaporeans.

4.2.3 Statistical analysis

All statistical analyses were carried out using SPSS ver. 17 (SPSS Inc, Singapore). The animals were nested within feed treatment, while treatment and panellist effects were arranged in a factorial manner with samples from every animal being evaluated by every panellist. The data were analysed as a nested-factorial design.

Treatment group effects were tested against animal effects. Both panellist and panellist x treatment effects were tested against panellist x animal (within treatment). Animal within treatment and panellist x animal (within treatment) effects were tested against the overall errors.

Scale marks from QDA were converted to intensity scores from 0 to 100 for each descriptor and analysed by ANOVA (Type I Sums of Squares) at 5% level of significance using the General Linear Model (GLM) procedures to determine differences among the groups. The Sharpiro-Wilk test for normality of the data indicated that 10 of the 18 attributes in the trained panellist data and two from the untrained panel required some kind of transformation ($p < 0.05$ for the Shapiro-Wilk test). Natural logs were used for 12 attributes (acid aroma, metallic aroma, mutton aroma, stale odour, acidic taste, metallic flavour, mutton flavour, stale flavour, bitter taste, and aftertaste for the trained panel, and mutton aroma, and mutton flavour for the untrained panel). Because these 12 attributes included some zero scores, the data were

analysed as $\log_e(x+1)$ where x = the untransformed value. To get the actual least-squares means, "1" was subtracted from the back-transformed least-squares means. The significance of differences between the least-squares means was assessed using the Least Significant Difference test. Relationships between attributes based on animal means were evaluated using Pearson's linear correlation coefficients. Discriminant analysis was performed using the scores of 14 aroma and flavour attribute from the trained panel in order to identify combinations of variables that discriminated best among the four treatment groups.

4.3 Results

4.3.1 Trained panel

Results in Table 4.3 indicate that 8 of the 18 attributes were not significantly different among the four groups. Many of the attributes (7 out of 14) had very low scores with means less than 4 on the 100-point scale. Brothy aroma scores were significantly higher for the NZP+ than NZA groups, with NZP and SG1 being in-between. Stale odour score was higher for NZP samples than those from NZA, but neither of these differed significantly from the other two groups. Meaty flavour scores were lower for the NZA group than for the other three groups. Brothy flavour was lowest for NZA, which differed significantly from NZP, but not the other two groups. Mutton flavour showed large differences between the groups with NZA having a higher score than NZP or NZP+, and these two groups had higher scores than SG1. Mutton flavour score for NZA was more than 20 times that for SG1. Mean stale flavour score for NZP+ was significantly higher than those for NZP and SG1, but not significantly different from that for NZA. Aftertaste was significantly higher for NZA than SG1 with the other two groups in-between.

Juiciness was lower for NZA than NZP. There was a significant positive correlation between acidic and metallic flavour scores ($r= 0.659$, $p=0.021$), both of which were highest for the NZA group although differences between NZA and NZP were not significant. Pork samples from the NZP and NZP+ groups were similar for all attributes except stale flavour, which was higher for NZP+. This similarity was reflected in the closeness of these two groups on the Function-1 scale of the discriminant analysis plot Fig. 4.1.

Table 4.3

Least squares means showing the effects of treatments on sensory attributes of pork as determined by a trained sensory panel

Sensory attribute [2, 3]	Treatment[1]				Effects	R^2(%), RSD[4]
	NZA	NZP	NZP+	SG1	(p-value)	
No. of animals	6	6	5	6		
Aroma & odour attributes:						
Meaty aroma	39.95	36.15	40.06	35.75	0.522	54.0, 23.63
Brothy aroma	31.41^{a}	34.31^{ab}	41.93^{b}	37.14^{ab}	0.025	56.2, 30.10
Metallic aroma[5]	1.07	1.07	1.08	1.44	0.058	64.9, 1.026
	(1.92)	(1.92)	(1.94)	(3.22)		
Acidic aroma[5]	0.596^{ab}	0.856^{ab}	0.573^{a}	0.922^{b}	0.046	71.7, 0.742
	(0.81)	(1.35)	(0.77)	(1.51)		
Mutton aroma[5]	2.38	2.29	1.86	1.95	0.108	60.2, 1.18
	(9.80)	(8.87)	(5.42)	(6.03)		
Stale odour[5]	0.639	0.921	0.619	0.646	0.095	73.6, 0.727
	(0.89)	(1.51)	(0.86)	(0.91)		
Flavour & taste attributes:						
Meaty flavour	47.66^{a}	55.53^{b}	53.13^{b}	54.72^{b}	0.002	77.8, 15.54
Brothy flavour	28.51^{a}	40.91^{b}	33.15^{ab}	32.93^{ab}	0.003	83.0, 14.64
Metallic flavour[5]	0.929	0.755	0.947	0.674	0.382	58.9, 0.830
	(1.53)	(1.13)	(1.58)	(0.96)		
Acidic taste[5]	0.629^{ab}	0.499^{ab}	0.860^{b}	0.439^{a}	0.027	71.2. 0.667
	(0.88)	(0.65)	(1.36)	(0.55)		
Mutton flavour[5]	3.42^{c}	1.96^{b}	2.17^{b}	0.897^{a}	<0.0001	67.4, 1.08
	(29.57)	(6.10)	(7.76)	(1.45)		
Stale flavour[5]	0.624^{ab}	0.448^{a}	0.849^{b}	0.343^{a}	0.001	72.2, 0.587
	(0.87)	(0.57)	(1.34)	(0.41)		
Bitter taste[5]	1.29^{b}	1.22^{b}	1.37^{b}	0.699^{a}	<0.0001	79.7, 0.683
	(2.63)	(2.39)	(2.94)	(1.01)		
Aftertaste[5]	3.57^{c}	3.10^{b}	3.22^{b}	2.20^{a}	<0.0001	67.2, 0.733
	(34.52)	(21.20)	(24.03)	(8.03)		
Other attributes						
Colour	37.89	42.56	34.58	39.07	0.353	79.3, 15.30
Lightness	31.30	36.13	32.01	32.15	0.707	62.1, 21.77
Juiciness	50.32^{a}	55.30^{b}	52.54^{ab}	52.35^{ab}	0.031	40.0, 13.32
Tenderness	60.08	58.75	60.49	56.13	0.114	52.4, 14.79

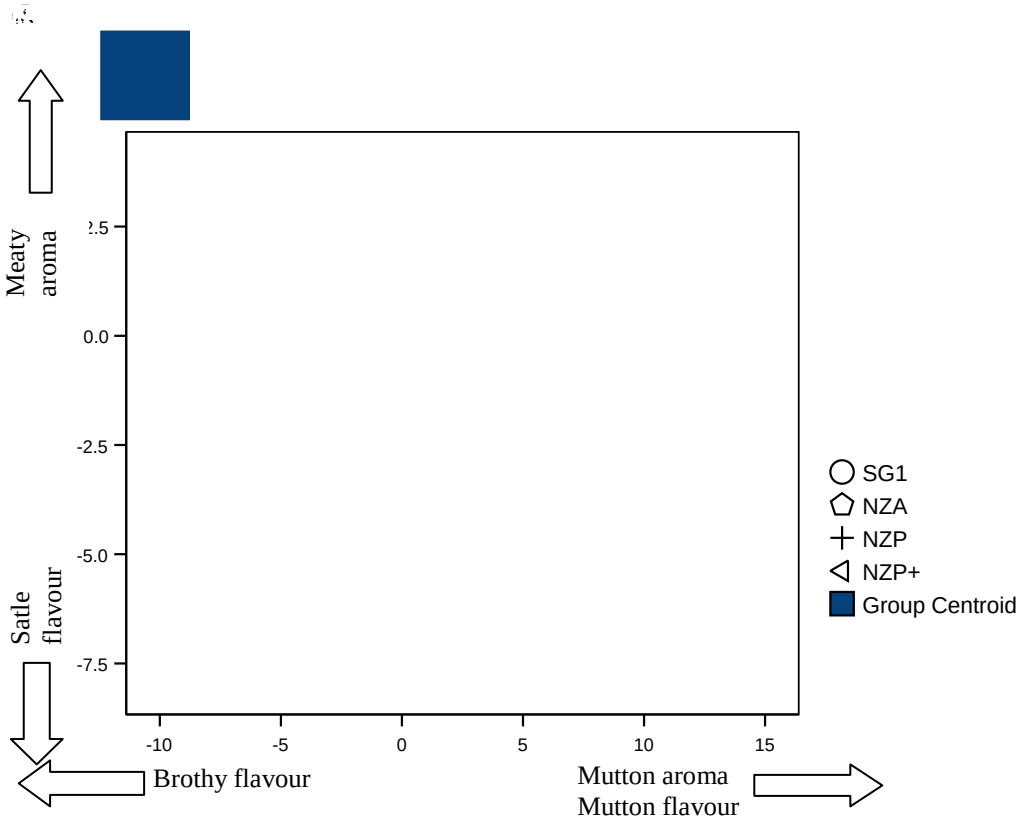
[1]NZA=diets with animal and plant products; NZP=diets with plant products only; NZP+=diet with plant products & Sanovite™; SG1=Indonesian pigs.

[2]All attributes were scored on a scale of 0-100 with higher values indicating a stronger note.

[3]Means in the same row with no letter or a common letter after them do not differ significantly ($P > 0.05$) as determined by Fisher's least significance difference (LSD) mean separation test.

[4] Measures of the overall goodness-of-fit for the model include the coefficient of determination [R^2(%)] and the residual standard deviation (RSD).

[5]The significance of differences (using LSD) was based on the transformed means ($\log_e$) of these attributes, and the back-transformed means are shown in brackets below the transformed means.



NZA=diet with animal and plant products
NZP=diet with plant products only
NZP+=diet with plant products & Sanovite™
SG1=Indonesian pigs

Fig. 4.1

A plot of function 1 versus function 2 from the discriminant analysis based on the 14 aroma and flavour attributes evaluated by the trained panel (Table 4.2). Points are shown for the 23 individual pigs as well as the treatment group centroids.

The first and second functions of the discriminant analysis accounted for 95.4% of the variance, with function 1 being related mainly to mutton aroma, meaty aroma, aftertaste, brothy flavour and mutton flavour, while function 2 reflected mainly meaty aroma, metallic aroma, bitter taste, stale flavour and metallic flavour

The first and second functions of the discriminant analysis (Figure 4.1) accounted for 95.4% of the variance, with function 1 being related mainly to mutton aroma, meaty aroma, aftertaste, brothy flavour and mutton flavour, while function 2 reflected mainly meaty aroma, metallic aroma, bitter taste, stale flavour and metallic flavour (Table 4.4). Function 1 clearly separated the treatments into 3 clusters with little separation of NZP and NZP+, but function 2 clearly separated NZP+ from the other three groups (Figure 4.1).

Table 4.4
The largest five coefficients for the first two discriminant functions from the discriminant analysis based on the 14 aroma and taste attributes (Table 4.2) assessed by the trained panel

Discriminant function number			
1 (83.7% of variation)		2 (11.7% of variation)	
Attribute	Discriminant coefficient	Attribute	Discriminant coefficient
Mutton flavour	1.560	Stale flavour	-1.580
Meaty aroma	1.417	Meaty aroma	1.250
Brothy flavour	-0.936	Metallic aroma	-1.212
Mutton aroma	0.918	Bitter taste	-1.106
Aftertaste	0.872	Metallic flavour	0.414

Correlations across all 23 samples revealed positive relationships between aftertaste and acidic taste ($r= 0.594$; $p<0.01$), mutton flavour ($r=0.884$; $p<0.01$), stale flavour ($r=0.429$; $p<0.05$) and bitter taste ($r=0.444$; $p<0.01$). Likewise, mutton flavour was positively related with stale flavour ($r=0.361$; $p<0.05$), and acidic taste ($r=0.584$; $p<0.01$), but negatively related to meaty flavour ($r=-0.728$; $p<0.01$). Meaty flavour was positively related with brothy flavour ($r=0.72$).

4.3.2 Untrained panel

Results of evaluations by the untrained panel (Table 4.5) showed no significant differences between the groups for juiciness, tenderness or overall acceptability. Both aroma and flavour of pork from the NZP group was preferred to that from the NZA group, with pork from the SG1 group having an aroma that was of similar acceptability to the NZP group, but a flavour that was significantly less acceptable than for that group. Flavour and aroma acceptability were not different between the NZP and NZP+ groups.

Table 4.5

Least squares means for treatment effects on acceptability and intensity scores as assessed by an untrained panel

Sensory attribute [2, 3]	Treatment[1]				Effect	R^2(%), RSD[4]
	NZA	NZP	NZP+	SG1	(p value)	
Measures of acceptability:						
Aroma	5.68[a]	6.33[b]	6.01[ab]	6.22[b]	0.007	49.4, 1.65
Flavour	5.58[a]	6.26[b]	5.80[ab]	5.82[a]	0.030	49.7, 1.74
Juiciness	5.70	6.26	5.80	5.82	0.094	49.2, 1.74
Tenderness	5.62	6.17	5.77	5.82	0.294	51.0, 1.84
Overall acceptability	5.64	6.11	5.85	6.05	0.247	50.3, 1.70
Measures of intensity:						
Mutton aroma[5]	0.414[c] (1.73[c])	0.298[b] (1.52[b])	0.261[ab] (1.47[ab])	0.199[a] (1.32[a])	0.005	48.8, 0.443
Mutton flavour[5]	0.670[c] (2.21[c])	0.335[b] (1.59[b])	0.387[b] (1.66[b])	0.186[a] (1.29[a])	<0.0001	52.1, 0.449

[1]NZA=diet with animal and plant products; NZP=diet with plant products only; NZP+=diet with plant products & Sanovite™; SG1=Indonesian pigs

[2] All acceptability scores were on a scale of 1 – 9 where 1 is "Dislike extremely" and 9 is "Like extremely", while intensity scores were on a scale of 1 – 5 where 1 is "None" and 5 is "Intense".

[3]Means in the same row with no letter after them or with a common letter after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[4] Measures of the overall goodness-of-fit for the model include the coefficient of determination [R^2(%)] and the residual standard deviation (RSD).

[5] The significance of differences (using LSD) was based on the transformed means ($\log_e$) of these attributes and the back-transformed means are shown in brackets below the transformed means.

Intensity scores for mutton aroma and flavour from the untrained panel (Table 4.5) were lowest for pork samples from the SG1 group and highest for the NZA group ($p < 0.01$). Scores for the NZP and NZP+ groups were intermediate. Scores from the untrained panel indicated that higher overall acceptability scores were associated with more acceptable aroma (r=0.906), juiciness (r=0.888), and tenderness (r=0.904), but with lower intensities of mutton aroma (r=-0.478) and flavour (r=-0.551).

4.4 Discussion

4.4.1 Dietary effects on pork flavour

The relatively low acceptability and high mutton-flavour scores for pork from the NZA group may have been due to the higher protein content of the diet for that group (20.6% vs 15.2% for NZP (Morel *et al.*, 2008)) and the fact that some of that protein came from meat and bone meal that is likely to have included meat and bone from sheep. The higher protein level would have resulted in more tryptophan being

available in the hind-gut (Tuomola, Vahva & Kallio, 1996; Henry & Chapman, 2002) for microbial degradation to form the flavourful compounds skatole and indole (Lane & Fraser, 1999; Lane, Fraser, Kolver, Rowan, Allen, Mills, Abraham & Olney, 2002). These indolic compounds have also been shown to be implicated in boar taint (Vold, 1970, Walstra & Maarse, 1970). The quantity of tryptophan reaching the hindgut was calculated using the NRC (1998) feedstuff gross tryptophan content and ileal digestibility coefficient. It is estimated that 20% more tryptophan would have reached the hindgut of NZA pigs relative to NZP pigs (0.53 vs 0.44 g per kg of feed, respectively). In addition to the possible extra synthesis of skatole and indole in the hind-gut of pigs in the NZA group, it is also likely that these compounds would have been present in the diet as they are present in sheep meat and fat (Young, Berdague, Viallon, Rousset-Akrim & Theriez, 1997; Young, Lane, Priola & Fraser, 2003; Schreurs, McNabb, Tavendale, Lane, Barry, Cummings, Fraser, Lopez-Villalobos & Ramırez-Restrepo, 2007; Schreurs, Lane, Tavendale, Barry & McNabb, 2008), and appear to be at least partly responsible for mutton flavour (Hoffman & Meijboom, 1968; Brennand & Linsay, 1982, Young *et al.*, 2003). These flavourful compounds may have been transferred to the pork, as has been reported for fish odours in several studies (Kjos *et al.*, 1999; Lauridsen *et al.*, 1999; Maw *et al.*, 2001; Jaturasitha, *et al.*, 2002).

Although not directly comparable because of differences in the cut used (current study vs. Janz et al study: loin vs. leg), cooking procedures (cooking in glass jars for 10 mins. vs. plastic bags for 9 mins.), and the flavour attributes evaluated (14 vs. 19 attributes), it is noteworthy that a trained New Zealand panel did not detect any significant differences between loin samples from the three groups of New Zealand pigs that were assessed in Singapore (Janz et al., 2008). These results suggest that the Singaporean panellists may have been more sensitive to some of the flavour characteristics, possibly due to the low consumption and poor acceptability of sheep meat in many Asian countries (Prescott, Young & O'Neill, 2001), including Singapore (Yeo, 1998), which may mean that mutton-like flavours are less acceptable and more apparent than in New Zealand (Crandall, 1985; Pliner, 1982). Prescott *et al.*, (2001) indicated that the low acceptability of sheep meat in Japan was also related to a low level of consumption in that country. In China, consumers describe the hedonically

negative cooking odour of sheep meat as *soo,* meaning sweaty or sour (Wong, Johnson & Nixon 1975).

4.4.2 Effects of nutrient supplements on pork flavour

The addition of CLA, vitamin E and selenium to the diet of pigs in the NZP+ group did not produce pork with significant differences in most of the sensory characteristics assessed by the Singapore panels, or by a New Zealand panel (Janz et al., 2008). The only significant difference ($p < 0.05$) was a higher stale flavour score for the NZP+ pork relative to the NZP pork, but the mean scores of 5.15 and 1.57, respectively, on the 100-point scale were very low (Table 4.3). This result and the assessment by the New Zealand trained panel (Janz *et al.*, 2008) of a slightly more frequent rancid odour for NZP+ compared to NZP, is in agreement with other reports indicating that incorporating CLA into finishing diets of pigs has no appreciable effects on the sensory quality of cooked pork and pork products (Dugan, Aalhus, Schaefer & Kramer, 1999; Wiegand, Sparks, Parrish Jr. & Zimmerman 2002; Corino, Magni, Pastorelli, Rossi & Mourot, 2003, Teye, Sheard, Whittington, Nute, Stewart & Wood, 2006). It is unlikely that the duration of feeding the supplement was too short to have an effect as it was fed from weaning to slaughter (Morel et al., 2008), but it may be that the amount of CLA added was not enough to cause an extensive oxidative reaction. Martin, Antequera, Muriel, Andres & Ruiz (2008) also demonstrated that CLA supplementation at doses lower than 1% in the diet of pigs did not affect lipid oxidation in loins.

The low levels of stale flavours in NZP+ (1.34) pork as well as NZP (0.57) pork in the current study and that of Janz et al. (2008) may be partly attributable to the antioxidant properties of vitamin E and selenium that were included in the diet. Vitamin E can stabilise the membrane-bound lipids against metmyogloblin/H_2O_2-initiated oxidation (Monahan, Buckley, Gray, Morrissey, Asghar, Hanrahan & Lynch, 1990; Asghar et al., 1991; Monahan, Buckley, Morrissey, Lynch & Gray, 1992).

4.4.3 Comparison of New Zealand and Indonesian pork

Differences reported here between the locally obtained Indonesian pork and that from New Zealand cannot be interpreted in any depth because there were a number of uncontrolled factors involved. These include the genetic makeup of the pigs, the diet they received, the composition of the pork (Purchas, Morel, Janz & Wilkinson, 2009), and the exact nature of the ways in which the pork was treated post mortem. The results indicate, however, that the Singapore panels detected significantly stronger mutton aroma and flavour (untrained panel); and mutton flavour but not aroma (trained panel), in the New Zealand samples, and particularly from pigs that received animal products in their diets (the NZA group).

4.5 Conclusions

Trained and untrained sensory panels in Singapore were able to detect differences in some flavour and aroma characteristics of pork from pigs raised on different diets in New Zealand with the pork from pigs with some animal products in their diet generally being less acceptable and having a stronger mutton flavour. It is suggested that these differences could be caused by the diet of NZA pigs which contained more protein and possibly some meat and fat from sheep.

The Singapore panels detected differences between pork from the three New Zealand groups that were not detected by a New Zealand panel, but there were some confounding factors in this comparison. These results, however, support previous evidence that sensory results from one population may not necessarily apply for populations in other countries even when trained panellists are involved.

Results showed that dietary supplementation with CLA (conjugated linoleic acid) increased the stale note in pork, but this effect was small, possibly because of the antioxidant effects of the additional vitamin E and selenium present.

Relative to locally-produced Indonesian pork the pork from New Zealand did not differ significantly in overall acceptability, but did have more intense mutton-like flavour attributes.

Chapter 5

A survey of the use of natural-flavoured plant materials by Singaporean consumers during cooking or consumption of pork

5.1 Introduction

Herbs and spices are natural-flavoured plant materials that have been used in cooking for many years throughout the world. They can be used in various forms: fresh or dehydrated, whole, or finely chopped. They have been shown to possess antimicrobial functions and could serve as a source of antimicrobial agents against food pathogens (Deans and Ritchie, 1987). Essential oils have natural antimicrobial properties with the potential to extend the shelf-life of food when used alone or in combination with other preservation techniques (Mejlholm and Dalgaard, 2002).

Besides having antimicrobial effects, some of these plant materials possess antioxidant properties (Sebranek *et al.*, 2005; Tanabe *et al*., 2002). Sebranek and co-workers (2005) demonstrated the antioxidant effects of rosemary extract in pork sausage while Tanabe (2002) outlined the antioxidant activities of various culinary herbs and spices with respect to lipid oxidation in pork.

Many studies have demonstrated that the incorporation of non-aromatic plant materials like oil seeds into the diets of meat-producing animals can alter the fatty acid profiles of these meats and also their sensory quality. Shackelford et al. (1990) has reported that the flavour profile of pork can be improved by changing the diets of pigs using canola oil. It is possible that pork from pigs fed special diets could have unique flavours and sensory characteristics imparted by the fats or oils in the diets. However, there has been little published work showing the influence of natural aromatic substances in the diets of meat-producing animals on the flavour or overall palatability of meat.

Janz et al. (2006) demonstrated a preference of pigs for a garlic-containing diet, based on higher total feed intakes. Horton, Blethen, and Prasad (1991) indicated that garlic is often added to pet foods to improve palatability. Garlic has also been added to the diets of race horses to increase intake and boost performance (Horton et. al 1991). Krusinski (2001) reported a feed consumption preference amongst finisher pigs for diets containing garlic within a herb mixture. Hansen et al.(2002) reported that the direct transfer of aromatic components from plant materials to the meat influences the sensory quality of pork (Hansen *et al*., 2002), just as direct transfer of aromatic components from

fish affects pork flavour (Janz *et al.*, 2006, Jaturasuitha *et al*., 2002; Kjos *et al*., 1999; Lauridsen *et al*., 1999).

The objective of this study was to investigate the popularity of various herbs, spices and natural-flavoured plant materials used during cooking or eating of pork by Singaporean consumers using a survey. This was done with a view to subsequently including one or more of the more popular plant materials into pork or into the diet of pigs to determine whether the flavour of pork can be modified to produce a product with enhanced consumer appeal.

5.2 Materials and methods

In total, 19 spices, 12 herbs and 8 other natural-flavoured plant materials were evaluated for frequency of usage and for cooking methods used by means of a survey. Hard and soft copies of the questionnaire were given out to respondents and they are required to complete the survey and return them via e-mailing, by-hand or through the mail.

The respondents were required to be pork eaters and to use natural-flavoured plant materials in cooking pork in order to be included in the survey. Other information obtained during the survey was with regard to gender, race, household income per month, dwelling type, and number of people in the household. The questionnaire included questions about eating and purchasing behaviour.

Frequencies of usage of plant materials in cooking pork and the usage of cooking methods were quantified on a scoring system from 0 to 4 where 0 was “none” and 4 was “always”. The frequency of usage of cooking methods was expressed as percentages of the total. Chi-square analysis was used to test for the significance of differences among plant materials and among cooking methods.

5.3 Results

The demographics of the 112 respondents (Table 5.1) indicated that the participants were mainly Chinese females with household incomes of more than S$5000 per month. There were almost equal proportions of married and single participants, mainly from the age group of more than 40 years. They stayed in Housing Development Board apartments (HDB) and most had four to five members in the household (>30%).

Almost half (46.2%) did the meat shopping, mainly at supermarkets (61.9%). Only 6% did the purchasing from meat speciality shops. Among the respondents, 78.3 % consumed pork more than once a week; while only 5.2% consumed pork less than once a month.

Table 5.1
Questions set out in the questionnaire together with a summary of the responses from the 112 respondents

Question	Response options					
What is your gender?	Male	Female				
	13%	87%				
What is your marital status?	Single/ widowed	Married				
	46.7%	43.3%				
What is your age group?	11-20;	21 - 30	31 - 40	>40		
	14%	22.1%	30.2%	33.7%		
What is your racial group?	Chinese;	Indian				
	90.4%	9.6%				
What is your household income per month?	Up to $1500	$1500 - $3000	$3001 - $5000	>$5000		
	17.4%	26.1%	23.9%	32.6%		
What is your dwelling type?	HDB	Private Apt/ Condo	Landed property			
	77.6%	16.3%	6.1%			
What is the number of people in the household?	1	2	3	4	5	>5
	0	6.9%	12.3%	30.1%	32.6%	18.1%
Are you the member in the household who shops and buys pork?	Yes	No				
	46.2%	53.8%				
Where do you buy the pork?	Wetmarkets	Supermarkets	Meat Speciality shops			
	32.1%	61.9%	6.0%			
How often do you eat pork?	Every day	>Once a week but not everyday	>Once a month but <once a week	<Once a month		
	9.1%	69.2%	16.5%	5.2%		

Fig. 5.1 shows the scores for the 20 items with the highest scores, with the six highest being garlic, onion, ginger, chilli, and white pepper. Garlic and ginger had very similar scores. By using Chi-square analysis, there was a significant difference among the top 20 plant materials ($p<0.05$). Singaporean consumers in this survey tended to use more natural-flavoured plant materials, like garlic, onion and ginger, than herbs or spices.

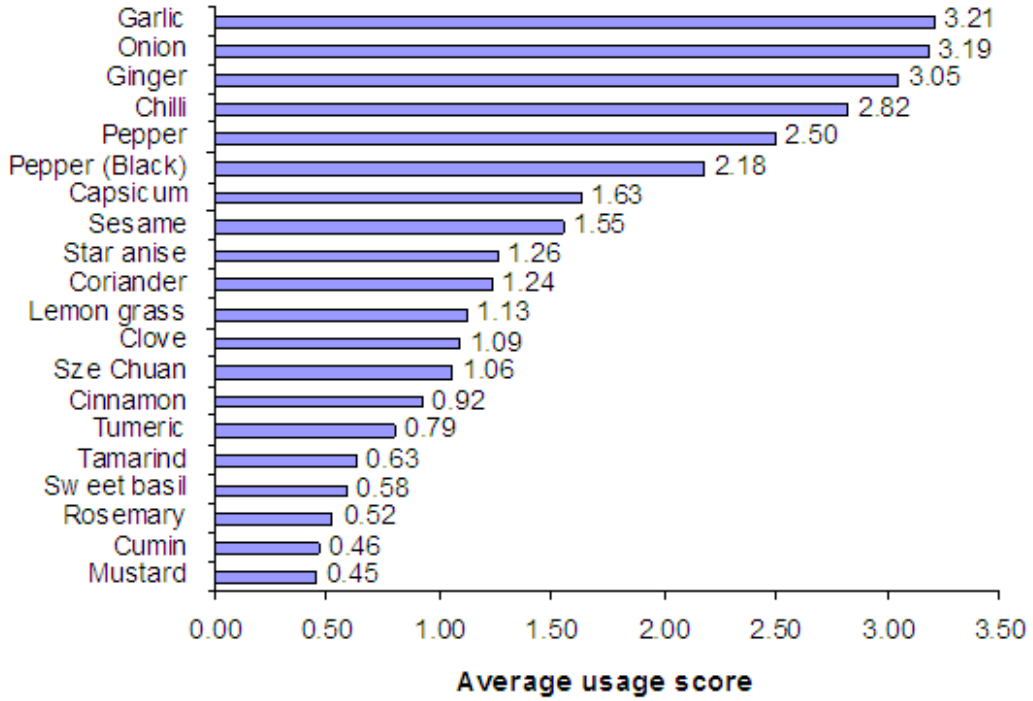


Fig. 5.1
The top 20 natural-flavoured plant materials and their average usage scores used in cooking pork in Singapore in terms of frequency of usage where 0 = "Not at all", and 4 = "Always".

The methods used for cooking pork with herbs, spices and other natural-flavoured plant materials (Table 5.2) indicated that stir frying was the most popular method for cooking spices with pork among the Singaporean consumers (Table 5.2), and this was followed by boiling (sauce and soup). The least frequently used method was microwaving. Table 5.3 illustrates the three most popular cooking methods for each type of spice when cooked with pork. Stir frying was the most popular method for pepper (Sze Chuan), pepper (black), sesame and cardamom.

The three most popular methods (Table 5.2) for cooking pork with herbs were boiling (sauce), followed by roasting and stir frying. Results in Table 5.3, show that the

most popular method for cooking pork with dill weed, spearmint, peppermint, rosemary and bay laurel was boiling (sauce). Among the herbs, lemongrass and sweet basil were considered as Asian-oriented to Singaporean consumers. They are often used in preparation of Thai-cuisine. Stir frying was the most frequently used cooking method for these two Asian herbs (Table 5.4).

For natural-flavoured plant materials, stir frying was the most popular cooking method (Table 5.2). This was followed by boiling (soup) and braising. Microwaving was the least frequently used method. Stir frying was the most popular method for cooking pork with garlic and onion (Table 5.5), the two most popular natural-flavoured plant materials used when cooking pork (Table 5.1).

Table 5.2
The cooking methods and their usage frequency in cooking pork with herbs, spices and other natural-flavoured plant materials[1]

Cooking methods for Herbs	Frequency (%)	Cooking methods for Spice	Frequency (%)	Cooking methods for Other plant materials	Frequency (%)
Boiling (in sauce)	21.1	Stir frying	21.5	Stir frying	29.7
Roasting	20.4	Boiling (soup)	14.2	Boiling (soup)	12.3
Stir frying	18.7	Boiling (in sauce)	14.1	Braising	11.2
Boiling (soup)	4.6	Braising	13.3	Boiling (sauce)	10.7
BBQ	14.5	BBQ	11.3	Steaming	9.9
Steaming	6.1	Roasting	9.9	BBQ	8.6
Braising	7.1	Steaming	9.4	Deep frying	7.8
Deep frying	5.7	Deep frying	4.2	Roasting	6.5
Microwaving	1.8	Microwaving	2.1	Microwaving	3.3
P value	<0.001	P value	<0.001	P value	<0.001

[1]A chi-square test for goodness of fit for types of cooking methods for pork yielded a p-value less than 0.05, thereby rejecting the null hypothesis

Table 5.3
The three most popular methods of cooking pork for 19 spices and their percentages of cooking event[1]

Spice	Three most popular methods in cooking pork		
	First rank	**Second rank**	**Third rank**
Aniseed	Braising (36.8%)	Boiling (sauce) (15.8%)	Stir frying (10.5%)
Caraway	Steaming (42.9%)	Stir frying (28.6%)	Boiling (sauce) (14.3%)
Cardamom	Stir frying (25%)	Boiling (sauce) (16.7%)	Steaming (8.3%)
Cinnamon	Braising (28.2%)	Boiling (sauce) (20.5%)	Stir frying (12.8%)
Clove	Boiling (sauce) (27.9%)	Braising (20.9%)	Stir frying (18.6%)
Coriander	Stir frying (22.2%)	Steaming (15.9%)	Boiling(sauce) (12.7%)
Cumin	Braising (26.3%)	Stir frying (sauce) (21.1%)	BBQ (15.8%)
Fennel	Boiling (sauce) (25%)	Stir frying (16.7%)	Boiling (sauce) (8.3%)
Fenugreek	Braising (37.5%)	Boiling (sauce) (25%)	Stir frying (12.5%)
Mace	Boiling (sauce) (27.2%)	Stir frying (18.2%)	Braising (9.1%)
Mustard seed	Boiling (sauce) (27.8%)	Steaming (16.7%)	Roasting (11.1%)
Nutmeg	Roasting (40%)	Boiling (sauce) (30%)	Stir frying (10%)
Pepper (Black)	Stir frying (30.6%)	Boiling (sauce) (18.4%)	BBQ (12.1%)
Pepper (Sze Chuan)	Stir frying (29.3%)	Boiling (sauce) (22.4%)	Roasting (10.3%)
Pepper (White)	Boiling (soup) (21.2%)	Stir frying (20.1%)	Braising (11.2%)
Saffron	Roasting (27.2%)	Boiling (sauce) (18.2%)	BBQ (9.1%)
Sesame	Stir frying (35.6%)	Roasting (16.1%)	BBQ (13.8%)
Star anise	Braising (32.6%)	Boiling (sauce) (21.7%)	Stir frying (17.4%)
Turmeric	Boiling (sauce) (24.1%)	Stir frying (20.7%)	Deep frying (13.8%)

[1]The percentages of cooking event was calculated based on the quotient of the number of times for a cooking method and the total number of times for all cooking methods for a particular spice

Table 5.4
The three most popular methods of cooking pork for 12 herbs and their percentages of cooking event[1]

Herb	Three most popular methods in cooking pork		
	First rank	**Second rank**	**Third rank**
Bay laurel	Boiling (sauce) (31.3%)	Roasting (18.8%)	Stir frying (12.5%)
Dill weed	Boiling (sauce) (25%)	Roasting (16.7%)	Stir frying (8.3%)
Lemongrass	Stir frying (26.3%)	Boiling (soup) (24.1%)	Braising (19.3%)
Marjarom	Stir frying (27.3%)	BBQ (18.2%)	Boiling (sauce) (9.1%)
Oregano	Roasting (27.8%)	BBQ (22.2%)	Boiling (sauce) (16.7%)
Peppermint	Boiling (sauce) (28%)	Boiling (soup) (14%)	BBQ (10.1%)
Rosemary	Boiling (sauce) (27.3%)	BBQ (24.2%)	Roasting (21.2%)
Sage	Roasting (33.3%)	BBQ (16.7%)	Boiling (sauce) (12%)
Spearmint	Boiling (sauce) (28.6%)	Boiling (soup) (13.8%)	BBQ (11.1%)
Sweet basil	Stir frying (25.7%)	Boiling (soup) (23%)	Roasting (14.2%)
Tarragon	Roasting (32.3%	BBQ (20.3%)	Boiling (sauce) (15.3%)
Thyme	Stir frying (26.3%)	BBQ (21.1%)	Roasting (15.8%)

[1]The percentages of cooking event was calculated based on the quotient of the number of times for a cooking method and the total number of times for all cooking methods for a particular herb

Table 5.5
The three most popular methods for cooking pork with eight natural-flavoured plant materials and their percentages of cooking event[1]

Natural flavoured plant material	Three most popular methods in cooking pork		
	First rank	**Second rank**	**Third rank**
Capsicum	Stirfrying (20.6%)	Boiling (sauce) (9.4%)	Roasting (8.2%)
Chilli	Stirfrying (29.3%)	Boiling (sauce) (13.2%)	Steaming (11.2%)
Galangal	Stirfrying (26.7%)	Boiling (sauce) (24.3%)	Deepfrying (9.8%)
Garlic	Stirfrying (36.7%)	Boiling (soup) (13.7%)	Braising (9.7%)
Ginger	Stirfrying (24.6%)	Steaming (19%)	Braising (17.1%)
Horseradish (Wasabi)	Stirfrying (27.7%)	Boiling (sauce) (22.2%)	Roasting (16.7%)
Onion	Stirfrying (31.2%)	Boiling (soup) (13.2%)	Braising (11.5%)
Tamarind	Stirfrying (24.5%)	Boiling (sauce) (28.4%)	Boiling (soup) (14.3%)

[1]The percentages of cooking event was calculated based on the quotient of the number of times for a cooking method and the total number of times for all cooking methods for a particular natural-flavoured plant material

5.4 Discussion

Garlic was selected from the results of the survey because of the popularity of this plant material among Singaporean consumers as a culinary herb. Garlic is also known for its antioxidant and antimicrobial properties (Ross et al., 2001). This strong aroma plant material contains an active compound known as allicin, which was identified by Cavallito and Bailey (1944) as the agent responsible for garlic's potent antibacterial properties. Allicin was found to be effective against a number of Gram-positive and Gram-negative bacteria (Cavallito and Bailey, 1944), as well as being antifungal (Small, Bailey and Cavallito, 1947) and antiviral (Tsai et al., 1985). Garlic may have a prebiotic effect due to its classification as a fructooligosaccharide (Gibson, 2001).

Besides possessing antimicrobial activities, garlic has been shown to increase feed palatability and thus feed intake (Horton, Blethen and Prasad, 1991). With this functional properties, pig farmers can consider the use of garlic in order to halt or minimise the use of antibiotics in the diets of pigs (Jost, 1996). There has been an increased concern over the use of antibiotics as growth promoters in animal feeds (Close, 2000). Much attention is being focussed on setting new regulations for more natural production methods that are friendly to animals, the consumer and the environment (Wenk, 2000). In addition, feeding garlic to weaned pigs may promote their growth and performance due to its antimicrobial, antioxidant and flavour enhancing properties (Jost, 1996).

5.5 Conclusions

Amongst 39, spices, herbs and natural-flavoured plant materials, garlic, onion and ginger were the three most widely used by Singaporean consumers when cooking pork. Stir frying was the most popular method used when these materials were cooked with pork.

With this information on the popularity of materials for use with pork in Singapore, it is suggested that the feasibility of incorporating appropriate items into pork directly or indirectly by including them in the diets of pigs should be investigated with respect to effects on product acceptability and possibly the levels of off-flavours present in pork.

Chapter 6

The production of pork with garlic flavour notes using garlic essential oil

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6.1 Introduction

Pork is the third most popular meat consumed in Singapore, and is imported mainly from Australia, Indonesia and Brazil. The amount of pork consumed is about 87,000 tonnes annually with a value of about $340 million (Kanagalingam, 2005). Singapore consumers often associate an undesirable mutton-like flavour with non-Indonesian pork according to the survey of Leong, Purchas, Morel & Wilkinson (2008a). The aversion to mutton flavour may be due to the low consumption of sheep meat in Singapore and many other Asian countries (Prescott, Young & O'Neill, 2001; Prescott, Young, Zhang & Cummings, 2004). There is some evidence that natural substances could be used to reduce the intensity of undesirable flavours in meat, including sheep meat and its products. Xylose, for example, was found to reduce sheep-meat flavours in casserole-type cooking of sheep meat (Young & Cummings, 2008), and the sheep-meat flavour of sausages made from the lamb shoulder cut was significantly "masked"/reduced by adding xylose. Prescott et al. (2001) suggested that adding herbs capable of suppressing or complementing sheep meat odour and flavour may be one possible strategy to improve the palatability of the meat.

Because garlic is one of the most popular flavoured plant materials used with pork in Singapore (Leong, Purchas, Morel & Wilkinson, 2008b), it was chosen to be tested for its ability to suppress undesirable mutton-like flavours in pork for the Singapore market. The addition of garlic has been investigated in some feeding studies with pigs. Janz, Morel, Wilkinson & Purchas (2006) demonstrated a preference by pigs for a garlic-containing diet, based on the total feed intake. Horton, Blethen & Prasad (1991) indicated that garlic is often added to pet foods to improve palatability. Diets with garlic added have been used for racing horses to increase intake and boost performance (Horton et al. 1991). Krusinski (2001) reported a preference amongst finisher pigs for diets containing garlic within an herb mixture. However, none of these studies looked into the possibility of suppressing undesirable flavours in pork by using garlic.

The first objective of the current research was to determine the detection threshold of garlic essential oil (GEO) when added to either rice bran oil or to minced pork using Singaporean and New Zealand panellists, and also to assess the acceptability

of flavour and the intensity of the garlic and mutton flavour of the minced pork with added GEO. The second objective was to determine the effect of GEO in the diet of pigs on aspects of pork flavour using Singapore and New Zealand consumer panellists.

6.2 Materials and methods

6.2.1 Materials for threshold tests

GEO (Garlic Oil Soluble, 80x,31-06) was obtained from Kalsec Corporation, USA. The shoulder muscles of Australian pork for the Singapore panel and New Zealand pork for the New Zealand panel were selected in local supermarkets.

Rice bran oil (Tong Seng Produce Pte Ltd – used for evaluation in Singapore; The 3 Mac Company Ltd – used for evaluation in New Zealand) was chosen as the carrier for the garlic essential oil because it imparted the least background aroma compared to other commercial cooking oils like peanut, olive, sunflower and canola oil.

6.2.2 Animals

Thirty two (Duroc x (Large White x Landrace)) female pigs weighing 48.3 (±3.15) kg (mean±SD) were obtained from a commercial farm, transported to Massey University and randomly allocated to one of eight dietary treatment groups. Four treatment groups received diets including both plant and animal products (mainly barley and meat and bone meal, with some tallow), while another four groups received diets containing plant products only (mainly barley and soybean meal with some soybean oil and linseed oil). The composition of the experimental diets was similar to the finisher diets without supplementation reported in Morel, Janz, Zou, Purchas, Wilkinson & Hendriks (2008). Both diets were supplemented with four levels (Table 6.1) of GEO. The GEO at the higher levels was increased in the diets gradually as the pigs became accustomed to the feed, but pigs on the AP diet found the diet unpalatable at a lower level of GEO than pigs on the P diet, presumably due to interactions between the diet components. As a result the GEO level in the diet and the total GEO intake was higher for the P_H group than the AP_H group (Table 6.1). Pigs were kept in pens of eight but were fed individually twice daily according to a fixed intake scale. Each pig received a

mean total of 107 kg of feed (SD = 5.6) until slaughter. Feed allowances were adjusted weekly. Feed refusals were collected daily and weighed, and pigs were weighed weekly. At the beginning the pigs were fed their respective control diets and were gradually introduced to the garlic-supplemented diets. The experiment was conducted in accordance with the "Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching" (Massey, 2008).

Table 6.1

The eight groups of pigs showing the base diet and the level of GEO added to the diet, together with the mean total garlic intake for pigs in each group

Group	No. of animals	Description of Base Diet	Level of GEO (g/kg feed)	Total GEO intake (g/pig) (± SE)
Animal and Plant Diets:				
AP_0	4	Plant and animal components	0 (zero)	0
AP_L	4	Plant and animal components	0.55 (Low, L)	59.9 ± 0.831
AP_M	4	Plant and animal components	1.44 (Medium, M)	155.5 ± 3.88
AP_H	3	Plant and animal components	1.84 (High, H)	173.2 ± 8.79
Plant Diet:				
P_0	4	Plant components only	0 (zero)	0
P_L	4	Plant components only	0.55 (Low, L)	58.0 ± 2.11
P_M	4	Plant components only	1.44 (Medium, M)	155.0 ± 3.22
P_H	4	Plant components only	2.15 (High, H)	236.6 ± 3.51

One pig was excluded from the trial because of poor growth. Slaughter and processing at a commercial abattoir took place after 57 days of feeding when the mean live weight (±SD) was 87.98 ± 3.66 kg. There were no significant differences between groups in growth rate (overall mean (±SD) = 0.721±(0.06) kg/d) or carcass weight (overall mean (±SD) = 66.9 (±4.0) kg). After overnight chilling (5.0 ±1.6^{o}C), the boneless loins (about 600 mm) from both sides of each carcass were removed, vacuum packed and frozen at ca -20°C within 36 hours *post mortem*. The left loins were transported to Massey University while the right loins were transported by air to Singapore and then to the Food Quality Laboratory at Singapore Polytechnic. Loins were kept frozen until being thawed just prior to preparation for sensory analysis.

The weights of the pork loins, including subcutaneous fat and skin, ranged from 3.2 to 3.5 kg.

6.2.3 Sample Preparation and Sensory Evaluation

6.2.3.1 Detection threshold test for GEO in rice bran oil

Procedures were similar in Singapore and New Zealand. Garlic essential oil was added to rice-bran oil to give final concentrations of 0, 25, 50, 75, 100, 125, 150, 175 and 200 ppm and mixed thoroughly using a high speed mixer (IKA; model no RW20 DZM.n). Ten mL of each concentration were dispensed into individual dark-coloured bottles with a volume of 12 mL and an opening with an internal diameter of 10.6 mm. They were screw-capped and stored at room temperature.

6.2.3.2 Detection threshold test for GEO in minced pork

The pork was minced through a plate with 6 mm diameter holes (Sammic brand, model no. 261 in Singapore; Kenwood brand, in New Zealand) at least two times in order to achieve a homogeneous texture. The essential oil was manually mixed thoroughly into the minced pork with final GEO concentrations of 0, 100, 125, 150 and 175 ppm.

For both Singapore and New Zealand, samples of approximately 150 g were placed into 115 mm x 230 mm plastic bags which were sealed using the attached metal twister, and then cooked in a 100^{o}C water bath for 15 minutes. After cooking, approximately 20 g of cooked samples were spooned into 30 mL plastic testing cups with an internal diameter of 40 mm and then covered with plastic lids. They were maintained at 60^{o}C for 30 mins in a forced-air oven to equilibrate the samples prior to tasting.

The 30 panellists in Singapore were mainly Chinese females, with the majority of the panellists being in the 31 – 40 age group (33.3%), and the 11 – 20 age group (26.7%). The 50 New Zealand panellists were mainly Caucasian female (98%), with the majority of them in the >40 age group (52%). The garlic and mutton flavour of a cooked sample was assessed by chewing a teaspoonful of pork. The intensity of garlic and mutton flavour was assessed on a 5-point intensity scale of 0 to 5 where 0 = “None”, 1 = “Barely detectable” to 5 = “Very intense”; and the degree of liking of garlic flavour was

based on a 9-point acceptability scale where 1 = "Dislike extremely" and 9 = "Like extremely".

6.2.3.3 Flavour of pork from pigs fed GEO

The sample preparation and cooking procedures were similar for the consumer panels in Singapore and New Zealand, and for the trained panel in Singapore, except that for the consumer panels all the pork from pigs within a treatment group was bulked together, while for the trained panel, the mince was prepared separately for pork from each individual pig.

Loin portions of approximately 1.5 kg were thawed overnight at 4°C, and trimmed of fat and skin to give approximately 1.3 kg of muscle, which was cut into chunks of about 80 – 100 g. Subcutaneous fat and muscle were minced separately using a plate with 6 mm diameter holes and were then mixed together in individual thermo pouches with 250 g of muscle and 25 g of fat, sealed (using the metal twister attached) and immersed in a 100^{o}C water-bath for 11 mins. The samples were stirred briefly (< 5 sec) every 3 min by introducing a spoon into the pouches to prevent clumping. When not stirring, the pouches were resealed using the metal twister attached to the pouches. After cooking for 11 min, the samples were transferred to small plastic cups (30 mL) which were covered with plastic lids. All samples were placed in a forced-air oven at 60^{o}C for 30 min to equilibrate samples prior to tasting. Plastic disposable spoons were used to scoop the samples and evaluate their flavour/taste.

For the untrained consumer panel testing, four sensory evaluation sessions were conducted over 2-days. Each session involved three triangle tests for each panellist with each treatment (either low, medium, or high level of GEO) tested against the control (no GEO). There were six triangle combinations, three with pork from pigs that had received animal and plant products in their diet and three with pork from pigs that had plant products only in their diet (Table 6.1). For each triangle test, panellists were asked to choose the odd sample on the basis of flavour. Each triangle combination was tested twice over the four sessions to give a total of 48 responses in Singapore and 60 responses in New Zealand. Within the triangle tests, panellists in Singapore only were also asked to assess the acceptability of flavour of pork from pigs fed the three levels of

garlic in comparison to the controls within the animal-plus-plant- and plant-diet groups on a scale of 1 = “Much less acceptable”, 2 = “Slightly less acceptable”, 3 = “Same acceptability”, 4 = “Slightly more acceptable” and 5 = “Much more acceptable”. Panellists were also asked to give comments about differences between the samples, particularly with regards to flavour.

The trained panel testing was conducted at the Food Quality and Sensory Evaluation laboratory of the Singapore Polytechnic. Quantitative descriptive analysis (QDA) was used with qualified panellists who were screened based on their sensory acuity, liking for pork, and their commitment to taste pork for 8 sessions over a period of 2 days. Triangle tests using different concentrations of sucrose, sodium chloride, citric acid and caffeine were used to perform the screening and ultimately the seven panellists selected participated in 5 discrimination trials over a period of 2 days. Under the direction of the panel leader, the panellists developed a sensory language to describe the products’ sensory properties. They grouped the attributes by modality order and then within a modality developed definitions for each attribute. There were three 1.5 hour training sessions. During training panellists became more confident with scoring the sensory attributes of pork by having samples presented at least two times per session to allow them to re-familiarise themselves with the typical flavour associated with each attribute.

During the actual sensory evaluation sessions, the panellists eventually evaluated 31 samples within eight sessions spread over two days (See evaluation form at Appendix 6.1). There were 15 to 16 samples evaluated per day, with 3 to 4 samples per session and breaks of at least 30 minutes between sessions. In the trained panel vocabulary there were 16 attributes of pork, each of which was assessed on a 150-mm unstructured scale which ranged from “None” to “Strong” (Table 6.2).

Table 6.2
Definitions of the sensory attributes of pork developed by the trained Singaporean panellists during training, together with the anchor points at the ends of each scale

Sensory attributes	Interpretation
Aroma attributes	
Meaty aroma	Aroma associated with cooked meat[a]
Brothy aroma	Aroma associated with pork cooked in water[a]
Garlic aroma	Aroma associated with raw garlic[b]
Metallic aroma	Aroma associated with presence of iron ions (blood)[a]
Acidic aroma	Aroma associated with presence of citric acid[b]
Mutton aroma	Aroma associated with presence of mutton[a]
Stale odour	A typical aroma generally associated with rancidity of meat and its fat[a]
Taste/flavour attributes	
Meaty flavour	Sensation associated with cooked meat[a]
Brothy flavour	Sensation associated with pork cooked in water[a]
Garlic flavour	Sensation associated with raw garlic[b]
Metallic flavour	Sensation associated with the presence of iron ions (blood)[b]
Acidic taste	Taste on the tongue associated with citric acid[b]
Mutton flavour	Sensation associated with cooked mutton[a]
Stale flavour	A typical taste generally associated with rancidity of meat and its fat[a]
Bitter taste	Taste on the tongue associated with caffeine[b]
Aftertaste	Sensation of lingering taste on the tongue after ingestion[b]

[a]Definitions as developed by the panellists
[b] Definitions of Meilgarrd et al. (1999)

6.2.4 Statistical analysis

All statistical analyses were carried out using SPSS ver. 18 (SPSS for Windows, version 18, SPSS Singapore Inc.)

Effects of GEO concentration (as a covariate), country, and the country x GEO concentration interaction on sensory attributes for rice bran oil and cooked pork mince were determined from type I ANOVA.

Sensory evaluation data obtained from the consumer panel for pork from pigs that had received varying quantities of GEO in their diet were analysed by comparing the number of correct triangle test responses within each treatment group, to the number required to achieve significance at $\alpha<=0.05$. The exact binomial probabilities which were obtained based on the number of correct responses vs. total number of responses were used to determine the effects of GEO level in the feed on the ability of consumers in Singapore and New Zealand to detect differences between the pork. For acceptability scores in comparison to the controls within the triangle tests, the Kruskal-Wallis test (SPSS Singapore Inc.) was used since the data was not normality distributed.

The data obtained from the trained panellists in the quantitative descriptive analysis (QDA) were analysed using a statistical model where the animals were nested within treatments, and treatment and panellist effects were arranged in a factorial manner with samples from every animal being evaluated by every panellist. Diet-type and GEO concentration within diet- type were tested against the animal term while the animal and panellists effects were tested against the overall error term. Scale marks on a 150 mm line scale from QDA attributes were converted to intensity scores from 0 to 100 for each sensory attribute and analysed by ANOVA (Type I Sum of Squares) at 5% level of significance using General Linear Model (GLM) procedures to determine differences among the treatments. The significance of differences between means was assessed using the Least Significant Difference test. Relationships between the amount of GEO consumed by pigs and the flavour attributes of pork from them were assessed by linear and quadratic regression models. Relationships between sensory attributes based on animal means were evaluated using Pearson's linear correlation coefficients.

6.3. Results and discussion

6.3.1 Aroma evaluation of garlic in cooking oil

Twenty-three out of 50 panellists from Singapore (46%) chose the threshold level for detection of garlic aroma as 75 ppm (Table 6.3), while 17 were able to detect the aroma at 50 ppm (Table 6.3). Fifty New Zealand panellists participated in this experiment, but the results of one was omitted as he did not detect any garlic flavour, even at 225 ppm. Thirty-one (63%) chose the threshold level for detection of garlic aroma as 50 ppm while a further 7 were able to detect the aroma at 75 ppm. These results were used as a reference to determine the concentration range of garlic essential oil to be used for threshold level determination in minced pork.

The Singapore panellists were able to detect the intensity of garlic aroma in oil very strongly, with mean intensity level increasing from 1.23 at 50 ppm to 4.03 at 225 ppm. There was no significant difference in degree of liking for garlic aroma over the garlic concentrations considered by Singaporean panellists ($p=0.064$).

For New Zealand panellists, the three highest concentrations (175, 200 and 225 ppm) samples had higher intensity scores than the lower concentrations, and the mean intensity score increased with increasing concentration (Table 6.3), except for the highest concentration (225 ppm) when the intensity score dropped slightly for most panellists. The hedonic score for garlic aroma showed no clear preference ($p=0.328$), with New Zealand panellists liking most of the concentrations of garlic oil presented to them.

Table 6.3

Number of panellists detecting each garlic concentration as the threshold level, and least squares means of hedonic and intensity scores for garlic aroma for different concentrations

Concentration of GEO in cooking oil (ppm)	Number (%) of panellists choosing the corresponding concentration as the threshold level		Intensity score of garlic aroma [1, 2, 3]		Hedonic score for garlic aroma [1, 2, 3]	
	Singapore (n=50)	New Zealand (n=49)	Singapore (n=50)	New Zealand (n=49)	Singapore (n=50)	New Zealand (n=49)
0	0	3 (6%)	NA	NA	NA	NA
50	17 (34%)	31 (63%)	1.23a	1.88ab	4.23	3.60
75	23 (46%)	7 (12%)	1.89ab	1.74ab	4.12	3.64
100	4 (8%)	4 (8%)	2.45bc	2.03abc	4.14	3.81
125	4 (8%)	2 (4%)	2.67bcd	1.91ab	4.54	4.24
150	2 (4%)	2 (4%)	2.98cd	2.17abc	4.22	4.16
175	0	1 (2%)	3.45cde	2.42bc	4.68	4.02
200	0	0	3.65de	2.79c	4.89	3.71
225	0	0	4.03e	2.50bc	5.02	4.10
Effect (P value)	NA	NA	<0.0001	<0.0001	0.064	0.328
R^2, RSD	NA	NA	61.3, 1.21	52.1, 1.12	56.8, 0.97	23.2, 1.35

[1] Intensity scale of 1-5 (1 being "barely detectable" and 5 being "very intense") and hedonic (acceptability) scale of 1-9 (1 being "dislike extremely" and 9 being "like extremely"). NA = "Not applicable".
[2] Means for intensity and hedonic scores with no letters after them or with a common letter after them within a column do not differ significantly ($P < 0.05$).
[3] Mean scores for garlic aroma intensity and hedonic scores are based only on the scores of those panellists who could detect the garlic aroma.

