ANTIOXIDATIVE EFFECTS OF MANGO WASTES ON SHELF LIFE OF PORK PRODUCTS

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Declaration

Some aspects of this thesis have been published and accepted for publication *(copies of the published and submitted papers are attached at the back of the thesis)* or presented at Conferences.

Publications

- Le, H.M., Mason, L.S., & Bickerstaffe, R. (2010). Total phenolic content of Tommy Atkins mangoes imported into New Zealand. Proceeding of the Nutrition Society of New Zealand, *34*, 34-40.
- Le, H.M., Mason, L.S., & Bickerstaffe, R. (2010). Total phenolic content of Tommy Atkins mangoes imported into New Zealand. *Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand 2009, Australasian Medical Journal* (Online). Doi 10.4066/AMJ.2010.209
- Mason, S.L., Le, H.M., & Bickerstaffe, R. (2011). The effects of antioxidants from mango on the shelf life of pork sausages. *Proceedings of the 57th International Congress of Meat Science and Technology* (ICoMST).
- Le, H.M., Mason, L.S., & Bickerstaffe, R. (2011). The relationship between mango physicochemical characteristics and mango kernel antioxidant activities. *Proceeding of the Nutrition Society of New Zealand* (In press).
- Le, H.M., Mason, L.S., & Bickerstaffe R. (2011). The relationship between mango physicochemical characteristics and mango kernel antioxidant activities. *Conference Abstracts Nutrition Society of Australia and Nutrition Society of New Zealand* 2011.
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Presentations

- Total phenolic content of Tommy Atkins mangoes imported into New Zealand. A poster presentation at the 44th Joint Annual Scientific Meetings of the Nutrition Society of New Zealand and the Nutrition Society of Australia at Newcastle, Australia in 2009.
- The relationship between mango physicochemical characteristics and mango kernel antioxidant activities. An oral and poster presentation at the 46th Joint Annual Scientific Meetings of the Nutrition Society of New Zealand and the Nutrition Society of Australia at Queenstown, New Zealand in 2011.
- The effects of antioxidants from mango on the shelf life of pork sausages. A poster presentation at the 57th International Congress of Meat Science and Technology (ICoMST) 2011, Ghent, Belgium.
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Abstract of a thesis submitted in partial fulfilment of
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Antioxidative effects of mango wastes on shelf life of pork products

by

Hung Minh Le

Peel and kernel often discarded during mango processing, are potential sources of antioxidants and could be utilised as additives to preserve meat products. The cultivar, physicochemical characteristics and stage of maturity are important factors that influence the antioxidant activity of mango flesh, peel and kernel. The cultivar Tommy Atkins, imported into New Zealand and the four cultivars; "Cat Hoa Loc", "Cat Chu", Ghep" and "Nam Dok Mai" grown in Vietnam were compared to determine which of the cultivars have antioxidant potential.

Tommy Atkins and Nam Dok Mai were less mature than the other three Vietnamese cultivars based on maturity score, firmness, total soluble solids (TSS), titratable acidity (TA) and Vitamin C. The antioxidant capacity of the mango flesh from all the cultivars ranged from 252.6 to 754.4 GAE $100g^{-1}$ DW (total phenolic content), 43.6 to 70.0 µmol TE g^{-1} DW (ABTS radical scavenging activity) and 30 to 51.6 µmol TE g^{-1} DW (DPPH radical scavenging activity). Mango peel antioxidant capacity was higher than flesh and ranged from 822.3 to 8084.9 GAE $100g^{-1}$ DW (total phenolic content), 406.3 to 1188.3 µmol TE g^{-1} DW (ABTS radical scavenging activity) and 210.6 to 735.4 µmol TE g^{-1} DW (DPPH radical scavenging activity). The antioxidant capacity values of mango kernel was the highest and ranged from 6286.0 to 13888.8 GAE $100g^{-1}$ DW (total phenolic content), 1066.3 to 2227.6 µmol TE g^{-1} DW (ABTS radical scavenging activity) and 667.4 to 2205.7 µmol TE g^{-1} DW (DPPH radical scavenging activity).

Flesh from Tommy Atkins and Cat Chu contained the highest antioxidant capacity than flesh from any other cultivar. Kernel from Tommy Atkins had the highest antioxidant capacity than any other cultivar whereas peel from Nam Dok Mai contained the highest antioxidant capacity than peel from any other cultivar. In all the cultivars investigated, kernel showed the highest

content of total phenolic content and antioxidant capacities followed by peel then flesh, except for Nam Dok Mai whose kernel and peel contained similar antioxidant capacities.

The tannin content was significantly higher in kernel (21.3 - 54.7 g kg^{-1} DW), than peel (7.9 - 22.9 g kg^{-1} DW) than flesh (0.9 - 1.1 g kg^{-1} DW) in all the cultivars. There were no oxalates detected in flesh in any of the cultivars. Small amounts of oxalates were detected in peel from all the cultivars and in kernel from Nam Dok Mai and Tommy Atkins. Peel and kernel from Cat Chu contained low levels of tannins and oxalates and are possible sources of antioxidants to prevent oxidative damage in food products.

Drying is usually used to produce dried products that can be mechanically incorporated into foods. Freeze, sun, vacuum and microwave drying reduced the hydrophilic antioxidants and antioxidant capacity of mango flesh whilst forced-air drying increased the total phenolic content (TPC) and ferric reducing antioxidant power (FRAP). In the case of peel, all the drying treatments reduced hydrophilic and lipophilic oxygen radical absorbance capacity (H-ORAC and L-ORAC) whilst microwave and freeze drying increased TPC and FRAP. For kernel, vacuum, followed by freeze drying increased the H-ORAC values and antioxidant capacity as measured by ABTS, DPPH and FRAP whilst the other drying treatments reduced the antioxidant activities.

The relationship between the physicochemical parameters (maturity) of Tommy Atkins mango fruits with the antioxidant capacity of their flesh, peel and kernel fractions as measured by their total phenolic content, ABTS, DPPH, FRAP, H-ORAC and L-ORAC activities was investigated. The physicochemical properties firmness, total soluble solids, titratable acidity, TSS:TA, vitamin C and moisture content were significantly correlated ($p < 0.05$) with the stage of maturity of the Tommy Atkins mango fruits. Thus, any one of the measured physicochemical parameters could be used as an indicator of fruit maturity in