The differences in the slope between the Singapore and New Zealand data were analysed using GLM by having country as the main effect and GEO concentration as a covariate (Table 6.4). Country effect and the interaction between country and the covariate were also analysed. Regression analysis (Table 6.4) indicated that for both attributes, the slope against GEO concentration was significantly greater for results from the Singapore panel, as shown by the significance of the interactions between country and GEO concentration ($p < 0.05$).

Increased GEO concentration had a positive influence on the garlic aroma intensity ($p<0.05$) for Singapore and New Zealand panellists. GEO concentration had a

positive influence on garlic aroma hedonic scores for Singapore consumers ($p<0.05$) but this effect was less evident for that of New Zealand consumers ($p=0.328$).

Table 6.4
Effects of GEO concentration, country, and country x GEO concentration interaction (as a measure of differences in slope) on the sensory attributes in rice bran oil and in cooked pork mince when GEO concentration was included as a covariate in the regression model

Sensory attribute (Y)	Regression coefficient (±SE) with conc. of GEO (X)		Effect (p value)		
	Singapore	New Zealand	GEO conc.	Country	Country x GEO conc.
Rice bran oil:					
Garlic aroma intensity	0.372±0.028	0.137±0.027	<0.0001	<0.0001	<0.0001
Garlic aroma acceptability	0.121±0.037	0.034±0.034	0.003	<0.0001	0.002
Cooked pork mince:					
Garlic taste intensity	0.680±0.052	0.333±0.057	<0.0001	<0.0001	<0.0001
Mutton taste intensity	-0.397±0.056	-0.122±0.046	<0.0001	0.005	<0.0001
Garlic taste acceptability	0.807±0.141	0.098±0.090	<0.0001	0.005	<0.0001

6.3.2 Flavour evaluation of garlic-treated cooked pork mince

Results in Table 6.5 show that with increasing GEO concentration in pork mince the Singapore panel detected significant increases in garlic flavour intensity ($p< 0.0001$), decreases in mutton flavour intensity ($p<0.0001$) and increases in garlic flavour acceptability ($p<0.0001$). A garlic concentration of 175 ppm had the most intense garlic flavour. All garlic-treated samples were significantly different from the control in intensity of garlic flavour; with garlic concentrations at 125, 150 and 175 ppm having significantly less intense mutton flavour compared to control samples. Garlic flavour intensity was negatively related to mutton flavour intensity ($r=-0.979$, $p=0.01$), and positively related with garlic flavour acceptability score ($r=-0.989$, $p=0.04$) for the Singapore panellists.

While the differences among garlic concentrations were distinctive for the Singapore panel, this was not the case for the New Zealand panel. They detected a difference in mutton intensity in pork mince between the non garlic and garlic added samples ($p<0.001$) but were not able to clearly differentiate amongst GEO concentrations from 100 to 175 ppm. Changes in garlic taste acceptability were not significant ($p=0.743$). The positive correlation between garlic flavour concentration and its hedonic score was less evident for New Zealand consumers ($p=0.276$). Regression analysis (Table 6.4) indicated that for all three attributes the slope against GEO concentration was significantly greater for results from the Singapore panel, as shown by the significance of the interactions between country and GEO concentration ($p < 0.0001$).

Table 6.5

Least squares means showing the effect of added GEO on sensory attributes and hedonic ratings of cooked pork mince using Singapore and New Zealand consumer panellists

Sensory attribute [1, 2]	Conc. of GEO in pork mince (ppm)					Effect (P values)	R^2, RSD[4]
	0	100	125	150	175		
Singapore panel:							
Garlic taste intensity	1.00a	2.73b	2.83b	3.53c	4.00c	<0.0001	73.2, 0.831
Mutton taste intensity [3]	2.70c	2.17bc	1.87b	1.27ab	1.16a	<0.0001	68.6, 0.73
Garlic taste acceptability	NA	4.78a	5.97b	7.03c	7.27c	<0.0001	64.8, 1.48
New Zealand panel:							
Garlic taste intensity	0.20a	2.42c	1.72b	1.79b	2.18bc	<0.0001	64.3, 0.953
Mutton taste intensity [3]	2.08b	1.67a	1.61a	1.55a	1.53a	0.046	57.5, 0.784
Garlic taste acceptability	NA	6.62a	6.47a	6.39a	6.72a	0.743	74.0, 0.109

[1] Sensory attributes were scored on a scale of 1-5 (1 being "none" and 5 being "very intense") and acceptability was scored on a scale of 1-9 (1 being "Dislike extremely" and 9 being "Like extremely"). NA = "Not applicable".

[2] Means in the same row with no letters after them or with a common letter after them within column do not differ significantly (P >0.05).

[3] The significance of difference (using LSD) was based on the transformed means (natural log) of these attributes, but the untransformed means are shown here.

[4]Measures of the overall goodness-of-fit for the model include the coefficient of determination [R2 (%)] and the residual standard deviation (RSD).

6.3.3. Evaluation of pork from pigs fed with GEO

6.3.3.1 Pig performance

Growth efficiency was unaffected by the different levels of garlic concentration in the diet (Table 6.6). Generally, when total feed intake was greater, the average daily gain (ADG) tended to increase. However, in this experiment when garlic intake increased, ADG decreased without an effect on feed conversion efficiency. An opposite trend was observed by Paschma (2000) and Paschma and Wawrzynski (2003) who demonstrated that by feeding a nine-herb supplement to pigs for 16 weeks at inclusion levels of 0 – 2 % of the total diet, the ADG increased with increased dose level. Similar results were also reported by Grela (2000) after feeding 90 pigs on a diet supplemented with 0%, 2%, or 4% of a six-herb mixture, without any effect on feed conversion efficiency.

Dietary supplementation with garlic had no significant effects on carcass weight or back fat thickness (Table 6.6). However, there was a significant difference in dressing percentage. Carcass dressing percentage was significantly lower for the P_H group compared to the other treatments. Previous studies have also demonstrated no effects on these carcass traits (Grela, 2000; Paschma, 2000; Paschma & Wawrzynski, 2003). Hence manipulation with aromatic plant materials could be exploited without risk of negative effects on animal performance or carcass traits.

Table 6.6

Least squares means showing the effects of diets on growth performance and carcass characteristics of female Duroc-cross pigs. Diet group abbreviations are explained in Table 6.1

	Dietary treatment group[1,2]								Pooled SEM	P
	Animal + Plant Diet				Plant Diet					
	AP_0	AP_L	AP_M	AP_H	P_0	P_L	P_M	P_H		
Number of animals	4	4	4	3	4	4	4	4		
Growth performance										
Initial liveweight (kg)	47.9	48.5	47.8	49.7	46.5	48.8	49.0	48.4	2.99	0.910
Total feed intake (kg)	110.5b	108.7b	107.6ab	99.7a	106.8ab	105.0ab	107.2ab	109.9b	4.31	0.057
Average daily gain (kg)	0.77	0.76	0.74	0.70	0.72	0.71	0.66	0.72	0.049	0.157
Total garlic intake (g)	0.00	59.9a	155.5b	173.2c	0.00	58.0a	155.0b	236.6d	5.71	<0.001
Carcass traits										
Carcass weight (kg)	70.0	68.3	67.8	67.5	65.2	68.0	64.7	63.6	3.08	0.213
Back fat thickness (mm)	10.8	11.0	11.0	10.3	10.8	10.5	9.8	9.5	0.861	0.263
Dressing percentage (%)	77.4b	75.6b	76.7b	76.7b	75.9b	77.3b	75.8b	72.4a	1.39	0.005

[1]AP=diet containing animal and plant products; P=diet containing plant products only; GEO at 4 levels from 0 to 4 where 0=zero, L=low, M=medium, H=high

[2] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05).

6.3.3.2 Sensory evaluation by a consumer panel

Results from triangle tests for comparisons between pork from groups of pigs receiving GEO in their diet and control groups are shown in Fig. 6.1. Results for pigs on a diet containing animal and plant products and those containing plant products only have been combined in Fig. 6.1 as the patterns were similar for the two groups. Both the Singapore and New Zealand panellists were able to detect clear differences in the pork from pigs receiving medium and high levels of GEO in their diets, and the low level differences were just detectable ($p=0.013$ and $p=0.051$), respectively. The Singapore panel did not detect any significant differences between the groups in acceptability of flavour when only correct assessments of the odd sample were considered.

There have been few reports on the sensory effects of feeding herb and spice extracts to pigs. Grela (2000) reported no effects on the ratings of smell, juiciness, tenderness or palatability of pork longissimus muscle from pigs fed diets supplemented at 0%, 2%, or 4% with a six-herb mixture, and Janz et al. (2006) reported that sensory panellists were unable to detect flavour or aroma differences between control pork and pork from pigs fed with 0.05% of essential oils or oleoresins of rosemary, garlic, oregano and ginger. Both Grela (2000) and Janz et al. (2006) suggested that supplementation levels were too low to effectively influence flavour, odour and overall palatability of pork.

With respect to acceptability scores for pork from pigs with some animal products in their diets, the Singapore consumer panel did not detect any significant differences between the groups in acceptability of flavour when only correct assessments of the odd sample were considered (acceptability was not scored by the New Zealand panel). Pork from the groups with animal plus plant items in their diets with no garlic (AP_0) was as acceptable as pork from pigs fed with low, medium and high levels of garlic (Fig. 6.2), but for pork from pigs receiving plant products only in their diets, the pork from the group with no garlic (P_0) was equally acceptable to P_M pork, but slightly less acceptable than P_L and P_H pork. Overall, the P samples were more acceptable than the AP samples. This observation is supported by the results of a study

by Leong et al. (2010a) where the acceptability level for pork from plant-fed pigs was significantly better than that from pigs with animal products in their diets.

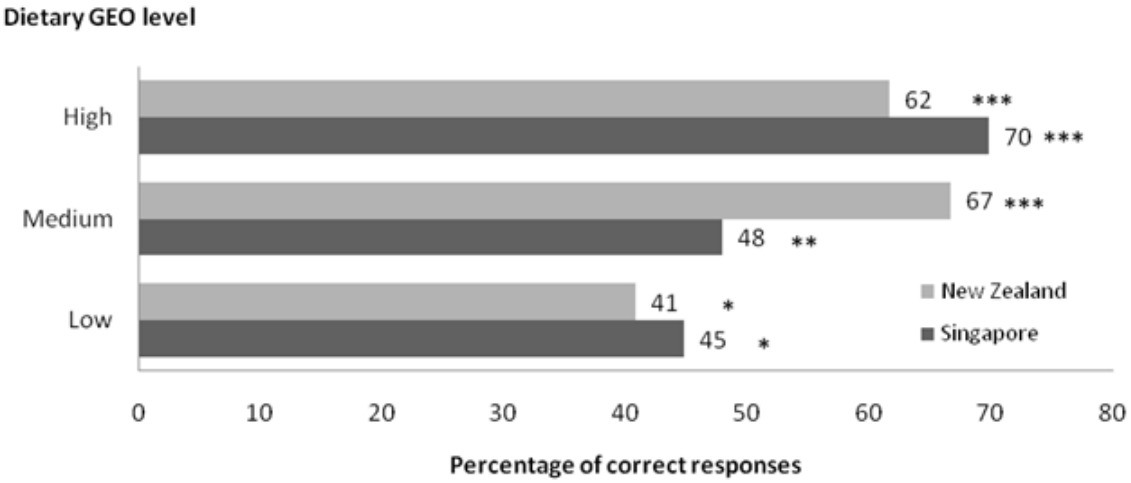
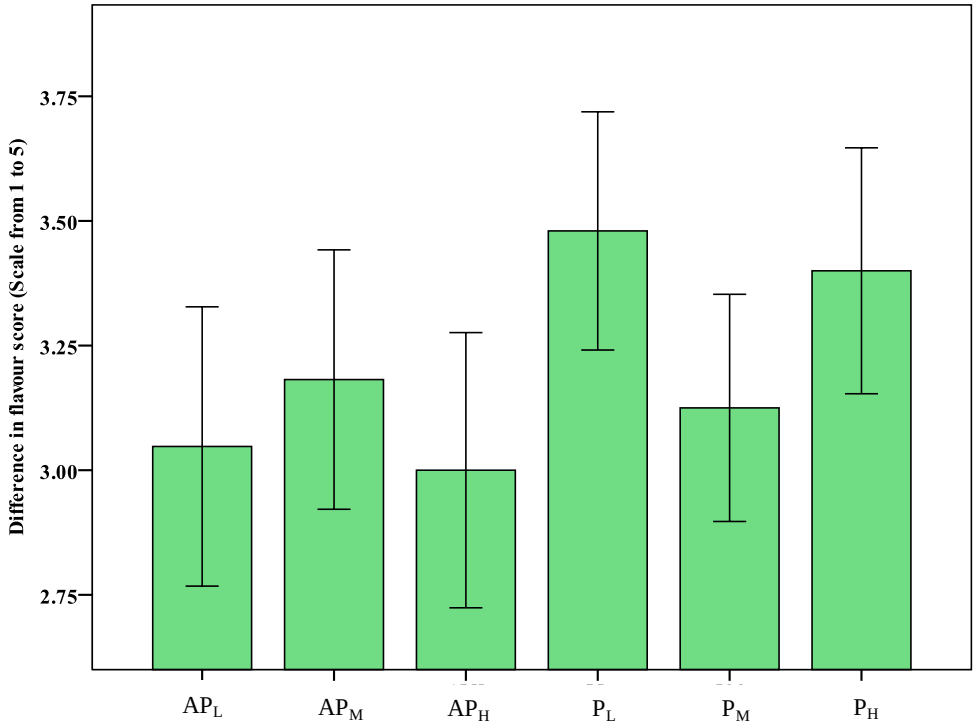


Fig. 6.1
The effects of the level of GEO in the diet (low, medium or high) of pigs on the ability of consumers in Singapore and New Zealand to detect differences between the pork from those pigs and that from pigs receiving no GEO using triangle tests (48 and 60 tests in Singapore and New Zealand, respectively). Significance of p values; *p< 0.05; ** p<0.01; ***p<0.001.



[1]3 = control and garlic treated sample equally acceptable; >3 = garlic treated sample was more acceptable; <3 = garlic treated sample was less acceptable

[2]AP=diet containing animal and plant products; P=diet containing plant products only; GEO at 4 levels from 0 to 4 where 0=zero, L=low, M=medium, H=high

Fig. 6.2
Mean (±SE) differences in scores for the acceptability of flavour of pork[1], as assessed by the Singapore consumer panel between groups receiving GEO[2] in their diet and the appropriate controls (i.e. either AP_0 or P_0).

6.3.3.3 Sensory evaluation by a trained panel

There were clear increases in the intensity of garlic aroma and garlic flavour, and a decrease in the intensity of mutton flavour of pork from pigs with increasing GEO in their diet (Table 6.7). Mutton aroma also decreased with increasing GEO level within both diet groups but the overall garlic effect was just significant (p=0.043). The mutton aroma scores for the AP_H, AP_M and AP_L groups were significantly lower than for the AP_0 group (Table 6.7). Aftertaste also increased with increasing level of GEO within the diet of the pigs (p = 0.003). Diet effects on flavour attributes were generally not significant (Table 6.7). Increased garlic flavour and decreased mutton flavour resulted for pork from pigs in the low-, medium-, and high-GEO groups for the plant-

only diet, but only in the medium-, and high-GEO groups for the animal-plus-plant diet (Table 6.7).

When the data was analysed with a model in which the amount of GEO consumed by individual pigs was fitted as a covariate (Fig. 6.3) a similar picture to that in Table 6.7 was obtained with increases in GEO consumption being associated with pork with more intense garlic flavour and aroma, and less intense mutton flavour and aroma. In the case of aroma scores there was no significant diet effect so there are only two lines in Fig. 6.3a, but for flavour attributes the presence of animal products in the diet of the pigs resulted in significantly higher mutton flavour scores ($p=0.002$) and significantly lower garlic flavour scores ($p=0.002$), to give the four lines in Figure 6.3b. These findings are in agreement with those from a garlic feeding study with sheep (Fraser, Lane, Kirk, Keogh & Cummings, 2007) in which meat from sheep that received a garlic powder supplement (>50 g/day) was described by trained panellists as 'garlic' and these samples were significantly less intense in 'sheep meat' flavours ($p<0.001$). Compared to those without garlic in the diet, the meat in that study from garlic-fed sheep also had increased 'barnyard' flavour (combination of sweaty, musty, animal, manure, dung and stale hay) in relation to those without garlic supplement in the diet.

Linear correlation coefficients across average flavour attributes for the 31 pigs indicated that mutton flavour was positively correlated with mutton aroma ($r=0.678$; $p<0.01$) and negatively correlated with garlic flavour ($r=-0.629$; $p<0.01$) and garlic aroma ($r=-0.629$; $p<0.01$). Garlic flavour was highly and negatively correlated with meaty aroma ($r=-0.666$), brothy aroma ($r=-0.502$), and mutton aroma ($r=-0.467$)

Table 6.7

Least squares means showing the effects of diet (Animal-Plant vs. Plant only) and GEO concentration (Table 6.1) on the sensory profile attributes of pork evaluated by a trained Singaporean sensory panel. GEO at 4 levels from 0 to 4 where 0=zero, L=low, M=medium, H=high

Sensory attribute	Animal-Plant (AP) diet groups				Plant-diet (P) groups				Effect (P values)		R^2%, RSD
	AP_0	AP_L	AP_M	AP_H	P_0	P_L	P_M	P_H	Diet	GEO conc. within diet	
Meaty aroma	49.77d	40.73bc	41.64bc	36.92d	47.07cd	46.67cd	34.02a	39.02ab	0.690	0.017	57.9, 12.99
Brothy aroma	36.43b	31.36ab	35.11ab	25.62a	34.65ab	33.37ab	28.20ab	32.55ab	0.883	0.364	70.9. 17.00
Garlic aroma[3]	0.739a	4.66c	4.97c	5.17cd	0.369a	3.50b	5.47cd	6.02d	0.909	<0.001	56.8, 2.10
	(0.546a)	(21.71c)	(24.71c	(26.73cd)	(0.136a)	(12.25b)	(29.92cd)	(36.24d)			
Metallic aroma[4]	1.24ab	0.878a	1.02ab	1.28b	1.26ab	1.25ab	1.25ab	1.29b	0.110	0.559	32.7, 0.767
	(2.46ab	1.41a	1.77ab	2.60b	2.53ab	2.49ab	2.49ab	2.63b			
Acidic aroma[4]	1.87c	1.26a	1.28a	1.57a	1.71abc	1.34ab	1.80bc	1.57ab	0.615	0.060	45.0, 0.906
	(5.49c	2.53a	2.60a	3.81a	4.53abc	2.82ab	5.05bc	3.81ab			
Mutton aroma[3]	6.29b	5.10a	4.61a	4.48a	5.02a	4.73a	4.81a	4.78a	0.210	0.043	13.7, 2.26
	(39.56b	26.01a	21.25a	20.07a	25.20a	22.37a	23.14a	22.85a			
Stale aroma[4]	2.70	2.40	2.74	3.12	2.72	2.49	2.49	3.01	0.841	0.529	30.0, 1.09
	(13.88	10.02	14.49	21.65	14.18	11.06	11.06	19.29			
Acidic taste[4]	2.04	2.02	2.30	2.38	2.41	2.21	2.01	2.31	0.583	0.355	49.9, 0.951
	(6.69	6.54	8.97	9.80	10.13	8.12	6.46	9.07			
Bitter taste[4]	0.832	0.852	1.008	0.815	0.990	0.935	1.39	0.815	0.272	0.293	31.5, 0.883
	(1.30	1.34	1.74	1.26	1.69	1.55	3.01	1.26			
Meaty flavour	47.62	48.34	43.64	42.14	46.19	45.42	39.82	41.72	0.274	0.450	54.4, 16.12
Brothy flavour	37.45	38.83	37.48	33.14	37.59	34.45	30.52	33.21	0.092	0.366	70.7, 17.83
Garlic flavour[4]	0.375a	2.59bcd	2.56bc	3.07d	0.323a	2.14b	2.85cd	2.96cd	0.899	<0.001	53.6, 1.12
	(0.45a	12.33bcd	11.94bc	20.54d	0.38a	7.50b	16.29cd	18.30cd			
Metallic flavour[4]	1.71	1.43	1.62	1.89	1.59	1.54	1.86	1.49	0.823	0.360	41.2, 0.997
	(4.53	3.18	4.05	5.62	3.90	3.66	5.42	3.44			
Mutton flavour[3]	5.20d	4.43c	2.91ab	3.66b	5.05cd	3.19ab	3.36ab	2.82a	0.132	<0.001	33.9, 1.71
	(27.04d)	(19.62c)	(8.47ab)	(13.40b)	(25.50cd)	(10.18ab)	(11.29ab)	(7.95a)			
Stale flavour[4]	2.40ab	2.13ab	1.99a	2.49ab	2.52ab	2.48ab	2.59b	2.09ab	0.252	0.401	37.0, 1.03
	(10.02ab)	(7.41ab)	(6.32a)	(11.06ab)	(11.43ab)	(10.94ab)	(12.33b)	(7.08ab)			
Aftertaste	37.48ab	45.59bcd	46.51cd	53.33d	38.48abc	34.02a	49.84d	50.21d	0.392	0.003	54.8, 18.02

[1]All attributes were scored on a scale of 0-100

[2] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[3] The significance of differences (using LSD) was based on the transformed means (square root) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

[4] The significance of differences (using LSD) was based on the transformed means ($\log_e$) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

Fig. 6.3
Regression lines showing changes in the intensity of garlic aroma and flavour and mutton aroma and flavouring pork (y) with increasing amounts of GEO consumed by the pigs (x).

The regression relationships for the AP and P diets (n=31) combined are:

(i) Garlic aroma intensity (y=0.157x+1.382), R^2=67.0%
(ii) Mutton aroma intensity (y=0.00060x^2-0.1825x+35.279), R^2=81.5%

The regression relationships shown separately for the AP (n = 15) and P (n = 16) diets are:
(i) garlic flavour intensity for AP diets (y=0.08x+1.37), R^2=67.0%
(ii) garlic flavour intensity for P diets (y=0.099x+3.07), R^2=84.0%
(ii) mutton flavour intensity for AP diets (y=0.00040x^2-0.2162x+50.377), R^2=83.1%
(iv) mutton flavour intensity for P diets (y=0.00060x^2-0.2093x+39.693), R^2=66.5%

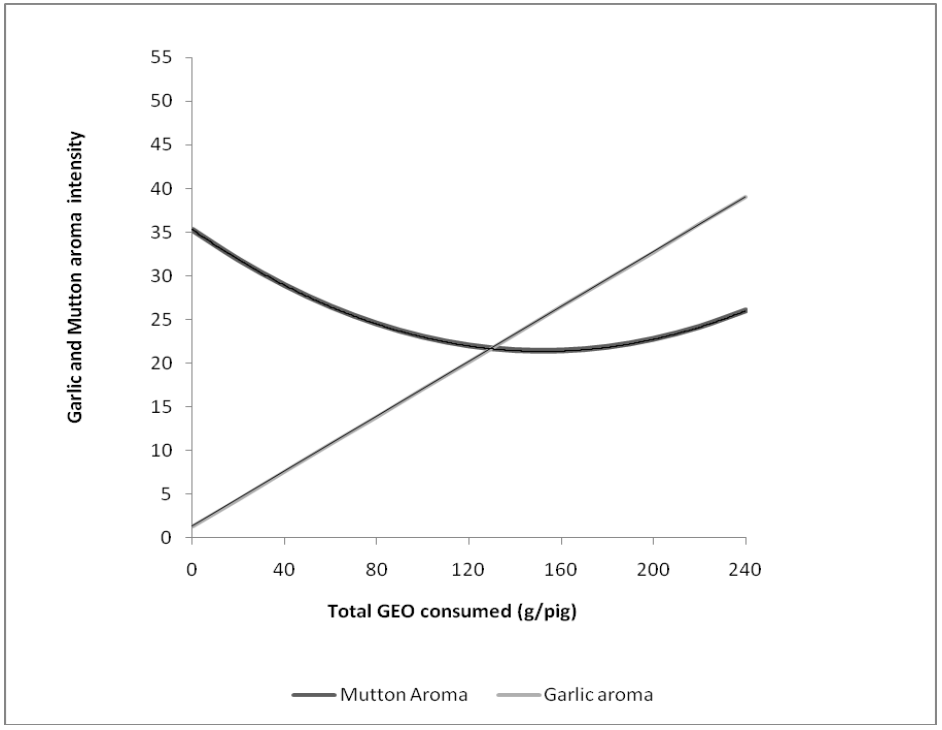


Figure 6.3 a

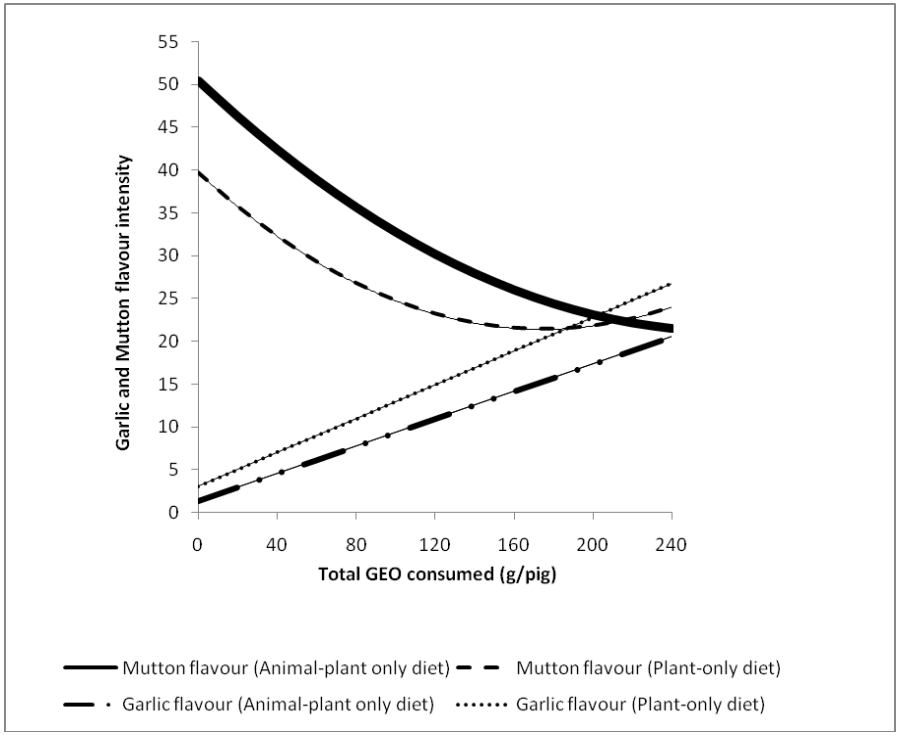


Figure 6.3 b

6.3.4 Relative costs of different forms of garlic

Results in Table 6.8 indicate that in order to acquire the same potency the costs would be lowest for GEO followed by garlic oleoresin (GO), and garlic powder (GP), with fresh garlic (FG) being the most expensive. It should be noted, however, that these estimates are totally dependent on the values used for the relative potency and relative cost values used.

In comparing the amount of GEO required to produce a similar effect when it is added to mince or to the diet of pigs, it can be seen from Table 6.3 that 125 ppm of GEO in pork mince resulted in a significant decrease in mutton taste and an increase in garlic taste for panellists in Singapore as well as New Zealand. The amount of GEO consumed by pigs on the plant-only diet to obtain a clear reduction in mutton flavour was about 150 g (Fig. 6.3). If it is assumed that this was evenly spread through about 47 kg of pork (70% yield from 67 kg carcasses), then the GEO required was about 3191 ppm (or $mg \cdot kg^{-1}$). Pigs on the animal-plus-plant diet required more GEO intake to achieve the same reduction in mutton flavour (Fig. 6.3). If this is taken as 220 g of GEO, then the effective GEO requirement was 4681 ppm. Thus, based on these estimates, and relative to the amount required when added to mince, the amount required when fed to pigs was 25 times as much for pigs receiving the plant-only diets and 37 times as much for the plant-plus-animal diet. The price of 150 and 220 g of GEO required for 47 kg of pork was \$15 and \$22, respectively, at a cost of \$0.10/g (\$100/kg). This will be equivalent to \$0.32 and \$0.47 per kg of pork or \$0.22 and \$0.33 per kg of carcass.

Table 6.8

Estimates of the relative costs of different forms of garlic including fresh garlic (FG), garlic powder (GP), garlic oleoresin (GO) and GEO based on differences in potency and costs

Item	Amount required (g) for the same effect (potency) as 1 g of GEO[1]	Cost per kg relative to FG that is set at \$2[2]	Cost for the same level of potency relative to FG set at 100
FG	360	2	100
GP	135	5.5	69
GO	4	80	30
GEO	1	100	14

[1] Based on estimates from Kalsec (2009).
[2] Based on relative prices in Singapore during July 2009.

6.3.5 General Discussion

Novel methods like the inclusion of GEO in the diet of pigs or mixing GEO with pork mince have the long-term goal of devising and characterising production and processing systems that improve the acceptability of pork for consumers in Singapore and possibly other countries as well. Although the inclusion of GEO in the diet of pigs improved pork flavour acceptability for Singapore consumers, the economics of this approach need to be considered, especially from the perspective of the pig producer. This was done in Section 3.4 above and indicated, first, that GEO was as cost effective as other forms of garlic based on the costs considered, and, secondly, that much larger amounts of GEO would be required to produce a similar reduction in mutton flavour notes when fed to pigs than when added to pork mince.

The relative potency of different forms of garlic varies quite widely. For example, the strength of GEO vs. fresh garlic used here was 360:1 (Kalsec, 2009) while Raghavan (2006) and Brewster & Rabinowitch (1990) estimated the ratio as 900: 1. The key compounds in garlic that are responsible for its flavour and aroma are diallyl and allyl di-and tri-sulphides (Lawson & Hughes, 1992; Shaath, Flores, Mohamed & Mohamed, 1995; Sowbhagya, Purima, Florence, Rao & Srinivas, 2009). These compounds are products from allicin which is a highly unstable and reactive compound that rapidly decomposes to other compounds. For this reason, no garlic product on the market contains a detectable amount of allicin (<1 μg/g) (Freeman & Kodera, 1995; Amagase, 2006). In the market, the GEO contains only oil-soluble sulphur compounds like allyl methyl trisulphide (0.324 mg g^{-1}, diallyl disulphide (0.340 mg g^{-1}) and diallyl trisulphide (0.282 mg g^{-1}); while the garlic powder contains alliin and a small amount of oil-soluble sulphur compound like allyl methyl trisulphide (0.020 mg g^{-1}, diallyl disulphide (0.008 mg g^{-1} and diallyl trisulphide (0.022 mg g^{-1}) (Yan, Wang & Barlow, 1992; Yan et al., 1993, Corzo-Martínez, Corzo & Villamiel, 2007). Because of the different active compounds in different forms of garlic, there appear to be no comparisons that have been made of the potency of the different forms based on concentrations of specific compounds or groups of compounds.

The use of GEO or GO is preferred to GP or FG as components of pig diets because they are free of microorganisms (Reineccius, 2006) and hence have longer shelf-lives. Therefore, the pigs are assured of a better quality feed if either GEO or GO is used. Moreover, GEO and GO do not introduce unwanted colour and moisture into the meat, and users do not have to worry about seasonal fluctuations in cost, quality and availability, or about the greater storage space needed for FG and GP.

The merits of adding GEO to the pig diets rather than to mince will include the fact that the pork from these animals can be regarded as a natural and unadulterated product, which may be important to some consumers. Besides, preparation work including mincing and mixing of garlic into the meat or possibly pumping the meat are not needed. With less handling required, it is expected that the pork will have a lower microbial load and hence better meat quality.

Finally, it is noted that garlic has been considered as antimicrobial due to the bioactive substance allicin which is also responsible for the strong odour of garlic (Block, 1985). Studies have shown that allicin has antibacterial, antiparasite and antifungal activities (Ross, O'Gara, Hill, Sleightholme & Maslin, 2001). Allicin also shows an inhibitory immunomodulatory effect on intestinal epithelial cells (Lang et al., 2004) and has the potential to attenuate intestinal inflammation. Therefore, dietary addition of garlic could be beneficial to immune-related parameters in pigs.

6.4. Conclusions

Threshold tests for garlic essential oil (GEO) in cooked pork mince showed that a significant increase in garlic taste was detected at 125 ppm of GEO, and this was associated with a significant reduction in mutton taste for both Singapore and New Zealand panellists, but an improved acceptability of garlic taste was shown by Singapore panellists only.

Significant increases in garlic flavour and aroma of pork from pigs fed diets containing GEO were also shown, but the amount of GEO required to produce a similar effect was more than 20 times as much as when it was added directly to mince prior to cooking. As with the mince, the increase in garlic flavour of pork from GEO-fed pigs was associated with a decrease in the intensity of mutton flavour.

At current prices of GEO, it is estimated that the extra cost of pork products to cover the 150-200 g of GEO that would need to be fed to pigs would not be more than $0.50/kg.

These results provide an example of where an aromatic plant material successfully masked an undesirable flavour note in pork by either adding it to minced pork or by including a larger amount in the diet of pigs.

Chapter 7

Effects of dietary components including garlic on concentrations of skatole and indole in subcutaneous fat of female pigs

This chapter has been partly published in Meat Science in 2011.
Leong, J., Morel, P. C. H., Purchas, R. W., & Wilkinson, B. H. P. (2011). Effects of dietary components including garlic on concentrations of skatole and indole in subcutaneous fat of female pigs. *Meat Science*. *88*, 45-50.

7.1 Introduction

Flavour is an important aspect of acceptance of pork (Leong, Purchas, Morel & Wilkinson, 2008a). Diets fed to pigs can lead to production of pork flavours that are considered acceptable for some consumers, but undesirable for other. A classical example of undesirable flavour in pork is boar taint which is due in part to the presence of skatole (Vold, 1970, Walstra,& Maarse, 1970; Hansson, Lundström, Fjelkner-Modig & Persson 1980). Singapore consumers often associate the presence of mutton flavour as an undesirable pork attribute that is more common in pork from western countries like Australia, Canada and New Zealand, than in pork from countries such as Indonesia and China (Leong et al., 2008).

Taints and foreign-flavours in meat can be the result of dietary components that result in the generation or transfer of undesirable compounds to the product. There have been a number of studies into the feasibility of changing the flavour profile of pork by feeding pigs with different plant and animal materials. These include the direct transfer of aromatic components rosemary, oregano, ginger, garlic or chicory that influence sensory quality (Janz, Morel, Wilkinson & Purchas, 2006; Hansen, Agerhem, Rosenvold & Jensen., 2002). A widely studied example is the transfer of aromatic components from fish that result in a fishy flavour in pork (Jaturasitha, Wudthithumkanaporn, Rurksasen & Kreuzer., 2002; Kjos, Skrede & Overland., 1999; Lauridsen, Anderson, Andersson, Danielsen, Engberg, & Jakobsen., 1999).

Several studies have shown that feeding rape seed meal or cake of poor quality to pigs and cows has resulted in inferior odour and flavour of the cooked meat (Andersen & Sørensen, 1985; Hertzman et al., 1988). There have also been reports of positive effects of certain feed components such as chicory roots (Jensen & Hansen, 2006) and potato starch (Claus, Losel, Lacorn, Mentschel & Schenkel, 2003; Zamaratskaia, Babol, Andersson, Andersson & Lundström. 2005) on the aroma and flavour of pork due to reduction of skatole. Ammonia and dust concentrations from the husbandry and housing system also cause off-flavours in pork products (Maw, Fowler, Hamilton & Petchey, 2001).

The objectives of this paper were, first, to establish the detection threshold levels of skatole and indole for Singapore consumers, and, secondly, to investigate the effects of diets, including those containing garlic, animal products, and several different lipids on the concentrations of skatole and indole in the adipose tissue of pigs.

7.2 Materials and methods

7.2.1 Threshold test for skatole and indole

Sensory thresholds were determined by using the forced-choice ascending concentration method of limits described by the American Society for Testing and Materials, designation E679-04 (ASTM, 2004). The test aims to determine a practical value close to the threshold, based on a minimum testing effort. It helps to determine a very approximate best estimate determination threshold for each panellist. The procedure involved presentation of three samples at a time with each set consisting of two blanks and one test sample. The odd sample was at all times the test sample under consideration. All evaluations were conducted in a sensory panel room containing 8 separated booths at 21 ± 1°C.

The panel consisted of three Chinese males and five Chinese females between 20 and 30 years of age initially selected using British Standards Institution (BSI) (British Standards Institution, 1993) methods. They received 2 weeks of training (5 sessions) on the discrimination and recognition of skatole and indole. All the panel members had at least two years in panel assessment of meat and meat products.

Samples for threshold tests were prepared by spiking a neutral lipid base of rice bran oil (Tong Seng Produce Pte Ltd, Singapore) with the required concentration of either indole or skatole (Sigma-Aldrich Co, Ltd, Poole, UK). Samples of 20 mL were prepared in 50 mL amber glass screw-top bottles that were heated to 60 – 65°C and held for 20 mins before evaluation by panellists to ensure that sufficient of the compounds volatilised within the headspace for sniffing. For sensory threshold tests, six ascending concentrations of skatole and indole in binary steps were used (0.0125, 0.025, 0.05, 0.10, 0.20, 0.40 μg/g). The concentrations were selected based on the range of 'First Approximation' thresholds obtained from the trained panel following earlier tests.

Panellists were individually seated in well-ventilated booths and were each presented with one blind-coded, 3-AFC set of samples that consisted of one level of the test sample and two blank samples. The order of presentation of the samples within a set of three samples was randomised. In addition to determining whether there was an odd sample in the set, when an odd sample was chosen correctly, panellists were also required to give a rating on the level of difference of the odd sample from the other two based on a scale from 1 to 10 with 1 = “least different” and 10 = “most different" (See evaluation form at Appendix 7.1). On each assessment day, six levels of indole or skatole were presented to the panellists in ascending order in six panel sessions separated 20 min apart. The assessments were replicated five times over a period of five weeks and were all carried out under red lights.

The Best Estimate Detection Threshold (BEDT) value for a panellist within a replicate was calculated as the geometric mean of the lowest concentration that was detected as being different and the next lowest concentration. The group BEDT detection values were calculated as the average geometrical means of the BEDT of the 5 replicates for each panellist and then by combining these across the 8 panellists. Reliability of BEDTs was estimated as inter-replicate correlations between individual means over the five replicates in each case.

The odour profile of skatole and indole were also determined by the trained panel. There were three parts to this experiment.

In the first part, the concentration of indole and skatole were derived based on the supra-threshold levels of indole and skatole, that were arrived at by consensus through a round table discussion. This discussion process took place for three hours.

The concentrations of the samples were as followed:

1. Indole (1.0 μg/g) (I)
2. Skatole (1.0 μg/g) (S)
3. Indole (1.0 μg/g) + Skatole (1.0 μg/g) (IS)

In the second part of the experiment, the characteristics relating to the odour of either indole and skatole were arrived by consensus by the same panel group. For each sample, panellists were expected to rate the perceived intensities of all the descriptors on a 15-cm unstructured scale. The descriptors were developed based on the supra-threshold levels of indole and skatole. In the final part, panellists were individually seated in well-ventilated booths and were presented with three single samples monadically (See Appendix 7.2 for evaluation form).

7.2.2 Pig feeding experiments

The experiments were conducted in accordance with the ‘‘Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching” (Massey, 2008).

7.2.2.1 Experimental design for Experiment A

The objective of this experiment was to investigate the effects of different levels of dietary garlic essential oil (GEO) on skatole and indole concentrations in pork subcutaneous fat. GEO (Product number: 31-05) was obtained from Kalsec Corporation, USA. Because garlic is one of the most popular flavoured plant materials used with pork in Singapore (Leong, Purchas, Morel & Wilkinson, 2008b), it was chosen to be tested for its ability to suppress undesirable mutton-like flavours in pork for the Singapore market.

The diets, animals, and experimental design were described by Leong et al., 2010. In short, GEO was added directly to the diets of the 31 female pigs (Duroc x (Large White x Landrace)) grown on diets containing either animal-plus-plant products (AP diet) or plant products only (P diet) with four levels of GEO: 0, 0.55, 1.44 and 1.84 g/kg feed for the AP diet, and 0, 0.55, 1.44 and 2.15 g/kg feed for the P diet.

7.2.2.2 Experimental design for Experiment B

In Experiment B, the effects of dietary fish oil, tallow and plant oils with either animal plus plant components in the diet or plant components only were investigated.

Forty-seven female pigs (PIC hybrids, with a mean starting live weight 18.90 kg ± 1.75 (mean±SD) from a single commercial operation in the North Island of New Zealand, were assigned to one of six dietary treatment groups (as shown in Table 7.1 with PFS having two sub groups as explained below). They grew at a mean rate of 851 ± 56 g/d over a period of 84 days to produce an average carcass weight of 72.1 ± 4.7 kg. Growth rates and carcass weights did not differ significantly between treatments ($p>0.05$). The pigs were kept in pens of six, but were fed individually twice daily. Water was available at all times. Individual feed intakes were measured daily and live weights recorded weekly.

In this experiment, the effects of (1) lipid type (soy bean oil, linseed oil, tallow and fish oil), (2) the period the fish oil was provided and (3) a dietary supplement (Sanovite^{TM}) containing conjugated linoleic acid (CLA), selenium, vitamin E and vitamin C on pig performance and pork quality were studied.

This experiment follows on from that reported by Janz et al. (2008) and Morel et al. (2008) in which the influence of diets supplemented with Sanovite^{TM}, with or without animal protein, on the growth performance, meat quality, and pork fatty acid profile from female pigs was studied.

The diet base was either a combination of animal and plant feedstuffs (AT and PTS), plant feedstuffs only (PO, POS) or plant feedstuffs combined with fish oil (PFS).

The diets also differed depending on the presence or absence of the nutritional supplement Sanovite™ and Vitamin C. Diet POS, PTS and PFS contained Sanovite™ and Vitamin C. The composition of the grower and finisher diets are given in Table 7.1.

The diets AT, PO, POS and PTS were fed over the whole experiment (84 days). The PFS was fed either between day 1 and 35 of the experiment (experimental group PFSe) or between day 36 and 56 of the experiment (experimental group PFSl). The total amount of fish oil fed per pig for groups PFSe and PFSl was the same at 2.31 kg. These two groups received the POS diet when diet PFS was not fed, which included the 28 days prior to slaughter.

Table 7.1

Ingredient composition of the diets on an as-fed basis for the grower and finisher periods for the five diets[1]. No values are given for PFS during the finishing period because no fish oil was fed during that period

	Grower period (days 1 to 56)					Finisher period (days 57 to 84)			
Ingredient (g/kg)	AT	PO	POS	PTS	PFS	AT	PO	POS	PTS
Barley	559.5	673.7	673.7	673.7	673.7	559.5	673.7	673.7	673.7
Wheat	100	0	0	0	0	100	0	0	0
Broll[2]	60	60	60	60	60	76	76	76	76
Soybean meal	70	160	160	160	160	70	160	160	160
Blood meal	30	-	-	-	-	30	-	-	-
Meat and bone meal	130	-	-	-	-	130	-	-	-
Tallow	44	-	-	44	-	28	-	-	28
Soybean oil	-	33	33	-	-	-	21	21	-
Linseed oil	-	11	11	-	-	-	7	7	-
Fish oil	-	-	-	-	44	-	-	-	-
Amino Acids	2.5	8.3	8.3	8.3	8.3	2.5	8.3	8.3	8.3
Mineral and vitamin	4	54	54	54	54	4	54	54	54
Sanovite™, [3]	0	0	6.14	6.14	6.14	0	0	6.14	6.14
Vitamin C	0	0	3.0	3.0	3.0	0	0	3.0	3.0
Calculated Composition									
Digestible energy (MJ/kg)	13.96	13.98	13.98	13.98	13.98	13.54	13.56	13.56	13.56
Ileal digestible lysine (g/kg)	9.1	9.7	9.7	9.7	9.7	9.2	9.7	9.7	9.7
Hindgut tryptophan[4] (g/kg)	0.57	0.48	0.48	0.48	0.48	0.57	0.48	0.48	0.48

[1]AT=diet containing animal and plant products; PO= diet containing plant products only; POS=diet containing plant feed products and Sanovite™; PTS=diet containing plant products with tallow; PFS=diet containing plant products and fish oil.

[2] Broll is a coarse product obtained whilst milling wheat flour and is a mixture of bran and pollard.

[3] Sanovite™ contained CLA, vitamin E (BASF,Auckland New Zealand), and organic Se (Alltech Inc., Nicholasville, KY) (Morel et al. 2008).

[4] An estimate of the amount of tryptophan that would have reached the hindgut per kg of diet.

7.2.3 Skatole and indole analysis in fat samples

Reference compounds indole, skatole, and the internal standard 2-methyindole were obtained from Sigma Aldrich, Singapore. They were of analytical reagent grade. Methanol and acetonitrile were HPLC grade (Sigma Aldrich, Singapore).

The samples were prepared as described by Tuomola, Vahva & Kallio (1996) with minor modifications. Liquid nitrogen was used to freeze a fat sample (2.4 – 2.5 g) which was then crushed to powder using a pestle in a mortar (Biase, 2002; Deveaud, 2007; Fraser, personal communication, 2008). The crushed sample was placed in a centrifuge tube containing methanol (5.0 mL) together with 50 μL volume of 2-methylindole as an internal standard (10 μg/mL in methanol). The tube was vortexed at high speed for 5 mins to ensure dispersion. After that, it was sent for sonication for 5mins before it was centrifuged (Eppendoft, model 5810R, Germany) at 3221 x g for 20 mins. The supernatant was passed through an activated and chilled Sep-Pak C18 column (Supelco, U.K.). The first 1 mL of the elutant was discarded and the next 2 mL collected. An aliquot of 20 μL was analysed by HPLC using a Shimadzu (Kyoto, Japan) HPLC system (Prominence series) consisting of a CMB-20A controller, two low pressure pumps (model LC-20A), an SIL-20A autosampler, and an RF-530 fluorescence monitor. The column used (a Superspher 100 RP18 (125 x 4 mm i.d. particle size 4 μm) was eluted with a mobile phase consisting of water:acetonitrile (60:40, v/v) at a flow rate of 1mL/min at 30°C. The native fluorescence of indolic compounds was monitored by using an excitation wavelength of 270 nm and an emission wavelength of 350 nm.

7.2.4 Skatole and indole analysis in commercial samples

Pork samples from the loin, belly, rib and shoulder cuts imported from Australia, Brazil, Indonesia, Canada and France were purchased from retail stores in Singapore (Fairprice and Cold Storage) for the analysis of skatole and indole. Samples from all four cuts were obtained for pork from Australia, Brazil and Indonesia, while samples from only shoulder and loin cuts were obtained for French pork and only from the shoulder cut for Canadian pork.

7.2.5 Statistical analysis

All statistical analyses were conducted using SPSS ver. 18 (SPSS for Windows, version 18, SPSS Inc, Singapore).

Effects of GEO intake (as a covariate), diet (animal-plant vs. plant only diet), and diet x GEO intake interaction on skatole and indole concentrations of pork back fat were determined from ANOVA (Type I Sums of Squares) at 5% level of significance using General Linear Model (GLM) to determine differences among the treatments. Effects of diets containing animal and plant products with or without a dietary supplement (S) , tallow (T) or fish oils (Fe & Fl)) (Table 7.1) on skatole and indole concentrations in the back fat from pigs were analysed by ANOVA with a focus on a set of five non-orthogonal contrasts that were selected *a priori* as follows:

- AT vs PO (a test of animal + plant diet vs a diet containing plant products only)
- PO vs POS (a test of a supplement effect)
- POS vs PTS (a test of the effect of tallow vs the plant oils)
- POS vs [PFSe + PFSl] (a test of fish oil vs plant oils)
- PFSe vs PFSl (a test of the early vs late consumption of fish oil by the pigs)

For the commercial pork samples, effects of country, product (belly, loin, rib, shoulder) and country x product were also analysed using ANOVA. The significance of differences between least squares means was assessed using the Fisher's Least Significant Difference test.

7.3. Results and Discussion

7.3.1 Threshold levels of skatole and indole by a Singapore panel

Individual BEDT for the eight panel members varied between 0.018 to 0.141 μg/g for skatole and 0.018 to 0.566 μg/g for indole. The group BEDT for skatole and indole were 0.028 μg/g and 0.051 μg/g, respectively (Table 7.2). Individual BEDTs for skatole and indole resulted in approximately 8-fold and 32-fold ranges, respectively, between panellists within a panel. The threshold value for skatole in relation to "boar taint" for the European Community has been reported as 0.20 μg/g by Mortensen and Sørensen (1984), which is about 8 times higher than that for the Singapore panel.

Sather (1995) reported a BEDT of 0.22 μg/g for skatole dissolved in sunflower oil with alternate-forced-choice presentations, while Moss et al. (1992) reported a much lower threshold of 0.08 μg/kg for skatole in a specially prepared meat puree mix. In a study by Annor-Frempong et al. (1997) the group BEDT for skatole spiked in sunflower oil was 0.026 μg/g. The threshold test was evaluated by ten females between 30 and 60 years of age. There was no mention of the race of the panel. No reports have been located that report threshold flavour levels for indole.

Table 7.2
Detection thresholds for skatole and indole as assessed by 8 panellists over a series of 5 replicated experiments

Panellist	Skatole threshold μg/g	Indole threshold μg/g
1	0.018	0.023
2	0.018	0.027
3	0.018	0.246
4	0.018	0.023
5	0.018	0.107
6	0.018	0.081
7	0.123	0.031
8	0.107	0.047
Group threshold	**0.028**	**0.051**

Inter-replicate correlations across panellists over the five replicates were mainly significant (Table 7.3) and were generally higher for skatole than for indole.

Table 7.3
Inter-replicate (Rep) correlations for skatole and indole thresholds across the eight panellists

	Skatole concentration				Indole concentration			
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
Rep 2	0.771***				0.258			
Rep 3	0.783***	0.765***			0.470**	0.651***		
Rep 4	0.710***	0.861***	0.843***		0.475**	0.743***	0.837***	
Rep 5	0.795***	0.825***	0.749***	0.853***	0.389*	0.633**	0.844***	0.887***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Scores for levels of difference in the threshold tests (Table 7.4) increased beyond 0.050 μg/g for both skatole and indole, which was around the group BEDT of 0.051 μg/g for indole. The mean levels of difference from the control for skatole were generally much higher than those for indole for all concentrations except at 0.0125 μg/g (Table 7.4).

Table 7.4
Mean levels of difference detected for increasing concentrations of skatole and indole in rice bran oil, using a scale from 1 to 10 where 10 is the most different

	Concentration μg/g [2]						Conc. Effects (p-values)	R^2	RSD
	0.0125	0.025	0.050	0.10	0.20	0.40			
Skatole	1.22a	2.74b	3.55bc	4.32c	5.70d	6.78e	<0.001	65.6	5.58
N[1]	9	39	40	40	40	40			
Indole	1.47a	2.10ab	2.22ab	2.86bc	3.25cd	3.87e	<0.001	41.8	1.62
N	15	29	32	36	39	40			
P-values for the difference between skatole and indole values	0.325	0.051	<0.001	<0.001	<0.001	<0.001			

[1]N = Number of times out of 40 (8 panellists x 5 replicates) when a difference was detected between the control sample and the sample with the relevant concentration of skatole or indole in rice bran oil.
[2]Means in the same row with no letters after them or with a common letter (a, b, c) after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

7.3.2 Odour profiles of skatole and indole

Based on the initial consensus profiling, a ten-descriptor profile was generated for indole and skatole. The important descriptors for the odour of indole included: Napthalene, sweaty, ammonia-like and mutton-like. The important descriptors for the odour of skatole were: Napthalene, sweaty, ammonia-like, mutton-like, musty, faecal, nauseating, pungent, sewage and stale.

Results showed a reasonably clear-cut distinction between indole and skatole. Scores for faecal, nauseating, pungent and sewage notes were significantly ($p<0.05$) higher for skatole than indole thereby providing a useful means of distinguishing between these two compounds (Table 7.5), with the highest R^2 of 0.613 for the nauseating note. The faecal, pungent and sewage notes were identified as significant ($p<0.05$) discriminating descriptors with R^2 of 0.448, 0.613 and 0.463 respectively (Table 7.5).

The intensity of mutton-liked, napthalene and sweaty notes increased in the mixture of indole and skatole as compared to individual pure samples of indole and skatole although this effect was small and not statistically significant.

Table 7.5
Least squares means of odour profile scores for indole only, skatole only and mixture of indole and skatole based on a scoring system of intensity scores from 0 to 100 where 0 is not intense and 100 is extremely intense. Measures of the overall goodness-of-fit for the model include the coefficient of determination [R^2(%)] and the residual standard deviation (RSD)

Odour note	Indole only[1]	Skatole only	Indole+ Skatole	R^2	RSD
Napthalene	49.10	55.20	57.74	12.8	29.8
Sweaty	22.09	35.17	38.42	28.9	24.88
Ammonia	21.88	32.68	31.89	20.7	28.96
Mutton-liked	20.01	23.82	25.02	34.1	17.60
Musty	19.81	28.92	22.99	30.2	24.52
Faecal	15.15a	41.32b	29.77ab	44.8	29.86
Nauseating	16.15a	54.93b	34.69ab	61.3	31.77
Pungent	19.01a	54.08b	35.17ab	61.1	28.71
Sewage	18.27a	43.91b	23.57a	46.3	26.01
Stale	18.80	34.90	21.08	30.2	24.69

[1] Means within a row followed by letters that do not include a common letter are significantly ($p<=0.05$) different

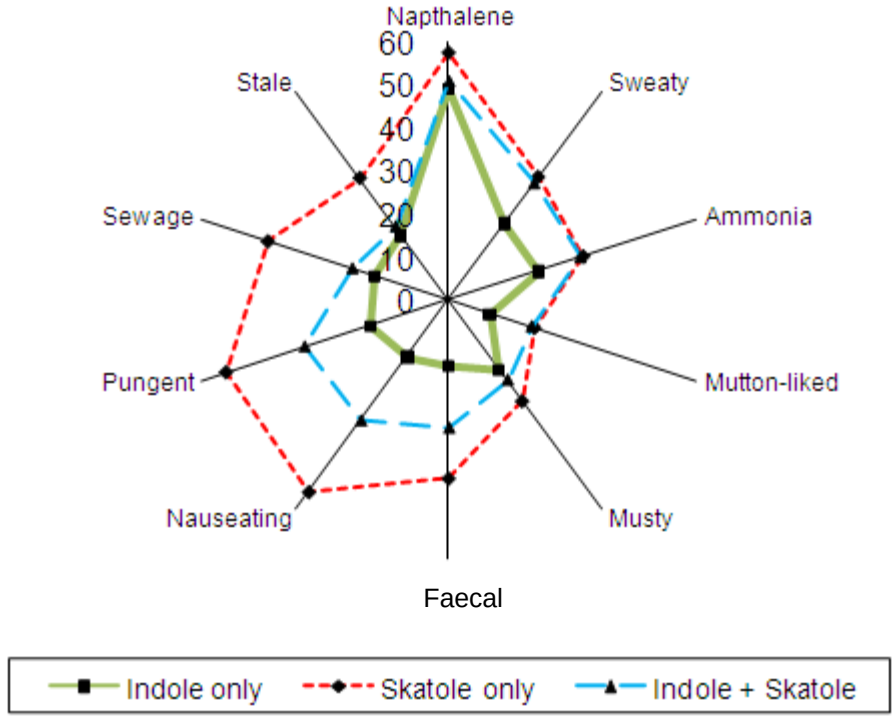


Fig. 7.1
Odour profiles for skatole and indole using a scale of 0 to 100 where 0 is not intense and 100 is extremely intense.

7.3.3 Effects of dietary garlic on skatole and indole concentrations in pork fat (Experiment A)

The concentrations of skatole and indole in back fat increased with increasing garlic concentration in the diet (Table 7.6), by more than 20-fold for skatole and indole at the highest garlic level on the AP diet and by more than 10-fold on the P diet ($P < 0.001$). Skatole and indole levels were higher for pigs on the AP diet than the P diet ($P < 0.05$). There was no interaction between diet and GEO intake for skatole and indole concentration. Garlic intakes (Table 7.6) were higher for pigs on the plant-only diet at the higher levels of GEO offered, which led to highly significant diet and GEO level effects, and also to a significant interaction between GEO level and diet.

The association between mutton flavour and skatole/indole levels appears to have been overridden by the flavour contributed by the GEO. A trend of increasing garlic note intensity and decreasing mutton flavour note intensity with increasing dietary garlic levels was observed for pork from the pigs of Experiment A. This suggests that despite higher levels of skatole and indole produced by the GEO, the scores for mutton flavour were lower rather than higher, probably due to the masking effects of the GEO flavour

The basic effects of garlic when used in cooking can be for masking other flavours and/or for injecting pungency to the food (Ravindran, Johny & Nimal Babu, 2002). This experiment shows that garlic can be used to suppress an undesirable flavour in pork. This could be due to the key compounds in garlic that are responsible for its flavour and aroma including diallyl and allyl di-and tri-sulphides (Lawson & Hughes, 1992; Shaath, Flores, Mohamed & Mohamed, 1995; Sowbhagya, Purima, Florence, Rao & Srinivas, 2009). Fraser et al. (2007) also reported significant effects of dietary garlic powder on sheepmeat flavour as well as significantly higher levels of skatole and indole in the subcutaneous fat ($p < 0.001$) of animals fed garlic powder. This concurs with results of the current study in showing that dietary garlic can lead to higher skatole and indole concentrations in fat.

The sulphur components in garlic have been hypothesised to have an effect on the liver metabolism of skatole and indole in sheep, thereby decreasing the removal of skatole and indole from the blood, which consequently can affect the sensory quality of

meat (Roy, Fraser, Lane, Sinclair & McNabb, 2004). The same mechanism could have been operating in this pig study where increasing levels of GEO in the diet were observed to give higher skatole and indole levels in pork fat. In addition, Yang, Chhabra, Hong & Smith (2001) showed that sulphur compounds in garlic can inhibit the P4502EI enzyme in rats. This enzyme has been shown to be involved in the hepatic metabolism of skatole in pigs (Babol, Squires & Lundstrom, 1998).

Table 7.6

Means showing the effects of diet (Animal+Plant vs. Plant only) and GEO concentration (Table 7.1) on levels of GEO intake[1] and the skatole and indole concentrations in back fat. For skatole and indole, untransformed means are given for the eight groups, but statistical analysis was carried out on natural logs so the RSD and slope values are in those units

	GEO Intake (g)	Skatole (ng/g)	Indole (ng/g)
Animal + plant diet:			
Control	0	39.6	35.8
Low GEO	59.8	131.8	84.8
Medium GEO	155.5	256.0	398.1
High GEO	179.1	1001.5	972.5
Plant-only diet:			
Control	0	23.1	18.91
Low GEO	58.0	47.6	53.7
Medium GEO	155.0	299.2	545.5
High GEO	236.6	435.0	823.1
$R^2(\%)^2$	99.5	76.8	84.8
RSD^2	6.5	0.719	0.658
Slope with GEO intake	-	+0.0136	+0.0173
Effects (P values):			
GEO group	<0.0001	-	-
Diet	<0.0001	0.007	0.030
GEO * Diet	<0.0001	-	-
GEO intake as a covariate	-	<0.0001	<0.0001
GEO intake*diet	-	0.45	0.81

[1] The average total amount of GEO consumed per pig (g/animal) over the 57 days of the experiment

[2] R^2% (coefficient of determination) and RSD (residual standard deviation) values are given as measures of the goodness of fit of the model.

7.3.4 Effects of dietary tallow, linseed and fish oil on skatole and indole concentrations in pork fat (Experiment B)

Results for Experiment B (Table 7.7) showed that skatole and indole concentrations in back fat from pigs on the AT diet were significantly higher than those on the PO diet ($p<0.05$). These results are consistent with those of Experiment A (Table 7.6) in showing that animal products in the diet can lead to higher concentrations of skatole and indole, and are also in line with the report of Leong et al. (2010) that pork samples from pigs fed with animal by-products like meat and bone meal had the highest scores for mutton flavour and aftertaste, possibly due to more tryptophan being available in the hind-gut (Tuomola et al., 1996; Henry et al., 2002) for microbial degradation to form flavourful compounds such as skatole and indole (Lane & Fraser, 1999; Lane et al., 2002). It is estimated that the tryptophan level reaching the hind gut of pigs whose diet included animal products (diet AT) was 0.57 g/kg of diet compared to 0.48 g/kg for the other diets (Table 7.1)

Although tallow is an animal by-product, its presence in diet PTS in the absence of meat and bone meal and blood meal did not increase the skatole and indole content in fat relative to the comparable group with plant products only in the diet (POS) (Table 7.7). Similarly, the presence of dietary fish oil did not increase the level of indolic compounds in the fat (Table 7.7), regardless of whether it was fed to the pigs early (PFSe) or late (PFSl). This suggests that it was protein, not fat (whether it was tallow or fish oil) that increased the production of indolic compounds in the hind gut of the pigs on the AT diet.

Several studies have investigated the effects of diet on skatole and indole concentrations in pork, including that of Hansen et al. (2006) where skatole concentrations were reduced to almost zero levels in blood plasma and back fat by including crude or dried chicory or inulin in the diet of pigs. Other studies involving different inclusion levels of non-starch polysaccharides (sugar beet and straw), however, showed no effects on skatole and indole concentrations in the back fat (Wiseman, Redshaw, Jagger, Nute, Whittington & Wood, 1999).

Table 7.7

Least squares means[1] showing the effects of diets[2] containing animal and plant products with or without a dietary supplement (S) , tallow (T) or fish oils (Fe & Fl)) on skatole and indole concentrations (ng/g) in the back fat from pigs

Indolic Compound	Dietary treatment group						Diet effects (P-values)	Contrast effect (P-values) [3]					R^2%, RSD
	AT	PO	POS	PTS	PFSe	PFSl		AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl	
Skatole (ng/g)	63.81b	37.94a	39.94a	40.72a	43.18a	41.40a	<0.001	0.004	0.719	0.865	0.633	0.777	91.0, 15.30
Indole (ng/g)	28.23b	17.25a	16.68a	15.37a	17.11a	16.60a	<0.001	0.021	0.801	0.575	0.941	0.859	87.4, 7.76

[1] Means in the same row with a common letter after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet containing animal and plant products; PO= diet containing plant products only; POS=diet containing plant feed products and Sanovite™; PTS=diet containing plant products with tallow; PFSe=diet containing plant products and fish oil (early stage of grower period); PFSl=diet containing plant products and fish oil (late stage of grower period).

[3]PO vs.POS - no supplementation vs. supplementation within plant
AT vs. PO – Animal vs. Plant with no supplementation
POS vs. [PFSe + PFSl] – no fish oil vs. fish oil within plant supplementation
POS vs. PTS – Tallow vs. plant oils within plant supplementation
PFSe vs. PFSl – Early fish oil vs. late fish oil within plant supplementation

The presence of high levels of skatole and indole in pork is a concern to Singaporean consumers due to their low detection thresholds (Table 7.2), which indicate they are able to readily detect these compounds. Singaporean consumers associate non-Indonesian pork with a mutton-like flavour which is not desirable to their palates (Leong et al. (2008a). Table 7.8 shows the skatole and indole concentrations in retail pork from Australia, Brazil and Indonesia. The country effect was significant ($p<0.001$), with pork from western countries like Brazil having the highest levels of skatole and indole; and Indonesian pork one of the lowest. Levels in Australian product were similar to those for Indonesian pork (Table 7.8). No significant product differences in indole concentration were observed for Australian, Brazilian or Indonesian pork. Ribs and shoulders from Brazil had higher skatole levels than loins, and ribs from Indonesia had higher skatole levels than shoulders or bellies. Shoulder pork from Brazil had the highest skatole concentration whereas that from France (result not reported in Table 7.8) was the next highest (80.01 ng/g). No significant differences among products were observed for Australian pork. Interestingly, Canadian loin (result not reported in Table 7.8) had the highest level of indole (17.96 ng/g) and the lowest level of skatole (13.59 ng/g). Results from Experiments A and B with skatole and indole concentrations for New Zealand pork of about 65 ng/g and 19 ng/g, respectively, suggest that levels were moderate to high compared with some other countries. The results presented here, however, suggest that these concentrations could be lowered to match those of Indonesian pork, thereby possibly improving the acceptability of New Zealand pork for Singaporean consumers.

Table 7.8
Least squares means[1, 2] for skatole and indole concentrations (ng/g) in the subcutaneous fats in commercial pork products imported into Singapore from Australia, Brazil and Indonesia

Indolic compound	Product (n=5)	Country of origin			Effects (P-values)			R^2(%), RSD
		Australia	Brazil	Indonesia	Country (C)	Product (P)	C * P	
Skatole	Belly	35.03b	73.05cxy	17.91axy	<0.001	<0.001	0.231	56.6, 26.8
	Loin	29.13	51.65x	26.30yz				
	Rib	36.50a	91.64by	36.53az				
	Shoulder	32.44a	102.53by	13.88ax				
Indole	Belly	6.40a	27.65b	8.29a	<0.001	0.160	0.284	61.9, 6.22
	Loin	5.65a	14.18b	7.69a				
	Rib	8.15a	21.61b	9.45a				
	Shoulder	8.80a	22.22b	7.31a				

[1] Means in the same row with no letters after them or with a common letter (a, b, c) after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2] Means in the same column within an indolic compound with no letters after them or with a common letter (x, y, z) after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

7.4 Conclusions

Tests with consumers in Singapore indicated that detection threshold levels of skatole and indole at 0.028 and 0.051µg/g, respectively, were lower for this group than previously published values. Diet composition was shown to affect skatole and indole concentrations in the fat tissue with moderate increases when animal products were included in the diet. Much greater increases resulted when garlic essential oil was included the diet. Previous reports suggest that this may have been an effect of the components in garlic oil on liver metabolism of the indolic compounds. Including only plant materials in the feed for pigs may be a practical means of reducing skatole and indole concentrations in pork fat.

Chapter 8

The influence of diets supplemented with various fatty-acid sources, selenium, and vitamins E and C with and without animal protein on the quality of pork from female pigs

Chapter 8

8.1 Introduction

Alpha-linolenic acid (ALA) is the precursor fatty acid of the omega-3 fatty-acid family. By consecutive elongation and desaturation, ALA is converted to eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), each of which play a major role in human health (Simopoulos, 1991) and tissue ontogenesis, especially for the central nervous system (Alessandri, Goustard, Guesnet & Durand, 1998; Bourre et al., 1989) and retina (Birch, Birch, Hoffman, & Uauy, 1992). The best known effect of n-3 fatty acids is the control of cardiovascular disease (Conquer & Holub, 1998), but they also have beneficial effects on hypertriglyceridemia (Jacotot, 1988; Simopoulos, 1991) and against inflammatory disease and specific cancers (colorectal cancer: Kimura et al., 2007). As a result of these benefits, it is now widely recommended that the consumption of long-chain n-3 polyunsaturated fatty acids (PUFA) be increased in the human diet and that the n-6/n-3 and the linoleic acid/α-linolenic acid ratios be lowered to between 1 and 4 (ANC, 2001; HMSO, 1984, 1994). Changing the diet of pigs provides an effective method of changing the fatty acid composition of pig fat depots. As the pig is a monogastric animal, it is relatively easy to modify fatty acid composition of adipose tissues by changing the dietary fatty acids (Mourot & Hermier, 2001), thereby, modifying the human dietary fat intake from pork (De Henauw et al., 2007; Howe, Meyer, Record, & Baghurst, 2006; Meyer & et al., 2003; Wood & Enser, 1997). In particular, the n-3 PUFA level can be increased in pork by feeding items such as linseed, which contains about one-third oil, of which more than 50% is 18:3n-3 (Enser, Richardson, Wood, Gill, & Sheard, 2000; Kouba, Enser, Whittington, Nute, & Wood, 2003; Kouba, Benatmane, Blochet & Mourot, 2008).

In Singapore, pork is a major dietary component of the Chinese who make up 75 percent of the 5.08 million population (Singapore Department of Statistics, 2010). The per capita consumption of pork and its products by Singaporeans is very high (20.6 kg/year, AVA, 2008). Therefore, the reduction of n-6/n-3 and the linoleic acid/α-linolenic acid ratios below 4 in cooked pork meats and products would result in significant human health

benefits, but no study has been undertaken on these products enriched with n-3 PUFA in Singapore.

The aim of the present work was to study the effects of animal and plant based diets on fatty acid composition of lean meat and subcutaneous back fat tissue from pigs. The study included evaluations of the effects of (1) lipid type (soy bean oil, linseed oil, tallow, and fish oil) in the diet, (2) the period over which the dietary fish oil was provided, and (3) a dietary supplement containing conjugated linoleic acid (CLA), selenium and vitamin E on pork quality. Quality characteristics assessed included ultimate pH, colour, lipid oxidation, fatty acid composition, mineral content, and sensory parameters assessed by trained and consumer panellists.

8.2 Materials and Methods

8.2.1 Animals, experimental design, and sample collection.

All animals were managed according to the Massey University Animal Ethics Committee and the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Forty-seven female pigs (PIC hybrids, mean live weight 18.90 kg ± 1.75 (mean±SD) that were obtained from a single commercial operation in the North Island of New Zealand, were assigned to one of six dietary treatment groups as shown in Table 8.1. The pigs were kept in pens of six, but fed individually twice daily. Water was available at all times. Individual feed intake was measured daily and live weight recorded monthly.

The experiment follows on from those of Janz et al. (2008) and Morel et al. (2008) in which the influence of diets supplemented with Sanovite™, with or without animal protein, on the growth performance, meat quality and pork fatty acid profile from female pigs was studied.

The diet base was either a combination of animal and plant feedstuffs (AT and PTS), plant feedstuffs only (PO, POS) or plant feedstuffs combined with fish oil (PFS). The diets also differed depending on the presence or absence of the nutritional supplement Sanovite™ and Vitamin C as outlined in Table 8.1. Further details of diet composition are given in Table 7.1 (p 118).

Table 8.1
Abbreviations for the six treatment groups, together with a brief description of the diets involved. More details regarding the composition of the different grower/finisher diets is given in Appendix 8.1

Abbreviation	Description
AT	Diets containing some animal products as well as plant products for the whole 84 days of the experiment. Animal products included meat and bone meal (13%), blood meal (3%), and tallow (4.4%).
PO	Diets containing plant products only for the whole experiment. Plant lipids included soybean oil (16%) and linseed oil (1.1%).
POS	As for PO but with the supplement Sanovite™ included at 0.614% of the diet plus vitamin C (0.30%). Sanovite™ contains CLA and vitamin E (BASF, Auckland , New Zealand) and organic selenium (Alltech Inc., Nicholasville , Kentucky).
PTS	As for POS except that some of the plant oils were replaced with tallow (4.4%).
PFSe	As for POS except that some of the plant oils were replaced with fish oil (a total of 2.31 kg) early in the experiment for the period from day 1 to day 35.
PFSl	As for PFSe except that the same amount of fish oil (2.31 kg) was fed later in the experiment from day 36 to day 56. Thus, no fish oil was fed for the last 28 days of the experiment.

Slaughter and processing at a commercial abattoir took place after 84 days of feeding to give a mean carcass weight (±SD) of 72.1 $\pm$ 4.7 kg. After chilling for 40±3 hours (5.0 ±1.6^{o}C), the loins (about 600 mm) from both sides of each carcass were removed, deboned, vacuum packed, and frozen. The left and right frozen loins were transported by air to Singapore and then to the Food Quality Laboratory at Singapore Polytechnic.

8.2.2 Preparation of lean meat and subcutaneous back fat samples

Samples from 47 animals were used for chemical and sensory evaluations as shown below. Three batches per animal were used for chemical analysis with each batch being analysed at each of 3 times of frozen storage (0, 3, & 6 months) except for the elemental analysis which was conducted on the 0-month samples only.

Pork fat (47 animals)

- Indole and skatole analysis (Chapter 7)
- Fatty acid analysis after storage times of 0, 3 and 6 months.
- TBARs analysis

Lean meat (47 animals)

- Fatty acid analysis
- TBARs analysis
- Analysis of minerals
- Ultimate pH
- Colour analysis in terms of L*, a*, & b* values
- Sensory evaluation

The weights of the pork loins, including subcutaneous fat and skin, ranged from 3.2 to 3.5 kg. They were cut into chunks of about 800 to 1000 g that were vacuum packed (Innovac, model : IV-251, Germany) into bags of five-layer PE–LD/ ADH /PA/ADH/PE–PD film (total thickness: 0.08 mm; PA layer thickness: 0.024 mm; oxygen permeability: 40 $mL/m^2/24$ h/bar; water vapour permeability: 10 $g/m^2/24$ h/bar). The vacuum-packed pork packs were placed in a temperature-controlled laboratory freezer with an external data logger, and held at -18 $\pm$ 2^oC in the dark for up to 6 months. Each package was labelled with an animal number and loin location. Left and right loin portions of each animal of approximately 2 kg were thawed overnight at 4°C, and trimmed of subcutaneous back fat and skin to give approximately 1.8 kg of muscle, which was cut into chunks of about 80 – 100 g. Subcutaneous fat and muscle were minced separately using a plate with 6 mm diameter holes (Sammic brand, model no. 261). One-third of the minced meat and

subcutaneous back fat were used for chemical analysis while the rest were used for sensory evaluation.

8.2.3 Sensory Evaluation

The sample preparation and cooking procedures were similar for the consumer panel and trained panel in Singapore. The mince was prepared separately for pork from each individual pig.

The minced subcutaneous back fat and lean meat were mixed together and placed in individual thermo pouches with 250 g of muscle and 25 g of subcutaneous back fat, sealed (using the metal twister attached) and immersed in a 100^{o}C water-bath for 11 mins. The samples were stirred every 3 mins by introducing a spoon into the pouches to prevent clumping. When not stirring, the pouches were resealed using the metal twister attached to the pouches. After cooking for 11 mins, the samples were transferred to small plastic cups (30 mL) which were covered with plastic lids. All samples were placed in a force-air oven at 60^{o}C to equilibrate for 30 mins prior to tasting. Plastic disposable spoons were used by panellists to scoop the samples and evaluate their flavour/taste.

The trained panel testing was conducted at the Food Quality and Sensory Evaluation laboratory of the Singapore Polytechnic. Quantitative descriptive analysis (QDA) was used with qualified panellists who were screened based on their sensory acuity, liking for pork, and their commitment to taste pork for 8 sessions over a period of 2 days. Triangle tests using different concentrations of sucrose, sodium chloride, citric acid and caffeine were used to perform the screening and ultimately the 7 panellists selected participated in 5 discrimination trials over a period of 2 days. Under the direction of the panel leader, the panellists developed a sensory language to describe the product's sensory properties. They grouped the attributes by modality order and then within a modality developed definitions for each attribute. There were three 1.5 hour training sessions. During training panellists became more confident with scoring the sensory attributes of pork by having samples

presented at least two times per session to allow them to re-familiarise themselves with the typical flavour associated with each attribute.

During the sensory evaluation sessions, the panellists evaluated 47 samples by a 7-membered trained panel over a period of 2 days, with 4 sessions per day and 6 samples per session. Each panellist received one minced pork sample from one animal. There were breaks of at least 30 minutes between sessions. The samples were evaluated for colour, aroma, flavour, tenderness, juiciness, and off-flavours using 150 mm line scales which ranged from "None" to "Strong". Full descriptions for the sensory characteristics of the pork that were evaluated are given in Table 8.2. The sensory questionnaire can be found in Appendix 8.2.

The pork samples were also evaluated by a 24-member consumer panel over a period of 2 days, with 4 sessions per day. In each session, panellists assessed one group of 4 pork samples (Group A – AT, PO, POS, PTS) and another group of 3 samples (Group B – POS, PSFe, PSFl). The reason for assessing two groups within a session was to reduce the possible carry-over effects of fishy flavour from the fish oil groups to the non-fish oil groups. There was a break of at least 15 mins between tasting samples from both groups. Each panellist attended each session and evaluated one pork sample from each of the 47 animals. Sample preparation of pork samples for consumer evaluation was similar to that for the trained panel. The acceptability level of aroma, flavour and overall acceptability of pork samples was assessed using a scale of 1 to 9, where 1 was "dislike extremely" and 9 was "like extremely". The questionnaire for consumer testing can be found in Appendix 8.3.

Table 8.2
Definitions of the sensory attributes of a mix of minced longissimus muscle and subcutaneous fat (9.1%) from the loin region developed by the trained panellists during training, together with the anchor points at each end of the scale

Sensory attributes	Interpretation	Anchor points
Colour attributes		
Colour	Degree of brownness[1]	Yellow / Brown
Colour saturation	Degree of darkness/lightness[1]	Light/Dark
Aroma attributes		
Meaty aroma	Aromatic associated with cooked meat[a]	None / Strong
Brothy aroma	Aromatic associated with pork cooked in water[a]	None / Strong
Fishy aroma	Aromatic associated with fish[a]	None / Strong
Metallic aroma	Aromatic associated with presence of iron ions (blood)[a]	None / Strong
Acidic aroma	Aromatic associated with presence of citric acid[b]	None / Strong
Mutton aroma	Aromatic associated with presence of mutton[a]	None / Strong
Rancid odour	Atypical aroma generally associated with deterioration of quality in fats and oils[b]	None / Strong
Taste/flavour attributes		
Meaty flavour	Sensation associated with cooked meat[a]	None / Strong
Brothy flavour	Sensation associated with pork cooked in water[a]	None / Strong
Fishy flavour	Sensation associated with fish[b]	
Metallic flavour	Sensation associated with the presence of iron ions (blood)[b]	None / Strong
Acidic flavour	Taste on the tongue associated with citric acid[b]	None / Strong
Mutton flavour	Sensation associated with cooked mutton[a]	None / Strong
Rancid flavour	Atypical taste generally associated with deterioration of quality in fats and oils[b]	None / Strong
Salty taste	Taste on the tongue associated with sodium chloride[b]	None / Strong
Aftertaste	Sensation of lingering taste on the tongue after ingestion[b]	None / Strong

[a]Definitions as developed by the panellists
[b]Meilgarrd and others (1999)

8.2.4 Laboratory measurements

(a) *Thiobarbituric acid reactive substances (TBARs)*

The samples were evaluated for oxidation (based on TBARs) at months 0, 3 and 6 of frozen storage. Samples were taken out of the chiller after thawing overnight for almost 24 hours and were minced using a food processor (Philip, model HR1396, Singapore) for 30 secs prior to analysis. The TBA assay was performed as described by Buege and Aust (1978). Duplicate samples of 0.5 g were mixed with 2.5 mL of stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis, Mo., U.S.A.), 15% TCA (Mallinckrodt Baker, Inc., Paris, Ky., U.S.A.), and 0.25-N HCl. The mixture was heated for 10 min in a boiling water bath (100^{o}C) to develop a pink colour, cooled in tap water, and then centrifuged (Eppendoft, model 5810, Germany) at 3150 g for 10 min. The absorbance of the supernatant was measured spectrophotometrically (Shimadzu Instruments, model UV2401, Japan.) at 532 nm against a blank that contained all the reagents except the pork sample. The malonaldehyde (MDA) concentration was calculated using an extinction coefficient of 156,000/mole/cm for the pink TBA-MDA pigment (Sinnhuber & Yu, 1958). The absorbance values were converted to ppm MDA by using the following equations:

(1) TBA (mg/kg) = Sample A532 × (1-M TBA Chromagen/156000) × [(1 mole/L)/M] × (0.003 L/0.5-g meat) × (72.07-g MDA/mole MDA) × (1000 g/kg) x 1000, or

(2) TBA (mg/kg) = Sample A532 × 2.77 (where MDA = malonaldehyde).

(b) *Colour measurement (L*, a*and b* values)*

The pork were bloomed for 1 h at 4°C prior to colour measurement. The colour of pork samples was measured at 3 sites on the surface of each pork sample after exposure to the atmosphere for approximately 15 min using a Minolta Chromameter (CR-300) reflectance spectrophotometer to provide lightness (L*), redness (a*), and yellowness (b*) values. The spectrophotometer was calibrated with a white porcelain plate (Y=94.5, x=0.3141, y=0.3207). The light source was from a pulsed xenon lamp with a measuring diameter of 50 mm.

(c) *Meat pH*

For pH determination, 10 g of a sample and 90 mL of distilled water were homogenized in a blender (Model D-500, Wiggenhauser, Germany,) for 60 s. The pH of the homogenate was measured using a pH meter with automatic temperature compensation, (pH 211, Hanna, England) standardised at pH 4.0 and 7.0 (Pexara *et al.* 2002). The pH was measured directly after homogenization and was read after stabilization. Two readings from different samples were taken.

(d) *Analysis of minerals*

Minced samples (2.0 g ±0.01 g) were weighed into a teflon vessel and 7 mL of nitric acid (65%) was added drop by drop followed by 1 mL of hydrogen peroxide (30%). The vessel was gently swirled to homogenise the sample with the liquids. The vessel was introduced into the rotor of the microwave digestion system (model: Ethos 1600, Milestone, USA) with the microwave program set to reach a temperature of 200^{o}C over 10 minutes and then maintained at that temperature for another 10 mins. The microwave power was about 1000 watts. When the microwave program was completed, the digestor was cooled until the solution inside the teflon vessel reached room temperature. The vessel was then opened and the solution was transferred into a volumetric flask that was topped up with ultrapure water to 100 mL. An ICPE spectrometer (Shimadzu model ICPE-9000) was used for the analysis using argon as a cooling, plasma, and carrier gas at flow rates of 14.0, 1.2, and 0.7 L/min, respectively. The sample was introduced via a coaxial nebulizer.

(e) *Fatty acid analysis*

The fatty acids in the pork samples were analysed based on the method described by O'Fallon, Busboom, Nelson & Gaskins (2007). Minced samples of lean muscle tissue or subcutaneous back fat (1.0 g ±0.01 g) were placed into 16 × 125 mm screw-cap Pyrex

culture tubes to which 1.0 mL of the C13:0 internal standard (0.5 mg of C13:0/mL of MeOH), 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added. The tube was incubated in a 55^{o}C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min to properly permeate, dissolve, and hydrolyse the sample. After cooling below room temperature in a cold water bath, 0.58 mL of 24 N H_2SO_4 in water was added. The tube was mixed by inversion and with precipitated K_2SO_4 present was incubated again in a 55^{o}C water bath for 1.5 h with hand-shaking for 5 s every 20 min. After FAME (fatty-acid methyl ester) synthesis, the tube was cooled in a cold tap water bath. When the tubes were cooled, 3 mL of hexane was added, and the tube was vortex-mixed for 5 min on a vortex. The tube was centrifuged for 5 min in a tabletop centrifuge, and the hexane layer, containing the FAME, was placed into a GC vial. The vial was capped and kept at –20 ^{o}C until GC analysis.

The fatty acid composition was determined by gas chromatography (Shimadzu GC2300, capillary column Supelco SP^{TM}-2560, 100m x 0.25mm ID, 0.2um film) with hydrogen as the carrier gas, and a flame ionisation detector (FID). The initial oven temperature was 140^{o}C, held for 5 min, subsequently increased to 240 ^{o}C at a rate of 4 ^{o}C min^{-1}, and then held for 20 min. Helium was used as the carrier gas at a flow rate of 0.5 mL.min^{-1}, and the column head pressure was 280 kPa. Both the injector and the detector were set at 260 ^{o}C. The split ratio was 30:1. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards : Supelco #47885-U: Supelco 37 component fatty acid methyl esters mix- (Sigma Aldrich, Singapore).

8.2.5 Statistical analysis

Statistical analysis was performed with the GLM procedure of SPSS ver. 17 using a statistical model where the animals were nested within treatments, included fixed effects of treatment and days of storage as repeat measures. Diet treatment were tested against the animal term while the storage time and interaction (treatment x storage time) effects were tested against the overall error term. The statistical model for all variables for pork samples (lean meat or subcutaneous back fat) included a treatment effect (n = 6), animal effect (n = 47) and storage effect (n = 3). The interaction between storage period and treatment was evaluated for all variables.

A set of 5 non-orthogonal contrasts between treatments was evaluated as follows:

1. A comparison between the group of pigs receiving a diet containing animal and plant components (the animal group) and the group receiving a diet containing plant components only (the plant group) [AT vs. PO].
2. A comparison between the control and supplemented diet groups within the plant group [PO vs. POS].
3. A comparison between the group receiving the diet containing tallow and that containing plant oils [PTS vs. POS].
4. A comparison between the groups receiving diets containing fish oil fed at an early or late stage and that containing plant components with supplemented diet [POS vs. [PFSe + PFSl]].
5. A comparison between the group receiving the diet containing fish oil fed early and that fed at a late stages [PFSe vs. PFSl].

Least squares means of six treatment groups across storage periods and least squares means for the three storage times across the six treatment groups were obtained. Means were considered statistically different at $p<0.05$.

8.3 Results and Discussions

8.3.1 pH

Pork pH decreased significantly as storage period increased (Table 8.4; $p<0.001$), but there were no significant differences between the dietary treatment groups (Table 8.3; $p=0.06$), and no interaction effect between treatment and storage time (Table 8.4). The decreased pH with storage time was possibly caused by the growth of bacteria in vacuum-packed samples during thawing. Thawing of frozen materials is important in food processing, while freezing is a convenient way of preserving food. Minimizing thawing times will reduce microbial growth, chemical deterioration and excessive water loss caused by dripping or dehydration (Taher & Farid, 2001). Muhammet & Mukerrem (2005) have shown that lactic acid bacteria, together with Micrococcus, Staphylococcus and Enterobactericeae are the bacteria group associated with the vacuum-packed beef frozen thawed at 10^{o}C for 24 hours. Nassos, King & Stafford (1988) reported lactic acid concentrations increased in freeze-thawed vacuum-packed ground beef at 7^{o}C. Another possible explanation of the low pH could be due to the presence of yeasts and moulds. In particular mould spoilage of meat has received considerable attention; yeasts are not normally considered to play a significant role in meat spoilage (Walker, 1977). However, the chances of such spoilage are low in the current study as moulds are recognised by the visible and often spectacular nature of the spoilage (Lowry & Gill, 1984).

8.3.2 Colour

There was no significant differences in L*, a* or b* among the diets except for a significant decrease in L* ($p=0.033$) in POS group compared to PO group (Table 8.3). Juárez, Dugan, Aldai, Aalhus, Patience, Zijlstra & Beaulieu (2011) showed that dietary flaxseed resulted in significantly lower L* ($P = 0.003$) indicating a shift to darker meat by feeding flaxseed. Previous studies have not reported any effect on meat colour when flaxseed was included in pig diets (Bee et al., 2008; Corino et al., 2008; Haak, De Smet, Fremaut, Van Walleghem, & Raes, 2008). Storage effects were significant (Table 8.4), with a slight increase in lightness (L*) and a significant increase in yellowness (b*) with time.

Redness (a*), however, decreased from month 0 to 6. Meats tend to turn brown (decreased redness) over time (Rosenvold & Andersen, 2003; Lindahl et al., 2005). Pork from pigs with selenium, vitamin E and CLA supplements (POS vs. PO) in their diets did not have improved colour stability. Some studies have shown improved colour stability after vitamin E supplementation (summarised in Table 2.7), whereas several studies have shown no effect on pork (Jensen, Lauridsen & Bertelsen, 1998). Faustman & Wang (2000) and Rosenvold & Andersen (2003) and also more recent studies show limited effects of this supplements on pork colour (Hasty et al., 2002; O'Sullivan et al., 2002; Geesink et al., 2004; Swigert et al., 2004).

Colour changes during air-exposed storage of pork are mainly related to oxidation of myoglobin to metmyoglobin (MetMb) (Lindahl et al., 2005, Papers VI, III). The accumulation of MetMb on the surface of pork during storage affects redness, which decreases with storage time (Rosenvold & Andersen, 2003; Lindahl et al., 2005), but this should not have been an important factor in the current study as the samples were vacuum packed.

8.3.3 TBARs analysis

There was a high positive correlation between back fat and lean meat with respect to TBARS ($r=0.823$, $p<0.05$) based on the group means of each treatment by storage time. In addition, the TBARS values of back fat and lean meat increase with storage period.

(a) *AT vs. PO*

TBARs for the AT group was significantly lower in back fat than for the PO group ($p<0.001$) (Table 8.5). Many studies have linked oxidative instability in meat and meat products with increasing concentrations of PUFA (Tables 2.4 and 2.5). Other authors (Teye et al., 2006) observed no effect of PUFA concentration on TBARs values, but these authors used high levels of vitamin E (250 mg/kg) in the diet.

(b) *PO vs. POS; POS vs. PTS*

Results in Table 8.5 showed that TBARs values for lean meat and subcutaneous back fat were higher in the PO diet than the POS and PTS ($p<0.05$). This shows that the vitamin C, E and Se in the supplemented diet had a positive effect by increasing the oxidative stability in pork samples. Zhan et al. (2007) reported that loin muscle of pigs receiving Se, regardless of source, had greater glutathione peroxidise (GSH-Px) activity and decreased malondialdehyde (MDA) content. Both the decrease in MDA content and the increase in GSH-Px activity in muscle were attributed to the capacity of Se to improve meat quality through enhancing its antioxidant ability. As for the effects of vitamin E on oxidative stability of pork, many studies have shown increased stability when the diets of pigs were supplemented with vitamin E, usually in the form of α–tocopherol (Table 2.7). Trefan et al. (2011) reported that at least 100 IU vitamin E/kg feed was required to significantly decrease lipid oxidation and every 1 μg α-tocopherol/g tissue decreased lipid oxidation by approximately 0.05 TBARS values in M. longissimus dorsi in pigs (Trefan et al., 2011).

TBARs between POS and PTS was significantly different in subcutaneous back fat; with PTS having a lower score than POS. Replacing plant oil with tallow and supplement (containing vitamin C, E and selenium) increased the oxidative stability in the pork samples as tallow which contains mainly saturated fatty acids is more stable than PUFAs containing plant oil (Table 2.5).

(c) *POS vs. [PFSe + PFSl]*

There were higher TBARs for groups PFSe and PFEl than in the POS group (Table 8.5). Numerous studies have shown the effects of feeding fish oil to pigs on the oxidative stability of pork. TBARs values were reported to be higher for pork from pigs fed with fish oil compared to those without (see a summary of studies in Table 2.4).

(d) *PFSe vs. PFSl*

Within the fish oil diets, there was a significant effect of stage of feeding (early vs. late) of the diet on TBARs of back fat ($p = 0.031$), but not the longissimus muscle. The results of feeding of fish oil to pigs were also reported in a study by Jaturasitha, Khiaosa-ard, Pongpiachan & Kreuzer (2009) where the TBARs value of pork from pigs fed with fish oil in the early stage of fattening was lower than those from the late stage, thereby resulting in better sensory flavour and overall acceptability.

Storage-time effects

Results in Table 8.6 showed that TBARs values increased from month 0 to month 3 of frozen storage, but a more significant increase was observed to month 6 for both lean meat and subcutaneous back fat during freezer storage at -18^{o}C. Subcutaneous back fat gave higher TBARs values than lean meat by almost 6-fold for months 0 and 3, and 2-fold for month 6. Both treatment and storage period had significant effects ($p<0.001$) on the oxidative status of lean meat and subcutaneous back fat. Treatment and storage period showed a slight significant interaction ($p=0.048$) on TBARs values in the lean meat (Fig 8.1) with the increase in value from 3 to 6 months being lower for the PTS group, which may be because the substitution of tallow for plant oil meant that there was less PUFA present. It might be expected that a smaller increase would also have been expected for the AT group, but this was not the case.

Table 8.3

Least squares means and the significance of 5 contrasts for pH and colour (L*, a* and b*) measurements for longissmus muscle in the loin region averaged across 3 storage periods (0, 3, and 6 months at -18°C). The pork was from pigs fed diets containing animal and plant products with or without a dietary supplement (S), tallow (T) or fish oils (Fe & Fl)). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation (RSD). Means for the 3 storage times are given in Table 8.4.

Item[1]	Treatment (Trt) group[2]						Trt Effect (p-values)	R^2(%), RSD	Contrast statistics[3] (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
pH	5.64ab	5.71b	5.68ab	5.60ab	5.57ab	5.56a	0.06	34.3, 0.19	0.37	0.73	0.19	0.05	0.76
L*	56.6ab	58.6b	54.9a	56.0ab	55.2ab	57.7ab	0.15	11.6, 5.38	0.27	0.033	0.51	0.07	0.05
a*	7.81	8.50	8.57	7.49	7.29	7.94	0.33	14.8, 2.30	0.34	0.93	0.12	0.14	0.27
b*	7.24	7.79	7.64	6.74	6.91	7.83	0.13	16.9, 1.72	0.26	0.77	0.10	0.19	0.08

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

[3]AT vs. PO – Animal vs. Plant with no supplementation

PO vs.POS - no supplementation vs. supplementation within plant

POS vs. PTS – Tallow vs. plant oils within plant supplementation

POS vs. [PFSe + PFSl] – no fish oil vs. fish oil within plant supplementation

PFSe vs. PFSl – Early fish oil vs. late fish oil within plant supplementation

Table 8.4

Least squares means and effects of treatment (Trt) group, frozen storage period (Time) and their interaction (Trt x Time) for pH and L*a*b* of longissmus muscle in the loin region made from pigs fed diets containing animal and plant products with or without a dietary supplement, tallow or fish oils. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Item[1]	Storage time (Months)			Effects (p-value)			R^2(%), RSD
	0	3	6	Trt	Time	Trt x Time	
pH	5.78c	5.61b	5.50a	0.06	<0.001	0.96	34.3, 0.19
L*	54.9a	56.8ab	57.9b	0.15	0.033	0.99	11.6, 5.38
a*	8.58b	7.82ab	7.44a	0.33	0.06	0.92	14.8, 2.30
b*	6.80a	7.93b	7.37ab	0.13	0.008	0.79	16.9, 1.72

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 8.5

Least squares means and the significance of 5 contrasts for TBARs measurements (mg MDA/kg) for longissmus muscle (LM) and subcutaneous back fat (FT) in the loin region averaged across 3 storage periods (months 0, 3, and 6). The pork was from pigs fed diets containing animal and plant products with or without a dietary supplement (S), tallow (T) or fish oils (Fe & Fl)). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation (RSD). Means for the 3 storage times are given in Table 8.6

Item[1, 2]	Treatment (Trt) group[3]						Trt effect (p-values)	R^2(%), RSD	Contrast statistics[4] (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
FT TBARs[3]	-0.594b	-0.340d	-0.476bc	-0.804a	-0.372cd	-0.202e	<0.001	69.2, 0.18	<0.001	0.018	<0.001	0.001	0.031
	(0.255b)	(0.457d)	(0.334bc)	(0.157a)	(0.425cd)	(0.628e)							
LM TBARs[3]	-1.14b	-0.926b	-1.08ab	-1.25a	-0.974b	-0.950b	<0.001	85.4, 0.17	0.08	0.22	0.10	0.46	0.83
	(0.072ab)	(0.119b)	(0.083ab)	(0.056a)	(0.106b)	(0.112b)							

[1] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2] Analysed as log values. The significance of differences (using LSD) was based on the transformed means (log) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

[3]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

[4]AT vs. PO – Animal vs. Plant with no supplementation

PO vs.POS - no supplementation vs. supplementation within plant

POS vs. PTS – Tallow vs. plant oils within plant supplementation

POS vs. [PFSe + PFSl] – no fish oil vs. fish oil within plant supplementation

PFSe vs. PFSl – Early fish oil vs. late fish oil within plant supplementation

Table 8.6

Least squares means for TBARs measurements (mg MDA/kg) and effects of treatment (Trt) group, frozen storage period (Time) and their interaction (Trt x Time) of longissmus muscle (LM) and subcutaneous back fat (FT) made from pigs fed diets containing animal and plant products with or without a dietary supplement, tallow or fish oils. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2 (%) and the residual standard deviation, RSD

Item[1,2]	Storage time (Months)			Effects (p-value)			R^2(%), RSD
	0	3	6	Trt	Time	Trt x Time[3]	
FT TBARs	-0.571a (0.269a)	-0.530a (0.295a)	-0.288b (0.515b)	<0.001	<0.001	0.204	88.5, 0.14
LM TBARs	-1.32a (0.048a)	-1.27a (0.054a)	-0.55b (0.282b)	<0.001	<0.001	0.048	72.1, 0.16

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2] Analysed as log values. The significance of differences (using LSD) was based on the transformed means (log) of these attributes, and the back-transformed means are shown in brackets below the transformed means.
[3]Interaction plots between treatment and storage period are given in Fig. 8.1

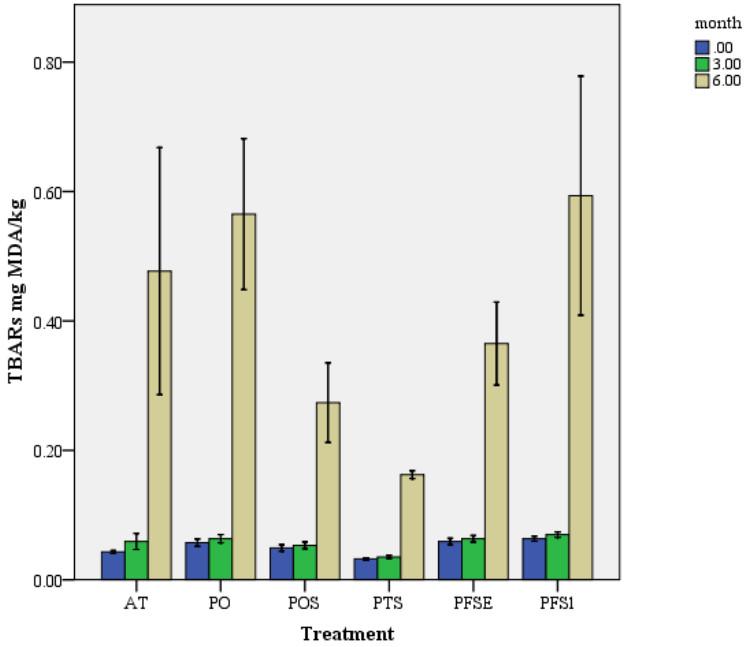


Fig 8.1
Interaction plots showing the effect of treatment group[1] and frozen-storage period (0, 3 and 6 months at -18°C) on TBARs values for longissmus muscle in the loin region (vertical bars show the standard error).
[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

8.3.4 Elements in pork

The concentrations of selected mineral elements (expressed as μg/g) in pork from pigs receiving different diets (Table 8.7) revealed no diet effects for the contrasts considered except that the concentration of Se was lower in PO than POS. Selenium supplementation significantly increased selenium in muscle tissue compared to the unsupplemented controls, AT and PO. Marked increments in Se were also observed in POS, PTS, PFSe and PFSl relative to AT and PO. Selenium content of muscle in the current study was increased as dietary Se level increased. Zhan et al. (2007), using finishing pigs, showed that the Se content of tissues were markedly improved as dietary Se level, in the form of sodium selenite or Se-methionine, increased. This result agrees with previous reports on swine (Morel et al., 2008; Kim & Mahan, 2001a,b; Mahan & Parrett, 1996; Mahan et al., 1999). In lambs, Yu et al. (2008) found that selenium supplementation as Se selenite significantly improved the concentration of Se in blood and liver with diets containing different polyunsaturated fatty acid sources. Therefore, it is clear that there is usually a response in Se deposition to dietary Se supplementation.

Table 8.7

Least squares means for concentrations of selected mineral elements (μg/g) in longissmus muscle in the loin region from pigs fed diets containing animal and plant products with or without a dietary supplement (S), tallow (T) or fish oils (Fe & Fl), as determined by inductively coupled plasma spectrometer. Element analysis was performed for month-0 samples only

Element[1]	Treatment (Trt) groups[2]						Trt Effects (p-value)	R^2, RSD	Contrast statistics (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Potassium	4193.75	4035.00	4133.75	3930.63	4097.86	4227.50	0.42	95.0, 304.23	0.36	0.57	0.24	0.79	0.30
Magnesium	202.19	211.19	212.50	211.31	217.93	215.38	0.64	84.6, 18.05	0.38	0.91	0.90	0.61	0.76
Sodium	513.56	538.63	509.31	479.94	510.79	515.25	0.53	53.7, 182.63	0.36	0.35	0.41	0.88	0.81
Phosphorus	2073.75	2055.63	2098.13	2036.87	2090.00	2043.75	0.93	34.4, 135.23	0.82	0.59	0.26	0.59	0.53
Calcium	49.61	49.71	48.66	42.51	45.96	48.62	0.65	43.1, 9.68	0.99	0.83	0.22	0.78	0.58
Copper	0.437	0.485	0.414	0.423	0.405	0.458	0.67	21.5, 0.105	0.34	0.13	0.88	0.65	0.30
Iron	6.57	5.85	5.22	5.27	4.68	5.56	0.13	49.9, 1.32	0.41	0.33	0.93	0.88	0.16
Selenium	0.232a	0.297a	0.397b	0.405b	0.412b	0.410b	0.08	82.0, 0.277	0.29	0.028	0.92	0.83	0.97
Zinc	16.33ab	18.79b	14.94ab	14.19ab	12.38a	16.93ab	0.25	40.8, 5.29	0.45	0.23	0.74	0.95	0.10

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

8.3.5 Fatty-acid concentrations

As a result of the different diets used in the current study, there was a significant differences in the total fatty acids as a percentage of intramuscular fat of the longissimus muscle (Appendix 8.4, p=0.046), with one (PFSe vs. PFSl) of the five contrasts significant. This significant difference in total fatty acids was not shown in subcutaneous back fat. The total fatty acids as a percentage of subcutaneous back fat were much higher than those from the intramuscular fat. In the current study, the TFAs (total fatty acids) per unit product weight in subcutaneous back fat in the current study (Appendix 8.5) were about 75 – 78% of total lipid per unit weight. Knight et al. (2003), for example, had percentages of 82.6 and 87.4 for their two experiments, but in a French study (De la Fuente et al. 2009) calculations indicated that the percentages ranged from 66% to 78%.

Dietary effects on levels of fatty acids in the subcutaneous back fat (Table 8.9) were generally greater than in longissimus muscle in the loin region (Table 8.8), possibly because the percentage of intramuscular fat in the longissimus muscle in the loin region was constant whereas the percentage of back fat was increasing with animal weight (D'Souza et al., 2000), thus allowing for a greater effect of dietary manipulation in the latter. There were 18 of 22 fatty acids in the subcutaneous back fat that showed a significant effect of the dietary treatment (Appendix 8.5), while only 12 were significantly affected in the longissimus muscle in the loin region (Appendix 8.4). Morel, McIntosh & Janz (2006) reported similar findings with five significant changes across seven fatty acids in subcutaneous fat, versus 3 differences in the longissimus muscle in the loin region. Enser et al. (2000) reported 15 and 9 significant differences in subcutaneous back fat and longissimus muscle in the loin region, respectively, across 17 fatty acids. In addition, larger treatment differences for individual fatty acids were also recorded in the subcutaneous fat due to differences in the pattern of deposition of particular fatty acids between tissues (Enser et al., 2000) or different rates of maturation of the fat depots with the subcutaneous depot maturing earlier than the intramuscular.

8.3.5.1 Diet effects

In this section, the individual contrasts between dietary treatments (AT vs. PO; PO vs. POS; POS vs. PTS; POS vs. (PFSe+PFSl); and PFSe vs. PSFI) will be discussed first, followed by storage-time effects.

(a) AT vs. PO

There were 11 significant differences across 22 fatty acids in subcutaneous back fat compared to five in longissimus muscle in the loin region; with significant changes in four fatty acids similar for these two tissues (Appendix 8.4 & 8.5). AT had a higher level in cis-9, trans-11 CLA than PO for both longissimus muscle in the loin region ($p=0.028$) and subcutaneous back fat ($p=0.009$). In contrast, the PO group had higher levels of α-linolenic acid, n-3 eicosatrienoic acid, and DPA ($p<0.05$) within the longissimus muscle in the loin region

From the current study, linoleic acid for the PO group that received a diet containing soya bean (16%) and linseed oil (1.1%) plus other plant materials was significantly higher than the AT group in the subcutaneous back fat for both tissues. Several papers have examined the effects of dietary oils containing a high proportion of linoleic acid on the fatty acid composition and quality of pork. Examples of such oils are soya, peanut, maize and sunflower. Concentrations of linoleic acid can easily be raised from basal levels of around 10–15% of fatty acids in intramuscular fat to over 30% (Hartman, Costello, Libal, & Walhlstrom, 1985; West & Myer, 1987), when these dietary oils are included in the feed. In terms of fatty acid composition in pork, linoleic acid was generally higher compared to the meat of sheep and cows, causing a higher P:S ratio. This is due to the high content of linoleic acid in the cereal-based diets consumed by pigs and can lead to an undesirably high n-6: n-3 ratio (Wood et al., 2003). Because the lipids in pig meat are relatively unsaturated, attempts to further increase concentrations of PUFA risk

the generation of lipid oxidation products, leading to off-odours and flavours and colour changes.

The concentration of n-6 eicosatrienoic acid was almost two times lower than n-3 eicosatrienoic acid for all dietary treatments in the current study. A significant difference was observed in n-3 eicosatrienoic acid between AT vs. PO ($p<0.05$). With increasing frozen storage time (Appendix 8.6 & 8.7) the proportion of both the eicosatrienoic acids decreased ($p<0.05$) except for n-3 eicosatrienoic acid in the intramuscular fat. Some studies have shown than n-6 eicosatrienoic acid was about 4 times lower than n-3 eicosatrienoic acid (Ahn, Luz & Sum, 1996). As α-linolenic acid in the diet increased, n-3 eicosatrienoic acid increased but not n-6 eicosatrienoic acid which remained almost constant. This could be due to the elongation of α-linolenic acid to n-3 eicosatrienoic acid by elongase. Other reports have also clearly shown that dietary linolenic acid increases n-3 eicosatrienoic acid in subcutaneous back fat (Realini et al., 2010; Juárez et al., 2010).

In general, feeding linseed containing diets prior to slaughter led to ($P < 0.05$) an increase in linoleic acid, α-linolenic acid, cis-9-trans-11 CLA, n-3 eicosatrienoic acid, DPA, total PUFA and reductions in arachidonic acid, total MUFA and n-6/n-3 (Appendix 8.4 and 8.5). Juárez et al. (2010) showed that presence of linseed in diet of pigs led to significant increases in α-linolenic acid and n-3 fatty acids in the back fat. Level of n-3 fatty acids was elevated when linseed oil was included in the pigs' diet (Realini et al., 2010)

The DHA levels in the intramuscular fats of longissimus muscle were 27% higher in PO diet compared to AT. This finding was similar to that of Enser et al. (2000) who demonstrated that feeding linseed high in α-linolenic acid (4.0 g/kg) increased DHA levels 50% in adipose tissue and 35% in muscle when compared with a control diet with only 1.9 g/kg α-linolenic acid. In contrast, this observation was not seen in work by Riley et al. (1998a), Riley et al. (1998b), Ahn, Lutz, and Sim (1996), and Specht-Overholt et al. (1997).

They proposed that much higher tissue concentrations of α-linolenic acid and EPA may result in competitive exclusion of DHA from tissue lipids, particularly from phospholipids.

The EPA, DPA and DHA in supplement-containing diets including POS, PTS, PFSe and PFSl, were higher compared to the AT diet, but less so for the PO diet.
This could be due to the presence of antioxidants like selenium, vitamin C and E. The fatty acid composition of mitochondrial fraction of the pork muscle from pigs fed with vitamin E and selenium in their diets had higher levels of n-3 fatty acids and PUFA compared to non-supplemented ones (Nuernberg et al., 2002). Morel et al. (2008) reported an increase in the n-3 fatty acids, particularly DHA ($p<0.05$) in pork from pigs fed with supplements containing plant oils, selenium, and vitamin E.

From Table 8.12, AT meats showed the highest SFA (36.57 in intramuscular fat; 35.58 in subcutaneous back fat) and MUFA proportion (46.54 in longissimus muscle in the loin region; 48.76 in subcutaneous back fat). Raes, De Smet, and Demeyer (2004) noted that as fatness increases, the levels of SFA and MUFA increase faster than the PUFA levels, leading to an increase in the relative proportions of SFA and MUFA. In our study, the highest TFAs (close to 87%) was found in the AT-diet group and the lowest proportion in PFSe and PFSl (78.7% and 77.4%), which explains, respectively, their highest and lowest SFA and MUFA proportions. In general, all plant diets showed an increase in PUFA, with either concomitant reductions in SFA and/or MUFA compared to AT.

(b) *PO vs. POS and POS vs. PTS*

For the PO vs. POS contrast, there were two and four significant differences across 22 fatty acids in subcutaneous back fat and in longissimus muscle in the loin region, respectively; with significant changes in two fatty acids similar for these two tissues: cis-9, trans-11 CLA and α-linolenic acid. A significant difference was observed in cis-9, trans-11 CLA with POS having a higher level than PO for longissimus muscle in the loin region

($p<0.001$) and subcutaneous back fat ($p=0.001$). Similarly, the POS group had a higher level in α-linolenic acid than the PO group in longissimus muscle in the loin region ($p<0.001$) and subcutaneous back fat ($p<0.018$).

For the POS vs. PTS contrast, there were eight significant differences across 22 fatty acids in subcutaneous back fat and in longissimus muscle in the loin region; with significant changes in two fatty acids that were similar for these two tissues: n-3 eicosatrienoic acid and α-linolenic acid. POS had higher levels of α-linolenic acid in the longissimus muscle in the loin region ($p<0.05$) and subcutaneous back fat ($p<0.001$) than PTS. The same trend was observed in n-3 eicosatrienoic acid in longissimus muscle in the loin region ($p<0.05$) and subcutaneous back fat ($p<0.001$).

In Appendix 8.5, by comparing PO with POS, dietary CLA led to increasing levels of saturated fatty acids (SFA) like myristic and stearic acid in the subcutaneous back fat, while decreasing those of MUFA like palmitoleic acid, oleic acid and elaidic acid. The apparent increase in the relative percentage of stearic acid and reduction in oleic acid indicated that delta-9 stearoyl-CoA desaturase was inhibited within the skeletal muscle and adipose tissue in the presence of dietary CLA. A similar finding was reported by Morel et al. (2008). The apparent inhibition of desaturase activity may contribute to the relative increase in the proportion of saturated: unsaturated fatty acids (Eggert et al., 1999). This observation was also reviewed by Dugan, Aalhus & Kramer, (2004) Martin, Muriel, Gonzalez, Viguera & Ruiz, (2008), Bee, (2001), and Demaree, Gilbert, Mersmann, & Smith, (2002). Such an increase in the ratio of SFA to unsaturated fatty acids could have negative health implications from a consumers' standpoint (Department of Health, 1994). Thus, including high levels of MUFA in pig diets when using dietary CLA could be a strategy for counteracting the decrease in MUFA caused by CLA. This work was followed up by Martin, Antequera, Muriel, Perez-Palacios & Ruiz (2011) by the combination of

dietary CLA with high MUFA diets for counteracting the increase in the ratio of SFA to unsaturated fatty acids caused by dietary CLA.

Direct effects of the supplement containing CLA, were shown in intramuscular fat in the longissimus muscle in the loin region (Appendix 8.4) and subcutaneous back fat (Table 8.9) with greater contents of CLA compared to the unsupplemented groups. The plant control group (PO) had very low CLA contents, but the increase with the supplemented plant diet resulted in CLA concentrations that were 2- to 4-fold higher in the subcutaneous back fat; and up to 2-fold higher in the longissimus muscle in the loin region. Dunshea et al. (2005) reviewed other studies in which dietary CLA led to elevated contents of these acids in pork and fat, and reported that the transfer of CLA from the diet to subcutaneous fat was more efficient than to intramuscular fat. In addition, α-linolenic acid showed a similar trend to CLA with the diet effect being more marked for subcutaneous back fat than intramuscular fat in the longissimus muscle.

Samples from the PTS group showed a decrease in α-linolenic acid, EPA, DPA and DHA because the amount of linseed fed was reduced and partially replaced by tallow. In vertebrates, the essential fatty acid α-linolenic acid can be converted to longer and more unsaturated n-3 PUFA, such as EPA and DHA (Sprecher, 2000). In fact, α-linolenic acid was positively correlated with n-3 long chain fatty acids such as EPA, DPA and DHA ($r=0.431$; $r=0.385$; $r=0.443$).

Among the plant-diet groups, PTS has the lowest omega-3 fatty acids level as some of the plant oils were replaced by tallow which has no association with the omega-3 fatty acids synthesis. Long chain fatty acid metabolism is controlled by a complex enzymatic system, consisting of desaturases and elongases (Raes et al., 2004; Mayes, 1996). These enzymes act both on the n-6 and n-3 fatty acids but have a preference for the n-3 group (Brenner, 1989). (see Fig.8.2 for PUFA metabolic pathway)

In addition, the concentration of n-6 eicosatrienoic acid was almost two times lower than n-3 eicosatrienoic acid for all dietary treatments in the current study. A significant difference was observed in C20:3n-3 between POS and PTS ($p<0.05$). The explanation is similar to the one given for AT vs. PO in the previous section.

(c) *POS vs. [PFSe + PSFl]*

There were 11 significant differences across 22 fatty acids in subcutaneous back fat compared to four in intramuscular fat of the longissimus muscle, with significantly lower levels of EPA, DPA and DHA ($p<0.001$) in the POS group for both these two tissues.

Hallenstvedt et al (2001) and Lauridsen et al. (1999) showed that feeding fish oil increased the level of DPA, EPA and DHA in pork. Apart from studies using fish oil as supplement in the diet of pigs, fish silages and fish fat have also been used. Kjos et al. (1999) showed that the total levels of omega-3 fatty acids were highest for the 5.5 and the 9.5 g per kg fish fat diets with 50 g per kg fish silage when they were fed to pigs until slaughter.

Increases in PUFA when feeding linseed and fish oil containing diets were inversely mirrored by reductions in MUFA (Table 8.12). This was reported in some plant groups for the study of Waters, Kelly, O'Boyle, Moloney & Kenny (2009), probably due to inhibition of delta-9 desaturase activity as the level of n-3 fatty acids increased in the diet.

The total PUFA and (EPA+DPA+DHA) proportions were significantly greater for the two fish oil groups compared to POS. It could be due to the incorporation efficiency of EPA and DHA from fish oil being much greater than the one of α-linolenic acid from linseed (Haak, Smet, Fremaut, Walleghem & Raes, 2008). This can be explained by three factors. First, there might be a greater digestibility of the fatty acids in the fish oil compared with those in linseed (Haak et al., 2008). Second, β-oxidation is faster for α-linolenic acid

compared with its products, which are more selectively incorporated into phospholipids within permanent cell structures (Leyton et al., 1987). Third, α-LNA has to compete with linoleic acid for incorporation and desaturation and elongation to its longer-chain metabolites in the tissues (Mohrhauer & Holman, 1963).

The increased level of DPA after fish oil supplementation as compared with the POS is in accordance with the results of Lauridsen et al. (1999). However, other studies have consistently shown an increased EPA and DHA deposition but no effect on the DPA concentration in pigs fed fish oil diets (Irie and Sakimoto, 1992; Morgan et al., 1992; Leskanich et al., 1997). Sprecher et al., 1995 found that DPA can be formed during elongation of EPA or by retroconversion of DHA.

(d) *PFSe vs. PFSl*

There were two significant differences across 22 fatty acids in subcutaneous back fat compared to six in longissimus muscle in the loin region.

The PFSl samples contained higher levels of EPA, DPA and DHA in the longissimus muscle and subcutaneous back fat in contrast to those in group PFSe. This result agrees with the work by Jaturasitha et al. (2008) when pigs were fed with tuna oil at early (35 – 60 kg) and late stages (75 – 90 kg) of fattening, with more DHA and EPA present in the fats for the latter stage group. The work by Valaja et al. (1992) also revealed that increasing fish meal and feeding period increased the levels of PUFA in the meat. Thus length and stage of feeding have effects on the levels of PUFA.

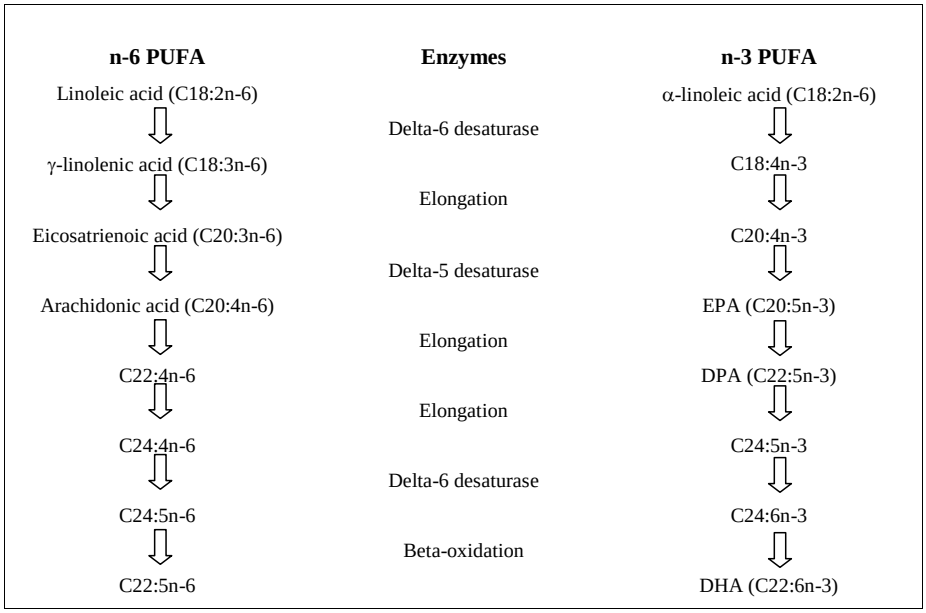


Fig 8.2

PUFA metabolic pathways (Mayes, 1996).

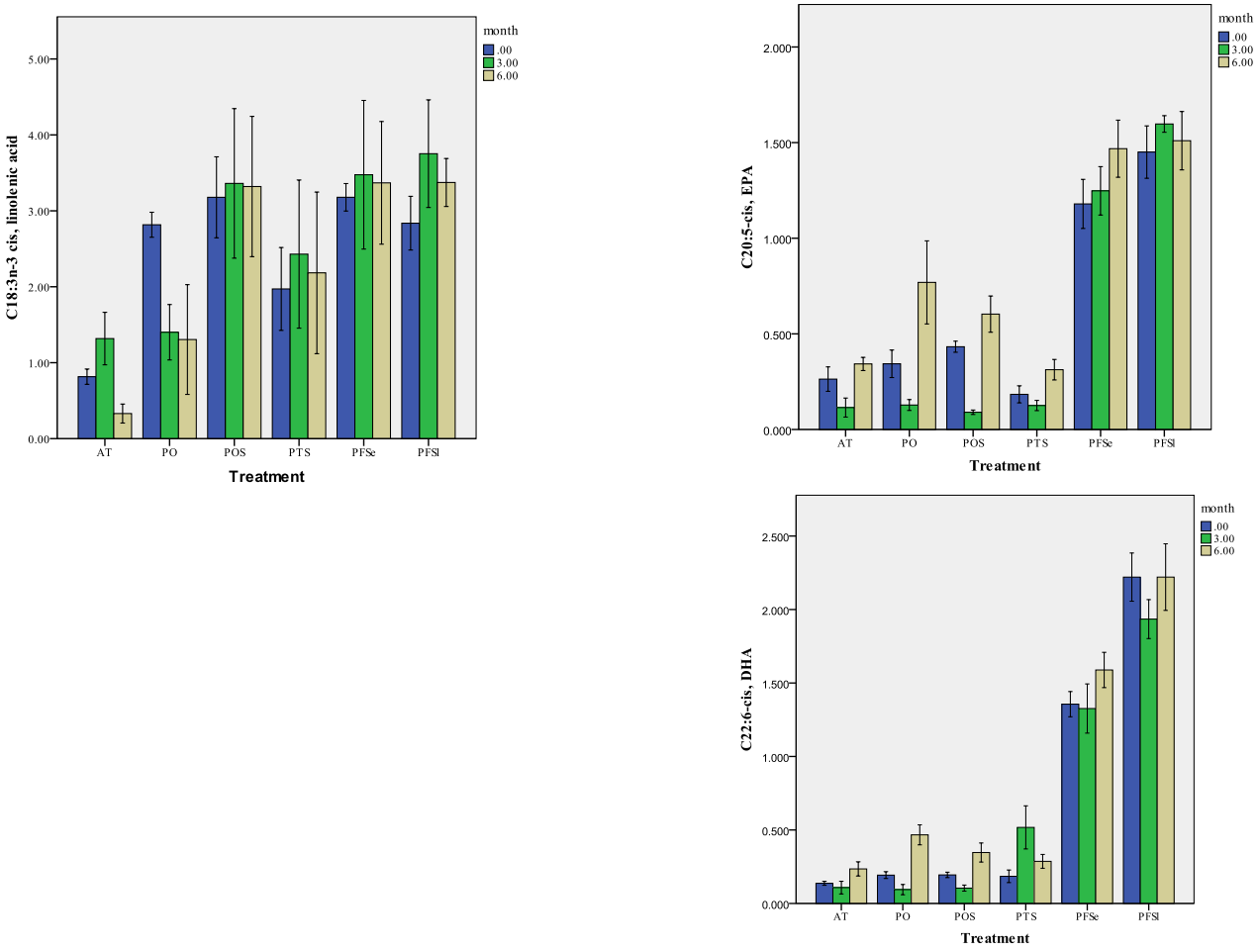


Fig. 8.3
Interaction plots showing the effect of treatment and frozen-storage period (0, 3 and 6 months) on fatty acids (%) in subcutaneous back fat (mean±SE).

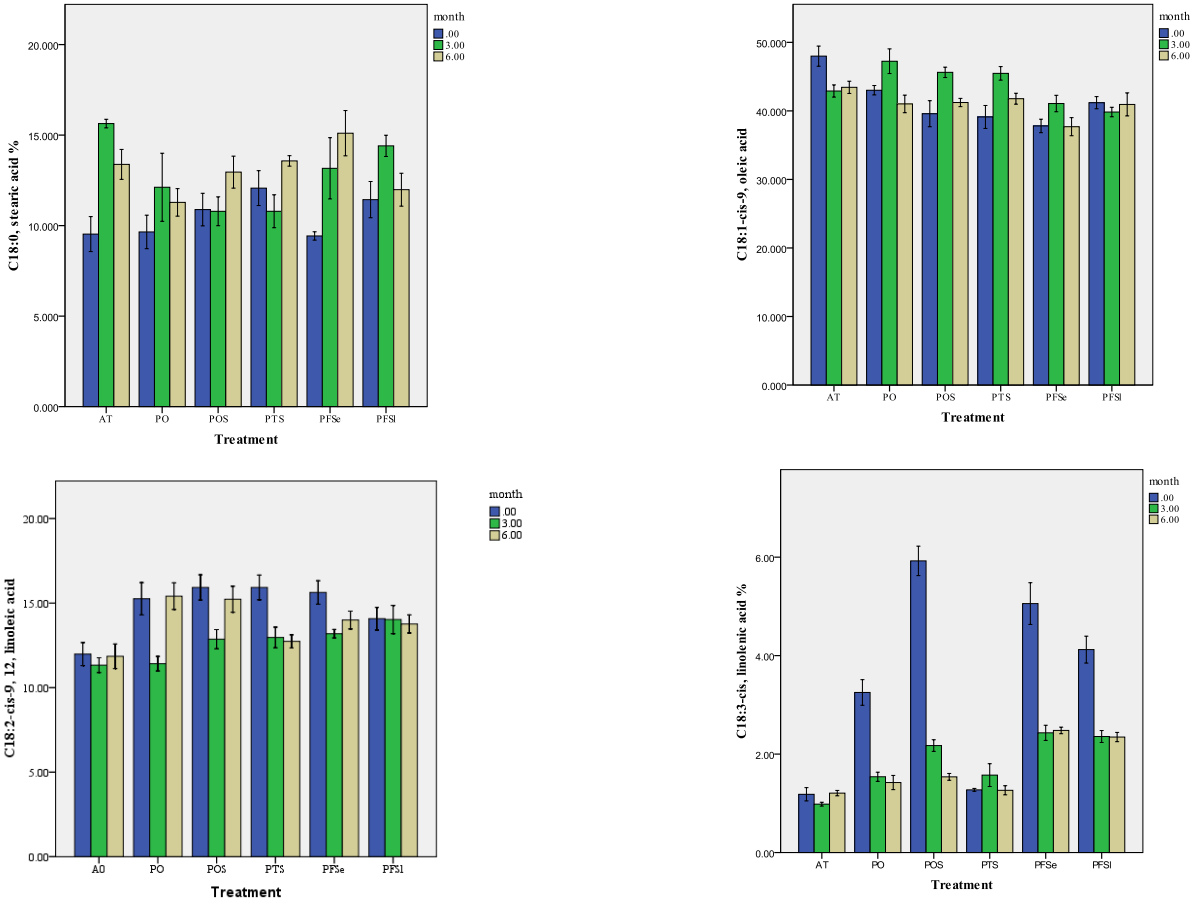


Fig. 8.4
Interaction plots showing the effect of treatment and frozen-storage period (0, 3 and 6 months) on fatty acids (%) in subcutaneous back fat (mean±SE).

Table 8.8

Least squares means of the fatty acid profiles in longissimus muscle and subcutaneous back fat in the loin region averaged across the 3 storage times (0, 3, and 6 months at -18°C) Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for the 3 storage times are given in Table 8.9

Item[1, 2]	Treatment (Trt) gp[3]						Trt effects (p-value)	R^2, RSD	Contrast (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PF8l
Longissimus muscle in the loin region													
SFA	36.57b	33.75b	33.73b	34.90b	34.43b	30.73a	0.002	20.1, 4.73	0.06	0.99	0.39	0.012	0.008
MUFA	46.54b	44.10b	43.33ab	44.57b	40.66a	43.07a	0.01	26.8, 5.16	0.17	0.68	0.42	0.9	0.12
PUFA	13.63a	17.01b	19.27c	17.32b	22.58d	23.25d	<0.001	62.1, 2.89	<0.001	0.007	0.024	<0.001	0.454
P:S	0.385a	0.520b	0.580b	0.516b	0.664c	0.771d	<0.001	51.3, 0.13	0.001	0.11	0.12	<0.001	0.009
L:LN	9.70c	6.62b	4.08a	6.19b	3.67a	3.39a	<0.001	48.2, 2.98	0.034	0.033	0.005	0.21	0.33
EPA+DHA+EPA	0.753a	1.25b	1.23ab	0.963ab	4.30c	5.55d	<0.001	88.0, 0.75	0.010	0.90	0.14	<0.001	<0.001
n-6/n-3	6.74d	4.19bc	3.29b	4.93c	1.87a	1.56a	<0.001	58.0, 1.69	<0.001	0.08	0.005	<0.001	0.011
ATT	1.35ab	2.18b	2.11b	0.994a	4.82c	6.34d	<0.001	73.8, 1.44	0.046	0.90	0.006	<0.001	0.042
L1	2.69	2.92	2.82	3.05	2.67	2.99	0.44	10.8, 0.76	0.30	0.68	0.36	0.19	0.07
Fats													
SFA	35.58b	32.92ab	32.86ab	34.97ab	33.42ab	32.25a	0.019	40.9, 3.67	0.09	0.96	0.044	0.10	0.41
MUFA	48.76d	47.62cd	45.77bc	46.40bcd	42.87a	44.56ab	<0.001	41.5, 3.63	0.39	0.15	0.060	0.004	0.12
PUFA	15.08a	18.77b	20.76c	17.97b	23.01d	22.54cd	0.001	70.6, 2.46	<0.001	0.08	0.010	<0.001	0.64
P:S	0.434a	0.592bc	0.645cd	0.521ab	0.707d	0.726d	<0.001	64.7, 0.12	<0.001	0.30	0.006	<0.001	0.74
L:LN	9.87c	6.97b	5.68a	10.24a	4.71a	4.95a	0.001	84.1, 1.25	<0.001	0.09	<0.001	<0.001	0.48
EPA+DHA+EPA	0.456a	0.599a	0.585a	0.486a	2.94b	3.31c	<0.001	94.2, 0.33	0.019	0.80	0.19	<0.001	0.024
n-6/n-3	7.35c	5.15b	4.56b	7.56c	2.50a	2.41a	<0.001	90.1, 0.80	<0.001	0.23	<0.001	<0.001	0.52
ATT	1.50a	1.65a	1.54a	1.65a	6.33b	7.91c	<0.001	73.1, 1.81	0.41	0.52	0.70	<0.001	0.12
L1	2.98a	2.02a	2.96a	2.79a	2.91a	3.28b	0.006	28.2, 0.43	0.66	0.81	0.19	0.008	0.025

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P:S: PUFA vs. SFA; L:LN: Linoleic acid vs linolenic acid; ATT: antithrombotic potential ; I1:The ratio between the not hypercholesterolaemic major fatty acids (C18:0 + C18:1) and the major hypercholesterolaemic fatty acid (C16:0)

[3]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 8.9
Least squares means of fatty acids profiles and effects of trt, time and trt x time of 6 treatment (trt) groups (AT, PO, POS, PTS, PFSe and PFSl) at 3 storage times (0, 3, and 6 months at -18°C) of longissimus muscle and subcutaneous back fat in the loin region made from pigs fed diets containing animal and plant products with or without a dietary supplement, tallow or fish oils. Means for treatment groups are given in Table 8.8

Item[1,2]	Storage time (months at -18°C)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
Longissimus muscle							
SFA	35.61b	33.13a	33.30a	0.002	0.02	0.98	20.1, 4.73
MUFA	44.89b	41.15a	45.40b	0.01	<0.001	0.40	26.8, 5.16
PUFA	18.32	18.49	19.47	<0.001	0.120	0.36	62.1, 2.89
P/S	0.523	0.585	0.605	<0.001	0.10	0.96	51.3, 0.13
L:LN	4.66a	5.50ab	6.79b	<0.001	0.004	0.009	48.2, 2.98
EPA+DHA+DPA	2.14a	2.09a	2.67b	<0.001	<0.001	0.49	88.0, 0.75
n-6/n-3	3.42	3.93	4.06	<0.001	0.16	0.57	58.0, 1.69
ATT[1]	2.05a	2.94ab	3.81b	<0.001	<0.001	<0.001	73.8, 1.44
I1[2]	2.84	2.81	2.93	0.44	0.70	0.90	10.8, 0.76
Subcutaneous back fat							
SFA	30.49a	34.69b	35.74b	0.09	<0.001	0.082	40.9, 3.67
MUFA	46.30ab	47.11b	44.77a	0.39	0.008	<0.001	41.5, 3.63
PUFA	22.42b	17.73a	18.70a	<0.001	0.001	0.007	70.6, 2.46
P/S	0.75b	0.522a	0.535a	<0.001	<0.001	0.172	64.7, 0.12
L:LN	6.14a	7.11ab	8.11b	<0.001	<0.001	<0.001	84.1, 1.25
EPA+DHA+DPA	1.52b	1.27a	1.29a	0.019	<0.001	0.06	94.2, 0.33
n-6/n-3	4.39a	5.04ab	5.49b	<0.001	<0.001	<0.001	90.1, 0.80
ATT[1]	4.01b	3.69ab	2.42a	0.41	<0.001	0.013	73.1, 1.81
I1[2]	3.17b	2.96a	2.80a	0.66	<0.001	0.13	28.2, 0.43

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2]SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P:S: PUFA vs. SFA; L:LN: Linoleic acid vs linolenic acid; ATT: antithrombotic potential ; I1:The ratio between the not hypercholesterolaemic major fatty acids (C18:0 + C18:1) and the major hypercholesterolaemic fatty acid (C16:0)

8.3.5.2 Storage-time effects on fatty-acid concentrations

With respect to effects of frozen storage period on fatty acid composition, there were 17 significant differences across 22 fatty acids in intramuscular fat of the longissimus muscle in the loin region and subcutaneous back fat, respectively, with 13 differences being similar for these two tissues (Appendices 8.6 and 8.7). Four significant (treatment x time) interactions were observed in the longissimus muscle compared to 11 such interactions in subcutaneous back fat. Selected interaction plots for intramuscular fat and subcutaneous back fat of the longissimus muscle are shown in Figures 8.3 and 8.4.

Comment [ITS1]: Are these now Appendix tables?

Comment [S2R1]: Amended on the labelling of these 2 tables as they are now in Appendix.

Storage period had a strong influence on the fatty acid ratios (Table 8.9), with 5 significant differences across 9 fatty acid ratios in longissimus muscle in the loin region, and all fatty acid ratios were statistically different in the subcutaneous back fat. Two significant (treatment x time) interactions were observed in longissimus muscle in the loin region compared to 5 such interactions in subcutaneous back fat.

EPA, DPA and DHA levels decreased over the frozen storage period of 6 months, possibly because these polyunsaturated fatty acids are highly susceptible to lipid oxidation (Whittington et al., 1986; Rhee et al., 1988). The trend was the same for cis-9 trans-11 CLA and trans-10 cis-12 CLA and oleic acid. Fatty acid profiles that decreased were MUFA, PUFA, P/S, Il and ATT. In contrast, levels of stearic acid, myristic acid, SFA and n-6:n-3 increased with the storage time (Appendix 8.6, 8.7, Table 8.9), but there is no clear explanation for these changes.

8.4.5.3 Principal Component analysis

The 9 fatty acid profiles used in the PCA analysis were those listed in Tables 8.8 and 8.9. The results of the PCA for subcutaneous back fat are shown in Figure 8.5. The first two principal components (PC) explained 98.5% of the total variation in fatty acid composition, and the first PC (PC1) explained 93.2%. The results of the PCA of fatty acid profiles for intramuscular fat of the longissimus muscle were very similar to that of

subcutaneous back fat. The first two principal components explained 97.4% of the total variation in fatty acid composition, and PC1 explained 80.2%. For both intramuscular fat and subcutaneous back fat, PC1 was mainly characterised by DHA+EPA+DPA, while PC2 was defined mainly by PUFA and L:LN. Pork from the PFSe and PFSl groups were clearly differentiated from the other diet groups and were located in the middle right half of the figure. The rest of the diet groups were located in the left half of the figure. AT and PTS were found in the lower left quadrant while the rest were in the upper half.

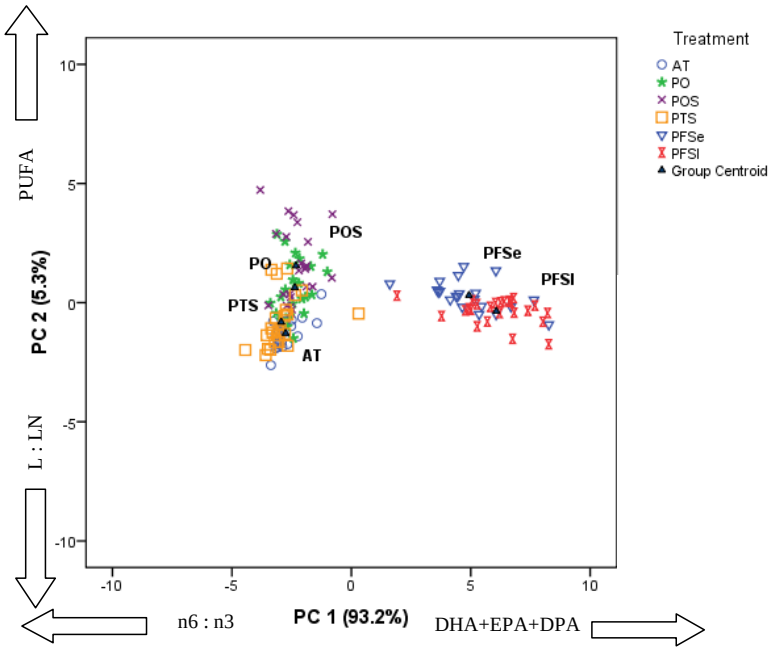


Fig. 8.5

Projection of fatty acid profiles of the 6 diet groups[1] in subcutaneous back fat based on the least squares means of the items in Tables 8.8 averaged for the 3 storage periods (0, 3, & 6 months at -18°C) studied in the plane defined by two principal components.

The first two principal components (PC) explained 97.4% of the total variation in fatty acid composition. PC1 explained 80.2% of the variability and was defined mainly by DHA+EPA+DPA, while PC2 explained 5.3% and was defined mainly by PUFA and L:LN (linoleic acid vs. linolenic acid) .

[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite^{TM}; PTS=diet with plant products, tallow & Sanovite^{TM} ; PFSe=diet with plant products, Sanovite^{TM} and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite^{TM} and fish oil (during the late grower period)

One of the most widely used parameters to evaluate the nutritional value of fat is the P/S ratio. The P/S ratio was higher for group PO than AT (Table 8.8) for both lean meat and subcutaneous back fat. PFSe and PFSl had the highest P/S ratio for lean meat, while PO and POS meat showed intermediate values. AT and PTS samples had the lowest ratios. From a consumer-health viewpoint, the recommended value for this ratio is 0.4 or higher (Department of Health, 1994). The only value below 0.4 was for lean of the AT group. The P/S ratio decreases as intramuscular fat increases according to De Smet et al. (2004) because, as intramuscular fat increases, triglycerides, which are rich in SFA, increase faster than phospholipids, which are rich in PUFA (Raes et al., 2004), leading to a decrease in the P/S ratio.

Both the n-6/n-3 and the linoleic acid/α-linolenic acid ratios were reduced in lean pork from pigs on plant diets (Table 8.8). The n-6/n-3 ratio was highest in AT and PTS, followed by PO and POS. The lowest n-6/n-3 ratio was observed in PFSe (1.87 in lean meat; 2.50 in subcutaneous back fat) and PFSl (1.56 in lean meat; 2.41 in subcutaneous back fat). The trend was similar for linoleic acid/α-linolenic acid ratio in lean meat and subcutaneous back fat. In fish oil fed samples, such as PFSe and PFSl, this ratio was beneficially low, compared to that in AT samples. From the human nutrition point of view, one of the most important indices is the n-6/n-3 ratio, which should have a value below 4 (Department of Health, 1994). The importance of the dietary ratio of linoleic acid /α-linolenic acid and that of n-6/n-3 ratio in the human diet has been well emphasized (Galli & Simopoulos, 1989; BNF, 1992). According to Choi, Enser, Wood, and Scollan (2000), the n-6/n-3 ratio is much more affected by feeding than by genetics. Linseed is the richest oilseed source of 18:3n-3 and feeding it to pigs has been used to lower the n-6/n-3 fatty acid ratio in pork, as reviewed by Nguyen, Nuijens, Everts, Salden, and Beynen (2003).

The antithrombotic potential (ATT) is the ratio between the sum of the antithrombogenic fatty acids, n-6 eicosatrienoic acid and EPA, and the thrombogenic fatty

acid, arachidonic acid (Enser, Hallett, Hewitt, Fursey, & Wood, 1996). The highest values for antithrombotic potential were observed in PFSe and PFSl, ranging from 4.82-7.91. All other diet groups displayed values between 1 and 2.2. The thrombotic tendency of blood depends largely upon the balance between antithrombogenic (n-6 eicosatrienoic acid and EPA) and thombogenic (arachidonic acid) fatty acids (ATT) (Enser et al., 1996).

8.3.5.4 Adequate intake of EPA, DPA and DHA

Pork from pigs fed the different diets contained different levels of EPA, DPA and DHA, with groups fed diets containing fish oil (PFSe and PFSl) having the highest EPA, DPA and DHA levels in loin muscle and subcutaneous back fat. These fatty acids play an important role in human nutrition. Figure 8.8 shows the amount of a pork product (g/d) varying in lean meat content that would need to be consumed to reach a daily intake of 160 mg (EPA+DPA+DHA). The (EPA+DPA+DHA) content in loin and back fat was determined as a percent of the total fatty acid content. To calculate the concentration of (EPA+DPA+DHA) (g/g) in the loin muscle, the intramuscular fat concentration (g/g) was multiplied by the concentration of (EPA+DPA+DHA) relative to total fatty acids (g/g). These calculations were based on the assumption that fatty acids made up 100% of intramuscular fat.

To calculate the concentration of (EPA+DPA+DHA) (g/g) in the back fat, the concentration of fat in back fat (g/g) was multiplied by the concentration of (EPA+DPA+DHA) in the total of all fatty acids (g/g). The fat percentage of the back fat was not determined, but was assumed to be 72% based on that determined for loin subcutaneous fat as shown in Table 4 in the study by Morel et al. (2008).

The following equation was used:

$$INT = \frac{AI}{\left(\frac{LN\%}{100} \times LN\_n3\right) + \left(\left(\frac{BF\%}{100} \times BF\_n3\right)\right.}$$

where:

INT = required intake of the pork product (g/d) to obtain 160 mg of (EPA+DPA+DHA)
AI = adequate intake of long-chain n-3 fatty acids (EPA+DPA+DHA) per day (set at 160 mg)
LN% = percentage of lean loin in the pork product
LN_n3 = concentration of (EPA+DPA+DHA) in the lean loin (g/g)
BF% = percentage of back fat in the pork product
BF_n3 = concentration of (EPA+DPA+DHA) in the back fat (g/g)

Using this equation it was shown that higher percentages of back fat in the pork product resulted in higher levels of (EPA+DPA+DHA) per gram of the product. As shown in Fig. 8.6, leaner products require higher amounts of daily intake to reach the target of 160 mg of (EPA+DPA+DHA) per day.

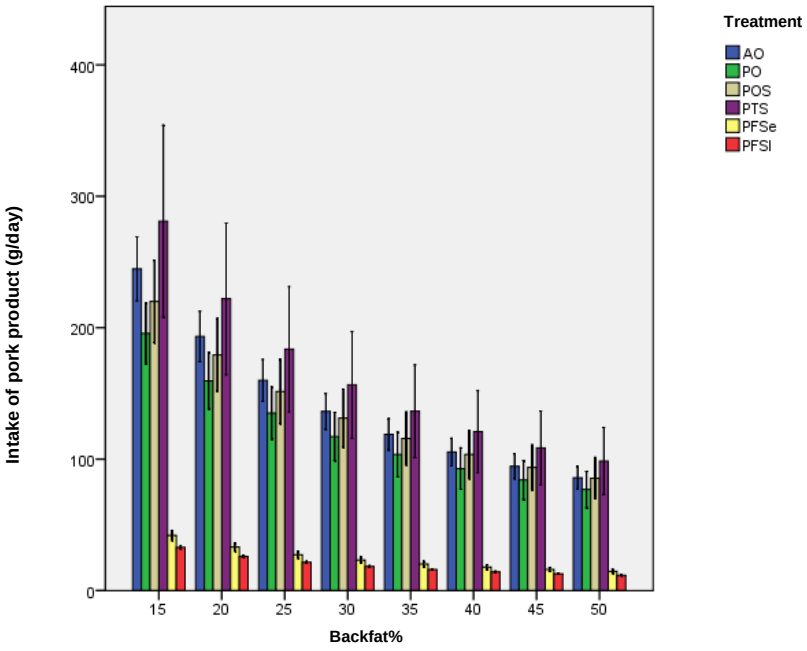


Fig. 8.6
The intake of a pork product (g/day; mean±SEM) required to achieve an intake of 160 mg of (EPA+DPA+DHA)/day as the percentage of back fat in that pork product increases from 15% to 50%, with the remainder of the product being lean meat.

If pork products with 15% back fat from pigs fed diet PFSe or PFSl were consumed, then a daily intake of 42 g and 33 g, respectively, would be sufficient to achieve an intake of 160 mg of (EPA+DPA+DHA)/day. Greater amounts would need to be consumed if pork products with 15% back fat were from pigs fed the AT (245 g/d), PO (196 g/d), POS (220 g/d) or PTS (281 g/d) diets.

Numerous recommendations for long-chain omega-3 fatty acid intakes have been made globally by many government agencies, professional groups, and scientists. The majority of recommendations have been issued on the basis of the amount of EPA+DHA

together, without specific guidance for each fatty acid. There is a difference in the levels of intake of the long-chain n-3 fatty acids (EPA, DPA, & DHA) recommended for humans for "adequate intake" (AI) and "suggested dietary targets" (SDT) (Howe et al. 2007). SDT levels are set to achieve health benefits, and as a result are between 3.8 and 4.8 times higher (respectively for females and males) than levels for AI targets (Howe et al. 2007). The National Health & Medical Research Council for Australia and New Zealand recommended AIs for the sum of EPA, DPA and DHA of 90 and 160 mg/day and SDTs of 430 and 610 mg/day for woman and men, respectively. The AI for men was used in calculating the values for Figure 8.6, because men require the higher amount of EPA, DPA and DHA per day.

The current study shows that pork with different levels of EPA, DPA and DHA in the intramuscular and subcutaneous fat can be produced from pigs fed different diets. A higher fat content in pork products results in higher levels of EPA, DPA and DHA per gram. Large amounts of lean pork need to be eaten on a daily basis when animal diets are not enriched with omega-3 PUFA to reach AI requirement of the long-chain n-3 fatty acids. If pork with 85% lean meat (15% back fat) from pigs on PFSe or PFSl diet were consumed, the daily intake to reach AI requirements reduces by 81.0% and 85.0%, respectively, compared to when lean loin meat from pigs fed diet POS was consumed. This shows that enriching animal diets with omega-3 PUFA helps in achieving standards for adequate intakes of these fatty acids.

Essential fatty acids such as omega-3 PUFA (found in salmon, canola and soya oil) may also help in lowering plasma triglyceride levels. The American Heart Association in its official Dietary Guidelines (2000) recommended that the target daily intake of DHA plus EPA for individuals with coronary heart disease should be 900 mg/day since this amount has been shown to be beneficial in decreasing coronary heart disease mortality rates in patients with coronary disease.

8.3.6 Sensory Evaluation

8.3.6.1 Diet effects

(a) Trained panel

Based on the results in Table 8.10 using the Singapore trained panel, it was deduced that all sensory attributes were consistent across treatment groups when averaged across 0, 3, and 6 months of frozen storage except for colour saturation, fishy and rancid odour; mutton, fishy, rancid, and metallic flavour, and aftertaste.

No significant differences were observed for AT vs. PO or POS vs. PTS for all attributes. This trend was similar for PO vs. POS except for colour saturation, which was lower for POS ($p < 0.001$) for no apparent reason. Relative to POS, the PFSe and PFSI groups had higher scores for colour saturation, fishy aroma, rancid odour, and fishy, and rancid flavours, and aftertaste ($p<0.05$), but lower scores for meaty aroma, and meaty, mutton and metallic flavours. Jaturasitha et al. (2008) also showed that pork from pigs fed fish oil (1.6kg) at a later stage had a stronger fishy flavour.

Mutton flavour decreased as fishy flavour increased ($r=-0.903$; $p<0.001$). Aftertaste increased with fishy aroma ($r=0.598$; $p<0.001$) and fishy flavour ($r=0.603$; $p<0.001$). Meaty and brothy aromas and flavours had strong positive correlations between them (meaty vs. brothy flavour; $r=0.946$; $p<0.001$; meaty vs. brothy aroma; $r=0.883$; $p<0.001$) and these characteristics decreased as the scores for the rancid attributes increased (meaty vs. rancid flavour; $r=-0.838$; $p<0.001$); (brothy vs. rancid flavour; $r=-0.781$; $p<0.001$). TBARS values in longissimus muscle in the loin region were negatively correlated with meaty aroma ($r= -0.694$), meaty flavour ($r=-0.746$), brothy aroma ($r=-0.787$) and brothy flavour ($r=-0.698$); all at $p<0.001$. Fig. 8.7 shows the negative relationship between TBARs and meaty flavour and the positive relationship between TBARs and rancid flavour.

The sample from PFSl diet seemed more brown and lighter in colour than PFSe. Meaty and brothy flavours were lowest for the PFSe and PFSl samples, possibly due to the masking effects from the rancid flavour and aftertaste by this fish-oil diet group. These masking effects agree with the results of Bryhni et al., (2002) and Gray, Gomaa & Buckley (1996) where oxidation products masked the meaty profiles of pork from pigs fed high PUFA diets.

Acidic aroma and taste as well as rancid odour and taste increased from month 0 to month 6 of frozen storage (Table 8.11). This observation is in agreement with the lower pH as storage period increased (Table 8.4). There was also a positive correlation between acidic aroma and rancid odour ($r=0.526$; $p=0.025$) and rancid flavour ($r=0.557$; $p=0.016$). Interaction effects between treatment and time was observed in acidic aroma ($p=0.001$). Rancid odour and flavour (Table 8.11) also increased for all dietary groups during the storage period ($p<0.001$). In addition, TBARS were positively correlated to acidic aroma ($r=0.721$; $p<0.001$) and taste ($r=0.592$; $p<0.001$). Fig. 8.7 shows the positive relation between TBARs and rancid flavour. In studies by Gray and Pearson (1987) and Watts (1962), a threshold value of 1 mg MDA/kg muscle for organoleptic detection of rancidity was reported. This is in excess of lipid oxidation values observed in the present study.

Mutton notes were stronger for the animal product groups like AT and PTS but less for the fish oil groups. Again, the fishy note from the fish oils could have contributed to the decrease in the mutton notes. There was a negative correlation between mutton flavour and fishy flavour ($r=-0.903$; $p<0.001$). Aftertaste was positively correlated to fishy aroma ($r=0.598$; $p< 0.001$) and fishy flavour ($r=0.603$; $p<0.001$).

Organoleptic changes have been reported in pork from pigs fed on fish oil and fish meal in the current trial and in trials by other researchers. In the present study, pigs in group PFSe and PFSl both received the same total amount of fish oil per pig (2.52 l/ 2.31 kg) which was 4.4% of the whole diet. The only difference was that fish oil was removed from

the PFSE group on day 36 onwards while PFSl group had theirs removed on day 57. Both groups received the POS diet thereafter until slaughter. Based on Appendix 8.4 and 8.5, the PFSl group which had higher levels of n-3 fatty acids compared to PFSe, also had stronger fishy off-flavour than PFSe. Interaction effects between treatment and time were observed in fishy aroma and flavour ($p<0.05$). Such long chain unsaturated n-3 fatty acids were susceptible to oxidation which led to fish off-flavour (Jonsdottir et al., 2003). A good feeding strategy is essential concerning the identification of the ideal proportion of fish oil with maximum effect on n-3 fatty acid incorporation but still acceptable pork flavour and oxidative susceptibility. It seems that 1% of fish oil in the diet causes only few problems in that respect, whereas greater concentrations trigger consumer complaints (Lauridsen et al., 1999; Jaturasitha et al., 2002).

In studies by other researchers, Maw et al. (2001) reported fish taints persisted in the bacon and that increased use of fish meat in the diet was associated with increased levels of fishy flavour. Similarly, diets containing 2.5 and 9.5 g kg^{-1} fish fat until slaughter caused off-flavour of vacuum-packaged bacon after both 1 and 6 months, and of loin muscle after six months of frozen storage at -16^{o}C (Kjos et al.,1999). Bryhni et al. (2002) observed more rancid loin and sausage products after one and eight months of freezer storage while Hertzman et al. (1988) reported increased off-flavour in pork as storage period increased from 0 to 9 months by a trained panel when 3% fish meal was used in the pigs' diet after frozen storage at -20^{o}C. Hallenstvedt et al. (2010) reported on reheating pork of long-term stored (12 months at -80^{o}C, 6 months at -20^{o}C) to produce warmed-over flavour (WOF) gave higher rancid and fish oil flavour of meat when 0.7% fish oil was used in the diet compared to the control.

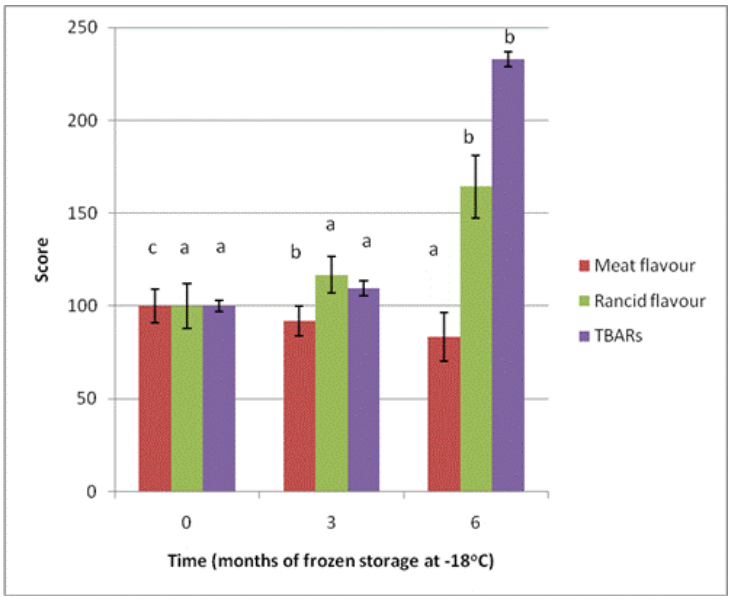


Fig. 8.7
Patterns of change in TBARs (in subcutaneous back fat) and rancid and meaty flavour scores for a mix of minced longissimus muscle (90.9%) and subcutaneous fat (9.1%) during frozen storage for 0, 3 or 6 months at -18°C. Meaty flavour was reduced by 9 and 17 % in months 3 and 6; whilst rancid flavour was increased by 20 and 60% relative to month 0. The meaty and rancid flavours were assessed by a Singapore trained panel. Common letters above the bars indicate non-significance ($p > 0.05$) in the scores amongst bars for the same characteristic.

(b) *Consumer panel*

The hedonic ratings were higher for PO relative to AT and higher for POS than PTS for most storage times, but were similar for POS and PTS after 6 months storage (Table 8.12). This concurred with a study by Leong et al. (2010) where pork from pigs fed a plant diet were better accepted than pork from animals on a diet containing animal products. The acceptability for the PO and POS groups was similar except that pork from the POS group was more acceptable at month 0 and the flavour was better liked at month 3 (Table 8.12). Within group B (Table 8.12), pork from the fish oil groups was consistently lower for hedonic scores relative to POS throughout the storage period with PFSl having a lower acceptability score than PFSe ($p<0.05$). Storage period had a strong influence on aroma liking, flavour liking and acceptability across the four treatments (AT, PO, POS, PTS) in Group A; and the three treatments (POS, PFSe, PFSl) in Group B ($p<0.001$) (Table 8.13).

As storage period increased, the negative responses from the consumer panel towards products increased for all dietary treatments. In addition significant interaction effects between treatment and time ($p<0.001$) were observed in aroma liking, flavour liking and acceptability for both group A and B (Table 8.13). Hedonic rating scores in group A ranging from 3 to 5.5, had larger changes over time; while those from group B with a much lower range from 1.8 to 3.6, had much smaller changes. The most acceptable product amongst the six dietary groups came from POS in which the highest liking score was 7.00 in month 0 (Table 8.12). Pork from the AT diet received similar negative responses to those for pork from the fish oil groups , but pork from the fish oil groups was generally more unacceptable.

Pork from the POS group was generally preferred to that from the PO group, possibly due to the presence of selenium, vitamin E and C which decreases oxidative rancidity of PUFA, resulting in less rancid off-flavour. Section 2.3.3.1c contains the references showing the anti-oxidative effects of dietary selenium, vitamin E and C on pork

and other types of meat. Campo, Nute, Wood, Elmore, Mottram, and Enser (2003) found that odour scores were often higher for linolenic acid than for linoleic acid, i.e. linolenic acid produces more intense odours. The pigs fed linseed had lower scores for acceptability of pork odour and flavour and higher scores for abnormal odour and flavour. These confirm the US data (Shackelford et al., 1990) showing that C18:3 concentrations of above about 3% in total lipid or neutral lipid can produce relatively undesirable flavours.

Off-flavours in terms of percentage detection are shown in Table 8.14. The percentage of panellists detecting rancid note in AT vs. PO diet from month 0 to 6 was 4.8% vs. 19%. This is indicating the panels' ability to pick up negative flavour attributes associated with increasing PUFA levels and resulting oxidative instability. The result was similar to the one conducted by Juárez et al., (2011) where the percentage of panellists detecting a stale note increased from 5% to 10% when 10% co-extruded flaxseed was in the diet in comparison to the control diet.

Table 8.15 shows that mutton notes were detected in all treatment groups regardless of period of storage (Table 8.15) and that mutton intensity decreased from month 0 to month 6 for all treatments in group A ($p<0.001$) as well as group B ($p=0.002$) (Table 8.16). This could be due to masking by the increasing rancid and fishy notes in groups A and B, respectively. Rancid notes were not detected in group A until month 3, the note appeared as early as month 0 for the fish oil diets in group B. This note increased in intensity in all treatments during the storage period ($p<0.001$), with PFSe and PFSl having the highest intensity in rancidity. Unsaturated fatty acids, especially PUFAs, are susceptible to oxidation and this has been shown in numerous studies where off-flavours have been observed when pigs were fed fishmeal and rapeseed (Hertzman, Goransson & Ruderus, 1988) and red clover silage (Jonsäll, Johansson, & Lundstrøm, 2000).

A bitter note was detected in all groups from month 0 onwards and was quite similar throughout all months. A similar trend was observed for metallic notes in Group A, but not for Group B where metallic intensity decreased with storage period. Apparently,

oxidation products could have masked the other milder characteristics. An acidic note was consistently present in all treatments and its intensity increased from month 0 to 6 for Groups A and B ($p<0.001$).

Fishy notes were only detected in PFSe and PFSl in group B (Table 8.15), with PFSl having significantly higher fishy intensity than PFSe from month 3 to 6 ($p<0.05$). This fishy attribute increased from month 0 to 6 ($p<0.001$). Rancid notes were markedly different between PFSe and PFSl for months 0, 3, and 6, with PFSI having the higher intensity. Rancid notes increased with storage period ($p<0.001$) in Group B. The combined fishy and rancid note in PFSe and PFSl could have masked the metallic and bitter notes which decreased during storage. Hertzman et al., (1988) observed off-flavours after 4 and 6 months of frozen storage of meat from pigs fed more than 3% dietary fishmeal. Arnkværn & Bronken Lein (1997) also showed that pork produced with a high amount of unsaturated fatty acids in the diet like marine fat, developed more rancid flavours and odours during freezer storage for 1, 4 and 8 months. Other studies have reported off-flavour when a fish meal or fish oil was included in the diet of the pigs (summarised in Table 2.4). Interactions between treatment and time were observed in intensity scores for mutton notes for both groups A and B. The interaction could have been due to the decrease in mutton intensity from month 0 to 3 and then increase from month 3 to 6. This arises possibly due to interferences of rancid and fishy notes with the mutton notes, thus causing misjudgement by the consumer panel. Interaction effects were also seen in rancid and fishy intensity scores in Group B. This could be due to a big increase in rancid note intensity from month 0 to 3; and a moderate increase from month 3 to 6 (Table 8.15). Fishy note intensity increased moderately from month 0 to 3 but there was a big increase from month 3 to 6.

Table 8.10

Least squares means of intensity score rating of a mix of longissimus muscle and subcutaneous fat in the loin region for the six treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the three storage times (0, 3, & 6 months at -18°C), on a scale from 0 to 100 with higher values indicating a stronger note, as assessed by a Singapore trained panel. Means for the 3 storage times are given in Table 8.11

Sensory terms[1]	Treatment (Trt) group[2]						Effects (p-value)	R^2, RSD	Contrast (p value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Colour	47.71	48.65	48.14	46.97	43.45	48.95	0.32	33.3, 22.9	0.71	0.84	0.64	0.08	0.040
Colour saturation	44.43c	45.35c	36.88ab	41.73bc	34.91a	42.49c	<0.001	48.0, 22.3	0.70	0.001	0.05	0.008	0.003
Meaty aroma	65.02	63.55	65.13	64.14	63.12	60.90	0.35	31.0, 19.2	0.49	0.46	0.60	0.16	0.35
Brothy aroma	43.06	43.11	43.56	42.75	42.63	40.74	0.95	12.0, 26.8	0.99	0.88	0.78	0.62	0.53
Fishy aroma	2.58a	3.00a	2.32a	2.34a	54.64b	61.83c	<0.001	74.3, 15.7	0.29	0.09	0.96	<0.001	0.024
Mutton aroma	25.81	23.35	21.92	23.04	22.85	21.52	0.56	31.0, 21.5	0.31	0.54	0.63	0.86	0.61
Metallic aroma	6.68	6.35	7.70	6.88	6.79	6.85	0.73	10.7, 7.70	0.68	0.16	0.39	0.58	0.95
Acidic aroma	6.72ab	6.44ab	6.83ab	6.41ab	5.91a	7.46b	0.26	9.3, 5.70	0.66	0.54	0.53	0.07	0.023
Rancid odour	16.05ab	15.64ab	14.32a	14.35a	17.84b	18.04b	0.043	80.0, 13.6	0.77	0.37	0.99	0.041	0.91
Meaty flavour	66.37ab	67.32b	67.75b	65.28ab	64.98ab	62.75b	0.06	9.7, 16.1	0.58	0.80	0.15	0.037	0.30
Brothy flavour	44.65	46.14	45.09	43.68	41.89	42.51	0.66	33.0, 25.3	0.59	0.71	0.61	0.49	0.83
Fishy flavour	4.64a	4.80a	4.84a	4.72a	56.07b	66.02c	<0.001	77.8, 14.4	0.72	0.93	0.79	<0.001	<0.001
Mutton flavour	33.26b	32.40b	29.84b	29.84b	21.44a	17.62a	<0.001	75.0, 22.1	0.73	0.31	0.99	<0.001	0.12
Metallic flavour	14.47ab	13.89ab	11.91a	11.84a	17.26b	15.77ab	0.025	24.0, 16.8	0.76	0.23	0.97	0.017	0.47
Acidic flavour	21.09	20.73	19.30	18.09	21.12	20.16	0.71	22.2, 19.9	0.87	0.50	0.57	0.73	0.67
Rancid flavour	18.11abc	16.56ab	15.61ab	15.14a	19.50bc	21.78c	0.004	64.3, 17.4	0.41	0.59	0.78	0.012	0.33
Salty taste	19.81	19.05	19.48	18.41	20.29	18.93	0.98	12.0, 23.1	0.76	0.86	0.67	0.88	0.61
Aftertaste	57.90bc	57.37bc	54.75ab	51.15a	61.71cd	65.29d	<0.001	59.6, 21.7	0.82	0.25	0.13	<0.001	0.16

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 8.11

Least squares means of the intensity score rating and effects of treatment (trt) group, time and (trt x time) of a mix of longissimus muscle and subcutaneous fat in the loin region; for the three storage times across the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) on a scale from 0 to 100 with higher values indicating a stronger note, as assessed by a Singapore trained panel. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment group are given in Table 8.10

Sensory terms[1]	Month			Effects (p-value)			R^2 , RSD
	0	3	6	Trt	Time	Trt x Time	
Colour	49.03b	49.60b	43.56a	0.32	0.001	0.17	33.3, 22.9
Colour saturation	43.54b	42.02b	37.72a	<0.001	0.003	0.59	48.0, 22.3
Meaty aroma	66.82b	64.12b	60.03a	0.35	<0.001	0.90	31.0, 19.2
Brothy aroma	44.34b	44.47b	39.12a	0.95	0.014	0.99	12.0, 26.8
Fishy aroma	20.31	22.91	18.00	<0.001	<0.001	<0.001	74.3, 15.7
Mutton aroma	21.99a	26.87b	20.39a	0.56	<0.001	0.36	31.0, 21.5
Metallic aroma	10.28b	5.61a	4.75a	0.73	<0.001	0.15	10.7, 7.70
Acidic aroma	5.21a	6.14b	8.57c	0.26	<0.001	0.001	9.3, 5.70
Rancid odour	11.00a	17.94b	19.07b	0.043	<0.001	0.92	80.0, 13.6
Meaty flavour	71.66c	65.87b	59.74a	0.06	<0.001	0.99	9.7, 16.1
Brothy flavour	49.59c	43.29b	39.22a	0.66	<0.001	0.99	33.0, 25.3
Fishy flavour	23.79	23.11	21.56	<0.001	0.12	0.003	77.8, 14.4
Mutton flavour	26.15	28.12	28.31	<0.001	0.38	0.56	75.0, 22.1
Metallic flavour	13.20a	16.08b	13.09a	0.025	0.035	0.95	24.0, 16.8
Acidic flavour	19.35ab	18.79a	22.04b	0.71	0.08	0.18	22.2, 19.9
Rancid flavour	14.21a	16.30a	22.72b	0.004	<0.001	0.81	64.3, 17.4
Salty taste	20.88	18.04	19.01	0.98	0.28	0.99	12.0, 23.1
Aftertaste	60.76b	56.48a	56.61a	<0.001	0.016	0.60	59.6, 21.7

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 8.12

Least squares means of hedonic rating of a mix of longissimus muscle and subcutaneous fat in the loin region from pigs on dietary treatment (trt) containing animal and plant products with or without a dietary supplement (S), tallow (T) or fish oils (Fe & Fl) assessed by a Singapore panel, on a scale from 1 to 9 with 1 being "Dislike Extremely" and 9 being "Like Extremely". The six treatment groups were divided into groups of 4 (group A) and 3 (group B) for evaluation with samples of POS in each group. Means for the 3 storage times are given in Table 8.13

Month	Sensory terms[1]	Group A Trt groups[2]				Trt effects (p-value)	R^2, RSD	Contrast (p-value)			Group B Trt groups[2]			Trt effects (p-value)	R^2, RSD	Contrast (p-value)	
		AT	PO	POS	PTS			AT vs. PO	PO vs. POS	POS vs. PTS	POS	PFSe	PFSl			POS vs. [PFSe]+ [PFSl]	[PFSe] vs.[PFSl]
0	Aroma liking	3.69a	7.00c	6.79c	4.62b	<0.001	94.0, 1.10	<0.001	0.38	<0.001	6.21c	2.00b	1.26a	<0.001	65.4, 0.97	<0.001	<0.001
	Flavour liking	2.64a	6.93c	7.12c	4.21b	<0.001	58.4, 0.93	<0.001	0.40	<0.001	6.93c	2.48b	1.38a	<0.001	76.9, 1.19	<0.001	<0.001
	Acceptability	2.90a	6.64c	7.31d	4.50b	<0.001	44.3, 1.31	<0.001	0.002	<0.001	7.24c	1.62a	1.26a	<0.001	80.5, 1.17	<0.001	0.025
3	Aroma liking	3.60a	4.07ab	3.93ab	4.33b	0.15	95.1, 1.48	0.17	0.64	0.17	4.31c	1.98b	1.21a	<0.001	70.2, 0.65	<0.001	<0.001
	Flavour liking	2.98a	4.60c	5.40d	3.95b	<0.001	81.3, 1.35	<0.001	0.009	<0.001	4.98c	2.33b	1.24a	<0.001	83.2, 0.96	<0.001	<0.001
	Acceptability	2.90a	4.86b	5.67c	3.29a	<0.001	91.3, 1.66	<0.001	0.05	<0.001	4.81b	1.45a	1.21a	<0.001	57.6, 0.78	<0.001	0.06
6	Aroma liking	2.71a	2.90ab	3.02ab	3.24b	0.06	54.3, 0.90	0.34	0.58	0.27	2.88c	1.85b	1.00a	<0.001	81.2, 1.10	<0.001	<0.001
	Flavour liking	2.67a	3.83b	3.95b	3.69b	<0.001	90.2, 1.14	<0.001	0.67	0.31	3.64c	2.10b	1.05a	<0.001	79.3, 0.93	<0.001	<0.001
	Acceptability	2.55a	3.57c	3.24bc	2.88ab	<0.001	67.4, 1.30	<0.001	0.29	0.23	3.05b	1.36a	1.07a	<0.001	64.3, 1.31	<0.001	0.007

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 8.13
Least squares means of the hedonic ratings and effects of treatment (trt) group, time and (trt x time) of a mix of longissimus muscle and subcutaneous fat in the loin region; for the three storage times across the 2 groups (A, B) where A consisted of 4 treatment groups (AT, PO, POS, PTS); and B consisted of 3 treatment groups (POS, PFSe and PFSl) on a scale from 1 to 9 with higher values indicating a stronger liking, as assessed by a Singapore consumer panel. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment group are given in Table 8.12

Sensory terms[1]	Group	Month			Effects (p-value)			R^2 , RSD
		0	3	6	Trt	Time	Trt x Time	
Aroma liking	A	5.52c	3.98b	2.97a	<0.001	<0.001	<0.001	56.9, 1.18
Flavour liking		5.23c	4.23b	3.54a	<0.001	<0.001	<0.001	60.7, 1.16
Acceptability		5.34c	4.10b	3.06a	<0.001	<0.001	<0.001	54.8, 1.43
Aroma liking	B	3.16c	2.50b	1.91a	<0.001	<0.001	<0.001	82.0, 0.77
Flavour liking		3.60c	2.85b	2.26a	<0.001	<0.001	<0.001	76.5, 1.04
Acceptability		3.37c	2.49b	1.82a	<0.001	<0.001	<0.001	83.1, 0.92

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 8.14

Percentage detection of off-flavour attributes of a mix of longissimus muscle and subcutaneous fat in the loin region by the Singapore consumer panel following 0, 3 or 6 months of storage at -18°C

Month	Off-flavour attribute detected	Group A				Group B		
		Treatment (Trt) groups[1]				Treatment (Trt) groups[1]		
		AT	PO	POS	PTS	POS	PFSe	PFSl
0	Rancid	0	0	0	0	0	23.8	33.3
	Mutton	100	100	100	100	100	100	100
	Fishy	0	0	0	0	0	100	100
	Metallic	9.5	14.3	7.1	9.5	9.5	16.7	0
	Acidic	11.9	7.1	7.1	7.1	7.1	33.3	33.3
	Bitter	19	16.7	21.4	11.9	19.0	16.7	21.4
3	Rancid	4.8	14.2	0	4.8	2.3	69.0	71.4
	Mutton	100	100	100	100	100	100	100
	Fishy	0	0	0	0	0	100	100
	Metallic	7.1	11.9	4.8	7.1	7.1	7.1	0
	Acidic	23.8	26.2	26.2	19.0	26.2	38.1	35.7
	Bitter	16.7	14.3	19.0	11.9	9.5	14.3	21.4
6	Rancid	4.8	19.0	4.8	4.8	7.1	71.4	73.8
	Mutton	100	100	100	100	100	100	100
	Fishy	0	0	0	0	0	100	100
	Metallic	2.4	9.5	2.4	4.8	4.8	11.9	0
	Acidic	26.2	28.6	33.3	19.0	33.3	40.5	42.9
	Bitter	11.9	9.5	14.3	9.5	9.5	16.7	21.4

[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 8.15

Least squares means of intensity scores of off-flavour attributes of a mix of longissimus muscle and subcutaneous fat in the loin region by the Singapore consumer panel based on a scale from 0 to 5 with 0, being not detected, 1 being low intensity and 5 being high intensity, if attribute was detected. Means for the 3 storage times are given in Table 8.16

Month	Attribute Detected[1]	Group A: Treatment (Trt) groups[2]				Trt effects (p-value)	Contrast (p-value)			Group B: Treatment (Trt) groups[2]			Trt effects (p-value)	Contrast (p-value)	
		AT	PO	POS	PTS		AT vs. PO	PO vs.POS	POS vs. PTS	POS	PFSe	PFSI		POS vs.[PFSe + PFSl]	PFSe vs. PFSl
0	Rancid	0	0	0	0	-	-	-	-	0a	0.60b	1.26c	<0.001	<0.001	0.05
	Mutton	4.12b	3.07a	3.00a	4.24b	<0.001	<0.001	0.37	<0.001	3.05b	1.79a	1.73a	<0.001	<0.001	0.67
	Fishy	0	0	0	0	-				0a	3.00b	3.30c	<0.001	<0.001	0.014
	Metallic	0.07	0.31	0.12	0.14	0.21	0.08	0.20	0.81	0.19ab	0.36b	0.10a	0.07	0.83	0.014
	Acidic	0.12	0.12	0.12	0.10	0.99	0.99	0.90	0.79	0.07a	0.52b	0.52b	0.002	0.001	0.99
	Bitter	0.24	0.24	0.26	0.12	0.56	0.97	0.85	0.15	0.14	0.24	0.24	0.62	0.33	0.99
3	Rancid	0.10	0.33	0.10	0.12	0.18	0.18	0.09	0.70	0.10a	2.10b	2.36b	<0.001	<0.001	0.45
	Mutton	2.21	2.45	2.52	2.38	0.28	0.16	0.63	0.38	2.50b	1.67a	1.69a	<0.001	<0.001	0.86
	Fishy	0	0	0	0	-	-	-	-	0a	3.24b	3.64c	<0.001	<0.001	0.004
	Metallic	0.02	0.14	0.07	0.07	0.47	0.12	0.43	0.99	0.17	0.10	0	0.13	0.10	0.10
	Acidic	0.55	0.67	0.79	0.45	0.52	0.61	0.65	0.17	0.79	0.93	0.95	0.80	0.51	0.93
	Bitter	0.26	0.21	0.29	0.14	0.67	0.72	0.59	0.23	0.14	0.24	0.33	0.35	0.21	0.51
6	Rancid	0.10	0.60	0.17	0.14	0.03	0.02	0.06	0.88	0.29a	3.29b	3.52b	<0.001	<0.001	0.62
	Mutton	2.76	2.62	2.62	2.83	0.52	0.44	0.99	0.19	2.74b	1.62a	1.67a	<0.001	<0.001	0.72
	Fishy	0	0	0	0	-	-	-	-	0a	4.19b	4.60c	<0.001	<0.001	0.018
	Metallic	0.10	0.17	0.10	0.07	0.51	0.27	0.30	0.99	0.15	0.12	0	0.11	0.19	0.021
	Acidic	0.95ab	0.81a	1.24b	0.60ab	0.30	0.67	0.24	0.07	1.10	1.31	1.36	0.74	0.45	0.90
	Bitter	0.24	0.24	0.26	0.12	0.56	0.99	0.85	0.15	0.14	0.24	0.24	0.62	0.33	-

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet containing animal and plant products; PO= diet containing plant products only; POS=diet containing plant feed products and Sanovite™; PTS=diet containing plant products with tallow; PFSe=diet containing plant products and fish oil (early stage of grower period); PFSl=diet containing plant products and fish oil (late stage of grower period).

Table 8.16

Least squares means of the intensity score rating and effects of treatment (trt) group, time and (trt x time) of a mix of longissimus muscle and subcutaneous fat in the loin region; for the 3 storage times across the 2 groups (A, B) where A consisted of 4 treatment groups (AT, PO, POS, PTS); and B consisted of 3 treatment groups (POS, PFSe and PFSl) on a scale from 0 to 10 with higher values indicating a stronger liking, as assessed by a Singapore consumer panel. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment group are given in Table 8.15.

Group	Sensory terms[1]	Month			Effects (p-value)			R^2 , RSD
		0	3	6	Trt	Time	Trt x Time	
A	Rancid	0a	0.15b	0.25b	0.003	0.001	0.14	37.3, 0.60
	Mutton	3.61c	2.39a	2.71b	<0.001	<0.001	<0.001	46.2, 0.68
	Fishy	0	0	0	-	-	-	-
	Metallic	0.16	0.08	0.10	0.03	0.17	0.95	10.4, 0.43
	Acidic	0.11a	0.61b	0.90c	0.14	<0.001	0.70	9.5, 1.13
	Bitter	0.21	0.23	0.21	0.15	0.97	0.99	11.2, 0.53
B	Rancid	0.62a	1.52b	2.37c	<0.001	<0.001	<0.001	42.8, 1.51
	Mutton	2.19b	1.95a	2.01ab	<0.001	0.002	0.044	46.7, 0.56
	Fishy	2.00a	2.29a	2.93b	<0.001	<0.001	<0.001	93.7, 0.46
	Metallic	0.21b	0.09a	0.09a	0.005	0.044	0.41	54.0, 0.43
	Acidic	0.37a	0.89b	1.25c	0.12	<0.001	0.93	57.4, 1.25
	Bitter	0.21	0.24	0.21	0.17	0.87	0.97	12.0, 0.54

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

8.5 Conclusion

In this study, it was found that a more unsaturated fatty acid profile may limit the shelf life of meat because PUFA are more prone to oxidation. This has been identified as a major problem in previous work using α-linolenic-rich plant materials or fish oils to enrich pork with n-3 PUFA. Those dietary treatments containing fish oil had lower oxidative stability and this was manifested as off-odours and flavours resulting from lipid oxidation. The significance of fish oil supplementation had an impact on the concentration of omega-3 fatty acids, with high levels DHA levels compared to those without fish oil. Fishy and acidic sensory scores were higher; with lower consumer acceptability for the pork when fish oil was introduced in a later phase even though no fish oil was fed during the four weeks prior to slaughter. On a positive note, feeding fish oil either earlier or later caused the n-6/n-3 ratio to conform to the recommendation of being <4.

This study has also confirmed that dietary plant materials and fish oil are effective means of altering the fatty acid composition of pork in order to provide consumers with a healthy product. Supplementing with higher levels of antioxidants like selenium and vitamin E in the feed, limiting fish oil amounts and strategising its phases of feeding need to be further investigated in order to produce pork with minimum off-flavours and off-odours. In addition, excluding animal products from the feed reduced undesirable mutton-like off-flavour. It should be kept in mind that pork quality is dependent on other factors besides the PUFA levels in the feed (e.g., duration of supplementation, dietary antioxidant levels, storage conditions, and processing conditions, as well as muscle and animal differences).

Chapter 9

Development of a low-fat, low-salt pork ball product for the Singapore market

Chapter 9

9.1 Introduction

Processed meat products often contain higher amounts of saturated fatty acids and sodium than recommended for a healthy diet. The reduction of fat and salt in foods is required because of their association with obesity, heart disease and high blood pressure issues that affect many people (Dahl, 1972; Law, Frost, & Wald, 1991; Davies, Bowey, Adlercreutz, Rowland, & Rumbsy, 1999).). Direct reduction of fats and sodium can lead to technological difficulties, however, because fat and salt have fundamental technological, microbiological and sensory functions to perform in meat products and there are many measures that have to be taken to offset any reduction in the content of these substances in a product.

Fat replacers and salt substitutes are widely used as ingredients in the formulation of low-fat and low-salt meat products. Fat imparts a wide range of characteristics to foods, including a desirable appearance, and improvements in flavour, aroma, texture and mouthfeel. Most of the currently available fat replacers can be divided by composition into those that are either protein-based, carbohydrate-based or fat-based. The protein- and carbohydrate-based fat replacers are known as fat mimetics since they cannot fully replace the functionality of fat in foods, while fat substitutes can replace fat in food on a one-to-one basis and can provide a range of sensory and functional qualities to low-fat products.

Salt reduction in meat products has adverse effects on water and fat binding, thereby impairing overall texture and increasing cooking loss as well as changing sensory quality, especially taste (Ruusunen, Vainionpa, Lyly, Lähteenmä, Niemistö, Ahvenainen, Puolanne, 2005). Salt substitutes (the term “salt” is used here to mean sodium chloride) like potassium chloride and tetrapotassium pyrophosphate have been used to replace sodium chloride for low salt meat products, but one of the biggest challenges is cost, as sodium chloride is one of the cheapest food ingredients available. Also, consumers have grown accustomed to salt through processed foods so it may be difficult to reduce without a decrease in acceptability. Pappa, Bloukas & Arvanitoyannis (2000) reported that low-fat frankfurters with a salt

content of 1.8-2.1% had a better acceptability by a consumer panel compared to those made with 1.3% of salt. In another meat product like ham, the optimal level of salt used in curing from the standpoint of consumer acceptability appears to be around 2.5% (Pearson et al., 1962).

The first option as a substitute for salt is potassium chloride, and a second approach is to use flavour enhancers, such as yeast extracts, nucleotides, and hydrolysed vegetable protein. A third alternative is to optimise the physical form of the salt to increase its taste bio-availability so that less salt is needed.

Currently, one of the most popular processed meat products in Singapore is the pork ball. There are about ten different brands of pork ball available for sale in Singapore, where they are popular ingredients for steamboats in which ingredients are cooked in a simmering pot of broth. This is a popular way of dining among the Chinese population in Singapore as well as elsewhere in East Asia. Other common steamboat ingredients include meat, leafy vegetables, mushrooms, tofu, noodles or seafood. As of 2009 (EDB, 2010) Chinese constituted 74.2% of the Singapore population, which means they are a huge target group for pork balls.

There are, however, no low-fat and low-salt pork balls available commercially in Singapore (as at mid 2010). The list of some existing commercial pork ball products and their ingredients can be found in Appendix 9.1. In most cases the sodium content is about 860 mg per 100 g, which is almost double that given in the Singapore Health Promotion Board healthy guidelines. Health Promotion Board (HPB) guidelines, state that in order for a product to be labelled as low fat and low salt, the amount of fat and sodium cannot exceed 10 g/100g and 450 mg/100g, respectively (HPB, 2010). It has been reported (SNDA, 2006) that 2 in 5 Singaporeans (42.7%) exceeded the recommended fat intake, and that the average Singaporean consumes 3527 mg of sodium a day when an average healthy adult requires no more than 2000 mg per day (Singapore Heart Foundation, 2009). Therefore, the availability of a low-fat and low-salt pork ball product would be a useful way of promoting low fat and sodium intake by Singaporeans.

The three experiments reported in this chapter and their objectives are as follows:

Experiment 1: The objective was to use consumer and trained sensory panellists to assess the overall acceptability and sensory parameters of pork balls varying in the content of fat and salt, and also in the levels of carrageenan (as a fat substitute) and potassium chloride (as a salt substitute).

Experiment 2: The objective was to refine the chosen recipe from Experiment 1 by evaluating two mixing methods (A and B) and two phosphate salt combinations when making pork balls.

Experiment 3: Using a low-fat, low-salt recipe from Experiment 1 and the refined formulation from Experiment 2, the objective was to use consumer and trained panels to investigate the sensory quality of pork balls made with pork from pigs fed diets with or without animal products and containing varying amounts of garlic essential oil (GEO).

9.2 Materials and Methods

9.2.1 Experiment 1: Development of low-fat and low-salt pork balls (16 formulations)

9.2.1.1 Pork Used and Experimental Design

Chilled Australian pork loins with fat were obtained from a supermarket (Fairprice). Australian pork was used as New Zealand pork was not readily available at the time of the study. In addition, Australian pork closely resembles New Zealand pork in terms of flavour and was widely available in the supermarkets in Singapore.

The lean and fat, after being separated and minced separately through a 6 mm plate (Sammic Mincer, USA), were stored at 4°C until used to make pork balls within 24 hours.

The formulations involved the use of carrageenan (CP Kelco, Singapore) as the fat replacer and potassium chloride as the sodium chloride substitute. In order to fulfil the low fat and low sodium guidelines provided by Singapore Health Promotion Board (HPB), the requirement was that the amount of fat and sodium not exceed 10 g of fat and 450 mg of sodium per 100 g of product, respectively.

The experimental design is shown in Table 9.1. Although this was run as a single experiment, the results were analysed in three groups to answer specific questions as follows:

1. Groups 1 to 4 were analysed as a 2 x 2 factorial to assess the effects of fat level (12.5 vs 10%) and NaCl level (1.0 vs. 0.6%) on the characteristics measured in the absence of any fat or NaCl substitutes.
2. Groups 4, 5 and 6 were analysed together to assess the effects of three levels of carrageenan (0, 1.0, 1.5%) on characteristics measured at constant levels of fat and NaCl, and without any KCl.
3. Groups 5 to 16 were analysed as a 2 x 6 factorial to assess the effects of 2 carrageenan levels and 6 KCl levels (0, 0.1, 0.2, 0.3, 0.4 and 0.5%) on the characteristics measured.

Table 9.1
The experimental design showing the 16 formulations of pork balls used in Experiment 1

Formulation	Fat (%)	NaCl (%)	Carrageenan (%)	KCL(%)	NaCl : KCL
1.	12.5	1.0	0	0	100 : 0
2.	10.0	1.0	0	0	100 : 0
3.	12.5	0.6	0	0	100 : 0
4.	10.0	0.6	0	0	100 : 0
5.	10.0	0.6	1.0	0	100 : 0
6.	10.0	0.6	1.5	0	100 : 0
7.	10.0	0.6	1.0	0.1	86 : 14
8.	10.0	0.6	1.5	0.1	86 : 14
9.	10.0	0.6	1.0	0.2	75 : 25
10.	10.0	0.6	1.5	0.2	75 : 25
11.	10.0	0.6	1.0	0.3	67 : 33
12.	10.0	0.6	1.5	0.3	67 : 33
13.	10.0	0.6	1.0	0.4	60 : 40
14.	10.0	0.6	1.5	0.4	60 : 40
15.	10.0	0.6	1.0	0.5	55 : 45
16.	10.0	0.6	1.5	0.5	55 : 45

9.2.1.2 Processing of Pork Balls

Based on the formulations given in detail in Table 9.2, 100 g of pork balls were prepared for each formulation using Method A shown in Fig. 9.1. In Experiments 1 and 3, 12 pork balls per formulation were used in the evaluation of physical characteristics, while 6 were used for the same purpose in Experiment 2. For sensory evaluation in Experiment 1, 12 pork balls per formulation were used for assessment by the 48 untrained panellists, while 8 pork balls per formulation were used for assessment by the 8-membered trained panel. In Experiment 3, 8 pork balls per formulation were used for assessment by the 8-membered trained panel, while 12 pork balls per formulation were used for assessment by the 12-membered untrained panel. Each trained panellist in the sensory panels in Experiments 1 and 3 evaluated one pork ball per formulation. The total sodium content of the formulations is also given in Table 9.2, and a complete listing of sodium contributions from each ingredient in the formulations is given in Appendix 9.2.

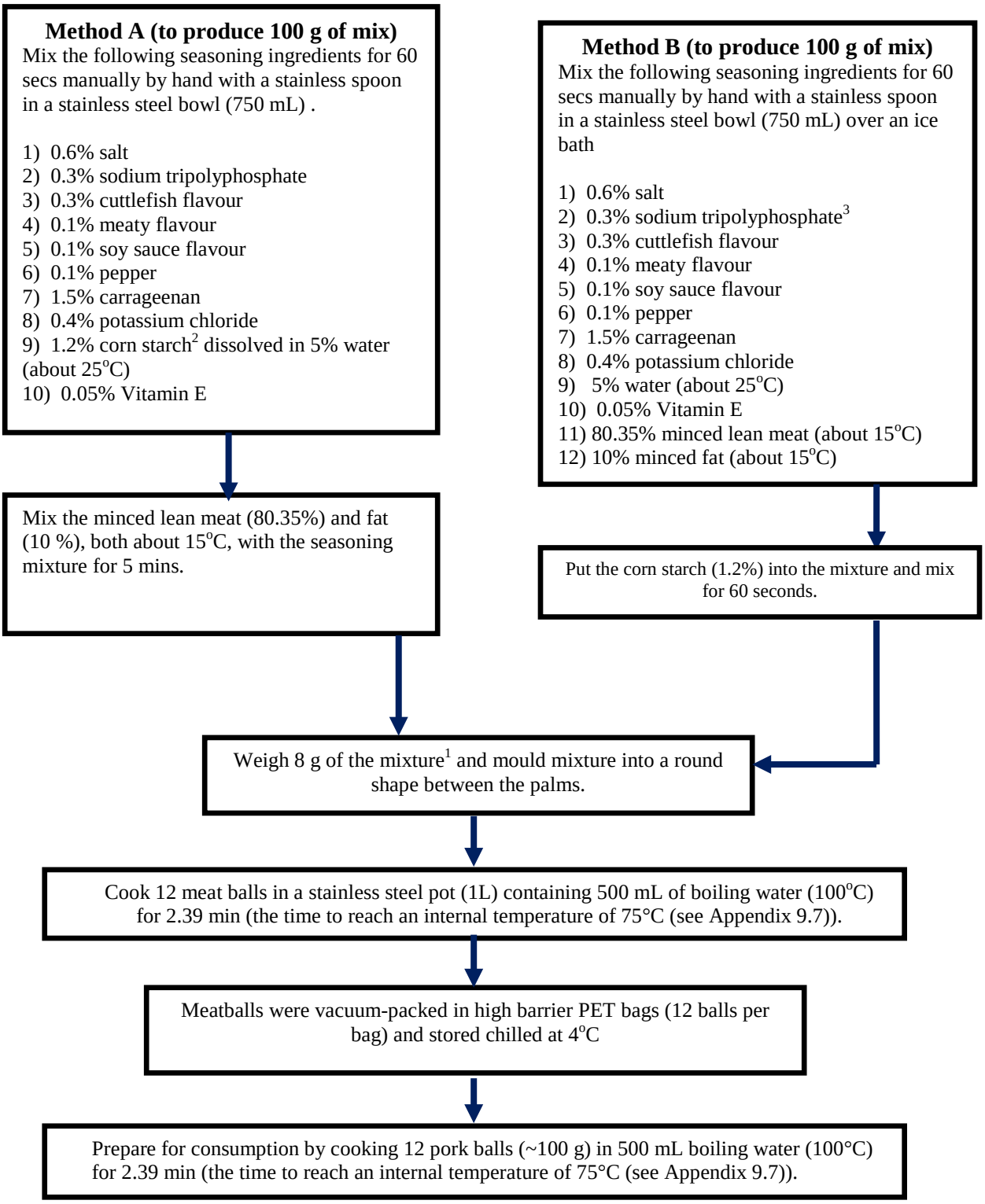


Fig. 9.1
A flowchart showing the steps followed for producing pork balls using Method A (Experiments 1 and 2) and Method B (Experiments 2 and 3).

Table 9.2
The composition of the 16 pork meatball formulations in Experiment 1 and their sodium content[1]

Ingredients	Formulation					
(weight%)	1	2	3	4	5	6
Pork lean	79.35	81.85	79.75	82.25	81.25	80.75
Pork back fat	12.50	10.00	12.50	10.00	10.00	10.00
Water	5.00	5.00	5.00	5.00	5.00	5.00
Corn Starch	1.20	1.20	1.20	1.20	1.20	1.20
Salt	1.00	1.00	0.60	0.60	0.60	0.60
Sodium tripolyphosphate	0.30	0.30	0.30	0.30	0.30	0.30
Cuttlefish flavour	0.30	0.30	0.30	0.30	0.30	0.30
Seasoning (Soy sauce)	0.20	0.20	0.20	0.20	0.20	0.20
Pepper	0.10	0.10	0.10	0.10	0.10	0.10
Carrageenan	0	0	0	0	1.00	1.50
Vitamin E	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Sodium (mg/100g)	603.06	604.64	445.98	447.56	446.93	446.61

Ingredients	Formulation					
(weight%)	7	8	9	10	11	12
Pork lean	81.15	80.65	81.05	80.55	80.95	80.45
Pork back fat	10.00	10.00	10.00	10.00	10.00	10.00
Water	5.00	5.00	5.00	5.00	5.00	5.00
Corn Starch	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.60	0.60	0.60	0.60	0.60	0.60
Sodium tripolyphosphate	0.30	0.30	0.30	0.30	0.30	0.30
Cuttlefish flavour	0.30	0.30	0.30	0.30	0.30	0.30
Seasoning (Soy sauce)	0.20	0.20	0.20	0.20	0.20	0.20
Pepper	0.10	0.10	0.10	0.10	0.10	0.10
Carrageenan	1.00	1.50	1.00	1.50	1.00	1.50
Potassium chloride	0.10	0.10	0.20	0.20	0.30	0.30
Vitamin E	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Sodium (mg/100g)	446.48	446.86	446.54	449.89	450.26	449.94

Ingredients	Formulation			
(weight%)	13	14	15	16
Pork	80.85	80.35	80.75	80.25
Fat	10.00	10.00	10.00	10.00
Water	5.00	5.00	5.00	5.00
Corn Starch	1.20	1.20	1.20	1.20
Salt	0.60	0.60	0.60	0.60
Sodium tripolyphosphate	0.30	0.30	0.30	0.30
Cuttlefish flavour	0.30	0.30	0.30	0.30
Seasoning (Soy sauce)	0.20	0.20	0.20	0.20
Pepper	0.10	0.10	0.10	0.10
Carrageenan	1.00	1.50	1.00	1.50
Potassium chloride	0.40	0.40	0.50	0.50
Vitamin E	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00
Sodium (mg/100g)	446.79	446.48	446.73	446.42

[1] Note that sodium chloride contains 39.33% sodium while sodium tripolyphosphate contains 31.24% sodium. Pork is taken to contain 63 mg of sodium per 100 g.

9.2.1.3 Sensory Evaluation for Experiments 1 and 3

A balanced incomplete blocked design (BIBD) was used for the sensory evaluation of pork balls by the untrained consumer panellists. There were 48 consumer panellists with samples from the 16 treatment groups evaluated by a separate set of 16 panellists during each of 3 tasting sessions over 2 consecutive days, with 4 samples assessed per panellist in each session. The panellists were students and staff from the School of Chemical and Life Sciences, Singapore Polytechnic. Panellists were instructed to rank the attributes of each sample from 1 to 4, where 1 was “Least acceptable” and 4 the “Most acceptable”. The sensory evaluation questionnaire for the ranking test is given in Appendix 9.4.

An 8-member sensory panel was trained according to AMSA (1978, 1995) procedures. All had participated in sensory evaluation of pork or pork products for 1 to 2 years. Panellists were male (n=3) and female (n=5) and ranged in age from 21 – 30 years. The trained panel testing was conducted at the Food Quality and Sensory Evaluation Laboratory of the Singapore Polytechnic. Quantitative descriptive analysis (QDA) was used to select panellists based on their sensory acuity, liking for pork, and their commitment to taste pork for 8 sessions over a period of 2 days. Triangle tests using different concentrations of sucrose, sodium chloride, citric acid and caffeine were used to perform the screening and ultimately the 8 panellists selected participated in 5 discrimination trials over a period of 2 days. Under the direction of the panel leader, the panellists developed a sensory language to describe the sensory properties of the products. They grouped the attributes by modality order and then within a modality developed definitions for each attribute. There were three 1.5 hour training sessions. During training, panellists became more confident with scoring the sensory attributes of pork by having samples presented at least 2 times per session to allow them to re-familiarise themselves with the typical flavour associated with each attribute. Pork balls were evaluated for the attributes described in Table 9.3 using a 150 mm line- scale. A sample questionnaire for the trained panel is shown in Appendix 9.5.

Table 9.3
Definitions of the sensory attributes of pork developed by the trained panellists in Experiment 1 during training, together with the anchor points at each end of the scale

Sensory attributes	Interpretation	Anchor points
Colour attributes		
Colour	Degree of brownness[1]	Yellow / Brown
Colour saturation	Degree of darkness/lightness[1]	Light/Dark
Aroma attributes		
Meaty aroma	Aromatic associated with cooked meat[1]	None / Strong
Brothy aroma	Aromatic associated with pork cooked in water[1]	None / Strong
Cuttlefish aroma	Aromatic associated with dry preserved cuttlefish[1]	None / Strong
Metallic aroma	Aromatic associated with presence of iron ions (blood)[1]	None / Strong
Acidic aroma	Aromatic associated with presence of citric acid[2]	None / Strong
Mutton aroma	Aromatic associated with presence of mutton[2]	None / Strong
Stale odour	Atypical aroma generally associated with deterioration of quality[2]	None / Strong
Garlic aroma[3]	Aromatic associated with garlic[1]	None / Strong
Taste/flavour attributes		
Meaty flavour	Sensation associated with cooked meat[1]	None / Strong
Cuttlefish flavour	Sensation associated with dry preserved cuttlefish[1]	None / Strong
Metallic flavour	Sensation associated with the presence of iron ions (blood)[2]	None / Strong
Acidic flavour	Taste on the tongue associated with citric acid[2]	None / Strong
Mutton flavour	Sensation associated with cooked mutton[1]	None / Strong
Stale flavour	Atypical taste generally associated with deterioration of quality[2]	None / Strong
Salty taste	Taste on the tongue associated with sodium chloride[2]	None / Strong
Bitter taste	Taste on the tongue associated with caffeine[2]	None / Strong
Aftertaste	Sensation of lingering taste on the tongue after ingestion[2]	None / Strong
Garlic flavour[3]	Sensation associated with garlic[1]	None / Strong
Texture attributes		
Cohesiveness	Degree of resistance to breakdown[2]	None / Strong
Chewiness	Amount of work to chew the sample for swallowing[1]	None / Strong
Hardness	The force required to bite through using molars[1]	None / Strong
Juiciness	Sensation of presence of moisture[2]	None / Strong

[1]Definitions as developed by the panellists
[2]Meilgarrd and others (1999)
[3]Definitions as developed by panellists for use in pork balls from pigs fed with garlic essential oil

9.2.1.4 Physical measurements

(a) pH:

For pH determination, 10 g of the sample (after 1st cooking) and 90 mL of distilled water were homogenized in a blender (Model D-500, Wiggenhauser, Germany,) for 60 s. The pH of the mixture was measured using a pH meter, (pH 211, Hanna, England) standardized at pH 4.0 and 7.0 (Pexara *et al.* 2002). The pH was measured directly after homogenization and was read after stabilization. Two readings from different samples of each formulation were taken.

(b) L*, a*and b* colour measurement:

The colour of the pork ball samples (after 1st cooking) was measured on three surfaces within the sample that had been exposed to the atmosphere for approximately 15 min using a Minolta Chromameter (Model CR-300, Konica Minolta, Tokyo, Japan) reflectance spectrophotometer to provide lightness (L*), redness (a*), and yellowness (b*) values. The spectrophotometer was calibrated with a white porcelain plate (Y=94.5, x=0.3141, y=0.3207). The light source was from a pulsed xenon lamp with a measuring diameter of 50 mm.

(c) Texture measurement:

After the 1st cooking and cooling to room temperature ($25^{o}C \pm 3^{o}C$), three whole pork balls per group were subjected to texture profile analysis using the Texture Analyzer (Model TA XT plus, Stable Micros System, Surrey, England). The pork ball was placed on the platform of the Texture Analyzer. A cylinder probe (P/5) of 6 mm diameter was attached to a 5 kg load cell and the sample was compressed to 80% of its original height at a cross head speed of 2.0 mm/s. Triplicate measures were performed on three samples of

the same formulation. Hardness (N) which is the peak force of the product at the first loading cycle in texture profile analysis was obtained (Ranganna, 2008).

(d) Cooking yield:

Cooking yield after the first cooking was calculated as the weight after the first cooking expressed as a percentage of the weight before cooking. Cooking yield after the second cooking was calculated as the weight after the second cooking expressed as a percentage of weight after the first cooking. A total cooking yield can be calculated as the product of the two cooking yields given in the tables. For example, with a cooking yield of 80% for each cooking, the overall cooking yield will be 64%.

(e) Moisture analysis and percent retention:

Moisture of the sample after the 1st cooking was analysed by a halogen moisture analyser (model HR73, Mettler Toledo, Greifensee, Switzerland). The pork ball sample was ground using a mortar and pestle before it was placed (about 3 g) onto an aluminium pan of the analyser. Duplicate readings from different samples of the same formulation were measured.

Moisture retention after the first cooking was calculated as the weight of moisture after the first cooking expressed as a percentage of the weight of moisture present before cooking. Moisture retention after the second cooking was calculated as the weight of moisture after the second cooking expressed as a percentage of the weight of moisture present after the first cooking.

(f) Fat analysis:

The Soxhlet system (Model: Soxtec 2050, FOSS, Sweden) was used and it follows the approved method AOAC 991.36 (meat) in this analysis. Samples (after the 1st cooking) were freeze-dried overnight, homogenised, and 2 g was weighed into thimbles. The Soxhlet system was then turned on and the temperature was set to 100^{o}C. Six thimbles containing

samples were inserted simultaneously into the extraction unit, 80 ml of petroleum ether (boiling point 40 to 60°C) was added into pre-weighed extraction cups and they were fixed with the holder into the extraction unit and locked firmly. The sample was first extracted for 10 minutes in the boiling position and thereafter, the thimbles were raised to the rinsing position and rinsed for 30 minutes. After the recovery valves were closed, the solvent was evaporated off and air pressure was used to remove the last traces of petroleum ether. The extraction cups were removed and dried for 30 minutes at 105^{o}C. After cooling in a dessicator for 10 minutes, they were weighed.

(g) Elemental analysis:

Minced samples (2.0 g ±0.01 g) were weighed into a teflon vessel. Then 7 ml of nitric acid (65%) was added drop by drop followed by 1 ml of hydrogen peroxide (30%). The vessel was gently swirled to homogenise the sample with the acids. The vessel was introduced into the rotor of the microwave digestion system (model: Ethos 1600, Milestone, USA). The microwave program was set to reach a temperature of 200^{o}C in 10 mins and then be maintained at that temperature for another 10 mins. The microwave power was about 1000 watts. When the microwave program was completed, the digestor was cooled until the solution inside the teflon vessel reached room temperature. The vessel was finally opened and the solution was transferred into a marked volumetric flask. The samples were topped up with ultrapure water to 100 mL. An ICPE spectrometer (Shimadzu model ICPE-9000) was used for the analysis using Argon as a cooling plasma, and carrier gas at flow rates of 14.0, 1.2, 0.7 L/min respectively. The sample was introduced via a coaxial nebulizer.

9.2.1.5 Refining the process in pork ball making (Experiment 2)

The objectives of this experiment were (1) to compare 2 mixing methods to improve the binding of ingredients in the pork ball and (2) to investigate the effects of tetrapotassium pyrophosphate and sodium tripolyphosphate on retention of elements in the

pork balls through minimisation of cooking loss. Cooking was carried out with either the pork balls directly in the water (F1 and F2) or with the pork balls in Thermo Bags that were immersed in the water (F3 and F4). Method A involved the mixing of all food ingredients with the lean meat and fat, while Method B required all ingredients except corn starch to be mixed with the lean meat and fat and then for starch to be added after mixing (Fig. 9.1).

Elemental analysis, cooking yield, and moisture retention before and after the first and second cookings were measured as described for Experiment 1. The ingredient composition and total sodium content of the two formulations are given in Table 9.4., and the sodium contribution of all ingredients in the formulations is given in Appendix 9.3.

Table 9.4
The ingredient level and total sodium content of three pork ball formulations evaluated in Experiment 2

Ingredients	Original formulation (%)	Improved formulation F1/F3[1] (%)	Improved formulation F2/F4[1] (%)
Pork lean	80.35	80.35	80.35
Pork back fat	10.00	10.00	10.00
Water	5.00	4.80	4.80
Corn Starch	1.20	1.20	1.20
Salt	0.60	0.60	0.60
Sodium tripolyphosphate	0.30	-	0.20
Tetrapotassium pyrophosphate	-	0.50	0.30
Cuttlefish flavour	0.30	0.30	0.30
Seasoning	0.20	0.20	0.20
Pepper	0.10	0.10	0.10
Carrageenan	1.50	1.50	1.50
Potassium chloride	0.40	0.40	0.40
Vitamin E	0.05	0.05	0.05
Total (mg/100g)	100.00	100.00	100.00
Sodium (mg/100g)	446.48	352.76	415.16

[1] F1 contained 0.5% tetrapotassium pyrophosphate; cooked in boiling water directly
F2 contained 0.2% sodium tripolyphoshate and 0.3% tetrapotassium pyrophosphate; cooked in boiling water directly
F3 contained 0.5% tetrapotassium pyrophosphate; cooked in a thermo-bag which was in boiling water
F4 contained 0.2% sodium tripolyphoshate and 0.3% tetrapotassium pyrophosphate; cooked in a thermo-bag which was in boiling water

The original formulation used 0.3% sodium tripolyphosphate, but because most panellists felt that the pork balls were not as cohesive as commercial ones, the total phosphate used was increased to 0.5% in Experiment 2. Thus F1/F3 contained 0.5% tetrapotassium pyrophosphate while F2/F4 contained 0.2% sodium tripolyphoshate and 0.3% tetrapotassium pyrophosphate. Sodium tripolyphosphate at 0.5% was not considered as this exceeded the 450 mg/100g limit in sodium level. 0.3% sodium tripolyphosphate and 0.2% tetrapotassium pyrophosphate was also not considered as the final sodium content was too close to 450 mg/100g. The combination of 0.2% sodium tripolyphosphate with 0.3% tetrapotassium pyrophosphate was used as one of the treatments as the final sodium contribution was further reduced. The recommended dosage level for tetrapotassium pyrophosphate is between 0.3 – 0.5%. Initially 3 batches of 5 pork balls per formulation were produced for evaluating mixing Methods A and B (Fig. 9.1). Then similar batch sizes were produced using Method B for the evaluation of the 4 treatments F1 to F4 outlined in Table 9.4.

9.2.2 Pig feeding experiment (Experiment 3)

The experiment was conducted in accordance with the ‘‘Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching” (Massey, 2008).

The objective of the experiment was to investigate the effects of different levels of dietary garlic essential oil (GEO) in the diet of pigs on the chemical and sensory quality of pork balls made from the pork and back fat from those pigs. The diets, animals, and the experimental design were described in Chapter 6, Section 6.2.2.

9.2.2.1 Processing of pork balls using New Zealand pork in Experiment 3

The method used to make pork balls for Experiment 3 was the best formulation and mixing method as determined in Experiments 1 and 2. This involved the formulation containing 10% fat, 1.5% carrageenan, 0.4% KCl, 0.6% NaCl, 0.2% sodium

tripolyphosphate, and 0.3% tetrapotassium pyrophosphate and mixing method B (Fig. 9.1). Pork balls, weighing 10 g, were made as this size was preferred to the 8 g pork balls used in Experiments 1 and 2. The questionnaire for sensory evaluation of preferred sizes of pork balls is given in Appendix 9.6. A preference test for the preferred size showed that, out of 42 panellists, 35 preferred 10 g to 8 g pork balls ($p < 0.05$).

9.2.2.2 Sensory evaluation for pork balls in Experiment 3

A trained panel consisting of 3 males and 5 females aged from 21 – 30 years was used in the sensory evaluation of pork balls in Experiment 3. The training procedure was as described in Section 9.2.1.3. The panellists evaluated 31 samples within 8 sessions spread over 2 days, with 15 to 16 samples evaluated per day, and 3 to 4 samples per session. There were breaks of at least 30 minutes between sessions. In the trained panel vocabulary there were 23 attributes of pork, each of which was assessed on a 150-mm unstructured scale which ranged from "None" to "Strong" (Table 9.5).

A separate 12-member untrained sensory panel tested the pork balls using a 150 mm line-scale for overall acceptability with the left end corresponding to the lowest acceptability and the right end to the highest. They evaluated 31 samples within 8 sessions (4 samples per session) over a period of 2 days.

9.2.3 Statistical analysis

9.2.3.1 Experiments 1 and 2

(a) <u>For physical measurements:</u>

ANOVA was used to measure the effects of batch, sodium, fats and their interactions as well as potassium chloride, carrageenan and their interaction. Orthogonal polynomial contrasts were used to evaluate the potassium chloride effect with linear,

quadratic and cubic components. The significance of differences between means of the physical characteristics was assessed using Fisher's Least Significant Different test.

(b) Sensory evaluation of pork balls (16 formulations)

Results of the untrained panel assessment of overall acceptability were analysed using a BIBD design. The Friedman-type statistic was used to rank the data (Durbin, 1951). The formula was:

$$T = [\frac{12}{p\lambda t(k+1)} \sum_{j=1}^{t} R_j^2] - [\frac{3(k+1)pr^2}{\lambda}] \qquad \text{[Equation 9.1]}$$

where:

T = Friedman-type statistic

t = total number of treatment groups (t= 16 for the current panel)

k = Number of samples evaluated by each panellist (k = 4 for the current panel)

r = Number of times a sample is evaluated within a replication (r = 4 for the current panel)

b = Number of blocks (with one block per panellist) to complete a single replication (b = 16 for the current panel)

p = Number of replications of the fundamental design (p = 3 for the current panel)

λ = Number of times pairs of treatment groups are evaluated within each replication (λ = 1 for the current panel)

R_j: Rank sum of the j'th sample

j = j'th treatment group within the total of t treatment groups.

If the results for equation 9.1 exceeded the upper-α critical value of a χ^2-statistic with 15 degree of freedom ($\chi^2_{15,\ 0.05} = 25.0$), then a multiple comparison procedure was performed using the nonparametric analog to Fisher's Latin Square Designs for rank sums from a BIBD (Durbin, 1951). Terms used in Equation 9.2 are the same as those used in Equation 9.1.

$$LSD_{rank} = z_{\frac{\alpha}{2}} \sqrt{p(k+1)(rk-r+\lambda)/6} \qquad \text{[Equation 9.2]}$$

where:

$z_{\frac{\alpha}{2}}$ = upper $\alpha/2$ critical value of a z distribution

For the data from the trained panel, scale marks from QDA questionnaires were converted to intensity scores from 0 to 100 for each descriptor. The significance of the differences between means of the sensory profiles was assessed using the Fisher's Least Significant Different test.

9.2.3.2 Sensory evaluation of pork balls from pigs fed GEO (Experiment 3)

The data from the 8 trained panellists in the quantitative descriptive analysis (QDA) and the 12 untrained panellists in the acceptability test were analysed using a statistical model where the animals were nested within treatments, and treatment and panellist effects were arranged in a factorial manner with samples from every animal being evaluated by every panellist. Diet-type, GEO concentration within diet- type, and the diet-type x GEO interaction were tested against the animal term while the animal and panellists effects were tested against the overall error term. Scores for each sensory attribute were analysed by ANOVA (Type I Sums of Squares) at 5% level of significance using a General Linear Model (GLM). The significance of differences between means was assessed using the Least Significant Difference test. Relationships between the amounts of GEO consumed by pigs and the flavour attributes of pork balls were assessed by linear and quadratic regression models. Relationships between sensory attributes based on animal means were evaluated using Pearson's linear correlation coefficients.

9.3 Results and Discussions

9.3.1 Pork ball formulation (Experiment 1)

9.3.1.1 Physical Characteristics for Experiment 1

Batch effects were very small for all physical characteristics tested.

(a) Hardness:

Results for hardness and other physical characteristics of the pork balls for Experiment 1 are shown in Tables 9.5, 9.6, and 9.7. Hardness was unaffected by a decrease in NaCl from 1% to 0.6%, by a decrease in fat content from 12.5% to 10.0% (Table 9.5), or by an increase in carrageenan from 0% to 1.5% (Table 9.6), although hardness was higher for 1.5% carragennan than 1.0% at some of the higher levels of KCl ($p = 0.027$) (Table 9.7). Results in Table 9.7 also show that hardness increased with increasing KCl levels in a linear manner ($p < 0.001$). There was also an interaction effect between carrageenan and potassium chloride ($p=0.035$). It was reported in a study on cooked hams that a 50% replacement with KCl gave superior bind (Frye, Hand, Calkins, & Mandigo, 1986). Conversely, in sectioned and formed hams, Collins (1997) stated that using a replacement of 30% KCl had no effect on tenderness compared to hams made with 100% salt. In fermented sausages, Gou, Guerrero, Gelabert, and Arnau (1996) found no significant alteration in texture of the products using KCL to replace NaCl.

(b) Colour parameters and pH:

Pork ball pH was not affected by levels of fat, NaCl, carrageenan or KCl level (Tables 9.5 – 9.7). Redness (a*) and yellowness (b*) were greater at the lower salt level ($p<0.001$), and b* was also higher at the lower fat level with a significant interaction effect (Table 9.5). There was a significant interaction between fat level and NaCl level for

lightness (L*, Table 9.5), with the higher fat level being more light at the higher salt level but less light at the lower salt level. The presence of carrageenan led to lower b* levels (Table 9.6), but no differences were shown between levels of 1 and 1.5% in the presence of KCl (Table 9.7). With KCl the higher carrageenan level was associated with lower a* levels (Table 9.7). With increasing KCl levels L*, a* and b* changed ($p<0.001$) but not in a linear manner (Table 9.7). There was a quadratic effect of KCl on b*.

The a* and b* values of the cooked products were inversely proportional to the salt content. When raw meat is cooked, so long as the temperature exceeds the denaturation temperature of myoglobin and haemoglobin, all three pigments, i.e. myoglobin, oxymyoglobin and metmyoglobin are converted to denatured metmyoglobin which is a greyish brown colour. The extra protein solubilised at the higher ionic strengths, plus the cell membrane breakdown brought about by the higher ionic strength could lead to more myoglobin on the surfaces of meat particles. This may lead to a decrease in L*, a* and b* with increasing ionic strength. Pietrasik & Li-Chan (2002) reported results similar to the current study in their study with pork batter with salt content reduced from 2.0 to 0.4%. Reduction of salt content from 2.5 to 1.5% in low-fat frankfurters was reported by Hand, Hollingworth, Calkins, & Mandigo, (1987) to produce an increase in L*, a decrease in b* and no change in a* values, while Jimenez-Colmenero, Fernandez, Carballo & Fernandez-Martin (1998) reported no relationship between ionic strength and yellowness of lowfat pork batters. Interaction effects between carrageenan and potassium chloride were observed for a* ($p=0.017$) and b* ($p=0.011$), but the nature of these effects is not clear-cut (Table 9.7).

(c) Cooking yield and moisture retention:

Results from Table 9.6 show that moisture retention was unaffected by carrageenan, but that it was associated with a higher cooking yield for the second cooking. Candogan and Kolsarici (2003) also noted that low-fat sausages, without carrageenan had a lower cooking yield compared to those using carageenan during processing, and an increased

cooking yield was also observed in studies on frankfurters by Cierach, Modzelewska-Kapituła, Kamil Szaciło (2009), Candogan & Kolsarici (2003), and Halloran, Rodgers, Dyer & Ellis (1997).

Cooking yield after the first cooking increased as KCl increased ($p<0.001$) and also after the second cooking ($p=0.041$) in a linear manner. Similarly, moisture retention after the first and second cookings increased linearly with KCl levels. Protein extraction is enhanced by high ionic strength which leads to increased solubilisation of myosin in the water phase and ultimately giving a better binding and higher water holding capacity (Hamm, 1971; Shults & Wiebicki, 1973). Sodium and potassium chloride exert effects on the ionic strength. Franks (1993) and Munasinghe & Sakai (2004) reported that KCl as well as NaCl have been widely used for protein extraction, though the latter was a better protein extractant. Thus the increased level of KCl may have improved moisture retention and cooking yield by increasing protein extraction.

(d) The content of fat, Na and K:

As expected fat content was significantly reduced in pork balls ($p=0.003$) when less back fat was used ($p=0.003$) (Table 9.5). When carrageenan was present (Table 9.6) in the formulation, fat contents were lower compared to when carrageenan was absent. This could be due in part to less lean meat (which contains intramuscular fat) used in the formulation. Significantly increased fat content with increasing KCl% (Table 9.7) did not have a clear pattern of change. At 1.0% carrageenan, fat exhibited a quadratic pattern with increasing KCl ($p=0.012$) while at 1.5%, the linear relationship was stronger ($p=0.010$).

Sodium and potassium levels in pork balls changed in the ways expected with increasing levels of NaCl (Table 9.5) or KCl (Table 9.7). There was a significant interaction between fat and NaCl for potassium content (Table 9.5), with higher fat levels being associated with more potassium for 1% NaCl, but less potassium at 0.6% NaCl. The NaCl concentration did not affect the fat contents of the pork balls ($p=0.45$). Perhaps the

concentration difference in NaCl was not high enough to reflect on the differences on fat content. It was reported that myofibrillar protein solubilisation requires a minimal ionic strength (0.15M NaCl) and increases when salt concentration is higher than 0.5M (Xiong, Lou, Wang, Moody & Harmon, 2000; Liu & Xiong, 1997). This increased protein extractability and protein solubilisation results in fat globules that are physically entrapped within a protein matrix (Barbut, 1998). This would have increased fat retention and reduced fat loss during cooking. When there are sufficient proteins to coat all the fat particles, a stable and firm texture is obtained (Mandigo, 2004).

Table 9.5

Effects of levels of fat, and sodium chloride and their interaction on physical characteristics of pork balls after the first cooking as determined by ANOVA based on a 2 x 2 factorial design. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Sample (Fat%: NaCl%)	Objective measures of pork ball physical characteristics											
	Hardness (N)	L*	a*	b*	pH	Moist Ret. 1st cooking (%)	Moist Ret. 2nd cooking (%)	Cooking Yield 1st cooking (%)	Cooking Yield 2nd cooking (%)	Fat (%)	Na (μgg^{-1})	K (μgg^{-1})
1. 12.5 : 1.0	8.93	75.27	5.37	12.52	6.04	74.99	77.30	79.20	80.39	9.75	6804	2895
2 10.0 : 1.0	8.51	69.41	5.35	14.33	6.04	75.18	74.85	78.13	78.10	6.01	6902	2600
3 12.5 : 0.6	8.47	71.05	5.74	14.66	6.03	79.45	75.62	82.25	78.65	9.08	4423	2589
4 10.0 : 0.6	7.75	73.58	5.82	15.36	5.96	80.09	76.28	82.82	76.69	7.37	4500	2754
Sources of Variation (p value)												
Fat	0.22	0.124	0.70	<0.001	0.20	0.68	0.68	0.96	0.08	0.003	0.66	0.19
NaCl	0.20	0.976	<0.001	<0.001	0.12	0.008	0.95	0.09	0.17	0.45	<0.001	0.14
Fat x NaCl	0.24	0.003	0.49	0.003	0.26	0.82	0.48	0.64	0.87	0.07	0.95	0.005
R^2%, RSD	74.7, 0.87	73.1, 1.68	84.7, 0.11	96.9, 0.23	66.9, 0.03	86.1, 1.34	16.8, 2.83	67.4, 1.99	66.7, 1.34	92.6, 0.59	97.6, 264	90.2, 58.3

Table 9.6

Effects of levels of carrageenan (0%, 1.0% and 1.5%) without KCl on physical characteristics of pork balls after the first cooking in Experiment 1 as determined from type I ANOVA. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Sample Carr. level (%)	Objective measures of pork ball physical characteristics											
	Hardness (N)	L*	a*	b*	pH	Moist Ret. 1st cooking (%)	Moist Ret. 2nd cooking (%)	Cooking Yield 1st cooking (%)	Cooking Yield 2nd cooking (%)	Fat (%)	Na (μgg^{-1})	K (μgg^{-1})
4. (0.0%)	7.75	73.58	5.82	15.36	5.96	80.09	76.28	82.82	76.69	7.37	4500	2754
5. (1.0%)	8.89	73.49	5.21	13.59	6.04	80.56	76.79	82.88	81.65	5.67	4489	2655
6. (1.5%)	8.48	73.79	5.02	13.63	5.91	81.26	78.89	81.27	80.50	5.62	4329	2722
Sources of Variation (p value)												
Carr.	0.215	0.124	0.699	<0.001	0.197	0.30	0.44	0.702	0.014	0.003	0.821	0.643
R^2%, RSD	74.7, 0.87	73.1, 1.68	84.7, 0.11	96.9, 0.23	66.9, 0.032	55.1, 2.09	41.9, 4.44	74.9, 1.76	94.2, 0.74	92.6, 0.59	12.3, 294	25.5, 99

Table 9.7

Effects of levels of carrageenan (carr.), potassium chloride (KCl) and their interactions on least squares means of physical characteristics of pork balls[1] after the first cooking in Experiment 1 as determined from ANOVA based on a 2 x 6 factorial design. Linear and quadratic effects of KCl were determined by orthogonal contrast polynomial. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Physical characteristic	Carr. level	KCl conc.(%)						Effects (p value)					R^2%, RSD
		0.0	0.1	0.2	0.3	0.4	0.5	Carr.	Overall KCl	KCl linear	KCl quadratic	Carr.x KCL	
Hardness	1.0%	8.89	8.16	8.10	10.29	9.97	10.85	0.027	<0.001	<0.001	0.451	0.035	70.9, 0.91
	1.5% .	8.48	7.85	10.75	9.39	10.68	11.14						
L*	1.0%	73.5	75.7	77.3	71.6	76.1	76.4	0.80	0.001	0.37	0.94	0.95	55.0, 2.00
	1.5%	73.8	75.8	77.4	72.2	74.4	75.8						
a*	1.0%	5.21	4.19	5.51	5.4	4.78	5.25	<0.001	<0.001	0.10	0.12	0.017	89.7, 0.19
	1.5% .	5.02	4.02	4.60	4.9	4.37	5.10						
b*	1.0%	13.6	12.7	13.4	14.7	12.3	12.3	0.63	<0.001	0.09	0.003	0.011	82.5, 0.47
	1.5%	13.6	12.4	12.8	14.5	13.9	12.2						
pH	1.0%	6.04	6.05	6.01	5.92	5.94	5.94	0.16	0.41	0.50	0.93	0.09	62.6, 0.06
	1.5% .	5.91	5.24	5.96	5.90	6.03	5.96						
Moist. ret (1^{st} cooking) %	1.0%	76.56	80.36	79.74	79.17	82.23	83.82	0.79	0.19	0.03	0.05	0.89	47.2, 3.12
	1.5%	80.00	82.07	79.12	78.22	80.81	83.74						
Moist. ret (2^{nd} cooking) %	1.0%	76.79	66.89	81.59	76.42	77.04	81.15	0.56	0.12	0.06	0.10	0.27	61.2, 6.13
	1.5%	70.89	73.69	79.75	81.82	88.41	77.06						
Cooking yield (1^{st} cooking) %	1.0%	82.3	83.6	82.6	81.8	84.7	86.4	0.49	0.49	0.05	0.13	0.47	45.7, 2.17
	1.5% .	81.3	82.9	82.1	85.2	83.1	83.5						
Cooking yield (2^{nd} cooking) %	1.0%	81.7	82.6	83.4	81.6	84.6	84.5	0.35	0.58	0.041	0.11	0.76	38.2, 2.73
	1.5%	80.5	81.7	80.0	83.4	81.6	84.6						
Fat (%)	1.0%	5.67	4.95	4.10	5.05	6.89	6.89	0.16	0.002	0.002	<0.001	0.73	78.7, 0.67
	1.5%	5.62	5.25	5.46	5.58	6.96	7.12						
Na ($\mu g \cdot g^{-1}$)	1.0%	4486	4300	4279	4291	4324	4638	0.17	0.72	0.75	0.53	0.42	46.4, 205
	1.5% .	4329	4174	4185	4450	4306	4144						
K ($\mu g \cdot g^{-1}$)	1.0%	2656	3870	5005	6271	7596	8657	0.39	<0.001	<0.0001	<0.001	0.92	99.6, 185
	1.5%	2722	3709	4831	6091	7511	8586						

Table 9.8

Effects of levels of fat, sodium chloride (NaCl) and the fat x sodium chloride interaction on least squares means of sensory attributes of pork balls[1] following the second cooking in Experiment 1 as determined by a trained panel using type I ANOVA based on a 2 x 2 factorial design. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Sensory Attributes	Formulation (Fat% : NaCl%)				Effects (P-values)			R^2%, RSD
	1 (12.5% : 1.0%)	2 (10.0% : 1.0%)	3 (12.5% : 0.6%)	4 (10.0% : 0.6%)	Fat	NaCl	Fat x NaCl	
Colour (brownness)	34.5abc	37.2abc	20.9ab	16.7a	0.91	0.011	0.59	17.2, 20.2
Colour saturation	29.6	30.5	21.1	15.3	0.67	0.06	0.58	10.9, 19.0
Meaty aroma	49.9	44.1	32.9	40.7	0.91	0.26	0.44	76.6, 27.9
Brothy aroma	55.7bc	47.5abc	24.6a	30.5ab	0.89	0.005	0.38	65.3, 25.2
Cuttlefish aroma	39.1ab	42.9b	16.8a	26.8ab	0.41	0.028	0.72	68.9, 26.5
Metallic aroma	27.2	19.2	8.86	12.4	0.76	0.09	0.43	72.1, 22.7
Acidic aroma	14.9	14.4	5.75	4.53	0.88	0.12	0.95	70.9, 18.7
Stale odour	14.9	7.86	4.93	5.33	0.40	0.12	0.35	82.1, 12.6
Meaty flavour	38.5	52.3	48.7	46.2	0.48	0.79	0.31	65.8, 25.0
Brothy flavour	38.1	54.2	39.9	39.7	0.29	0.40	0.28	56.2, 23.3
Cuttlefish flavour	18.1	27.3	20.1	23.3	0.42	0.89	0.69	64.9, 24.0
Metallic flavour	14.5	8.67	5.24	5.01	0.47	0.13	0.50	71.6, 13.1
Acidic flavour	12.5	15.7	17.6	17.1	0.87	0.69	0.83	84.2, 25.9
Stale flavour	5.94	8.13	3.96	5.33	0.50	0.37	0.88	69.2, 8.34
Salty taste	42.3bcd	52.5b	24.3ab	25.1ab	0.36	<0.001	0.43	75.5, 22.5
Bitter taste	0.00	0.00	0.286	0.043	0.18	0.07	0.18	23.5, 0.23
Chewiness	46.9	62.0	48.5	61.7	0.06	0.93	0.89	58.9, 18.9
Cohesiveness	41.1ab	59.1abc	38.4a	50.5abc	0.010	0.99	0.73	59.3, 22.5
Juiciness	43.2	50.3	45.2	48.4	0.56	0.99	0.83	55.6, 23.7
Hardness	33.7	42.3	33.9	34.1	0.50	0.53	0.51	66.7, 27.3
Aftertaste	16.6	24.7	26.6	28.1	0.51	0.35	0.64	54.8, 20.0

[1] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 9.9
Effects of levels of carrageenan (carr. 0%, 1.0% and 1.5%) without KCl on sensory attributes of pork balls following the second cooking in Experiment 1 as determined by a trained panel using type I ANOVA. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Sensory attributes	Carr.			Effects (p-value)	R^2%, RSD
	4. (0.0%)	5. (1.0%)	6. (1.5%)		
Colour (brownness)	16.7	24.8	31.2	0.25	10.0, 18.8
Colour saturation	15.3	18.8	21.9	0.61	31.8, 14.6
Meaty aroma	40.7	48.3	47.2	0.83	58.2, 29.8
Brothy aroma	30.5	44.2	47.9	0.35	15.0, 27.4
Cuttlefish aroma	26.8	36.8	31.6	0.66	55.1, 24.2
Metallic aroma	12.4	10.6	18.6	0.69	61.4, 20.8
Acidic aroma	4.53	11.9	20.0	0.25	10.3, 20.4
Stale odour	5.33	8.21	12.7	0.46	23.2, 13.1
Meaty flavour	46.2	51.4	55.5	0.67	36.9, 23.2
Brothy flavour	39.7	46.2	53.7	0.39	18.0, 22.5
Cuttlefish flavour	23.3	25.5	33.1	0.57	34.6, 21.5
Metallic flavour	5.01	7.19	13.2	0.47	26.7, 15.2
Acidic flavour	17.1	14.9	23.3	0.75	66.5, 25.2
Stale flavour	5.33	5.39	14.1	0.27	21.6, 13.5
Salty taste	25.1	17.5	24.9	0.40	78.7, 14.1
Bitter taste	0.043	0.157	0.214	0.73	34.0, 0.41
Chewiness	61.7	62.5	66.7	0.84	61.5, 20.2
Cohesiveness	50.5	61.7	68.9	0.20	66.7, 18.5
Juiciness	48.4	46.2	59.6	0.45	42.6, 24.0
Hardness	34.1	39.7	52.2	0.12	69.0, 19.4
Aftertaste	28.1	31.9	17.9	0.50	52.0, 27.3

Table 9.10

Effects of levels of carrageenan (carr.), potassium chloride (KCl) and their interaction on least squares means of sensory attributes of pork balls[1] following the second cooking in Experiment 1 as determined by a trained panel using an ANOVA based on a 2 x 6 factorial design. Linear and quadratic effects of KCl were determined by orthogonal polynomial contrast. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation , RSD

Sensory Attributes	Carr.	KCl concentration (%)						Effects (P values					R^2%, RSD
		0.0	0.1	0.2	0.3	0.4	0.5	Carr.	Overall KCl	KCl linear	KCl quadratic	Carr.x KCl	
Colour (brownness)	1.0%	24.8	28.3	42.7	33.9	30.5	27.9	0.33	0.32	0.89	0.09	0.97	61.4, 23.1
	1.5% .	31.2	39.1	45.8	31.2	32.9	32.6						
Colour saturation	1.0%	18.8	26.1	42.7	33.9	30.5	27.9	0.85	0.70	0.24	0.29	0.79	56.4, 21.0
	1.5%	21.9	29.3	24.5	29.5	29.8	32.3						
Meaty aroma	1.0%	48.3	48.3	41.9	58.1	49.3	50.7	0.79	0.64	0.28	0.76	0.94	75.0, 26.9
	1.5% .	47.2	43.9	42.3	49.9	55.3	53.4						
Brothy aroma	1.0%	44.2	42.8	38.4	56.3	41.9	47.2	0.92	0.73	0.69	0.56	0.77	51.3, 23.3
	1.5%	47.9	40.1	41.3	44.1	51.9	48.1						
Cuttlefish aroma	1.0%	36.8	39.1	36.6	35.3	35.7	39.7	0.42	0.93	0.89	0.88	0.99	63.9, 22.9
	1.5% .	31.6	33.3	31.8	32.9	30.6	41.8						
Metallic aroma	1.0%	10.6	4.91	9.62	12.1	5.53	4.63	0.43	0.48	0.44	0.38	0.56	55.1, 14.8
	1.5%	18.6	5.33	9.87	4.70	11.9	10.5						
Acidic aroma	1.0%	11.9	13.6	7.79	9.83	9.53	7.53	0.80	0.53	0.13	0.36	0.87	61.9, 18.6
	1.5% .	20.0	8.07	5.21	6.74	9.96	5.35						
Stale smell	1.0%	8.21	5.21	5.33	5.46	3.06	7.53	0.23	0.31	0.89	0.02	0.86	78.1, 9.4
	1.5%	12.7	5.26	5.34	4.66	7.60	14.5						
Meaty flavour	1.0%	51.4	53.7	46.1	58.3	56.6	56.2	0.42	0.42	0.14	0.93	0.94	64.8, 23.9
	1.5% .	55.5	47.3	51.9	64.7	64.3	59.5						
Brothy flavour	1.0%	46.2	52.9	47.8	54.9	50.6	56.7	0.26	0.14	0.03	0.64	0.48	76.2, 19.6
	1.5%	53.7	49.4	43.2	59.5	67.3	58.9						
Cuttlefish flavour	1.0%	25.5	28.4	20.2	31.3	34.9	43.4	0.35	0.18	0.03	0.23	0.90	65.0, 22.9
	1.5% .	33.1	31.3	28.2	32.1	45.1	37.7						

[2] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P > 0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 9.10 (continued)

Sensory attributes	Carr.	KCl concentration (%)						Effects (p values)					R^2%, RSD
		0.0	0.1	0.2	0.3	0.4	0.5	Carr.	Overall KCl	KCl linear	KCl quadratic	Carr.x KCl	
Metallic flavour	1.0%	7.19	3.26	3.80	7.80	4.60	4.74	0.51	0.50	0.34	0.46	0.80	61.2, 12.5
	1.5%	13.2	4.94	4.53	4.64	9.47	3.03						
Acidic flavour	1.0%	14.9	11.6	6.74	10.4	11.9	15.1	0.94	0.49	0.51	0.05	0.95	74.2, 19.8
	1.5%	23.3	11.7	7.99	5.96	9.59	13.9						
Stale flavour	1.0%	5.39	2.33	5.53	4.01	2.11	5.40	0.25	0.24	0.17	0.11	0.29	59.2, 9.39
	1.5%	14.1	4.60	2.94	3.74	7.52	3.24						
Salty flavour	1.0%	17.5	24.3	25.3	30.1	26.7	39.4	0.53	0.031	0.34	0.42	0.95	55.2, 26.2
	1.5%	24.9a	26.3a	23.0a	29.1ab	26.5a	48.7b						
Bitter flavour	1.0%	0.157a	0.228a	0.543ab	1.03b	1.83c	4.20d	0.22	<0.001	<0.001	<0.001	0.88	78.9, 0.79
	1.5%	0.214a	0.257a	0.671a	1.71b	2.13b	4.29c						
Chewiness	1.0%	62.5a	57.2a	69.7ab	80.8b	70.7ab	69.9ab	0.04	0.030	0.007	0.10	0.45	57.6, 18.2
	1.5%	66.7a	70.4ab	74.8ab	75.6ab	79.5b	76.5ab						
Cohesiveness	1.0%	61.7	63.7	65.6	69.5	59.7	71.0	0.03	0.50	0.63	0.86	0.57	52.9, 22.1
	1.5%	68.9	69.1	74.6	73.4	74.6	67.6						
Juiciness	1.0%	46.2a	59.3ab	54.3ab	70.5b	49.5a	55.1ab	0.20	0.27	0.26	0.31	0.36	69.5, 22.4
	1.5%	59.6	53.8	60.9	62.9	61.2	62.9						
Hardness	1.0%	39.7	46.6	56.7	53.2	57.3	55.0	0.030	0.05	0.007	0.32	0.85	74.2, 24.3
	1.5%	52.2a	47.9a	67.9b	64.9ab	58.2ab	77.1b						
Aftertaste	1.0%	31.9	27.0	24.7	23.3	24.2	38.4	0.040	0.03	0.478	0.86	0.45	72.3, 23.4
	1.5%	17.9a	22.4ab	24.0ab	27.1ab	30.6ab	41.9b						

[2] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

9.3.1.2 Trained Sensory Panel Results for Experiment 1

Results in Table 9.8 showed that lower levels of fat were associated with higher cohesiveness, and that lower levels of NaCl decreased colour brownness, brothy aroma, cuttlefish aroma and salty taste. Increased carrageenan (Table 9.9) had no effect on sensory characteristics alone, but in conjunction with KCl, the higher level of carrageenan increased chewiness, cohesiveness, hardness, and aftertaste at high KCl levels (Table 9.10). The main effect of increased levels of KCl was a more bitter flavour (Table 9.10), but they were also associated with a greater saltiness, chewiness, and hardness, and with inconsistent changes in aftertaste (Table 9.10). At the highest levels of KCl the saltiness scores were similar to those for formulations containing 1% sodium chloride (Table 9.8), but the bitterness and aftertaste scores were appreciably higher.

Potassium chloride is currently the most commonly used material to replace sodium in foods (Dotsch, Busch, Batenburg, Liem, Tareilus, Mueller & Meijer, 2009) as it produces a salt-like taste. Thus, increased levels of KCl used will lead to greater intensity of saltiness. In addition, as its dosage level increased, potassium chloride has been reported to elicit a bitter/metallic taste (Van der Klaauw & Smith, 1995). This is thought to occur via the non-sodium specific channel of taste receptor cells (Chandrashekar, Kuhn, Oka, Yarmolinsky, Hummler, Ryba & Zuker, 2010). When potassium chloride is used in high concentrations the bitter/metallic taste dominates over the salty taste, often causing foods to become unpalatable, thus limiting the use of potassium chloride as a salt substitute (Ainsworth & Plunkett, 2007). With regard to the increase in chewiness and hardness as levels of KCl increases, this could have been due to the increase in myofibrillar protein extraction, particularly myosin, which in turn would have led to increased water binding and ultimately to increased binding between adjacent pieces of meat (Hamm, 1986). Chloride ions (Cl^-) bind to the protein resulting in negative charges, which cause repulsion between the myofibrillar proteins. This results in a swelling of myofibrils due to repulsion between individual molecules and finally an increase in water-holding capacity (Hamm,

1986). Frye, Hand, Calkins, & Mandigo (1986) reported in a study on cooked hams that a 50% replacement with KCl gave a superior bind in the meat.

9.3.1.3 Untrained Sensory Panel Results for Experiment 1

Table 9.11
Rank sum of acceptability scores for sensory attributes of the 16 formulations of pork balls following the second cooking in Experiment 1 using an untrained panel

Formulation	Variable ingredients (%)				Colour	Aroma	Flavour	Texture	Overall Acceptability
	Fat	NaCl	Car.	KCl					
1	12.5	1.0	0	0	49ab	63cd	58ab	58ab	55ab
2	10.0	1.0	0	0	47a	65cd	65bc	53a	53ab
3	12.5	0.6	0	0	50ab	47a	51a	55a	53ab
4	10.0	0.6	0	0	53ab	46a	54ab	59ab	59ab
5	10.0	0.6	1.0	0	58ab	48ab	62ab	61ab	62b
6	10.0	0.6	1.5	0	67c	55ab	67bc	60ab	62b
7	10.0	0.6	1.0	0.1	61c	53ab	70bc	74b	63bc
8	10.0	0.6	1.5	0.1	64c	61c	62ab	70b	50a
9	10.0	0.6	1.0	0.2	66c	62cd	70bc	69b	57ab
10	10.0	0.6	1.5	0.2	64c	67cd	70bc	70b	62b
11	10.0	0.6	1.0	0.3	61c	70cd	68bc	69b	70bc
12	10.0	0.6	1.5	0.3	65c	67cd	69bc	69b	64bc
13	10.0	0.6	1.0	0.4	48ab	69cd	63b	69b	66bc
14	10.0	0.6	1.5	0.4	58ab	73d	75c	69b	74c
15	10.0	0.6	1.0	0.5	59bc	58bc	65bc	71b	67bc
16	10.0	0.6	1.5	0.5	60bc	64cd	66bc	69b	65bc

[1]Means within a column followed by a common letter are not significantly different at the 5% significance level (LSD rank = 11.72)

All t-values for colour, aroma, flavour and overall acceptability for the 16 formulations exceeded $\chi^2_{15.0,\ 0.05}$=25.0, indicating that there were significant differences in the consumer acceptability of these sensory attributes. Formulation 14 had the highest rank scores for aroma, flavour and overall acceptability (Table 9.11), and had scores that were significantly different from 6 samples for aroma and flavour acceptability and 9 samples for overall acceptability. This suggests that the panellists found the extra bitterness of the formulations with higher KCl levels quite acceptable. In addition, this formulation met the low-fat and low-sodium criteria. Thus, formulation 14 was used for all subsequent pork ball

batches. Formulation 14 had 40% of NaCl replaced with KCl. Lilic, Matekalo-Sverak & Borovic (2008) reported that sausages with 20% and 40% replacement of NaCl with KCl were acceptable, while those with 60% of NaCl replaced were at the limit of acceptability, and products with 80% KCl were unacceptable. The current results are also in accordance with results of Askar, El-Samahy, Shehata and Tawfik (1994) in showing that the replacement is possible between 20% and 40% replacement of NaCl by KCl without significant influences on taste.

9.3.2 Experiment 2: Refining Pork Ball Processing

In this experiment Formulation 14 from Experiment 1 was used to evaluate the effects of 2 mixing methods (A and B, Fig. 9.1), 2 cooking methods, and 2 phosphate types on the retention of minerals and cooking yields. Using mixing Method B resulted in non-significantly higher percentage retention levels of elements than Method A, except for potassium and sodium (Table 9.12). The cooking yields after the first and second cookings were higher for Method B than Method A, but this was statistically significant for the second cooking only.

Sodium chloride and phosphate increase protein solubility and protein extractability in meat systems (Prusa & Bowers, 1984), and high ionic strength salt solutions are employed to extract proteins for meat batter preparations (Gordon & Barbut, 1992). An increase in extracted myosin will significantly increase the binding strength of the protein matrix with the meat surface. Thus in Method B, the purpose of adding salt and spices before adding corn starch was to increase the ionic strength and protein solubility. Once the solubility was maximised, corn starch was added to adsorb the moisture lost by the meat proteins during cooking. The amylase and amylopectin that are extracted from the starch granules during swelling do help to bind adjacent pieces of meat in cold meatballs. By adding all dry ingredients at one time may not achieve high protein solubility as corn starch may dilute the salting-in effect. Temperature also plays an important role in protein extraction. Gillett, Meiburg, Brown & Simon (1977) reported that the optimum temperature

for protein extraction from three beef and two pork sources using a 7.5% sodium chloride solution was 7.2°C, with temperatures above 7.2°C, resulting in less extractable protein.

Table 9.12
True mean retention of elements (%)[1] in cooked pork ball prepared using two mixing methods[1] and cooked in boiling water directly in Experiment 2. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2 (%) and the residual standard deviation, RSD

Item	Mixing method[2]		Effects (p-value)	R^2%, RSD
	A	B		
Number of pork balls	3	3		
True mean retention of elements				
Ca	89.47	89.70	0.98	25.0, 10.8
Cu	84.31	91.19	0.41	17.8, 9.06
Fe	74.38	74.78	0.95	24.9, 7.58
K	79.70	74.35	0.14	46.6, 3.51
Na	81.78	78.86	0.54	52.1, 5.72
Se	82.23	82.25	0.99	25.0, 9.58
Zn	84.37	87.85	0.66	54.0, 8.87
Cooking Yield (%):				
After 1st cooking	82.10	85.03	0.18	40.1, 2.20
After 2nd cooking	78.03	83.40	0.024	75.6, 1.87

[1]True mean retention of an item (expressed here as a %) refers to the weight of that item in the product after cooking relative to the weight of the same item in the product before cooking.
[2] Method A (Fig. 9.1) - a method that required the addition of all ingredients all at once with the pork. Method B (Fig. 9.1) - a method that required the addition of salt and phosphate and spices right at the start before adding corn starch. Mixing was done in a mixing bowl that was chilled in an ice-bath.

There was generally no significant effect of phosphate salt (F1 vs. F2) or cooking method (Table 9.12) on the true retention of elements cooking yields, except that the retention of potassium from the F2 formulation cooked in a thermo-bag was significantly higher. Thus either formulation could be used for the manufacture of pork balls. Incorporation of phosphates in raw meat emulsions increases protein solubility and improves emulsion stability and water holding capacity according to Kerry, Kerry & Ledward (2002). Knipe, Olson and Rust (1985) showed that tetrapotassium pyrophosphates resulted in higher protein solubility than sodium or potassium tripolyphosphate. Tetrapotassium pyrophosphate is more expensive than sodium tripolyphosphate and is less

popular in the meat processing industry, partly due to its lower solubility (Hamm & Neraal, 1977). F2 was chosen as the method to make pork ball for future batches as it included some potassium (better stability and water retention) and some sodium phosphates (lower costs).

For pork balls cooked in thermo-bags the retention of potassium was better for F2 only (Table 9.14). As the most popular way currently is to cook pork balls directly in boiling water/stock (steam boat style), cooking pork balls in a thermo-resistant bag was not considered in future trials.

In conclusion, a low-fat, low-salt pork ball was developed for the Singapore market with fat and sodium levels below the recommended maximum levels of 10 g/100g and 450 mg/100 g, respectively. The nutrient content of the cooked pork ball (based on F2 formulation after the first cooking) is shown in Table 9.13.

Table 9.13

Nutrient content of pork ball (based on the F2 formulation given in Table 9.4).

Fat (g/100g)	7.7
Protein (g/100g)	16.7
Carbohydrate (g/100g)	4.4
Sodium (mg/100g)	431
Energy kcal/100g	154

Table 9.14
Effects of phosphate formulations (P)[1] and cooking methods (C)[2] and their interactions on least squares means of true mean retention of elements (%) and cooking yield for pork balls in Experiment 2 as determined from ANOVA based on a 2 x 2 factorial design. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Item	Cooking method (C)	Phosphate salt (P)		Effects (p-value)			
		F1	F2	C	P	C x P	R^2%, RSD
True mean retention of elements (%)							
Ca	Boiled directly[a]	82.73	84.13	0.78	0.67	0.49	25.0, 9.01
	Boiled in bag[b]	87.98	81.92				
Cu	Boiled directly	81.88	93.17	0.99	0.05	0.84	39.7, 7.82
	Boiled in bag	82.90	92.27				
Fe	Boiled directly	82.86	83.37	0.28	0.77	0.69	67.1, 7.71
	Boiled in bag	89.85	86.69				
K	Boiled directly	87.23	86.24	0.022	0.019	0.012	77.1, 2.69
	Boiled in bag	86.96	93.50				
Na	Boiled directly	86.78	81.61	0.20	0.64	0.99	21.4, 4.24
	Boiled in bag	91.30	91.10				
Se	Boiled directly	75.52	78.34	0.52	0.77	0.79	73.0, 8.32
	Boiled in bag	80.12	80.23				
Zn	Boiled directly	95.73	88.85	0.84	0.16	0.11	41.2, 3.58
	Boiled in bag	92.46	92.97				
Cooking Yield (%)							
After 1st cooking	Boiled directly	82.07	80.77	0.19	0.81	0.25	31.2, 1.52
	Boiled in bag	82.23	83.10				
After 2nd cooking	Boiled directly	79.50	77.90	0.36	0.42	0.38	24.1, 1.55
	Boiled in bag	79.53	79.61				

[1] Phosphate formulation : F1-0.5% tetrapotassium pyrophosphate; and F2- 0.2% sodium tripolyphoshate and 0.3% tetra potassium pyrophosphate;
[2]Cooked in boiling water directly and cooked in a thermo-bag in boiling water
[a] The porkballs were cooked directly in boiling water for 2.39 minutes to achieve an internal temperature of 75°C.
[b] The pork balls (one pork ball per bag) were cooked in thermo-bags which were placed in boiling water for 8.5 minutes to achieve an internal temperature of 75°C.

9.3.3 Experiment 3: Garlic pork ball evaluation

9.3.3.1 Physical characteristics of the pork balls in Experiment 3

Diet and GEO concentration had no significant effects on the physical characteristics shown in Table 9.15.

9.3.3.2 Sensory attributes of pork balls in Experiment 3

Pork balls made from pork and back fat from pigs fed on a diet containing some animal products had a higher colour saturation score but other sensory attributes in Table 9.16 were unaffected. Neither mutton aroma nor flavour scores was significantly reduced by excluding animal products from the diet (Table 9.16) in the way they were in the study of Leong et al. (2010b) with pork samples rather than pork balls.

Higher GEO concentrations in the diet significantly increased both stale odour ($p=0.032$) and stale flavour ($p=0.011$) (Table 9.16). Significant effects of GEO concentration on colour brownness was accompanied by a significant interaction between GEO level and diet, because increased GEO was associated with increased brownness for the P diet, but a decreased brownness for the AP diet (Table 9.16). Although scores for garlic aroma and garlic flavour both increased with increasing GEO concentration (Table 9.15), these changes were not statistically significant. This suggests that other components in the pork balls largely masked any differences in garlic flavour or mutton flavour in the pork used for the trial. Interactions between diet and GEO level were not significant for any attribute except for colour ($p=0.032$).

Table 9.15

Least squares means showing the effects of diet[1,2] (Animal-Plant vs. Plant only) and GEO concentration (zero, low, medium, or high) on the physical and chemical attributes of pork balls in Experiment 3. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Diet	Objective measures of physical characteristics									
	Hardness (N)	L*	a*	b*	pH	Moist. Ret.aft 1st cooking (%)	Moist. Ret. aft 2nd cooking (%)	Cooking Yield aft 1st cooking (%)	Cooking Yield aft 2nd cooking (%)	Fat (%)
AP_0	11.58	65.78	3.45	10.13	6.01	75.93	72.85	80.71	76.66	6.54
AP_L	11.98	64.62	3.29	9.38	5.98	74.11	72.73	80.22	76.75	5.95
AP_M	12.64	66.17	3.37	9.70	5.93	75.01	75.56	79.79	77.59	5.78
AP_H	12.44	65.70	3.26	9.86	5.95	74.85	75.62	80.38	76.20	6.19
P_0	13.13	65.65	3.33	9.50	5.89	72.69	70.73	80.25	76.36	6.11
P_L	12.25	66.13	3.46	10.06	5.98	74.08	74.16	79.92	76.00	5.98
P_M	11.59	65.64	3.58	9.96	6.00	75.93	74.72	80.74	78.64	5.90
P_H	11.44	65.63	3.29	9.79	5.97	74.57	72.65	80.31	78.11	5.98
Effects (p-values)										
Diet	0.935	0.723	0.267	0.756	0.717	0.45	0.14	0.892	0.465	0.468
GEO conc.	0.180	0.588	0.208	0.164	0.123	0.68	0.07	0.184	0.148	0.606
R^2%, RSD	30.6, 1.87	21.2, 2.24	42.0, 0.24	30.6, 0.82	33.9, 0.11	26.3, 3.40	17.5, 3.56	61.2, 0.98	14.5, 2.34	32.7, 0.63

[1]AP=diet containing animal and plant products; P=diet containing plant products only; GEO at 4 levels from 0 to 4 where 0=zero, L=low, M=medium, H=high

[2] There were 4 animals per group except for AP_H where there were 3 animals.

Table 9.16

Least squares means showing the effects of diet[1] (Animal-Plant (A) vs. Plant only (P)) and GEO concentration (0, L, M, or H) on the sensory profile attributes of pork balls following the second cooking evaluated by a trained Singaporean sensory panel in Experiment 3. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Sensory Attributes[2, 3]	Sources of variation (Probability)								Effect (P values)			R^2%, RSD
	AP_0	AP_L	AP_M	AP_H	P_0	P_L	P_M	P_H	Diet	GEO conc.	Diet x GEO	
Colour (brownness)	61.57c	57.18c	61.98c	51.90ab	40.62a	46.05abc	41.41a	47.70abc	0.09	0.010	0.032	45.9, 24.5
Colour saturation	48.61bc	45.05abc	53.54c	40.07abc	30.46a	34.50ab	44.92abc	37.21abc	0.002	0.20	0.52	69.0, 26.5
Meaty aroma	65.30	65.08	67.60	62.41	62.32	59.10	61.61	65.13	0.16	0.86	0.53	56.4, 16.6
Brothy aroma	59.21	59.24	61.25	54.76	56.23	50.64	53.69	56.97	0.10	0.86	0.49	61.4, 19.4
Cuttlefish aroma	38.48	45.26	41.24	38.16	37.53	36.96	31.97	40.87	0.28	0.84	0.58	65.1, 25.3
Mutton aroma	23.97	19.66	15.36	13.05	17.79	15.35	14.31	13.36	0.69	0.81	0.61	40.4, 16.7
Garlic aroma	1.60	5.43	5.93	6.70	1.83	5.09	6.89	7.90	0.46	0.20	0.56	42.2, 9.02
Metallic aroma	11.95	18.61	15.10	16.76	19.19	17.19	11.88	19.76	0.51	0.62	0.52	63.8, 19.5
Acidic aroma	8.78	8.16	10.14	12.28	5.06	10.74	8.88	11.51	0.77	0.42	0.74	64.7, 15.0
Stale odour	8.45a	14.64abc	16.12abc	20.61c	9.63ab	11.26abc	13.39abc	20.13bc	0.75	0.032	0.36	52.3, 17.0
Meaty flavour	63.41	62.46	63.94	63.47	60.76	63.26	63.26	61.38	0.60	0.97	0.95	54.2, 16.9
Brothy flavour	59.84	57.38	58.15	52.82	56.09	53.08	55.74	56.25	0.46	0.84	0.76	55.8, 20.0
Cuttlefish flavour	38.15	36.73	28.44	35.87	33.24	32.59	27.78	34.66	0.45	0.28	0.95	50.5, 22.9
Mutton flavour	35.74	19.70	17.93	15.14	25.29	18.88	17.09	13.16	0.45	0.51	0.27	54.4, 20.8
Garlic flavour	5.38	7.17	9.01	10.35	5.95	8.76	10.01	12.05	0.66	0.34	0.98	54.0, 12.8
Metallic flavour	10.76	15.36	15.19	14.38	15.71	19.69	17.45	22.81	0.09	0.59	0.92	65.1, 22.0
Acidic flavour	10.57	12.45	12.91	11.02	10.56	13.81	11.59	11.64	0.99	0.89	0.99	66.5, 18.9
Stale flavour	7.33a	14.86abc	15.48abc	19.64bc	10.70ab	11.48ab	12.76abc	23.69c	0.70	0.011	0.39	51.7, 18.5
Salty taste	35.61	30.43	26.00	32.84	28.43	34.36	34.15	24.64	0.80	0.75	0.19	76.9, 22.9
Aftertaste	31.01	35.01	32.65	33.58	26.41	32.89	29.93	33.81	0.52	0.88	0.64	48.8, 16.3
Chewiness	72.84	71.86	71.54	69.57	67.12	70.36	72.74	70.12	0.82	0.27	0.26	50.4, 19.9
Cohesiveness	63.76	63.99	67.59	70.67	60.76	71.65	72.07	64.44	0.86	0.63	0.82	38.6, 19.4
Juiciness	59.38	54.56	51.38	56.64	56.08	54.38	55.02	54.35	0.41	0.05	0.80	66.9, 21.8
Hardness	39.12	44.45	49.17	43.75	38.22	39.53	51.97	39.53	0.49	0.59	0.96	50.7, 23.0

[1]AP=diet containing animal and plant products; P=diet containing plant products only; GEO at 4 levels from 0 to 4 where 0=zero, L=low, M=medium, H=high

[2]All attributes were scored on a scale of 0-100

[3] Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Linear correlation coefficients across average scores for flavour attributes for the 31 pigs indicated that mutton flavour was positively correlated with mutton aroma (r=0.921; p<0.01; n=31).The negative effect of GEO on mutton flavour was supported by the negative correlation between garlic and mutton flavour (r=-0.885; p< 0.01) and between garlic aroma vs. mutton odour (r=-0.809; p<0.05). There was a strong positive correlation between stale and garlic flavour (r=0.849; p < 0.01) and between stale odour vs garlic aroma (r=0.851; p<0.01). Garlic flavour was highly and negatively correlated with meaty aroma (r=-0.666), brothy flavour (r=-0.503), and mutton aroma (r =-0.828). These findings are in broad agreement with those from a garlic feeding study with sheep (Fraser, Lane, Kirk, Keogh & Cummings, 2007) in which meat from sheep that received a garlic powder supplement (>50 g/day) was described by trained panellists as 'garlic' and these samples were significantly less intense in 'sheep meat' flavours (p<0.001).

Results in Fig. 9.2 show that there were diet (p=0.001) and garlic-concentration (p=0.002) effects as well as an interaction effect (p=0.008) on overall acceptability of pork balls. The interaction reflected the pattern whereby pork balls made with pork from pigs with animal products in their diet was less acceptable than for the plant group when no GEO was fed, but this diet difference disappeared when low or medium levels of GEO were included in the diet so that all those groups (AP_L, AP_M, P_L, & P_M in Fig 9.3) had a moderately high level of acceptability. However, the acceptability dropped regardless of diet when high levels of GEO were fed. Results also showed that overall acceptability was negatively correlated to garlic aroma (r=-0.347, n=31), garlic flavour (r=-0.462), mutton flavour (r=-0.754), stale flavour (r=-0.702) and aftertaste (r=-0.82); but positively correlated to meaty aroma (r=0.604), meaty flavour (r=0.815), brothy aroma (r=0.525) and brothy flavour (r=0.651). A quadratic garlic level effect was apparent for overall acceptability (p=0.011) where acceptability increased when a low level of GEO (0.55 g/kg feed) was used but decreased after medium level of GEO (1.44 g/kg feed) was in the diet of the pigs. Leong et al. (2010b) reported that consumer panellists in Singapore did not find pork from the high-GEO group to be less acceptable than that from the medium-GEO group.

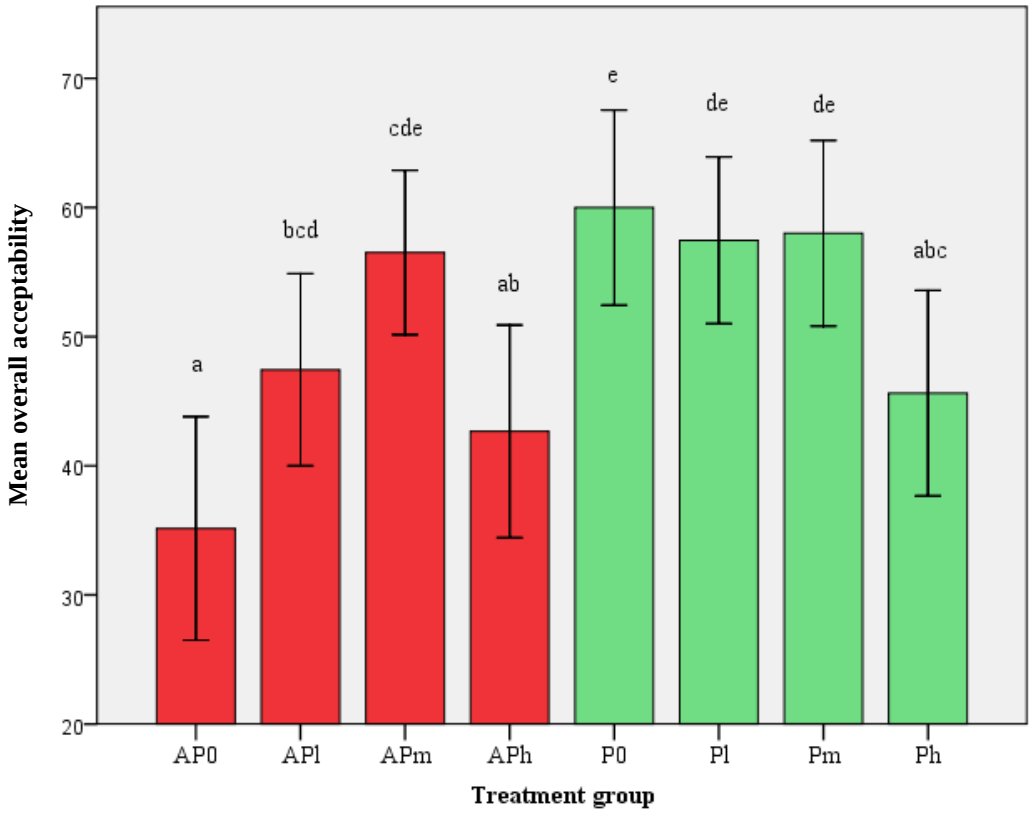


Fig. 9.2

Least-squares means (±SE) for overall acceptability of pork balls following the second cooking as assessed by an untrained panel with SE bars from pigs fed with three levels of garlic relative to the appropriate controls within groups receiving either animal or plant diets[1] in Experiment 3. A line scale of 150 mm was used where anchor points at the ends of the scale were "Lowest acceptability and "Highest acceptability". There were 4 animals per group except for AP_h where 3 animals were used. Each panellist evaluated one pork ball produced from pork and back fat from each pig. Bars with a common letter above them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[1]AP=diet containing animal and plant products; P=diet containing plant products only; GEO at 4 levels from 0 to 4 where 0=zero, l=low, m=medium, h=high

9.4. Conclusions

A low-fat, low-salt pork ball was developed for the Singapore market with fat and sodium levels below the recommended maximum levels of 10 g/100g and 450 mg/100 g, respectively, and without any loss in acceptability or deterioration in cooking yield or other physical characteristics of the pork balls. Carrageenan was used to replace some of the fat in this product and KCL replaced some of the NaCl. These ingredients improved the cooking yield and moisture retention.

Low-fat, low-salt pork balls prepared with lean meat and back fat from pigs fed a diet containing moderate levels of garlic essential oil (GEO) were more acceptable than when there was no GEO in the diet of the pigs when the base diet contained animal products, but not when the diet contained plant products only. High levels of GEO in the diet of pigs resulted in pork balls that were less acceptable regardless of the base diet.

It is concluded that it is possible to produce low-fat, low-sodium pork balls without a decrease in acceptability for Singapore consumers, and that GEO may be useful as a means of masking undesirable flavours like mutton flavour in pork balls.

Chapter 10

Development of low-fat, low-sodium pork balls using functional ingredients for the Singapore market

10.1 Introduction

Pork contains large amounts of oleic acid but polyunsaturated fatty acids (PUFA) are scarce. The consumption of diets high in saturated fatty acids has been shown to increase plasma cholesterol in man which has been implicated in the aetiology of coronary heart disease (Mattson and Grundy, 1985). Conversely, unsaturated fatty acids, in particular eicosapentaeonoic acid (EPA) and decosahexaenoic acid (DHA) appear to play a role in the amelioration of coronary heart disease. As the composition of the body fat of the pig tends to reflect that of the dietary fat intake, the PUFA fraction of pork products can be manipulated by dietary PUFA supplementation (Van Oeckel and Boucque, 1996; Koch *et al*., 1968). Previous studies have shown that dietary PUFA supplementation has been accompanied by adverse effects upon meat quality (Whittington *et al*., 1986; Rhee *et al*., 1988). Other studies have shown that a diet containing 20% rapeseed oil plus 1% fish oil results in no appreciable differences in carcass characteristics or overall organoleptic stability (Leskanich *et al*., 1994). Consumer awareness of the potential health benefits of increased monounsaturated fatty acids (MUFA) and PUFA in the diet means that the meat industry is under pressure to produce products that are perceived as 'healthy'. Therefore, the reduction of the n-6:n-3 ratio to below 4 in cooked pork would result in significant human health benefits.

The aim of the present work was to study the effects of feeding animal and plant based diets to pigs on fatty acid composition of pork balls made from the resulting pork. Specifically, the aims were to assess the effects on pork quality (pH, colour, lipid oxidation, fatty acids, microbiological status, sensory characteristics, and elemental concentrations) of:

(1) Lipid type (soy bean oil, linseed oil, tallow, and fish oil),
(2) The period over which the fish oil was provided, and,
(3) A dietary supplement containing conjugated linoleic acid (CLA), selenium and vitamin E.

10.2 Materials and Methods

10.2.1 Pork

Details of the feeding regime and the dietary treatment groups were given in section 8.2.1, with a summary of the main features of the 6 treatment groups (7 or 8 pigs per group) in Table 8.1.

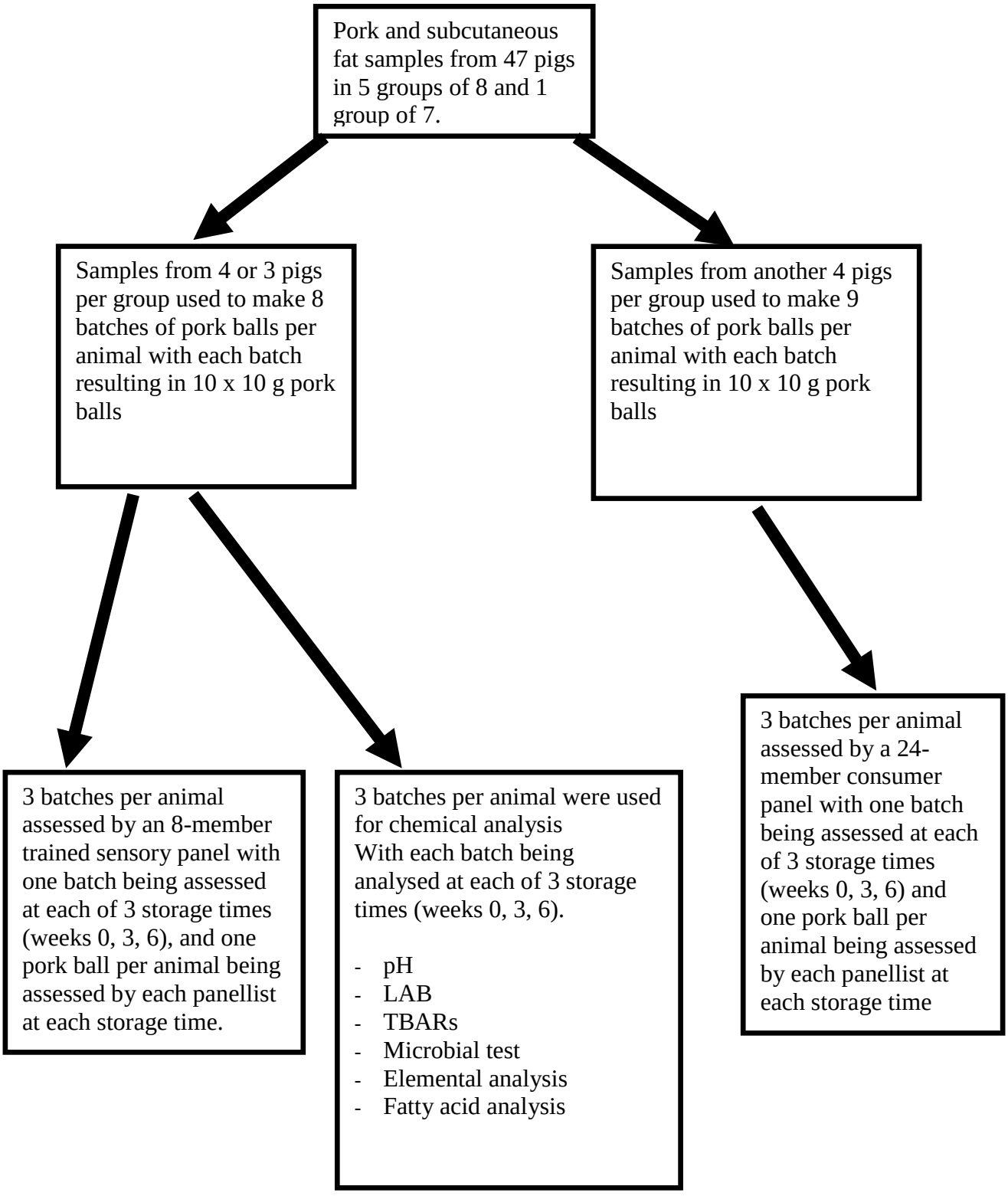
10.2.2 Processing of pork balls

Pork from the longissimus muscle of 47 animals was used in pork ball processing based on the methods described in Fig. 9.1. Samples from 23 animals (4 pigs per treatment – with 3 pigs in one treatment) were used to make 8 x 100 g batches of pork balls with each pork ball weighing 10 g (Fig. 10.1). Two batches of pork balls for each treatment were randomly selected for sampling on weeks 0, 3, and 6; one was assigned to determine the pH, colour, microbiological analysis, fatty acid profile and elemental content and another for sensory evaluation by a trained panel. The last 2 batches were considered as spares.

The remaining 24 animals (4 pigs per treatment) were used to make 9 x 100 g batches of pork balls. Three batches were used for sensory evaluation by a consumer panel following 0, 3, and 6 weeks of chiller storage at 4°C.

All batches of samples were vacuum packed (Innovac, model : IV-251, Germany) into bags of five-layer PE–LD/ ADH /PA/ADH/PE–PD film (total thickness: 0.08 mm; PA layer thickness: 0.024 mm; oxygen permeability: 40 $mL/m^2/24$ h/bar; water vapour permeability: 10 $g/m^2/24$ h/bar). The vacuum-packed pork balls were placed in a temperature-controlled laboratory refrigerator with an external data logger, and held at 4 ± 1^oC in the dark for up to 6 weeks.

Fig. 10.1 Animal involvement in pork ball processing.



10.2.3 Sensory Evaluation

Sensory evaluation was conducted by an 8-membered trained panel over a period of 2 days, with 3 sessions per day, and 4 samples per session. Each panellist received one pork ball per animal. Before consumption, the pork balls were removed from the packaging and were placed in a pot containing boiling water. They were taken out once the internal temperature reached 75°C. The samples were evaluated for colour, aroma, flavour, tenderness, juiciness, and off-flavours using a 150 mm line scale.

After cooking for 2.5 mins to an internal temperature of 75^{o}C, the samples were transferred to small plastic cups (30 mL) which were covered with plastic lids. All samples were placed in a forced-air oven at 60^{o}C to equilibrate prior to tasting. To evaluate the aroma of the pork ball, the panellists opened the plastic lids and smelled the head space of the samples. Plastic disposable spoons were used to scoop the samples and evaluate the flavour/taste of the samples. The full descriptions for the sensory characteristics of the pork ball are given in Table 10.1 and Appendix 10.1.

The pork balls were also evaluated by a 24-membered consumer panel over a period of 2 days, with 3 sessions per day and 4 samples per session. Each panellist evaluated one pork ball per animal. The fish oil supplemented samples were evaluated at the third (last) session for each day as these were much stronger flavoured products compared to the others. Sample preparation of pork balls for consumer evaluation was similar to that for the trained panel. The acceptability level of aroma, flavour and overall acceptability of pork balls was assessed using a scale of 1 to 9, where 1 was dislike extremely and 9 was like extremely.

10.2.4 Chemical analysis

The procedures for TBARs, pH, colour measurement, fatty acid and elemental analysis were as described in section 8.2.4.

10.2.5 Microbiological Analysis

A 10-g minced sample of each product was taken aseptically and placed in a sterile stomacher bag containing 90 mL of Ringer's solution. The sample in the bag was blended in a laboratory blender (Stomacher 400, Seward Limted, London, UK) for 2 min and submitted to serial dilutions with the same diluent. One mL of each dilution was placed in Petrifilm^{TM} AC (3M Microbiology. St. Paul, MN, USA) and YM for total aerobic count and yeast and mould count respectively, following the manufacturer instructions. For the enumeration of total aerobic count, two plates were incubated at 35 ± 1ºC for 48 ± 3h for aerobic mesophilic microorganism counts, and two at 7 ± 1ºC for 10 days for psychrotrophic microorganism counts. For the enumeration of yeast and mould count, two petrifilms were incubated at 35 ± 1ºC for 60 ± 3h for aerobic mesophilic microorganism counts. Petrfilms presenting 25 to 250 red colonies were selected and counted. All microbial counts were reported as colony forming units per gram of samples. Microbial populations in the samples were determined in triplicate at their raw state, and after the first cooking and second cooking after storage at 4 ± 1^{o}C for 0, 3 and 6 weeks.

10.2.6 Statistical analysis

Statistical analysis was performed with the GLM procedure of SPSS ver. 17. The statistical model included fixed effects of treatment (n=6), an animal effect (n=24) and days of storage (n=3) as repeat measures. The interaction between storage period and treatment was evaluated for all variables.

A set of 5 non-orthogonal contrasts between treatments was evaluated as follows:

1. A comparison between the group of pigs receiving a diet containing animal and plant components (the animal group) and the group receiving a diet containing plant components only (the plant group) [AT vs. PO].

2. A comparison between the control and supplemented diet groups within the plant group [PO vs. POS].

3. A comparison between the group receiving the diet containing tallow and that containing plant oils [PTS vs. POS].
4. A comparison between the groups receiving diets containing fish oil fed at an early or late stage and that containing plant components with supplemented diet [POS vs. [PFSe + PFSl]].

5. A comparison between the group receiving the diet containing fish oil fed early and that fed at a late stages [PFSe vs. PFSl].

Least squares means of six treatment groups across storage periods and least squares means for the three storage times across the six treatment groups were obtained. Means were considered statistically different at $p<0.05$.

Table 10.1

Definitions of the sensory attributes of pork ball developed by the trained panellists during training, together with the anchor points at each end of the scale

Sensory attributes	Interpretation	Anchor points
Colour attributes		
Colour	Degree of brownness[1]	Yellow / Brown
Colour saturation	Degree of darkness/lightness[1]	Light/Dark
Aroma attributes		
Meaty aroma	Aromatic associated with cooked meat[a]	None / Strong
Brothy aroma	Aromatic associated with pork cooked in water[a]	None / Strong
Cuttlefish aroma	Aromatic associated with dry preserved cuttlefish[a]	None / Strong
Metallic aroma	Aromatic associated with presence of iron ions (blood)[a]	None / Strong
Acidic aroma	Aromatic associated with presence of citric acid[b]	None / Strong
Mutton aroma	Aromatic associated with presence of mutton[a]	None / Strong
Rancid odour	Atypical aroma generally associated with deterioration of quality fats and oil[b]	None / Strong
Taste/flavour attributes		
Meaty flavour	Sensation associated with cooked meat[a]	None / Strong
Brothy flavour	Sensation associated with pork cooked in water[a]	None / Strong
Cuttlefish flavour	Sensation associated with raw garlic[b]	
Metallic flavour	Sensation associated with the presence of iron ions (blood)[b]	None / Strong
Acidic flavour	Taste on the tongue associated with citric acid[b]	None / Strong
Mutton flavour	Sensation associated with cooked mutton[a]	None / Strong
Rancid flavour	Atypical taste generally associated with deterioration of quality of fats and oils[b]	None / Strong
Salty taste	Taste on the tongue associated with sodium chloride[b]	None / Strong
Aftertaste	Sensation of lingering taste on the tongue after ingestion[b]	None / Strong
Texture attributes		
Cohesivesness	Degree of resistance to breakdown[2]	None / Strong
Chewiness	Amount of work to chew the sample for swallowing[1]	None / Strong
Hardness	The force required to bite through using molars[1]	None / Strong
Juiciness	Sensation of presence of moisture[2]	None / Strong

[a]Definitions as developed by the panellists

[b]Meilgarrd and others (1999)

10.3 Results and Discussions

10.3.1 Microbiological analysis

The pork ball as developed for this project, is a pre-cooked product which requires storage at chilled temperature. This is a ready-to-cook product that has gone through one round of thermal processing. A second round of cooking is necessary before consumption. The shelf life of commercial pork balls is about two months under chilled storage conditions or six months under frozen conditions.

Based on the Singapore Food Regulation (edition 2002), the maximum allowable bacterial count for processed meat products is 10^6 per g of food sample. The initial contamination of the samples with mesophilic bacteria (Table 10.2) was $10^3 – 10^5$ cfu/g (raw meat balls). More aerobes in the samples were destroyed during the second cooking. The counts of mesophilic bacteria before the second cooking increased as storage period increased. Yilmaz *et al.* (2005) observed a reduction in the count of mesophilic bacteria following heat treatment (grilling, microwave heating, traditional cooking) of meat balls.

The counts of psychrotrophic bacteria increased in the samples as storage period increased but counts were low compared to mesophilic bacteria. Throughout, the counts of psychrotrophic bacteria remained at a similar level in the samples for all treatments. There may be sublethal damage of some psychrotrophic bacteria during heating with recovery during cold storage (Farber, 1991). Some psychrotrophic bacteria, which are relative anaerobes, may develop even at a substantially reduced oxygen concentration. Most often, these are micrococci and lactic acid bacteria, whose metabolites have no negative effect on the sensory properties of foods. Lin and Lin (2002) did not observe a significant effect of storage time on the count of psychrotrophic bacteria in vacuum-packed sausages. Yeasts were totally destroyed during thermal processing. No fungal growth was recorded over storage. Yilmaz *et al.* (2005) also observed the destruction of fungi in heat-treated meat balls.

Table 10.2

The effect of 3 storage times (0, 3 and 6 weeks at 4^{o}C) on the microbial load of vacuum-packed pork balls. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD.

Item[1,5]	Treatment (trt) groups[2]						Trt effects (p-value)	R^2, RSD
	AT	PO	POS	PTS	PFSE	PFSl		
Total mesophilic[3,4] bacteria (cfu/g)								
Week 0	75	68	57	50	20	32	0.060	54.7, 21.5
Week 3	567	469	351	254	359	326	0.288	37.0, 163
Week 6	875	728	952	837	1267	870	0.568	25.1, 355
Total psychrotrophic[4] bacteria (cfu/g)								
Week 0	21	12	30	9	8	12	0.104	49.5, 12.3
Week 3	204	165	155	176	127	136	0.767	17.4, 68.1
Week 6	375	225	260	297	358	342	0.708	19.7, 133

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2]AT=diet containing animal and plant products; PO= diet containing plant products only; POS=diet containing plant feed products and Sanovite^{TM}; PTS=diet containing plant products with tallow; PFSe=diet containing plant products and fish oil (early stage of grower period); PFSl=diet containing plant products and fish oil (late stage of grower period).
[3]Mesophilic bacteria was $10^3 - 10^5$ cfu/g for raw pork ball; with none detectable (ND) after first cooking
[4]Microbial load as shown here was before 2nd cooking after storage periods of 0, 3, and 6 weeks.
[5]Not Detected for yeast and mould count for week 0, 3, and 6

10.3.2 pH

There was a significant decrease in pH as storage period increased (Table 10.4) ($p<0.001$), probably due to the growth of lactic acid bacteria in the vacuum-packed samples. There were no significant differences in pH among the treatment group (Table 10.3) ($p=0.60$).

According to Borch et al. (1996) and Korkeala & Björkroth (1997), lactic acid bacteria are the major bacteria group associated with the spoilage of cooked, cured meat products packed in vacuum and stored at chill temperatures. Samelis et al. (2000) showed a very good correlation between lactic acid bacteria growth and the product pH. According to Kuo and Chu (2003) and Santos et al. (2005), the counts of lactic acid bacteria increase in vacuum-packed meat products during storage, which results in lactic acid accumulation followed by a reduction in pH. The results of the present study are consistent with those obtained by other authors (Houben & Van-Dijk, 2001; Jeun-Horng et al., 2002; Pexara et al., 2002; Karpin'ska-Tymoszczyk, 2007; Kuo and Chu, 2003; Santos et al., 2005; Michaelsen et al. 2006) who reported a gradual decrease in pH values of vacuum-packed meat products as the time of cold storage was prolonged.

10.3.3 Colour

Storage period had an impact on the colour indices (Table 10.4), with a slight increase in lightness and a significant increase in yellowness, and redness from week 0 to week 3 and then a decrease to week 6. Lightness tended to increase with storage time, whereas yellowness remained constant from week 3 and 6. A similar outcome was observed in work by Rosenvold & Andersen (2003) and Lindahl et al., (2006).

Meat tends to turn brown (decreased in redness) over time. Colour changes during storage of pork are mainly related to oxidation of myoglobin to MetMb (Lindahl et al., 2006a, 2006b). The accumulation of MetMb on the surface of pork upon further storage affects redness, which decreases with storage time (Rosenvold & Andersen, 2003; Lindahl et al., 2005).

Pork balls with selenium, and vitamin E in the diet of the pigs that the pork came from did not further improve the colour stability. Some studies have shown improved colour stability after vitamin E supplementation, whereas several studies have shown no effect on pork as reviewed by Jensen, Lauridsen & Bertelsen, (1998), Faustman & Wang (2000) and Rosenvold & Andersen (2003). More recent studies with vitamin E supplementation have also shown limited effects on pork colour (Hasty et al., 2002; O'Sullivan et al., 2002; Geesink et al., 2004; Swigert et al., 2004).

10.3.4 TBARs analysis

The results for TBARs analysis for pork balls were similar to those for the longissimus muscle and subcutaneous back fat as reported in Section 8.4.3.

There was a significantly lower oxidative stability of pork balls from the AT group compared to the PO group. TBARs values were also higher for the PO diet group than the POS group (0.516 vs. 0.309 mg MDA/kg at $p<0.001$) and PTS (0.270 mg MDA/kg at $p<0.001$). This showed that the supplemented diet containing vitamins C, and E and Se had a positive effect in increasing the oxidative stability of pork balls. As for the effects of vitamin E on oxidative stability of pork, many studies (summarised in Table 2.7) have reported increased stability when diets of pigs have been supplemented with vitamin E, usually in the form of α-tocopherol.

The significantly lower oxidative stability of the pork balls from the fish oil diet group compared to the POS group was probably due to the higher content of PUFA in the pork used. There was a marked difference in TBARs between pork balls made from pork from pigs on a fish oil diet and those not (POS). Besides fish oil, other types of PUFA in plant oils reduce oxidative stability in meat and meat products. Many studies have also linked this oxidative instability with increasing concentrations of PUFA (summarised in Table 2.5).

Table 10.3

Least squares means for pH, colour indices (L*, a* and b*) and TBARs (mg MDA/kg) measurements averaged across storage periods (0, 3, and 6 weeks at 4°C) and their contrast statistics for pork balls from pigs fed diets containing animal and plant products with or without a dietary supplement (S), tallow (T) or fish oils (Fe & Fl)). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation , RSD

Item[1]	Treatment (Trt) group[2]						Diet Effect (p-values)	R^2, RSD	Contrast statistics [3] (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
pH	5.73	5.72	5.75	5.83	5.81	5.85	0.60	53.0, 0.23	0.89	0.74	0.35	0.54	0.65
L*	72.04	71.83	73.87	73.57	72.44	72.37	0.74	39.0, 3.93	0.93	0.27	0.72	0.17	0.94
a*	3.35	2.93	3.23	3.12	3.48	3.57	0.06	14.8, 0.54	0.06	0.25	0.67	0.30	0.70
b*	10.33	10.15	10.19	10.50	10.84	10.34	0.32	10.3, 0.79	0.56	0.89	0.31	0.18	0.18
TBARs	0.400bc	0.516d	0.309ab	0.270a	0.395bc	0.474cd	<0.001	38.7, 0.11	0.033	<0.001	0.14	0.007	0.17

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

[3]AT vs. PO – Animal vs. Plant with no supplementation

PO vs.POS - no supplementation vs. supplementation within plant

POS vs. PTS – Tallow vs. plant oils within plant supplementation

POS vs. [PFSe + PFSl] – no fish oil vs. fish oil within plant supplementation

PFSe vs. PFSl – Early fish oil vs. late fish oil within plant supplementation

Table 10.4
Least squares means for pH, colour indices (L*, a* and b*) and TBARs measurements (mg MDA/kg) for the 3 storage times (week 0, 3, and 6 at 4°C) average across the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl);and effects of treatment (Trt) group, storage period (Time), and their interaction (Trt x Time) of pork balls made from pigs fed diets containing animal and plant products with or without a dietary supplement, tallow or fish oils. Measures of the overall goodness-of-fit for the model include the coefficient of determination [R^2(%)] and the residual standard deviation, RSD

Item[1]	Storage time (Weeks)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
pH	6.02c	5.79b	5.53a	0.60	<0.001	0.47	53.0, 0.23
L*	71.18a	73.36b	73.53b	0.74	0.041	0.68	39.0, 3.93
a*	3.07a	3.42b	3.35ab	0.06	0.003	0.31	14.8, 0.54
b*	9.88a	10.60b	10.69b	0.32	<0.001	0.12	10.3, 0.79
TBARs	0.308a	0.423b	0.451b	<0.001	<0.001	0.17	38.7, 0.11

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

10.3.5 Fatty profile analysis

10.3.5.1 Diet effects

In this study, the highest total fatty acid (close to 87%) was found for the AT diet group and the lowest proportion for the PFSe and PFSl groups (Table 10.5) (78.7% and 77.4). In general, all plant-diet groups showed higher PUFA and lower SFA and MUFA compared to AT, but many of these comparisons were not statistically significant (Table 10.7).

(a) *AT vs. PO*

Pork balls made from pork from the AT group had a higher level of palmitic acid than PO (Table 10.5) ($p=0.02$). In contrast, the PO group had higher levels of n-6 eicosatrienoic acid, DPA and DHA ($p<0.05$). From Table 10.7, AT pork balls showed higher levels of SFA and lower PUFA compared to PO. Raes, De Smet, and Demeyer (2004) noted that as fatness increases, the levels of SFA increased faster than the PUFA levels, leading to an increase in the relative proportions of SFA.

A significant difference was observed in n-6 eicosatrienoic acid between AT vs. PO ($p<0.05$). The concentration of n-6 eicosatrienoic acid was almost two times lower than n-3 eicosatrienoic acid in the current study (Table 10.5). The results were similar to that in intramuscular fats and subcutaneous back fat in the longissimus muscle as shown in Tables 8.8 and 8.9.

The DHA levels in the pork balls were 45% higher for the PO group compared to the AT group. This finding was similar to that of Enser et al. (2000) who demonstrated that feeding linseed high in α-linolenic acid (4.0 g/kg) increased DHA levels 50% in adipose tissue and 35% in muscle when compared with a control diet with only 1.9 g/kg α-linolenic acid. In contrast, this observation was not seen in studies by Riley et al. (1998a), Riley et al.

(1998b), Ahn, Lutz, and Sim (1996), and Specht-Overholt et al. (1997). The DPA levels in the pork balls were 65% higher for the PO group compared to the AT group, which is consistent with the results obtained for intramuscular fat in the longissimus muscle and subcutaneous back fat (Tables 8.8 and 8.9).

The EPA, DPA and DHA levels in pork balls made with pork from the AT diet group were significantly lower than that from the PO diet group. This could be due to the presence of antioxidants like selenium, vitamin C and E. The findings were similar to those for intramuscular fat in the longissimus muscle (Section 8.4.5.1a).

In terms of fatty acid profiles, the AT group had higher SFA ($p=0.033$), and n-6/n-3 ratios ($p=0.013$) than the PO group, while the PO group had higher levels of (EPA+DPA+DHA) ($p=0.003$) compared to the AT group (Table 10.7).

(b) *PO vs. POS*

Relative to the PO group, pork balls from the POS group had significantly lower levels of cis-11-vaccenic acid, trans-11-vaccenic acid, n-3 eicosatrienonic acid and n-6 eicosatrienoic acid (Table 10.5). The levels of linoleic and α-linolenic acid in pork balls were not significantly different between the POS and PO groups (Table 10.5) as they were in intramuscular fats of longissimus muscle and subcutaneous back fat (Table 8.8 and 8.9).

In addition, the concentration of n-6 eicosatrienoic acid was almost two times lower than n-3 eicosatrienoic acid for all dietary treatments in the current study. The trend is similar to the one given for AT vs. PO in the previous section (a) above.

(c) POS vs. PTS

The PTS group showed a significant increase in myristic acid, stearic acid, palmitoleic acid, elaidic acid, cis-11 vaccenic acid and trans-11 vaccenic acid compared to the POS group. This reflects the replacement of plant oil by tallow which comprises about 13 kinds of fatty acids, including 45% of unsaturated fatty acids of which 40.5% are monounsaturated fatty acids (Berliz, Grosch & Schieberle, 2009).

The PTS group showed a significant decrease in DPA as the amount of linseed fed was reduced and partially replaced by tallow (Table 10.5). In vertebrates, the essential fatty acid α-linolenic acid can be converted to longer and more unsaturated n-3 PUFA, such as EPA, DPA and DHA (Sprecher, 2000). Among the plant groups, PTS had the lowest omega-3 fatty acids level as some of the plant oils had been replaced by tallow which would contain very little omega-3 fatty acids. .

POS showed a significantly higher P:S ratio ($p=0.029$) and (EPA+DPA+DHA) ($p=0.03$) compared to PTS which in turn had higher SFA than the POS group ($p=0.026$) (Table 10.7).

(d) POS vs. [PFSe + PSFl]

Levels of EPA, DPA and DHA in the pork balls were from 2 to 6 times higher in the PFSe and PFSl groups compared to the POS group ($P<0.001$; Table 10.5). This is in accordance with the LC-omega-3 fatty acids levels in intramuscular fats in the longissimus muscle and subcutaneous back fat (Table 8.8) as well as with the results of Hallenstvedt et al (2001), Lauridsen et al. (1999) and Kjos et al. (1999).

Increases in PUFA when feeding linseed and fish oil containing diets were inversely mirrored by reductions in MUFA in some plant groups for the study of Waters, Kelly, O'Boyle, Moloney & Kenny (2009), probably due to inhibition of delta-9 desaturase activity as the level of n-3 fatty acids increased in the diet (Table 10.7).

The total PUFA, n-6/n-3 ratio, and (EPA+DPA+DHA) proportion in the pork balls was significantly greater for the two fish oil groups compared to POS (Table 10.7), which is consistent with the greater incorporation efficiency of EPA and DHA from fish oil than of α-linolenic from linseed (Haak, Smet, Fremaut, Walleghem & Raes, 2008). The findings for these fatty acid proportions were similar to those for intramuscular fats in the longissimus muscle and subcutaneous back fat (Table 8.12). In this study, the highest P/S ratio was found for the PFSe and PFSl groups, as a consequence of their higher PUFA and lower SFA values Table 10.7). The lowest n-6/n-3 ratio was also shown for these two groups. From a consumer-health viewpoint, the recommended value for P:S ratio is 0.4 or higher, as achieved by pork balls of all groups (Table 10.7), and 4 or below for the n-6/n-3 ratio, as achieved by pork balls of all groups except AT (Table 10.7) (Department of Health, 1994).

(e) PFSe vs. PFSl

In Table 10.5, the PFSl samples contained higher levels of EPA and DHA in the pork balls in contrast to those in group PFSe ($p<0.05$). This result agrees with those for the intramuscular fat of the longissimus muscle and subcutaneous back fat (Tables 8.8, 8.9) and it also agrees with the work by Jaturasitha et al. (2008) who reported that more DHA and EPA were present in the fats when fish oil was fed at later rather than earlier stages of pig growth. Valaja et al. (1992) also revealed that length and stage of feeding had effects on the levels of PUFA.

In addition, SFA was significantly higher in PFSe than in PFSl ($p=0.006$) while ATT was significantly higher for the PFSl group than the PFSe group ($p=0.009$) (Table 10.7).

(f) Storage effects

There were storage effects on arachidic acid, palmitoleic acid, cis-11 vaccenic acid, trans-11 vaccenic acid, cis-9, trans-11 CLA, γ-linolenic acid, cis-11, 14 eicosadienoic acid

and arachidonic acid ($p<0.05$) with mainly the MUFA and PUFA decreasing (except for trans-11 vaccenic acid) as storage period increased (Table 10.6). These fatty acids, especially the PUFAs, are more highly susceptible to lipid oxidation than MUFAs or SFAs (Higgins, Carroll, O'Brien & Morrissey, 1999; Whittington et al., 1986; Rhee et al., 1988) due to the increased degree of unsaturation..

Interactions between treatment and time was observed for trans-11 vaccenic acid ($p=0.01$), γ-linolenic acid ($p=0.028$) and cis-11, 14 eicosadienoic acid ($p=0.036$).

From the interaction plots (Fig. 10.2), the POS group had the least changes, while the PFSe group had the largest changes over 6 weeks for trans-11 vaccenic acid, where levels increased as storage period increased. In addition, γ-linolenic acid in the AO group increased as storage period increased, but the opposite trend was observed for the rest of the five dietary treatments. An increasing trend with time in levels of cis-11, 14 eicosadienoic acid was shown in the AO and POS groups, for a decreasing trend was observed for the other four groups. Changes in cis-11, 14 eicosadienoic acid were the least over the storage period as compared to trans-11 vaccenic acid and γ-linolenic acid.

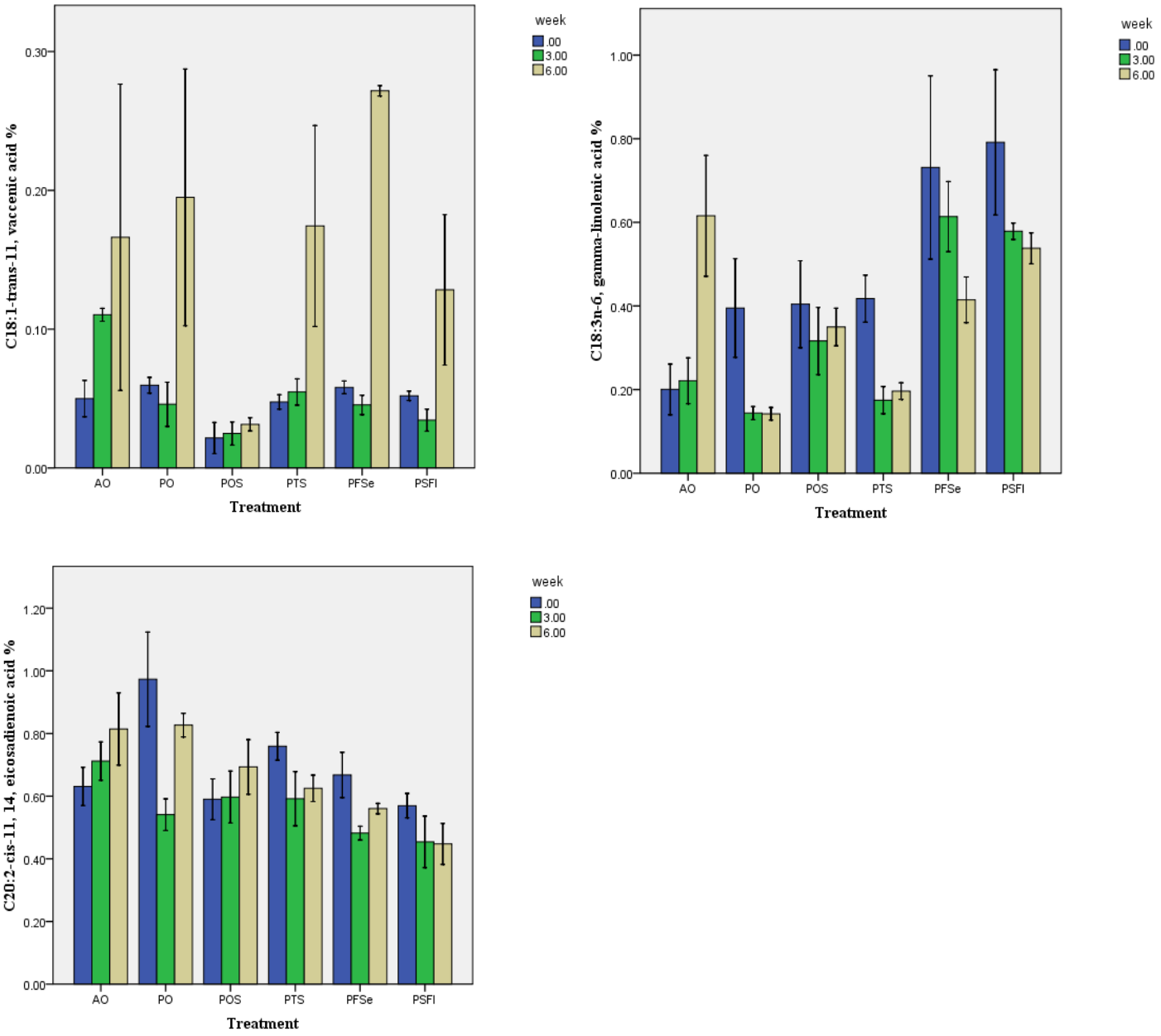


Fig. 10.2
Interaction plots showing the effect of treatment[1] and storage period (0, 3 and 6 weeks at 4^{o}C) on trans-11 vaccenic acid, γ-linolenic acid and cis-11, 14 eicosadienoic acid (%) in pork balls (mean±SE)

[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite^{TM}; PTS=diet with plant products, tallow & Sanovite^{TM}; PFSe=diet with plant products, Sanovite^{TM} and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite^{TM} and fish oil (during the late grower period).

Table 10.5

Least squares means for total fatty acids (total fatty acids as a % of total lipid) and fatty acid contents (% of total fatty acids) in pork balls for the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the 3 storage times (week 0, 3, 6 at 4°C). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for the 3 storage times are given in Table 10.6

Item[1]	Treatment (Trt) group						Effects (p-value)	R^2, RSD	Contrast (p value)				
	AT	PO	POS	PTS	PFSe	PFSl	Trt		AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Total fatty acids (%)	86.8c	83.6bc	80.6ab	81ab	78.7ab	77.4a	0.003	53.7, 0.30	0.14	0.17	0.27	0.12	0.32
C14:0 myristic acid	1.66b	1.45ab	1.35a	1.66b	1.64b	1.35a	0.05	61.8, 1.66	0.13	0.56	0.028	0.031	0.002
C16:0, palmitic acid	19.70b	17.68a	16.76a	18.23a	17.65a	16.76a	0.001	62.0, 1.76	0.02	0.31	0.07	0.25	0.022
C18:0, stearic acid	8.60a	8.22a	8.97ab	10.90c	11.13c	10.22bc	0.003	54.4, 0.16	0.65	0.49	0.026	0.015	0.06
C20:0 arachidic acid	0.370	0.371	0.284	0.338	0.315	0.235	0.11	57.0, 0.19	0.98	0.29	0.58	0.50	0.10
C16:1- cis-9, palmitoleic acid	0.675a	0.578a	0.567a	0.860b	0.636a	0.640a	0.006	68.0, 4.21	0.32	0.90	<0.001	0.47	0.96
C18:1- cis-9, oleic acid	46.5c	46.73c	47.56c	44.57bc	40.23a	41.74ab	0.009	63.8, 0.10	0.90	0.72	0.20	0.002	0.34
C18:1- trans-9, elaidic acid	0.315b	0.252ab	0.199a	0.316b	0.273ab	0.200a	0.034	65.6, 0.08	0.23	0.22	0.014	0.05	0.030
C18:1- cis-11, vaccenic acid	0.109	0.100	0.026	0.092	0.125	0.072	0.09	64.0, 0.14	0.87	0.046	0.030	0.011	0.17
C18:1- trans-11, vaccenic acid	0.273ab	0.309b	0.151a	0.267ab	0.285ab	0.214ab	0.002	57.0, 2.19	0.63	0.019	0.003	0.15	0.37
C18:2-cis-9, 12, linoleic acid	12.9	13.11	13.71	12.86	13.96	14.57	0.65	65.6, 0.19	0.87	0.54	0.34	0.57	0.503
C18:2-cis-9, trans-11, CLA	0.493	0.514	0.561	0.714	0.634	0.591	0.14	64.1, 0.07	0.77	0.65	0.14	0.80	0.69
C18:2-trans-10, cis-12, CLA	0.211	0.167	0.138	0.174	0.154	0.112	0.26	73.4, 0.19	0.32	0.42	0.23	0.24	0.04
C18:3n-6 cis-6, 9, 12, γ-linolenic	0.346a	0.227a	0.357a	0.263a	0.587b	0.636b	<0.001	65.3, 1.18	0.21	0.07	0.12	0.011	0.64
C18:3n-3 cis-9, 12, 15, α-linolenic	2.50a	3.11ab	4.00bc	3.58abc	4.34c	4.58c	0.042	51.9, 0.44	0.36	0.17	0.45	0.45	0.44
C20:1-cis-11, eicosanoic acid	0.931abc	1.22bc	0.742a	0.859ab	1.20bc	1.275c	0.022	73.0, 0.14	0.27	0.08	0.26	<0.001	0.43
C20:2-cis-11, 14, eicosadienoic	0.719cd	0.780d	0.627abc	0.659bcd	0.570ab	0.490a	0.006	55.6, 0.12	0.50	0.09	0.59	0.05	0.13
C20:3n-6 cis-8, 11, 14, eicosatrienoic	0.342ab	0.463c	0.325ab	0.376bc	0.348b	0.244a	0.002	67.2, 0.21	0.002	0.019	0.45	0.09	0.006
C20:3n-3 cis-11, 14, 17, eicosatrienoic	0.757bc	0.907c	0.597ab	0.692b	0.610ab	0.479a	0.007	57.2, 0.11	0.19	0.007	0.21	0.33	0.19
C20:4n-6 cis-5, 8, 11, 14, arachidonic	0.112	0.195	0.129	0.101	0.109	0.068	0.16	89.0, 0.20	0.21	0.38	0.53	0.30	0.08
C20:5n-3 cis-5, 8, 11, 14, 17, EPA	0.117a	0.229a	0.284a	0.157a	0.888b	1.10c	<0.001	85.0, 0.26	0.05	0.58	0.15	<0.001	0.037
C22:5-cis-7, 10, 13, 16, 19, DPA	0.308a	0.508b	0.588b	0.379ab	1.24c	1.28c	<0.001	97.9, 0.10	0.021	0.40	0.010	<0.001	0.80
C22:6n-3-cis-4, 7, 10, 13, 16, 19, DHA	0.191a	0.277b	0.233ab	0.202ab	1.15c	1.33d	<0.001	53.7, 0.30	0.025	0.313	0.476	<0.001	0.011

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test

[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 10.6
Least squares means for fatty acids contents of pork balls for the 3 storage times (week 0, 3, 6 at 4°C) averaged across the 6 treatment groups(AT, PO, POS, PTS, PFSe and PFSl). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment groups are given in Table 10.5

Item[1]	Time (week)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
C14:0 myristic acid	1.51	1.52	1.54	0.05	0.95	0.85	53.7, 0.30
C16:0, palmitic acid	17.53	17.84	18.02	0.001	0.59	0.09	61.8, 1.66
C18:0, stearic acid	9.59	9.35	10.08	0.003	0.35	0.66	62.0, 1.76
C20:0 arachidic acid	0.417b	0.340b	0.200a	0.11	<0.001	0.91	54.4, 0.16
C16:1- cis-9, palmitoleic acid	0.74b	0.653ab	0.585a	0.006	0.027	0.73	57.0, 0.19
C18:1- cis-9, oleic acid	43.53	45.22	44.91	0.009	0.34	0.06	68.0, 4.21
C18:1- trans-9, elaidic acid	0.294	0.230	0.254	0.034	0.07	0.10	63.8, 0.10
C18:1- cis-11, vaccenic acid	0.304a	0.291a	0.154b	0.09	<0.001	0.38	65.6, 0.08
C18:1- trans-11, vaccenic acid	0.048a	0.053a	0.161b	0.002	0.001	0.01	64.0, 0.14
C18:2-cis-9, 12, linoleic acid	13.90	13.51	13.15	0.65	0.50	0.47	57.0, 2.19
C18:2-cis-9, trans-11, CLA	0.704b	0.466a	0.584ab	0.26	0.001	0.30	65.6, 0.19
C18:2-trans-10, cis-12, CLA	0.185	0.137	0.156	0.14	0.06	0.11	64.1, 0.07
C18:3n-6 cis-6, 9, 12, γ-linolenic	0.490b	0.341a	0.376a	<0.001	0.024	0.028	73.4, 0.19
C18:3n-3 cis-9, 12, 15, α-linolenic	4.11	3.66	3.28	0.042	0.06	0.98	65.3, 1.18
C20:1-cis-11, eicosanoic acid	1.08	0.932	1.10	0.022	0.35	0.88	51.9, 0.44
C20:2-cis-11, 14, eicosadienoic	0.698b	0.563a	0.661ab	0.006	0.004	0.036	73.0, 0.14
C20:3n-6 cis-8, 11, 14, eicosatrienoic	0.372	0.333	0.343	0.002	0.52	0.40	55.6, 0.12
C20:3n-3 cis-11, 14, 17, eicosatrienoic	0.742	0.654	0.625	0.007	0.15	0.11	67.2, 0.21
C20:4n-6 cis-5, 8, 11, 14, arachidonic	0.092a	0.174b	0.091a	0.16	0.015	0.40	57.2, 0.11
C20:5n-3 cis-5, 8, 11, 14, 17, EPA	0.459	0.497	0.431	<0.001	0.53	0.48	89.0, 0.20
C22:5-cis-7, 10, 13, 16, 19, DPA	0.743	0.775	0.633	<0.001	0.16	0.31	85.0, 0.26
C22:6n-3-cis-4, 7, 10, 13, 16, 19, DHA	0.562	0.585	0.541	<0.001	0.34	0.08	97.9, 0.10

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 10.7
Least squares means of fatty acid profiles in pork balls for the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the 3 storage times (week 0, 3, 6 at 4°C). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for the 3 storage times are given in Table 10.8.

Item[1, 2]	Treatment (Trt) gp[3]						Trt effects (p-value)	R^2, RSD	Contrast (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
SFA	30.33b	27.73a	27.36a	31.12b	30.74b	28.57a	0.004	54.9, 3.01	0.033	0.82	0.026	0.041	0.006
MUFA	48.79c	49.19c	49.25c	46.96bc	42.75a	44.14ab	0.011	66.7, 4.16	0.84	0.98	0.30	0.004	0.37
PUFA	19.01a	20.49a	21.55a	20.16a	24.58b	25.47b	0.010	67.9, 3.39	0.40	0.53	0.34	0.015	0.51
P:S	0.631a	0.753ab	0.812b	0.654a	0.802b	0.895b	0.017	58.4, 0.16	0.12	0.49	0.029	0.25	0.08
L:LN	6.18b	4.51ab	4.94ab	3.85ab	3.00a	2.96a	0.048	66.6, 0.99	0.09	0.95	0.68	0.05	0.64
EPA+DHA+EPA	0.62a	1.01b	1.10b	0.74ab	3.27c	3.71d	<0.001	83.6, 0.65	0.003	0.61	0.030	<0.001	0.08
n-6/n-3	4.39c	3.24b	3.07b	3.15b	2.00a	1.90a	<0.001	77.7, 0.78	0.013	0.66	0.84	<0.001	0.17
ATT	10.31a	9.21a	8.92a	8.88a	16.82b	28.59c	<0.001	71.6, 7.29	0.55	0.87	0.98	<0.001	0.009
L1	2.86a	3.20ab	3.48b	3.10ab	2.96a	3.14ab	0.040	55.5, 0.45	0.10	0.28	0.12	0.037	0.16

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2]SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P:S: PUFA vs. SFA; L:LN: Linoleic acid vs linolenic acid; ATT: antithrombotic potential ; I1:The ratio between the not hypercholesterolaemic major fatty acids (C18:0 + C18:1) and the major hypercholesterolaemic fatty acid (C16:0)
[3]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 10.8
Least squares means of fatty acid profiles for the 3 storage times (week 0, 3, 6 at 4°C) averaged across 6 treatment (trt) groups (AT, PO, POS, PTS, PFSe and PFSl); and effects of trt, time and trt x time on fatty acid profile of pork balls made from pigs fed diets containing animal and plant products with or without a dietary supplement, tallow or fish oils. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment groups are given in Table 10.7

Item[1,2]	Storage time (Weeks)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
SFA	29.05	29.04	29.84	0.004	0.58	0.28	54.9, 3.01
MUFA	45.99	47.38	47.17	0.011	0.47	0.07	66.7, 4.16
PUFA	23.06	21.70	20.87	0.010	0.09	0.75	67.9, 3.39
P/S	0.801	0.763	0.709	0.017	0.17	0.99	58.4, 0.16
L:LN	3.77	4.07	4.88	0.048	0.20	0.84	66.6, 0.99
EPA+DHA+EPA	1.76	1.86	1.61	<0.001	0.10	0.13	83.6, 0.65
n-6/n-3	2.80	2.85	3.22	<0.001	0.13	0.76	77.7, 0.78
ATT[1]	14.52	14.15	12.85	<0.001	0.65	0.55	71.6, 7.29
I1[2]	3.10	3.13	3.14	0.040	0.96	0.08	55.5, 0.45

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2]SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; P:S: PUFA vs. SFA; L:LN: Linoleic acid vs linolenic acid; ATT: antithrombotic potential ; I1:The ratio between the not hypercholesterolaemic major fatty acids (C18:0 + C18:1) and the major hypercholesterolaemic fatty acid (C16:0)

(g) Principal Component Analysis (PCA)

The results of the PCA based on means across the three storage times for all the fatty acids listed in Table 10.5 are shown in Fig. 10.3. The first two principal components (PC) explained 93.6% of the total variation in fatty acid composition. PC1 explained 66.4% of the total variability in fatty acid composition. This component is mainly characterised by DHA, EPA, DPA, cis-9 trans-11 and α-linolenic acid. PC2 explained 27.2% of the variability. It is defined by linoleic acid, arachidonic acid, myristic acid, stearic acid, palmitoleic acid and cis-11-vaccenic acid. Pork balls from the PFSe and PFSl group were clearly differentiated from the other diet groups and were located on middle right half of the figure. The rest of the diet groups were located in the left half of the figure. PO was found in the lower left quadrant while the rest in the upper half.

In summary, pork balls from AT and PTS groups were clustered together, PFSe and PFSl were within another group, while PO was on its own. POS seems to be closer to AT and PTS for the first PCA and closer to PFSe and PFSl on the second PC. A similar pattern for subcutaneous back fat of the longissimus muscle was shown in Fig. 8.5.

(h) Adequate intake of EPA, DPA and DHA

Details of the method for calculating adequate intake can be found in Section 8.5.4.5. A higher percentage of back fat in the pork product results in higher levels of (EPA+DPA+DHA) per gram pork product. Leaner products require higher amounts of daily intake to reach the target of 160 mg of (EPA+DPA+DHA) per day.

If pork products such as pork balls with 10% back fat from pigs fed diets PFSe or PFSl were consumed, then a daily intake of 59 g (equivalent to 6 pork balls) and 48 g (equivalent to 5 pork balls), respectively, would be sufficient to achieve an intake of 160 mg of (EPA+DPA+DHA)/day. Greater intakes would be needed if similar pork balls made from pork and back fat from pigs fed AT (343 g/d), PO (265 g/d), POS (296 g/d) or PTS (393 g/d) diets were consumed (Fig. 10.4).

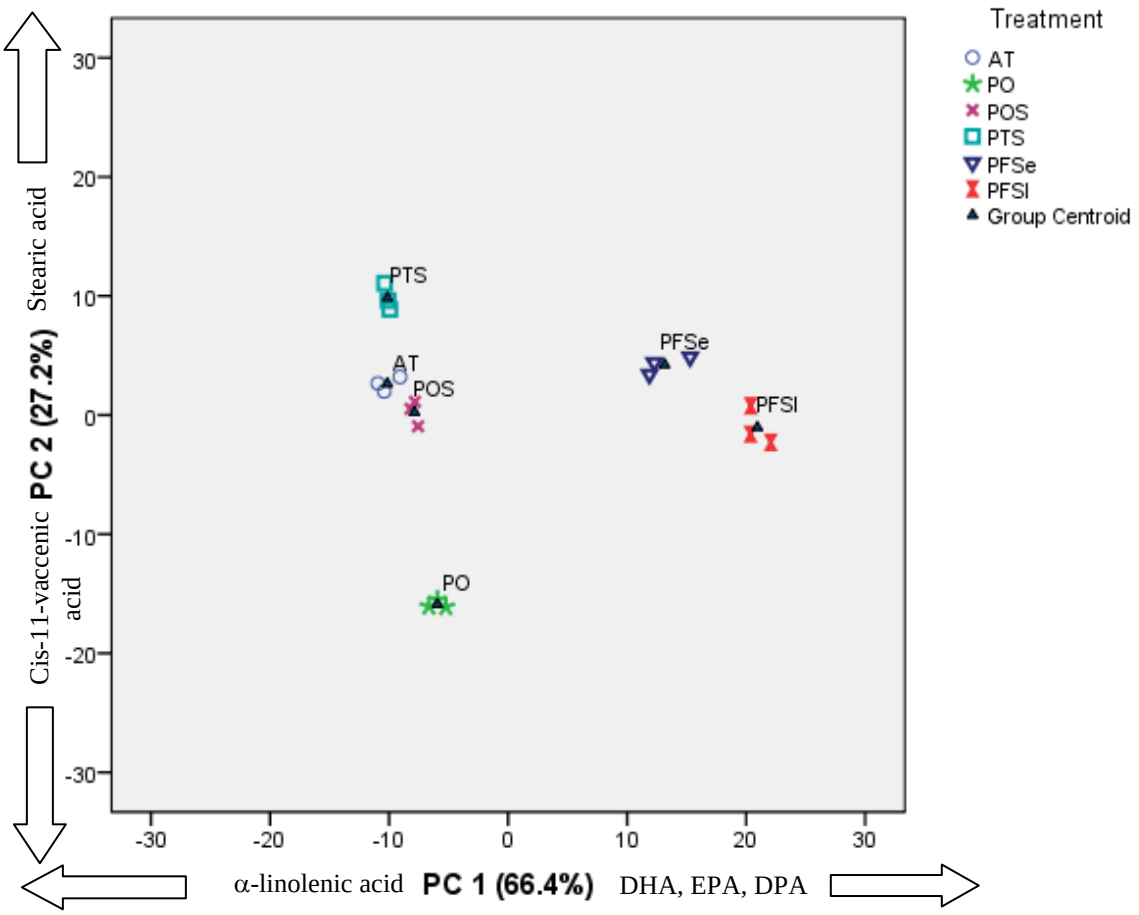


Fig. 10.3

Projection of fatty acids of the 6 diet groups[1] (based on the least squares means over the 3 storage periods of 0, 3, & 6 weeks at 4°C) studied in the plane defined by two principal components . Values are shown for individual animals (n = 4/treatment group) as well as the group centroids.

The first two principal components (PC) explained 93.6% of the total variation in fatty acid composition.
PC1 explained 66.4% of the total variability in fatty acid composition and was defined mainly by DHA, EPA, DPA, cis-9 trans-11 CLA and α-linolenic acid.
PC2 explained 27.2% of the variability and was defined by mainly linoleic acid, arachidonic acid, myristic acid, stearic acid, palmitoleic acid and cis-11-vaccenic acid.

[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™ ; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period)

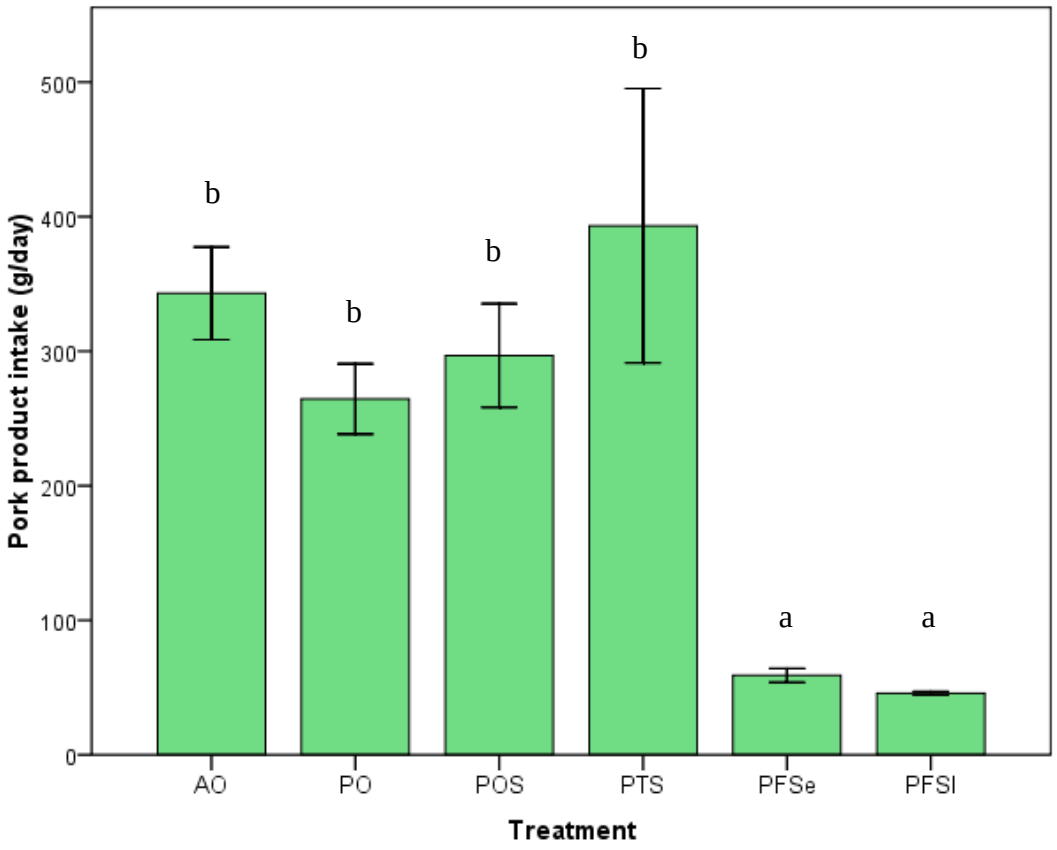


Fig. 10.4

The intake of a pork balls (g/day) (mean±SEM) for 6 dietary treatment groups[1] that was required to achieve an intake of 160 mg of (EPA+DPA+DHA)/day when percentage of back fat in the pork balls is 10%.

[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

10.3.6 Element analysis

The concentrations of mineral elements (expressed as μg g^{-1}) in pork balls from pigs receiving different diets (Table 10.9) showed no significant diet-group effects except for selenium which was higher for the groups that had received fish oil. Potassium chloride was one of the ingredients in pork balls, and hence the potassium concentrations were high compared to the concentration in the longissimus muscle alone at (3900-4300 μg g^{-1} (Table 8.7). Levels in the same muscle ranged from 4000 to 4500 μg g^{-1} in the report of Purchas et al. (2009). Magnesium concentrations in the pork balls were very high compared to those in the longissimus muscle at 202-218 μg g^{-1} (Table 8.7) or the range of 200-300 μg g^{-1} reported by Purchas et al. (2009). This could possibly be attributed to the presence of magnesium in the flavouring ingredients used. Its presence does not pose an issue as magnesium is not a pro-oxidant like most transition elements (Packer, 1995; Marnett, 2000). Concentrations of potassium and magnesium were significantly different between the pork balls made from pork from the PFSe and PFSl groups, with higher values for the PFSl group (Table 10.9). The difference was not observed for the longissimus muscles (Table 8.7). Selenium content of muscle in the current study was markedly increased as dietary Se level increased. This finding agrees with the elemental analysis of longissimus muscle in Section 8.4.4. Selenium supplementation significantly increased selenium in the longissimus muscle in the two fish oil groups with relative to the POS group ($p<0.05$; Table 8.7). Furthermore, selenium content was higher as dietary fish oil was fed later ($p<0.05$; Table 10.9; PFSe vs PFSl).

10.3.7 Sensory Evaluation

(a) *AT vs. PO*

For the trained panel, the AT group had a stronger meaty flavour but a lower acidic aroma compared to the PO group (Table 10.10; $P < 0.04$). In contrast when the longissimus muscles from these two groups were tasted, no significant differences were shown for these two attributes (Table 8.14).

As for the comparison between pork balls from the AT and PO groups among the consumer panellists, aroma liking was significantly different in weeks 3 ($p<0.05$) and 6 ($p<0.001$) with PO having a higher aroma liking score in week 6. Overall, the acceptability scores for the PO group were higher than the AT group. This is in agreement with the study in Chapter 8 with longissimus muscle (Section 8.4.6.1b) and in Leong et al, (2010) where pork from pigs fed a diet containing some animal products was less preferred than that from pigs fed a diet with plant items only.

(b) *PO vs. POS*

From the contrast between PO and POS by the trained panel, it can be seen that the supplementation with CLA, vitamin E, selenium, and vitamin C had an appreciable effect on mutton aroma only ($p=0.04$) with the PO group having a higher mutton intensity than POS group. This result agreed with that for longissimus muscles from these groups (Table 8.14). The presence of supplementation could have reduced metabolite formation due to oxidation, which may have contributed to the mutton note.

The consumer panel (Table 10.12), significantly preferred pork balls from the POS group than the PO group in week 0, possibly due to the presence of selenium which prevents oxidative rancidity of PUFA, resulting in rancid off-flavour. Aroma was significantly preferred for POS than PO in week 3 ($p=0.02$) but PO was preferred to POS in

week 6 ($p<0.001$). There is no obvious explanation for these apparently contradictory results.

(c) POS vs. PTS

Results with the trained panel showed that mutton aroma, mutton flavour, cohesiveness and aftertaste were more intense for pork balls of the PTS group than the POS group ($p<0.05$). Aftertaste was positively correlated to mutton aroma ($r=0.486$), and mutton flavour ($r=0.739$).

Aroma and flavour liking as well as acceptability scores were significantly higher for the POS group than the PTS group in week 0 ($p<0.001$), as rated by the consumer panel (Table 10.12). Aroma liking was also significantly preferred in week 3 ($p=0.001$) for the POS group. In the final week, POS was still more acceptable than PTS ($p=0.003$). This could be due to the stronger mutton aroma and flavour in PTS (Table 10.10) which led to lower acceptability scores compared to the POS group and suggests that tallow in the diet was at least partly responsible for differences between AT and PO.

(d) POS vs. [PFSe+PFSl]

There was a significant difference in cohesiveness, chewiness and hardness ($p<0.05$) between POS vs. [PFSe+PFSl] ($p<0.05$) with POS samples being less chewy and cohesive, but harder. The pork balls from the PFSe and PFSl groups had higher aftertaste scores than POS samples ($p<0.001$), and aftertaste was positively correlated to rancid aroma ($r=0.635$), rancid flavour ($r=0.872$), mutton aroma ($r=0.486$), mutton flavour ($r=0.739$), cuttlefish aroma ($p=0.739$) and cuttlefish flavour ($p=0.873$). Cuttlefish aroma and flavour were strongest for the fish oil groups (PFSl > PFSe) due to the strong fishy note from the fish oil that was fed to the pigs. The presence of the fishy attributes has enhanced the cuttlefish character in the pork balls. Jaturasitha et al. (2008) also showed that pork

from pig fed fish oil at a later stage has a stronger fishy flavour. Meaty and brothy flavours were lowest for the PFSe and PFSl, possibly due to the masking effects from the rancid flavour and aftertaste for those groups. Meaty and brothy aromas and flavours, which were strongly correlated, seemed to decrease as the scores for the rancid attributes increased. Amongst the plant diets, it was shown that meaty and brothy attributes were negatively correlated with cuttlefish and mutton attributes. There was a negative correlation between mutton flavour and cuttlefish flavour (r=-0.679).

Aroma liking was significantly preferred by the consumer panel for pork balls in the POS group than the two fish oil groups in week 3. The presence of fishy notes could have led to the negative response by the consumer panel in the current study. Many studies had linked the presence of fishy notes in pork to low acceptability by consumers as summarised in Table 2.4 of Section 2.3.3.

(e) *PFSe and PFSl*

Organoleptic evaluation of the pork balls for the PFSe and PFSl groups by the trained panel revealed no major differences in sensory quality except in chewiness ($p=0.006$) and cohesiveness ($p=0.018$), both of which were greater for the PFSl group.

The consumer panel preferred the aroma of pork balls of the PFSe group relative to the PFSl group in week 3 (Table 10.12; $P = 0.016$), which is similar to the result with the longissimus muscle that was unflavoured (Table 8.16). Flavourings used in the processing of pork balls could have masked the intrinsic flavour of the pork and make differences more difficult to detect by the consumer and even the trained panel.

(f) *Storage effects*

The intensity scores for cuttlefish aroma and flavour, mutton aroma and flavour, and rancid odour and flavour increased during the storage period ($p<0.001$), especially from those in the fish oil groups. These observations were associated with the lower pH as storage period increased. There was also a positive correlation between acid aroma and

rancid odour (p=0.639) and rancid flavour (r=0.807). In contrast, meaty and brothy aroma and flavour attributes decreased in their intensity during the six week storage period. Interactions between treatment and time was observed for mutton aroma (p=0.004), cuttlefish flavour (p=0.028), mutton flavour (p=0.01) and acidic flavour (p=0.001).

As storage period increased, the negativity from the consumer panel towards products from all dietary groups increased (Table 10.13). Pork balls from the fish oil groups had low liking scores for aroma, flavour and acceptability throughout the six weeks. The lowest score recorded was 2.21 for PFSe in week 6 (Table 10.13). The most acceptable product amongst the six dietary groups came from the POS group with an acceptability score of 7.06 in week 0. Pork balls from the plant diet with tallow (PTS) received similar negative responses from the consumer panel to those for the fish oil groups, although samples from the fish oil groups were usually more unacceptable.

Highly significant interactions between treatment and time were observed in aroma liking, flavour liking and overall acceptability ($p<0.001$). From Fig. 10.5, the scores for aroma liking had the least changes while those from the flavour liking and overall acceptability had the greatest changes over the storage period of six weeks at 4^{o}C. Week 6 had the greatest plunge in the acceptability scores in terms of flavour liking and overall acceptability, possibly due to a significant reduction in pH from week 3 to 6 (Table 10.4) and significant increase ($p<0.05$) in cuttlefish aroma, cuttlefish flavour, rancid odour, rancid flavour and aftertaste (Table 10.11). The decline in acceptability can also be supported by the increment in TBARs values which increased from 0.308 to 0.451 mg MDA/kg from week 0 to 6 (Table 10.4). In studies by Gray and Pearson (1987) and Watts (1962), a threshold value of 1 mg MDA/kg muscle for organoleptic detection of rancidity was reported. This is in excess of lipid oxidation values observed in the present study. This suggests that the rancid note was detected more readily by Singaporeans compared to the panellists from those two studies.

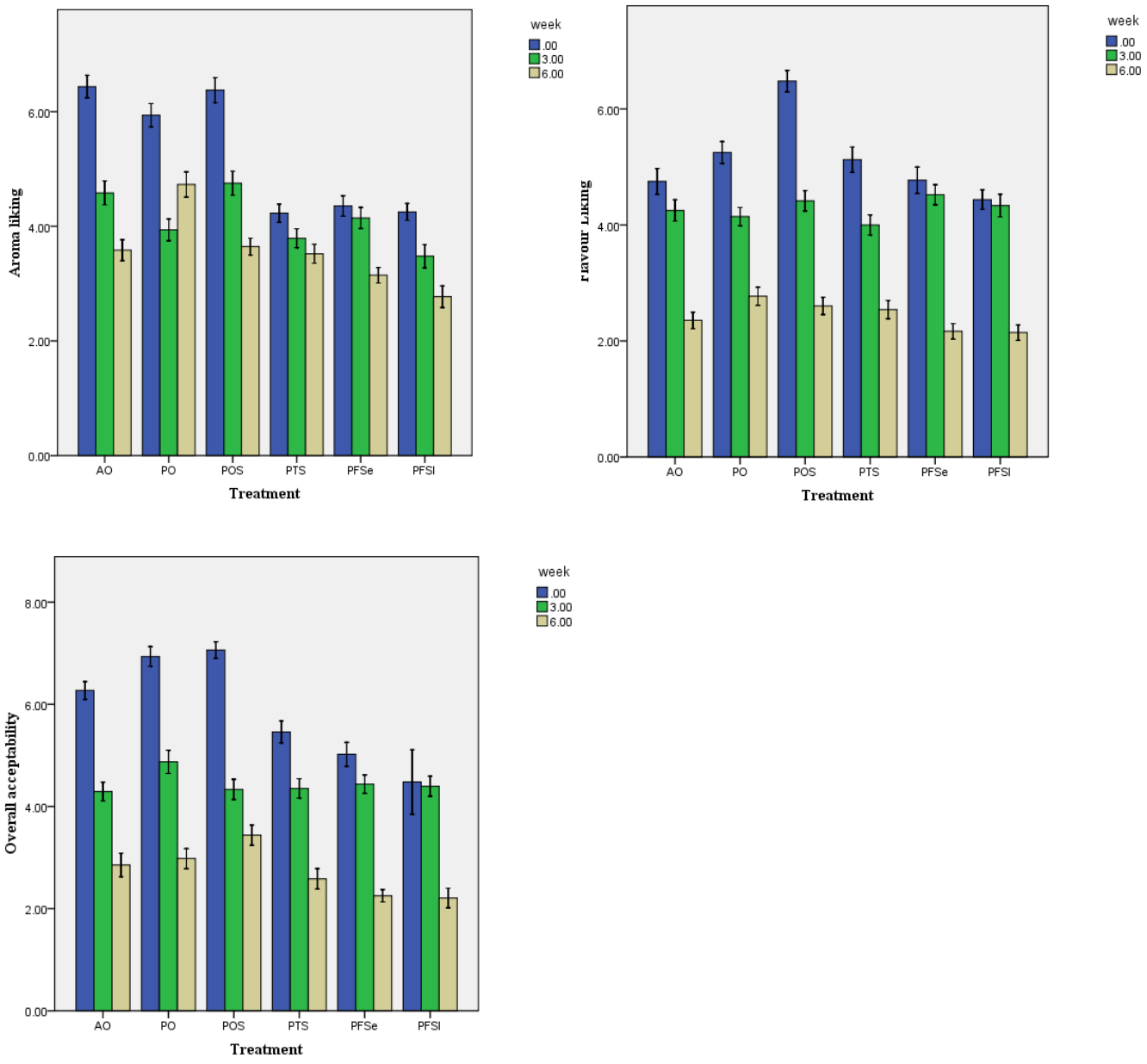


Fig. 10.5
Interaction plots showing the effect of treatment[1] and storage period (0, 3 and 6 weeks at 4°C) on consumer acceptability scores of pork balls (mean±SE) on a scale from 1 to 9 with 1 being "Dislike Extremely" and 9 being "Like Extremely".

[1]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 10.9
Least square means of element concentrations (μg/g) in pork balls from pigs fed diets containing animal and plant products with or without a dietary supplement (S), tallow (T) or fish oils (Fe & Fl), as determined by inductively coupled plasma spectrometer. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD

Element[1]	Treatment (Trt) groups[2]						Trt Effects (p-value)	R^2, RSD	Contrast (p-value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Potassium	7727	8510	8411	8276	7598	8632	0.417	20.6, 835	0.207	0.870	0.864	0.599	0.047
Magnesium	2613	2636	2690	2753	2470	2813	0.641	25.1, 290	0.912	0.834	0.828	0.810	0.020
Sodium	4333	4284	4014	4319	4435	4415	0.653	41.0, 371	0.852	0.452	0.372	0.088	0.924
Phosphorus	2108	2089	2153	2009	2056	1998	0.705	71.0, 155	0.894	0.690	0.142	0.192	0.583
Calcium	83.46	101.88	97.14	86.48	88.19	105.29	0.587	34.3, 20.45	0.139	0.775	0.549	0.978	0.301
Copper	0.490	0.512	0.560	0.513	0.558	0.549	0.639	70.6, 0.071	0.676	0.464	0.446	0.873	0.832
Iron	6.585	7.170	7.124	6.569	7.236	7.071	0.898	36.8, 1.08	0.347	0.969	0.616	0.969	0.775
Selenium	0.132a	0.152ab	0.244abc	0.320bc	0.401c	0.684d	<0.001	93.8, 0.122	0.608	0.158	0.316	0.030	0.033
Zinc	13.42	13.05	14.35	14.36	17.13	16.80	0.067	40.5, 2.16	0.765	0.429	0.993	0.127	0.872

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Table 10.10
Least squares means showing intensity score ratings of pork balls for the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the 3 storage times (week 0, 3, 6 at 4^{o}C), on a scale from 0 to 100 with higher values indicating a stronger note, as assessed by a Singapore trained panel. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for the 3 storage time are given in Table10.11

Sensory terms[1]	Treatment (Trt) group						Trt effects (p-value)	R^2, RSD	Contrast (p value)				
	AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Colour	41.23	39.36	39.04	43.58	43.61	44.10	0.51	26.8, 20.21	0.60	0.92	0.16	0.25	0.89
Colour saturation	37.93	34.11	35.41	37.86	41.90	40.87	0.13	26.5, 18.40	0.26	0.66	0.43	0.09	0.75
Meaty aroma	67.34	66.63	64.80	69.82	65.37	69.79	0.37	82.8, 18.35	0.82	0.56	0.09	0.15	0.11
Brothy aroma	53.30	51.01	49.41	53.31	50.50	52.30	0.82	37.8, 21.92	0.49	0.63	0.27	0.69	0.59
Cuttlefish aroma	22.90	23.30	24.02	22.93	24.38	23.95	0.99	83.8, 19.97	0.90	0.82	0.74	0.99	0.89
Mutton aroma	9.89	7.88	5.46	9.16	7.56	7.50	0.19	69.1, 8.42	0.79	0.04	0.016	0.28	0.97
Metallic aroma	9.02	10.37	10.54	8.69	10.32	10.14	0.95	70.1, 14.85	0.57	0.95	0.43	0.99	0.94
Acidic aroma	6.09	9.28	9.35	7.80	9.99	6.80	0.45	81.8, 14.27	0.04	0.97	0.40	0.47	0.31
Rancid odour	15.46	15.70	14.95	16.36	17.65	20.17	0.68	49.3, 15.39	0.92	0.77	0.59	0.14	0.35
Meaty flavour	72.76d	67.79bc	65.82bc	70.02cd	64.02ab	60.40a	0.005	32.3, 15.68	0.04	0.45	0.09	0.22	0.18
Brothy flavour	54.91	49.31	63.09	52.40	49.11	48.96	0.34	48.1, 43.06	0.13	0.20	0.32	0.26	0.38
Cuttlefish flavour	30.59	29.78	27.33	29.95	31.12	38.90	0.38	36.8, 23.02	0.72	0.54	0.50	0.012	0.73
Mutton flavour	15.73	11.29	9.78	15.51	10.76	8.15	0.52	37.7, 14.83	0.06	0.47	0.049	0.25	0.23
Metallic flavour	13.17	13.98	14.76	10.98	12.20	12.25	0.70	59.8, 15.88	0.76	0.76	0.12	0.49	0.98
Acidic taste	11.07	10.10	10.33	13.07	8.25	10.30	0.30	78.7, 11.97	0.62	0.90	0.21	0.47	0.27
Rancid flavour	20.89	19.24	19.48	21.01	20.65	22.49	0.94	66.1, 19.26	0.63	0.94	0.65	0.64	0.57
Salty taste	55.14	53.54	47.08	50.52	50.82	54.65	0.26	73.8, 24.07	0.68	0.10	0.38	0.13	0.29
Chewiness	52.82b	48.86ab	50.77ab	53.57b	46.88a	54.34b	0.04	54.0, 16.58	0.14	0.49	0.33	0.024	0.006
Cohesiveness	50.96ab	49.82ab	45.15a	53.65b	45.75a	52.59b	0.009	63.3, 17.43	0.69	0.11	0.003	0.016	0.018
Juiciness	42.23	42.13	39.94	44.26	44.44	46.31	0.22	88.6, 16.70	0.97	0.40	0.122	0.037	0.46
Hardness	46.17ab	50.46bc	51.77bc	54.02c	42.87a	48.20abc	0.001	58.0, 15.60	0.12	0.62	0.39	0.008	0.07
Aftertaste	59.11bc	54.03ab	49.33a	55.89abc	57.11bc	61.49c	0.003	83.6, 19.26	0.12	0.15	0.04	<0.001	0.21

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.
[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite^{TM}; PTS=diet with plant products, tallow & Sanovite^{TM}; PFSe=diet with plant products, Sanovite^{TM} and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite^{TM} and fish oil (during the late grower period).

Table 10.11
Least squares means showing the effects of treatment (trt) group, time and (trt x time) on the intensity score rating of pork balls for the 3 storage times across the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) on a scale from 0 to 100 with higher values indicating a stronger note, as assessed by Singapore trained panel. Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment group are given in Table 10.10

Sensory terms[1]	Week			Effects (p-value)			R^2 , RSD
	0	3	6	Trt	Time	Trt x Time	
Colour	29.03a	49.33b	48.17b	0.51	<0.001	0.09	26.8, 20.21
Colour saturation	26.65a	44.40b	43.90b	0.13	<0.001	0.51	26.5, 18.40
Meaty aroma	65.91a	71.26b	62.25a	0.37	0.008	0.84	82.8, 18.35
Brothy aroma	49.97	53.40	51.81	0.82	0.39	0.99	37.8, 21.92
Cuttlefish aroma	17.57a	23.14b	29.96c	0.99	<0.001	0.97	83.8, 19.97
Mutton aroma	6.26a	5.72a	11.42b	0.19	<0.001	0.004	69.1, 8.42
Metallic aroma	10.07b	14.03c	6.04a	0.95	<0.001	0.999	70.1, 14.85
Acidic aroma	7.89	8.32	8.45	0.45	0.93	0.16	81.8, 14.27
Rancid odour	9.22a	16.21b	24.64c	0.68	<0.001	0.50	49.3, 15.39
Meaty flavour	63.75a	70.10b	67.02ab	0.005	0.002	0.13	32.3, 15.68
Brothy flavour	54.63	53.47	50.86	0.34	0.72	0.08	48.1, 43.06
Cuttlefish flavour	20.00a	26.50a	55.24b	0.38	<0.001	0.028	36.8, 23.02
Mutton flavour	6.51a	7.46a	21.02b	0.52	<0.001	0.01	37.7, 14.83
Metallic flavour	9.97a	15.72b	13.39ab	0.70	0.006	0.91	59.8, 15.88
Acidic taste	6.94a	8.70a	15.66b	0.30	<0.001	0.001	78.7, 11.97
Rancid flavour	10.71a	18.88b	32.03c	0.94	<0.001	0.78	66.1, 19.26
Salty taste	47.45a	56.57b	52.52ab	0.26	0.004	0.86	73.8, 24.07
Chewy	46.60a	56.68c	51.11b	0.04	<0.001	0.77	54.0, 16.58
Cohesiveness	44.77a	54.30b	50.55b	0.009	<0.001	0.75	63.3, 17.43
Juiciness	41.22	45.41	43.34	0.22	0.09	0.41	88.6, 16.70
Hardness	50.24b	51.44b	45.42a	0.001	0.001	0.27	58.0, 15.60
Aftertaste	49.31a	54.34b	64.76c	0.003	<0.001	0.35	83.6, 19.26

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

Table 10.12

Least square means of hedonic rating of pork balls for the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the three storage times (week 0, 3, 6 at 4^{o}C) on a scale from 1 to 9, with 1 being "Dislike Extremely" and 9 being "Like Extremely". Means for the 3 storage times are given in Table 10.13

Time (Week)	Attribute[1]	Treatment (Trt) groups[2]						Trt effects (p-value)	R^2, RSD	Contrast (p-value)				
		AT	PO	POS	PTS	PFSe	PFSl			AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe +. PFSl]	PFSe vs. PFSl
0	Aroma liking	6.44b	5.94b	6.38b	4.23a	4.35a	4.25a	<0.001	58.2, 1.28	0.15	0.08	<0.001	<0.001	0.65
	Flavour liking	4.75ab	5.25b	6.48c	5.13b	4.77ab	4.44a	<0.001	81.2, 1.41	0.81	0.10	<0.001	<0.001	0.34
	Overall acceptability	6.27cd	6.93d	7.06d	5.45bc	5.02ab	4.48a	<0.001	66.1, 2.17	0.62	0.011	<0.001	<0.001	0.42
3	Aroma liking	4.58cd	3.94ab	4.75d	3.79ab	4.14bc	3.48a	<0.001	56.7, 1.34	0.005	0.02	0.001	<0.001	0.016
	Flavour liking	4.25	4.15	4.42	4.00	4.52	4.33	0.34	67.6, 1.23	0.25	0.67	0.10	0.96	0.47
	Overall acceptability	4.29	4.88	4.33	4.35	4.44	4.40	0.31	72.1, 1.36	0.08	0.05	0.94	0.72	0.88
6	Aroma liking	3.58b	4.73c	3.65b	3.52b	3.15ab	2.77a	<0.001	68.4, 1.22	<0.001	<0.001	0.57	0.001	0.11
	Flavour liking	2.35abc	2.77c	2.60bc	2.54abc	2.17ab	2.15a	0.012	70.5, 1.01	0.44	0.05	0.77	0.009	0.91
	Overall acceptability	2.85b	2.98bc	3.43c	2.58ab	2.25a	2.21a	<0.001	69.6, 1.33	0.10	0.68	0.003	<0.001	0.86

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite^{TM}; PTS=diet with plant products, tallow & Sanovite^{TM}; PFSe=diet with plant products, Sanovite^{TM} and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite^{TM} and fish oil (during the late grower period).

Table 10.13

Effects of treatment (trt) group, time and (trt x time) and least square means of consumer acceptability scores of pork balls made from pigs fed diet AO, PO, POS, PTS, PFSe and PFSl, with measures of the overall goodness-of-fit for the model including the coefficient of determination, R^2(%)] and the residual standard deviation, RSD. Means for treatment groups are given in Table 10.12

Attribute[1, 2]	Time (Week)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
Aroma liking	5.26c	4.11b	3.57a	<0.001	<0.001	<0.001	41.0, 1.27
Flavour liking	5.14c	4.28b	2.43a	<0.001	<0.001	<0.001	51.1, 1.22
Overall acceptability	5.87c	4.45b	2.72a	<0.001	<0.001	<0.001	45.5, 1.65

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2] All attributes were scored on a scale of 1 – 9 with higher values indicating a stronger liking.

10.4 Conclusions

A low-fat low-sodium pork-ball product with a desirable n-6/n-3 ratio of less than 4 was produced by supplementing the diets fed to pigs with PUFA from plant materials and fish oils, thereby manipulating the fatty acid composition of intramuscular fat and subcutaneous back fat.

The pH of pork balls decreased significantly, while TBARs increased significantly during six weeks of storage in vacuum packs at 4°C. This shows that the increased contents of PUFAs in pork may be beneficial from a human nutrition point of view, but they are more prone to lipid oxidation. Intensity of sensory attributes like rancid, acidic, mutton and fishy notes increased for all the dietary treatments during storage while meaty and brothy notes decreased. It was found through the use of a trained sensory panel that fish oil was able to mask the mutton aroma and flavour, which are generally considered as undesirable notes in New Zealand pork by Singapore consumers who prefer pork from pigs fed on plant materials only rather than on a mix of animal and plant materials.

Based on evaluations by a consumer panel, decreases in the liking of aroma and flavour and in overall acceptability occurred for all dietary groups as storage period increased. Pork balls from the fish oil groups had particularly low liking scores in these three aspects throughout the six weeks. The acceptability of the product especially the one based on pork from the fish oil groups was greatly compromised due to the fishy and rancid aftertaste. Despite the early withdrawal of fish oil from the pigs' feed 49 days prior to slaughter (the PFSe group), the pork ball products received a low acceptability score which was comparable to the group that had fish oil withdrawn for the last 28 days prior to slaughter. To overcome the off-flavour problem, terminating fish oil feeding for a sufficiently long period prior to slaughter could be a possible strategy. However, the loss of n-3 in that period would need to be monitored. Alternatively, other plant oils with the extra use of dietary natural antioxidants such as vitamins E could be used to enrich long chained omega-3 fatty acids in pork.

Chapter 11

Summary and Conclusions

In this Chapter, the main results of the research are summarised based on the objectives that were set out in the first chapter and conclusions are drawn.

Objective 1

To characterise the perception of Singaporean consumers towards pork from different countries by means of a survey (Chapter 3).

Anecdotal comments from Singapore over several years have suggested that there are aspects of the flavour of pork from western countries like New Zealand and Australia that make it less acceptable than pork from pigs from Indonesia. The problem flavour note has been described as "milky" or sometimes "mutton-like". In order to investigate this formally, a survey (n=202) was conducted amongst pork-consuming Singaporeans. It found that, relative to pork from Indonesia, New Zealand pork:

- Was less frequently purchased because of its less acceptable taste (33.8% of respondents vs. 82.7% for Indonesian pork)
- Had "flavour" as the reason for not liking the pork (70.2% of respondents vs. 20.9%)
- Had "Mutton-like flavour", "Stale flavour" and "Milky flavour" as flavour attributes associated with undesirable flavours.

On a positive note, New Zealand pork was perceived as more safe to be consumed compared to Indonesian pork (90.3% of respondents vs. 67.9%)

To overcome the flavour issues, methods of minimising these undesirable aspects of the pork flavour were investigated. One of the approaches was to conduct feeding trials with pigs to investigate how different dietary components affect the flavour of New Zealand pork. Hence the next objective was:

Objective 2

To determine the effects of dietary treatments (animal-plant vs. plant only diets and the supplement Sanovite™) on the sensory quality of New Zealand pork using Singaporean panels, and also to compare New Zealand pork with a local reference pork sample from Indonesia (Chapter 4).

Trained and untrained sensory panels in Singapore were able to detect differences in some flavour and aroma characteristics of pork from pigs raised on different diets in New Zealand (NZA, NZP, NZP+) with the pork from pigs with some animal products in their diet generally being less acceptable and having a stronger mutton flavour. It is suggested that these differences could be caused by the diet of NZA pigs which contained more protein and possibly some meat and fat from sheep. The Singapore panels detected differences between pork from the three New Zealand groups that were not detected by a New Zealand panel, but there were some confounding factors in this comparison. These results, however, support previous evidence that sensory results from one population may not necessarily apply for populations in other countries even when trained panellists are involved.

Results showed that dietary supplementation with CLA (conjugated linoleic acid) increased the stale note in pork, but this effect was small, possibly because of the antioxidant effects of the additional vitamin E and selenium present.

New Zealand pork did not differ significantly in overall acceptability compared to locally-produced Indonesian pork, but did have more intense mutton-like flavour attributes as detected by the Singaporean panel. With the same trial, a trained sensory panel in New Zealand detected no significant differences in a range of flavour and odour notes between pork from pigs that had or had not received animal products in their diets (Janz et al., 2008). This suggests that even after training, the Singapore panellists were more sensitive to the mutton flavour note than those in New Zealand.

The next approach to overcome the flavour issue was to investigate ways of masking the undesirable flavours by adding materials like herbs and spices and other flavourful plant materials. Any plant materials to be tested for their potential to mask undesirable flavours were required to be natural products that are normally consumed with pork in Singapore. To identify a suitable plant material, a survey was formally conducted on Singaporean consumers based on this objective:

Objective 3

To determine the usage of herbs, spices and other plant materials in the cooking of pork by Singaporean consumers by means of a survey, with a view to using an

aromatic plant material to improve the flavour of New Zealand pork (Chapter 5).

In this survey consisting of 112 consumers, the extent of use of 39 plant-based flavourful items was determined. On a scale where 0 indicated no use and 4 indicated that an item was used always when cooking or consuming pork, garlic received the highest average score (3.21), closely followed by onion and ginger. On the basis of these results the use of garlic oleoresin to improve the acceptability of NZ pork was investigated.

Investigations into the feasibility of incorporating garlic into pork directly or indirectly by including it in the diets of pigs formed the basis of the next two objectives.

Objective 4

To determine the threshold levels of garlic in rice bran oil and pork mince by means of both New Zealand and Singaporean panels of consumers (Chapter 6).

The amount of garlic essential oil (GEO) that was required in order to impart the desired flavour notes was initially assessed in an oil base with minimal flavour (rice bran oil), and then the results obtained were used to determine the amounts that should be added to pork mince. Threshold levels were determined for consumers from both New Zealand as well as Singapore over a range of eight concentrations in rice bran oil, and four concentrations within pork mince. Threshold tests for GEO in cooked pork mince showed that a significant increase in garlic taste was detected at 125 ppm of GEO, and this was associated with a significant reduction in mutton taste for both Singapore and New Zealand panellists, but an improved acceptability of garlic taste was shown by Singapore panellists only.

The next approach was to determine the amount of GEO that needed to be fed to pigs in order to improve the flavour of pork as outlined under the next objective.

Objective 5

To determine the effects of different levels of garlic essential oil in the diets of pigs on the sensory quality and acceptance of pork (Chapter 6).

GEO was added at three levels to the diet of pigs for a period of 57 days. Half the pigs received a diet without animal products to give a total of eight groups. Triangle tests with consumers in both New Zealand and Singapore were unable to distinguish between the control pork and low garlic group pigs. However they were able to detect the difference at the medium and high garlic levels for diets with or without animal products.

Significant increases in garlic flavour and aroma of pork from pigs fed diets containing GEO were also shown, but the amount of GEO required to produce a similar effect was more than 20 times as much as when it was added directly to mince prior to cooking. The strength of garlic flavour increased and the strength of mutton flavour decreased regardless of whether the diets had included animal products. However, the strength of mutton flavour was assessed as being greater when animal products were included in the diet of the pigs. At the current prices of GEO, it was estimated that the extra cost of pork products to cover the 150-200 g of GEO that would need to be fed to pigs would not be more than $0.50/kg.

These results from Objectives 4 and 5 provided an example of where an aromatic plant material successfully masked an undesirable flavour note in pork by either adding it to minced pork or by including a larger amount in the diet of pigs. Interest in determining the extent to which the undesirable mutton-like flavour may be linked to concentrations of skatole and indole in pork fat led to the next objective:

Objective 6

To evaluate the effects of garlic or n-3 PUFA in the diets of New Zealand pigs on the concentrations of indole and skatole in the fat with pork (Chapter 7).

The threshold concentrations of skatole and indole in rice-bran oil for Singaporean consumers were shown to be 0.028 μg/g and 0.051 μg/g, respectively. The effect of diet on concentrations of skatole and indole in subcutaneous fat of pigs was assessed in two experiments. In Experiment A, 31 female pigs were fed with diets based on plant products only (P) or plant plus animal by-products (AP), with added levels of garlic essential oil from zero to 2.15 g/kg feed. Concentrations of skatole and indole increased with increasing garlic concentration ($P<0.001$). Previous reports suggest that

this may have been an effect of the components in garlic oil on liver metabolism of the indolic compounds. In Experiment B, P and AP diets were fed to 47 female pigs with different dietary lipid sources (fish oil, tallow, and a mix of linseed oil and soya oil). Skatole and indole concentrations were higher in back fat of pigs fed with the AP diet ($P<0.05$), but were unaffected by the type of lipid. It was concluded that including only plant materials in the feed for pigs may be a practical means of reducing skatole and indole concentrations in pork fat.

With the knowledge of reducing indole and skatole production through diet manipulation in pigs' feed, the next objective was to produce a premium product that had improved nutritional qualities without compromising the sensory aspects. The rationale for focussing on a premium product was because of the difficulty for New Zealand pork to compete directly on price with pork from countries such as Indonesia, China and Australia. This led us to the following objective:

Objective 7

To determine the effects of including n-3 polyunsaturated fatty acids (PUFA) and Sanovite™ in the diet of pigs on the sensory and nutritional quality of pork, and to evaluate the effects of storage period on these characteristics (Chapter 8).

In this study, it was found that a more unsaturated fatty acid profile may limit the shelf-life of meat, because PUFA are more prone to oxidation. This had been identified as a major problem in previous work using α-linolenic-rich plant materials or fish oils to enrich pork with n-3 PUFA. Those dietary treatments containing fish oil had lower oxidative stability and this was manifested as off-odours and flavours resulting from lipid oxidation. The significance of fish oil supplementation had an impact on the concentration of omega-3 fatty acids, with high levels DHA levels compared to those without fish oil. Fishy and acidic sensory scores were higher; with lower consumer acceptability for the pork when fish oil was introduced in a later phase even though no fish oil was fed during the four weeks prior to slaughter. On a positive note, feeding fish oil either earlier or later caused the n-6/n-3 ratio to conform to the recommendation of being less than four.

Supplementing with higher levels of antioxidant like selenium, vitamin C and E in the feed, limiting fish oil amounts and strategising its phases of feeding, need to be further investigated in order to produce pork with minimum off-flavours and off-odours. In addition, excluding animal products from the feed will reduce undesirable mutton-liked off-flavour.

Having developed methods of manipulating the diet of pigs in order to reduce indole and skatole production and to produce a premium pork of improved sensory quality, the next objective was to develop a popular pork product using pork from these pigs. As Singaporeans are getting more health conscious, the availability of a healthy meat product that is low in fat, and low in sodium is desirable for the market. Thus the next objectives were to develop a pork ball to meet the demands of healthier products by the Singapore market.

Objective 8

To develop a healthy low-fat and low-salt pork ball product for Singaporean consumers (Chapter 9).

A low-fat, low-salt pork ball was developed for the Singapore market with fat and sodium levels below the recommended maximum levels of 10 g/100g and 450 mg/100 g, respectively, and without any loss in acceptability or deterioration in cooking yield or other physical characteristics of the pork balls. Carrageenan was use to replace some of the fat in this product and KCL replaced some of the NaCl. These ingredients improved the cooking yield and moisture retention. The next step was to develop two similar pork ball products of good sensory and nutritive quality from pigs fed diets containing either (a) GEO, or (b) n-3 polyunsaturated fatty acids (PUFA) and Sanovite™. Therefore the next objective of the product-development work was:

Objective 9

To evaluate the effects of storage time on physical, nutritional, and sensory characteristics of low-fat, low-salt pork balls produced for Singaporean consumers using pork from New Zealand pigs fed either (a) GEO (Chapter 9), or (b) n-3 PUFA and Sanovite™ (Chapter 10).

(a) The low-fat, low-salt pork balls prepared with lean meat and back fat from pigs fed a diet containing moderate levels of GEO was more acceptable than when there was no GEO in the diet of the pigs when the base diet contained animal products, but not when the diet contained plant products only. High levels of GEO in the diet of pigs resulted in pork balls that were less acceptable regardless of the base diet. Diet and GEO concentration had no significant effects on the physical characteristics like pH, colour, moisture retention, cooking yield and fat content. Thus it is possible to produce low-fat, low-salt pork balls without a decrease in acceptability for Singapore consumers. Inclusion of GEO may be useful as a means of masking undesirable flavours like mutton flavour in pork balls.

(b) A healthy low-fat and low-sodium pork-ball product with a desirable n-6/n-3 ratio of <4 was produced through supplementation of the diets fed to the pigs with PUFA from plant materials and fish oils, thereby manipulating the fatty acid composition of intramuscular fat and subcutaneous back fat. Physical attributes of pork balls like pH decreased significantly while TBARs increased significantly during six weeks of storage in vacuum packs at 4°C. This showed that the increased contents of PUFAs in pork may be beneficial from a human nutrition point of view but they are more prone to lipid oxidation. More DHA and EPA were present in the fats of samples in which fish oils were fed at a latter stage (up to 28 days vs. 49 days prior to slaughter). The DHA levels in the pork balls were 45% higher for the plant-only diet group compared to the animal-plant group.

Sensory attributes in the pork balls from all the dietary treatments changed during a storage period of 6 weeks at 4^{o}C, with rancid, acidic, mutton and fishy notes increasing while meaty and brothy notes decreased. It was also found that fish oil was able to mask the mutton aroma and flavour, which were considered as undesirable notes in New Zealand pork by Singapore consumers who preferred pork from pigs fed on plant materials rather than on a mix of animal and plant materials. In addition, the negativity from the consumer panel towards products from all dietary groups increased as storage period increased. The acceptability scores for the plant-only group were higher than the animal-plant group. Pork balls from the fish oil groups had low liking

scores in these three aspects throughout the six weeks. The acceptability of the product especially the one based on pork from the fish oil groups was greatly compromised due to the fishy and rancid aftertaste.

Recommendations for future work

Masking of undesirable flavour notes in pork

Based on the results from Chapters 4 and 5, the addition of garlic essential oil into the diets of pigs has provided an example of the successful addition of an aromatic plant material to mask an undesirable flavour note in pork. Other popular aromatic plant materials like ginger and onion as indicated by the consumer survey in Chapter 3 can be further investigated either by addition to the minced pork or by inclusion into the diet of pigs. An optimisation study may be warranted to see the effect of duration of feeding of aromatic plant materials on the masking effect. The effect of inclusion of aromatic plant materials and their period of feeding on the production of indole and skatole can be further investigated.

Reduction of indole and skatole

Eating quality of pork will be enhanced if levels of indole and skatole are reduced. Thus another area worth investigating for future study will be the effects of feeding regime (restricted protein diet) and antioxidant supplementation (vitamin C and E and/or other plant materials with antioxidant properties) in the diets of the pigs on the production of indole and skatole. Prebiotics and lactic acid bacteria which influence intestinal microflora can be explored. Manipulation of indole and skatole production through the diet has been shown possible through reductions in dietary sulphur, protein, and indigestible fractions. These methods remove substrate available for the microbes that produce odorants. Inclusion of low-fiber feedstuffs from some herbs and spices or even traditional Chinese herbs like ginseng, licorice, and root of Astragalus membranaceus can perhaps alter the microbial population and thus decrease the production of odorants and improve the overall flavour of pork.

Enhancing omega-3 fatty acids in pork with better sensory quality

The period of feeding of omega-3 fatty acids to the pigs with incorporation of dietary antioxidants like vitamin E to reduce the fishy note and optimise the omega-3 fatty acid composition in pork, is an area that warrants further investigation. Further research into the effects of combining vitamin E along with other natural antioxidants such as extracts from green tea, turmeric, rosemary and sage, in the diets of pigs on the sensory quality of pork is recommended.

Overall conclusion

It is concluded that the problem of flavour acceptability of pork from New Zealand by Singapore consumers, which was shown here by means of a consumer survey to be due to mutton-like flavour notes, can be minimised by removing animal co-products from the diets of pigs, and/or by including flavourful items such as garlic or fish oil in the diet to mask the undesirable flavour notes. The possible association between the mutton-like flavour in pork and skatole and indole levels in fat suggests that these compounds may be responsible in part for this undesirable flavour note. It is also concluded that the development of acceptable, healthy pork balls with low fat and low salt levels together with elevated levels of health-promoting fatty acids for the Singapore market is feasible, and that such products may be a means of obtaining premium prices in the market place provided flavour acceptability can be assured. Further research needs to be carried out in order to devise sustainable and profitable production and processing systems that lead to pork products that can satisfy consumer demands in Singapore."

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Appendices

Appendix 6.1
Sensory evaluation questionnaire for QDA for pork balls using a trained panel of Singaporeans in Experiment 1

Name : _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Instructions : Please taste the given sample and mark your evaluation on each of the line scales.

Sample Code : ☐

Aroma Characteristics

Meaty aroma

None Strong

Brothy aroma

None Strong

Garlic aroma

None Strong

Metallic aroma

None Strong

Acidic aroma

None Strong

Mutton aroma

None Strong

Stale odour

None Strong

Taste/Flavour Characteristics

Meaty flavour

None Strong

Brothy flavour

None Strong

Garlic flavour

None Strong

Metallic flavour

None Strong

Mutton flavour

None Strong

Stale flavour

None Strong

Acidic taste

None Strong

Bitter taste

None Strong

Aftertaste

None Strong

Comments :

Appendix 7.1
Evaluation questionnaire for threshold detection of skatole and indole by a Singaporean trained panel

Name _____

Age group: 15 – 20 21 – 30 31 – 40 >40
(circle one please)

Race: Chinese Indian Others _____ (please state)

1. You are given a tray of 18 samples for odour evaluation. There are altogether 6 rows. In each row, there are three samples.
2. Please evaluate all samples, starting from order 1 which has an orange dot on the cap.
3. For each row, open the bottle and sniff the headspace.
4. Decide whether there is any difference among the 3 samples.
    a. If there is a difference, circle the code number of odd sample within each row and define the level of difference with 1 being least different and 10 being most different.
    b. If there is no difference, just circle **ND**

Order	**Sample code**	**Sample code**	**Sample code**	**If odd, state the level of difference from 1 to 10**	
1	110	135	185		ND
2	214	295	282		ND
3	303	321	346		ND
4	426	435	469		ND
5	596	513	543		ND
6	628	637	693		ND

Thank you for your participation!

Appendix 7.2

Sensory evaluation questionnaire for QDA for skatole and indole using a Singapore trained panel

Name : _____

Age Group :	11-20	21-30	31-40	>40
Race :	Chinese	Malay	Indian	Others
Smoking Habits :	Yes	No		

Instructions: Please taste the given sample and mark your evaluation on each of the line scales.

Sample Code : ☐

Napthalene

None ——— Strong

Sweaty

None ——— Strong

Ammonia

None ——— Strong

Mutton-like

None ——— Strong

Musty

None ——— Strong

Faecal

None ——— Strong

Nauseating

None Strong

Pungent

None Strong

Sewage

None Strong

Stale

None Strong

Comments :

Appendix 8.1

Ingredient composition of the five diets during the grower and finisher periods. The PFS diet was fed at either an early (PFSe) or late (PFSl) period within the grower period, and was not fed at all during the 28-day finisher period

Ingredient in % of diet	Grower					Finisher			
	AT	PO	POS	PTS	PFS	AT	PO	POS	PTS
Barley	55.95	67.37	67.37	67.37	67.37	55.95	67.37	67.37	67.37
Wheat	10	-	-	-	-	10	-	-	-
Broll	6	6	6	6	6	7.6	7.6	7.6	7.6
Soybean meal	7	16	16	16	16	7	16	16	16
Blood meal	3	-	-	-	-	3	-	-	-
Meat and bone meal	13	-	-	-	-	13	-	-	-
Tallow	4.4	-	-	4.4	-	2.8	-	-	2.8
Soybean oil	-	3.3	3.3	-	-	-	2.1	2.1	-
Linseed oil	-	1.1	1.1	-	-	-	0.7	0.7	-
Fish oil[a]	-	-	-	-	4.4	-	-	-	-
Lysine	-	0.37	0.37	0.37	0.37	-	0.37	0.37	0.37
Methionine	0.15	0.26	0.26	0.26	0.26	0.15	0.26	0.26	0.26
Threonine	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2
Dicalcium phosphate	-	3	3	3	3	-	3	3	3
Limestone	-	1.6	1.6	1.6	1.6	-	1.6	1.6	1.6
Sodium chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Disodium phosphate	-	0.4	0.4	0.4	0.4	-	0.4	0.4	0.4
Vitamin-mineral grower	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Conjugated linoleic acid	-	-	0.250	0.250	0.250	-	-	0.250	0.250
Selplex	-	-	0.024	0.024	0.024	-	-	0.024	0.024
Vitamin E	-	-	0.040	0.040	0.040	-	-	0.040	0.040
Vitamin C	-	-	0.300	0.300	0.300	-	-	0.300	0.300

[a] *To prevent oxidation, the fish oil was stored in a fridge and added to the mash diet freshly every feed by the use of a syringe. Water was added to the mixture afterwards.*

Appendix 8.2
Sensory evaluation questionnaire for QDA for pork using a Singapore trained panel

Name : _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Instructions : Please taste the given sample and mark your evaluation on each of the line scales.

Sample Code : ☐

Appearance

Colour

Grey Brown

Colour saturation

Light Dark

Aroma Characteristics

Meaty aroma

None Strong

Brothy aroma

None Strong

Fishy odour

None Strong

Mutton aroma

None Strong

Metallic aroma

None Strong

Acidic aroma

None Strong

Rancid odour

None Strong

Taste/Flavour Characteristics

Meaty flavour

None Strong

Brothy flavour

None Strong

Fishy flavour

None Strong

Mutton flavour

None Strong

Metallic flavour

None Strong

Acidic flavour

None Strong

Rancid flavour

None Strong

Salty taste

None Strong

Aftertaste

None Strong

Comments :

Appendix 8.3
Sensory evaluation questionnaire for consumer analysis of pork using a Singapore consumer panel

Name: _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Sample Code : ☐

1. How do you like the overall aroma of the pork based on a scale from 1 – 9 where 1 is "dislike extremely", and 9 is "like extremely"? _____

   (a) Can you detect any off odour? (Yes; No)
   If yes, please go to question 1 (b). If not, please go to question (2)

   (b) Please mark in the appropriate box for the possible off-notes in the sample and indicate the level of intensity of these flavour off-notes in the brackets, from 1 to 5 where 1 is the weakest and 5 is the strongest.

   - ☐ Fishy note ( )
   - ☐ Rancid note ( )
   - ☐ Metallic note ( )
   - ☐ Mutton note ( )
   - ☐ Acidic ( )

- Bitter ( )
- Others (Please specify)_____ ( )

2. How do you like the overall flavour of the pork based on a scale from 1 – 9 where 1is "dislike extremely" and 9 is "like extremely"? _____

(a) Can you detect any off flavour? (Yes; No)
If yes, please go to question 2 (b). If not, please go to question (3)

(b) Please mark in the appropriate box for the possible off-notes in the sample and indicate the level of intensity of these flavour off-notes in the brackets from 1 to 5, where 1 is the weakest and 5 is the strongest.

- Fishy note ( )
- Rancid note ( )
- Metallic note ( )
- Mutton note ( )
- Acidic ( )
- Bitter ( )
- Others (Please specify)_____ ( )

3. What is the overall acceptability of the pork based on a scale from 1 – 9 where 1 is "dislike extremely" and 9 is "like extremely"? _____

Thank you for your participation

Appendix 8.4

Least squares means of fatty acid (% of total fatty acids) and the total fatty acids contents (total fatty acids as a % of fresh weight) in intramuscular fat of the longissimus muscle in the loin region for the 6 treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the 3 storage times (0, 3, and 6 months at -18°C). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for the 3 storage times are given in Appendix 8.6

Item[1,2]	Treatment (Trt) group[3]							R^2, RSD	Contrast (p value)				
	AT	PO	POS	PTS	PFSe	PFSl	Effects (p-value)		AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Total fatty acids (%)	1.11a	1.61ab	1.37ab	1.72ab	1.89b	1.14a	0.046	23.4, 0.55	0.10	0.45	0.27	0.022	0.011
C14:0 myristic acid	1.63	1.40	1.66	1.49	1.64	1.45	0.22	17.3, 0.45	0.08	0.056	0.18	0.27	0.22
C16:0, palmitic acid	21.90b	20.28b	19.63ab	19.74ab	19.96ab	17.91a	0.017	13.4, 3.70	0.22	0.60	0.91	0.051	0.018
C18:0, stearic acid	12.78ab	11.71ab	12.08ab	13.32b	12.54ab	11.10a	0.09	20.2, 2.78	0.29	0.64	0.12	0.08	0.039
C20:0 arachidic acid	0.259	0.359	0.368	0.358	0.305	0.270	0.28	32.4, 0.21	0.11	0.88	0.91	0.21	0.52
C16:1- cis-9, palmitoleic acid	1.33	1.06	1.20	1.07	1.30	1.33	0.37	22.9, 0.59	0.17	0.51	0.41	0.79	0.86
C18:1- cis-9, oleic acid	41.19b	40.12b	39.22ab	39.11ab	35.75a	38.00ab	0.02	24.0, 5.31	0.56	0.64	0.95	0.10	0.15
C18:1- trans-9, elaidic acid	0.248	0.238	0.256	0.209	0.234	0.227	0.92	16.4, 0.15	0.82	0.66	0.33	0.80	0.90
C18:1- cis-11, vaccenic acid	3.24bc	2.14a	2.16a	3.72c	2.76ab	2.92abc	0.001	24.4, 1.41	0.010	0.97	0.001	0.14	0.71
C18:1- trans-11, vaccenic acid	0.266	0.267	0.256	0.346	0.326	0.248	0.12	38.1, 0.15	0.99	0.81	0.07	0.11	0.06
C18:2-cis-9, 12, linoleic acid	9.85a	11.15ab	12.69c	12.15bc	12.82c	12.18bc	<0.001	27.1, 2.28	0.09	0.054	0.42	0.57	0.32
C18:2-cis-9, trans-11, CLA[2]	-0.952 (0.112ab)	-1.127 (0.075a)	-0.695 (0.202c)	-0.811 (0.154ab)	-0.692 (0.203bc)	-0.533 (0.293c)	<0.001	43.2, 0.14	0.028	<0.001	0.022	0.29	0.044
C18:2-trans-10, cis-12, CLA[2]	-0.625 (0.237)	-0.701 (0.199)	-0.597 (0.253)	-0.565 (0.272)	-0.598 (0.252)	-0.495 (0.320)	0.56	19.2, 0.18	0.536	0.31	0.62	0.72	0.602
C18:3n-6 cis-6, 9, 12, γ-linolenic[2] acid	-0.759 (0.174)	-0.770 (0.170)	-0.786 (0.164)	-0.691 (0.204)	-0.706 (0.197)	-0.652 (0.223)	0.43	12.0, 0.22	0.321	0.28	0.65	0.25	0.38
C18:3n-3 cis-9, 12, 15, α-linolenic acid	0.821a	1.87b	3.29c	2.19b	3.34c	3.32c	<0.001	58.9, 0.91	<0.001	<0.001	0.002	0.98	0.94
C20:1-cis-11, eiconsanoic acid[2] acid	-0.760 (0.174)	-0.720 (0.191)	-0.714 (0.193)	-0.896 (0.127)	-0.633 (0.233)	-0.466 (0.342)	0.004	27.5, 0.19	0.761	0.52	0.007	0.20	0.36
C20:2-cis-11, 14, eicosadienoic[2] acid	-0.362 (0.435)	-0.332 (0.466)	-0.343 (0.454)	-0.363 (0.434)	-0.403 (0.395)	-0.432 (0.370)	0.43	18.7, 0.24	0.44	0.64	0.85	0.30	0.57

Appendix 8.4 (continued)

C20:3n-6 cis-8, 11, 14, eicosatrienoic[2] acid	-0.955	-0.957	-0.908	-0.923	-0.949	-0.921	0.91	16.7, 0.12	0.46	0.96	0.68	0.72	0.57
	(0.111)	(0.110)	(0.124)	(0.120)	(0.112)	(0.120)							
C20:3n-3 cis-11, 14, 17, eicosatrienoic acid	0.305ab	0.471c	0.361abc	0.227a	0.427bc	0.347abc	0.002	25.0, 0.21	0.029	0.11	0.011	0.41	0.21
C20:4n-6 cis-5, 8, 11, 14, arachidonic[2] acid	-0.601	-0.634	-0.568	-0.418	-0.492	-0.521	0.08	22.2, 0.18	0.75	0.29	0.001	0.08	0.67
	(0.251)	(0.232)	(0.270)	(0.382)	(0.322)	(0.301)							
C20:5n-3 cis-5, 8, 11, 14, 17, EPA[2]	-0.742	-0.587	-0.569	-0.708	0.099	0.166	<0.001	80.0, 0.32	0.09	0.72	0.009	<0.001	0.041
	(0.181)	(0.259)	(0.270)	(0.196)	(1.26)	(1.47)							
C22:5-cis-7, 10, 13, 16, 19, DPA	0.353a	0.587bc	0.634c	0.426ab	1.53d	1.77e	<0.001	77.3, 0.33	0.002	0.61	0.039	<0.001	0.043
C22:6n-3-cis-4, 7, 10, 13, 16, 19, DHA[2]	-0.825	-0.721	-0.757	-0.511	0.139	0.315	<0.001	89.4, 0.28	0.06	0.49	0.09	<0.001	<0.001
	(0.150)	(0.190)	(0.175)	(0.308)	(1.38)	(2.07)							

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]Analysed as log(value). The significance of differences (using LSD) was based on the transformed means (log) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

[3]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite^{TM}; PTS=diet with plant products, tallow & Sanovite^{TM}; PFSe=diet with plant products, Sanovite^{TM} and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite^{TM} and fish oil (during the late grower period).

Appendix 8.5

Least squares means of fatty acid contents (% of total fatty acids) and the total fatty acids (total fatty acids as a % of fresh weight) in subcutaneous back fat in the loin region for the six treatment groups (AT, PO, POS, PTS, PFSe and PFSl) averaged across the three storage times (0, 3, and 6 months at -18°C). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for the 3 storage times are given in Appendix 8.7.

Item[1,2]	Treatment (Trt) group[3]							R^2, RSD	Contrast (p value)				
	AT	PO	POS	PTS	PFSe	PFSl	Effect (p value)		AT vs. PO	PO vs. POS	POS vs. PTS	POS vs. [PFSe + PFSl]	PFSe vs. PFSl
Total fatty acids (%)	75.3	76.4	71.3	77.4	77.7	77.2	0.57	24.0, 7.76	0.80	0.20	0.037	0.21	0.92
C14:0 myristic acid	1.72bc	1.42a	1.56ab	1.83c	1.77c	1.67bc	0.010	27.1, 0.25	0.001	0.15	<0.001	0.038	0.27
C16:0, palmitic acid	20.62c	20.26bc	19.53bc	20.81c	18.88ab	17.78a	0.001	45.8, 2.07	0.64	0.36	0.041	<0.001	0.16
C18:0, stearic acid	12.85	11.02	11.54	12.15	12.57	12.61	0.27	32.0, 2.56	0.07	0.56	0.41	0.58	0.97
C20:0 arachidic acid	0.191a	0.228b	0.225b	0.186a	0.209ab	0.198ab	0.014	49.1. 0.03	0.011	0.86	0.033	0.07	0.44
C16:1- cis-9, palmitoleic acid[2]	-0.091c	-0.222ab	-0.272a	-0.090c	-0.147bc	-0.253ab	0.015	44.3, 0.15	0.018	0.60	0.006	0.15	0.08
	(0.811c)	(0.600ab)	(0.535a)	(0.813c)	(0.713bc)	(0.558ab)							
C18:1- cis-9, oleic acid	44.78d	43.76cd	42.15bc	42.13bc	38.86a	40.66ab	0.001	40.4, 3.18	0.40	0.21	0.99	0.001	0.07
C18:1- trans-9, elaidic acid	0.332b	0.296b	0.235a	0.307b	0.185a	0.240a	<0.001	54.8, 0.05	0.31	0.008	0.007	0.014	0.021
C18:1- cis-11, vaccenic acid	2.45	2.60	2.47	2.76	2.73	2.73	0.25	34.5, 0.51	0.36	0.42	0.13	0.12	0.99
C18:1- trans-11, vaccenic acid	0.272	0.279	0.272	0.292	0.289	0.287	0.36	46.0 0.06	0.74	0.73	0.40	0.07	0.93
C18:2-cis-9, 12, linoleic acid	11.72a	14.03b	14.67b	13.88b	14.27b	13.96b	0.13	29.9, 1.75	0.001	0.39	0.23	0.031	0.56
C18:2-cis-9, trans-11, CLA[2]	-0.615b	-1.13a	-0.492b	-0.401b	-0.383b	-0.563b	0.17	55.3, 0.38	0.009	0.001	0.22	0.49	0.57
	(0.243b)	(0.074a)	(0.322b)	(0.397b)	(0.414b)	(0.274b)							
C18:2-trans-10, cis-12, CLA	0.177a	0.188a	0.286b	0.312b	0.335b	0.296b	0.31	43.5, 0.11	0.69	0.011	0.60	0.61	0.24
C18:3n-6 cis-6, 9, 12, γ-linolenic acid	0.140a	0.265c	0.266c	0.163a	0.200b	0.211b	<0.001	40.0, 0.06	<0.001	0.95	<0.001	<0.001	0.35
C18:3n-3 cis-9, 12, 15, α-linolenic[2] acid	0.042a	0.274b	0.428c	0.120a	0.490c	0.448c	<0.001	88.2, 0.09	<0.001	0.018	<0.001	<0.001	0.30
	(1.10a)	(1.88b)	(2.68c)	(1.32a)	(3.09c)	(2.81c)							
C20:1-cis-11, eiconsanoic acid	0.055c	0.041ab	0.048abc	0.048c	0.035a	0.041ab	0.062	40.1, 0.02	0.038	0.27	0.88	0.07	0.32
C20:2-cis-11, 14, eicosadienoic acid	0.513a	0.588b	0.524ab	0.544ab	0.591b	0.558ab	0.27	19.3, 0.11	0.015	0.12	0.62	0.31	0.26
C20:3n-6 cis-8, 11, 14, eicosatrienoic acid	0.133	0.131	0.114	0.148	0.154	0.161	0.10	11.5, 0.08	0.91	0.37	0.14	0.05	0.77
C20:3n-3 cis-11, 14, 17, eicosatrienoic acid	0.261a	0.588b	0.533b	0.313a	0.508b	0.498b	0.003	51.2, 0.14	<0.001	0.38	0.001	<0.001	0.84

Appendix 8.5 (continued)

C20:4n-6 cis-5, 8, 11, 14, arachidonic acid	0.152	0.152	0.139	0.156	0.137	0.131	0.53	29.1, 0.03	0.97	0.25	0.19	0.26	0.59
C20:5n-3 cis-5, 8, 11, 14, 17, EPA[2]	-1.15a	-0.999b	-1.09ab	-1.14a	-0.226c	-0.142c	<0.001	83.6, 0.21	0.07	0.10	0.99	<0.001	0.06
	(0.070a)	(0.100b)	(0.082ab)	(0.072a)	(0.594c)	(0.721c)							
C22:5-cis-7, 10, 13, 16, 19, DPA[2]	-0.048ab	-0.517bc	-0.445c	-0.679a	0.017d	0.061d	<0.001	62.8, 0.25	0.01	0.41	0.13	<0.001	0.55
	(0.225ab)	(0.304bc)	(0.359c)	(0.209a)	(1.04d)	(1.15d)							
C22:6n-3-cis-4, 7, 10, 13, 16, 19, DHA[2]	-0.973b	-1.01ab	-1.12a	-0.990ab	0.055c	0.123c	<0.001	87.6, 0.21	0.98	0.42	0.75	<0.001	0.015
	(0.106b)	(0.098ab)	(0.076a)	(0.102ab)	(1.14c)	(1.33c)							

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]Analysed as log(value). The significance of differences (using LSD) was based on the transformed means (log) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

[3]AT=diet with animal and plant products; PO= diet with plant products only; POS=diet with plant feed products and Sanovite™; PTS=diet with plant products, tallow & Sanovite™; PFSe=diet with plant products, Sanovite™ and fish oil (during the early grower period); PFSl=diet with plant products, Sanovite™ and fish oil (during the late grower period).

Appendix 8.6

Least squares means of fatty-acids contents (% of total fatty acids) and effects of treatment (trt) group, time and (trt x time) of intramuscular fat of longissimus muscle in the loin regionfor the three storage times (0, 3, and 6 months at -18°C) averaged across the six treatment groups(AT, PO, POS, PTS, PFSe and PFSl). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment groups are given in Appendix 8.4

Item[1]	Time (month)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
C14:0 myristic acid	1.38a	1.68b	1.57b	0.22	0.006	0.64	27.1, 0.25
C16:0, palmitic acid	20.29	19.74	19.67	0.017	0.67	0.95	45.8, 2.07
C18:0, stearic acid	13.65b	11.52a	11.58a	0.09	<0.001	0.97	32.0, 2.56
C20:0 arachidic acid	0.291b	0.192a	0.478c	0.28	<0.001	0.71	49.1. 0.03
C16:1- cis-9, palmitoleic acid	1.45b	1.25b	0.94a	0.37	<0.001	0.20	44.3, 0.15
C18:1- cis-9, oleic acid	39.56b	36.72a	40.62b	0.02	0.002	0.33	40.4, 3.18
C18:1- trans-9, elaidic acid	0.22a	0.189a	0.25b	0.92	0.004	0.38	54.8, 0.05
C18:1- cis-11, vaccenic acid	3.05	2.67	2.75	0.001	0.40	0.15	34.5, 0.51
C18:1- trans-11, vaccenic acid	0.317b	0.151a	0.384c	0.12	<0.001	0.88	46.0 0.06
C18:2-cis-9, 12, linoleic acid	11.46	11.58	12.32	<0.001	0.15	0.23	29.9, 1.75
C18:2-cis-9, trans-11, CLA	-0.674b	-0.805ab	-0.935a	<0.001	<0.001	0.73	42.7, 0.29
	(0.212b)	(0.157ab)	(0.116a)				
C18:2-trans-10, cis-12, CLA	-0.506b	-0.736a	-0.548b	0.09	<0.001	0.85	25.0, 0.24
	(0.312b)	(0.183a)	(0.283b)				
C18:3n-6 cis-6, 9, 12, γ-linolenic acid	-0.725ab	-0.800a	-0.657b	0.43	0.026	0.30	16.6, 0.26
	(0.188ab)	(0.158a)	(0.220b)				
C18:3n-3 cis-9, 12, 15, α-linolenic acid	2.47	2.60	2.29	<0.001	0.25	0.015	88.2, 0.09
C20:1-cis-11, eiconsanoic acid	-0.584b	-0.913a	-0.568b	0.001	<0.001	<0.001	47.2, 0.29
	(0.261b)	(0.122a)	(0.270b)				
C20:2-cis-11, 14, eicosadienoic acid	-0.331b	-0.493a	-0.291b	0.44	<0.001	0.97	23.8, 0.19
	(0.467b)	(0.321a)	(0.511b)				
C20:3n-6 cis-8, 11, 14, eicosatrienoic acid	-0.848b	-1.090a	-0.855b	0.99	<0.001	0.79	20.1, 0.28
	(0.141b)	(0.081a)	(0.140b)				
C20:3n-3 cis-11, 14, 17, eicosatrienoic acid	0.412	0.320	0.332	0.002	0.07	0.13	51.2, 0.14
C20:4n-6 cis-5, 8, 11, 14, arachidonic acid	-0.393b	-0.654a	-0.572a	0.032	<0.001	0.39	30.1, 0.24
	(0.405b)	(0.222a)	(0.268a)				
C20:5n-3 cis-5, 8, 11, 14, 17, EPA	-0.303b	-0.745a	-0.192b	<0.001	<0.001	<0.001	82.0, 0.22
	(0.498b)	(0.180a)	(0.643b)				
C22:5-cis-7, 10, 13, 16, 19, DPA	0.809	0.791	1.01	<0.001	0.002	0.91	62.8, 0.25
C22:6n-3-cis-4, 7, 10, 13, 16, 19, DHA	-0.420	-0.492	-0.280	<0.001	<0.001	<0.001	87.1, 0.20
	(0.380)	(0.322)	(0.525)				

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]Analysed as log(value). The significance of differences (using LSD) was based on the transformed means (log) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

Appendix 8.7

Least squares means of fatty-acids contents (% of total fatty acids) and effects of treatment (trt) group, time and (trt x time) of subcutaneous back fat in the loin region for the three storage times (0, 3, and 6 months at -18°C) averaged across the six treatment groups(AT, PO, POS, PTS, PFSe and PFSl). Measures of the overall goodness-of-fit for the model include the coefficient of determination, R^2(%) and the residual standard deviation, RSD. Means for treatment groups are given in Appendix 8.5

Item[1]	Time (month)			Effects (p-value)			R^2, RSD
	0	3	6	Trt	Time	Trt x Time	
C14:0 myristic acid	1.72b	1.51a	1.74b	<0.001	<0.001	0.12	41.9, 0.26
C16:0, palmitic acid	18.05a	20.19b	20.76c	<0.001	<0.001	0.25	41.2, 2.17
C18:0, stearic acid	10.52a	12.91ab	13.00b	<0.001	<0.001	<0.001	50.6, 2.61
C20:0 arachidic acid	0.20b	0.18a	0.24c	0.006	<0.001	0.022	41.7, 0.045
C16:1- cis-9, palmitoleic acid[2]	-0.063b	-0.276a	-0.199a	<0.001	<0.001	0.09	44.3, 0.15
	(0.865b)	(0.530a)	(0.632a)				
C18:1- cis-9, oleic acid	41.533	43.75	41.09	<0.001	0.044	<0.001	39.8, 3.56
C18:1- trans-9, elaidic acid	0.33b	0.26a	0.22a	<0.001	<0.001	<0.001	69.9, 0.06
C18:1- cis-11, vaccenic acid	3.09	2.27	2.50	0.18	0.15	0.34	15.7, 1.56
C18:1- trans-11, vaccenic acid	0.35b	0.24a	0.26a	0.17	<0.001	0.001	51.1, 0.05
C18:2-cis-9, 12, linoleic acid	14.78c	12.62a	13.83b	<0.001	<0.010	0.008	43.7, 1.85
C18:2-cis-9, trans-11, CLA[2]	-0.259b	-0.634a	-0.911a	<0.001	<0.001	0.15	55.3, 0.38
	(0.551b)	(0.232a)	(0.123a)				
C18:2-trans-10, cis-12, CLA	0.33b	0.21a	0.25a	<0.001	<0.001	0.002	46.2, 0.11
C18:3n-6 cis-6, 9, 12, γ-linolenic acid	0.21	0.22	0.20	<0.001	0.43	0.90	45.9, 0.05
C18:3n-3 cis-9, 12, 15, α-linolenic acid[2]	0.450b	0.233a	0.206a	<0.001	<0.001	<0.001	88.2, 0.09
	(2.82b)	(1.71a)	(1.61a)				
C20:1-cis-11, eiconsanoic acid	0.039a	0.046ab	0.05b	0.007	0.006	<0.001	34.6, 0.01
C20:2-cis-11, 14, eicosadienoic acid	0.55	0.56	0.55	0.10	0.91	0.07	18.3, 0.11
C20:3n-6 cis-8, 11, 14, eicosatrienoic acid	0.18b	0.12a	0.12a	0.28	0.001	0.45	20.3, 0.08
C20:3n-3 cis-11, 14, 17, eicosatrienoic acid	0.52b	0.45ab	0.38a	<0.001	<0.001	<0.001	52.6, 0.15
C20:4n-6 cis-5, 8, 11, 14, arachidonic acid	0.14	0.15	0.15	0.14	0.09	0.029	21.9, 0.03
C20:5n-3 cis-5, 8, 11, 14, 17, EPA[2]	-0.691b	-0.798ab	-0.921a	<0.001	<0.001	0.17	83.6, 0.21
	(0.204b)	(0.159ab)	(0.120a)				
C22:5-cis-7, 10, 13, 16, 19, DPA[2]	-0.398	-0.423	-0.309	<0.001	0.084	0.321	62.8, 0.25
	(0.400)	(0.378)	(0.491)				
C22:6n-3-cis-4, 7, 10, 13, 16, 19, DHA[2]	-0.596	-0.697	-0.709	<0.001	0.021	0.62	87.6, 0.21
	(0.254)	(0.201)	(0.195)				

[1]Means in the same row with no letters after them or with a common letter after them do not differ significantly (P >0.05) as determined by Fisher's least significance difference (LSD) mean separation test.

[2]Analysed as log(value). The significance of differences (using LSD) was based on the transformed means (log) of these attributes, and the back-transformed means are shown in brackets below the transformed means.

Appendix 9.1
A comparison of three different pork ball products available in the market in Singapore with the recipe for the current low-fat, low-salt product

Brands	**Ping Pong**	**Fair Price**	**Lion Dance**	**New Recipe** (used in Experiments 2 & 3)
Salt Content (mg/100 g)	860	860	535	431
Fat Content (g/100 g)	12.2	12.2	12.0	7.7
Ingredients				
Pork lean	✓	✓	✓	✓
Pork backfat	✗	✗	✓	✓
Water	✓	✓	✗	✗
Starch	✓	✓	✓	✓
Vegetable Oil	✓	✓	✗	✗
Dried Cuttlefish	✓	✓	✗	✗
Sugar	✓	✓	✓	✓
Salt	✓	✓	✓	✓
Spices	✓	✓	✓	✓
Sodium tripolyphosphate	✓	✓	✓	✓
Cuttlefish Seasoning	✗	✗	✓	✓
Egg	✗	✗	✓	✗
Monosodium Glutamate (MSG)	✗	✗	✓	✓

✓ presence of ingredient; ✗ absence of ingredient

Appendix 9.2

Sodium contributions (mg/100g) from individual ingredients within the 16 pork-ball formulations in Experiment 1

Ingredients	Formulation					
(Sodium/mg)	1	2	3	4	5	6
Pork lean	50.03	51.61	50.28	51.86	51.23	50.91
Pork backfat	-	-	-	-	-	-
Water	-	-	-	-	-	-
Corn Starch	-	-	-	-	-	-
Salt	393.33	393.33	236.00	236.00	236.00	236.00
Sodium tripolyphosphate	93.60	93.60	93.60	93.60	93.60	93.60
Cuttlefish flavour	34.20	34.20	34.20	34.20	34.20	34.20
Seasoning (Soy sauce)	31.90	31.90	31.90	31.90	31.90	31.90
Pepper	-	-	-	-	-	-
Carrageenan	-	-	-	-	-	-
Vitamin E	-	-	-	-	-	-
Total (mg/100g)	603.06	604.64	445.98	447.56	446.93	446.61

Ingredients	Formulation					
(Sodium/mg)	7	8	9	10	11	12
Pork lean	50.79	51.04	50.72	50.77	51.02	50.70
Pork backfat	-	-	-	-	-	-
Water	-	-	-	-	-	-
Corn Starch	-	-	-	-	-	-
Salt	236.00	236.00	236.00	236.00	236.00	236.00
Sodium tripolyphosphate	93.60	93.72	93.72	93.60	93.72	93.72
Cuttlefish flavour	34.20	34.20	34.20	37.62	37.62	37.62
Seasoning (Soy sauce)	31.90	31.90	31.90	31.90	31.90	31.90
Pepper	-	-	-	-	-	-
Carrageenan	-	-	-	-	-	-
Potassium chloride	-	-	-	-	-	-
Vitamin E	-	-	-	-	-	-
Total (mg/100g)	446.48	446.86	446.54	449.89	450.26	449.94

Ingredients	Formulation			
(Sodium/mg)	13	14	15	16
Pork lean	50.98	50.66	50.91	50.60
Pork backfat	-	-	-	-
Water	-	-	-	-
Corn Starch	-	-	-	-
Salt	236.00	236.00	236.00	236.00
Sodium tripolyphosphate	93.72	93.72	93.72	93.72
Cuttlefish flavour	34.20	34.20	34.20	34.20
Seasoning (Soy sauce)	31.90	31.90	31.90	31.90
Pepper	-	-	-	-
Carrageenan	-	-	-	-
Potassium chloride	-	-	-	-
Vitamin E	-	-	-	-
Total (mg/100g)	446.79	446.48	446.73	446.42

[1] Note that sodium chloride contains 39.33% sodium while sodium tripolyphosphate contains 31.24% sodium. Pork is taken to contain 63 mg of sodium per 100 g.

Appendix 9.3

A comparison of the contribution of sodium (mg/100 g) from individual ingredients in the three pork ball formulations in Experiment 2

Ingredients	Sodium(mg)		
	Original formulation	Improved formulation F1/F3	Improved formulation F2/F4
Pork lean	50.66	50.66	50.66
Pork backfat	-	-	-
Water	-	-	-
Corn Starch	-	-	-
Salt	236.00	236.00	236.00
Sodium tripolyphosphate	93.72	-	62.40
Tetrapotassium pyrophosphate	-	-	-
Cuttlefish flavour	34.20	34.20	34.20
Seasoning	31.90	31.90	31.90
Pepper	-	-	-
Carrageenan	-	-	-
Potassium chloride	-	-	-
Vitamin E	-	-	-
Total (mg/100g)	446.48	352.76	415.16

Appendix 9.4

Questionnaire for sensory evaluation of pork ball using a Singapore consumer panel for Experiment 1

Name : _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Instructions:

1. Please evaluate the four samples by smelling and tasting.
2. Please rank the four samples for their acceptability level of the sensory attributes using a scale from 1 to 4 where 1 is the least acceptable and 4 is the most acceptable. No tie score is allowed.

Sample code	154	365	427	632
Colour				
Aroma				
Flavour				
Texture				
Overall acceptability				

Comments (if any):

_____

_____

_____

_____

Thank you for your participation.

Appendix 9.5
Sensory evaluation questionnaire for QDA for pork balls using a trained panel of Singaporeans in Experiment 1

Name : _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Instructions : Please taste the given sample and mark your evaluation on each of the line scales.

Sample Code : ☐

Appearance

Colour

Grey Brown

Colour saturation

Light Dark

Aroma Characteristics

Meaty aroma

None Strong

Brothy aroma

None Strong

Cuttlefish aroma

None Strong

Mutton aroma

None Strong

Metallic aroma

None Strong

Acidic aroma

None Strong

Garlic aroma

None Strong

Stale odour

None Strong

Taste/Flavour Characteristics

Meaty flavour

None Strong

Brothy flavour

None Strong

Cuttlefish flavour

None Strong

Mutton flavour

None Strong

Metallic flavour

None Strong

Acidic flavour

None Strong

Stale flavour

None Strong

Salty taste

None Strong

Bitter taste

None Strong

Garlic flavour

None Strong

Texture

Chewiness

Not Chewy Very Chewy

Cohesiveness

Very Brittle Very Compact

Juiciness

None Strong

Hardness

Very Soft Very Hard

Aftertaste

None Strong

Comments :

Appendix 9.6

Questionnaire for sensory evaluation of preferred size of pork ball using a Singapore consumer panel

Name : _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Instructions:

1. There are two samples on the tray.
2. Please DO NOT EAT the samples.
3. Please put a tick in the box for the preferred sample size.

Sample code	147	615
Which do you prefer in terms of size?		

Comments (if any):

_____

_____

_____

_____

Thank you for your participation.

Appendix 9.7

Changes in mean internal temperatures of pork balls weighing either 8 or 10 g with increasing time when cooked in boiling water

Time (min) (Y)	Temperature (^{o}C) (X)	
	8 g pork balls (n=3)	10 g pork balls (n=3)
0	4.0	4.0
0.5	30.5	30.1
1.0	47.1	46.5
1.5	59.5	57.4
2.0	70.1	68.1
2.5	77.7	75.2
3.0	84.7	83.4
3.5	89.8	87.4
4.0	99.0	95.0
4.5	100	100
Time at 75°C[1]	2 min 39 sec	2 min 45 sec

[1] Estimated from regressing temperature on time to give the following equations:

For 8 g balls: $Y = 2.79X - 49.82$; $R^2 = 92.5$

For 10 g balls: $Y = 2.87x + 50.42$; $R^2 = 93.0$

Appendix 10.1

Sensory evaluation questionnaire for QDA for pork balls made with pork and back fat from diet treatment groups AT, PO, POS, PTS, PFSe and PFSl using a trained panel of Singaporeans

Name : _____

Age Group : 11-20 21-30 31-40 >40

Race : Chinese Indian Others

Smoking Habits : Yes No

Instructions : Please taste the given sample and mark your evaluation on each of the line scales.

Sample Code : ☐

Appearance

Colour

Grey — Brown

Colour saturation

Light — Dark

Aroma Characteristics

Meaty aroma

None — Strong

Brothy aroma

None — Strong

Cuttlefish aroma

None — Strong

Mutton aroma

None — Strong

Metallic aroma

None Strong

Acidic aroma

None Strong

Rancid odour

None Strong

Taste/Flavour Characteristics

Meaty flavour

None Strong

Brothy flavour

None Strong

Cuttlefish flavour

None Strong

Mutton flavour

None Strong

Metallic flavour

None Strong

Acidic flavour

None Strong

Rancid flavour

None Strong

Salty taste

None Strong

Texture

Chewiness

Not Chewy Very Chewy

Cohesiveness

Very Brittle Very Compact

Juiciness

None Strong

Hardness

Very Soft Very Hard

Aftertaste

None Strong

Comments :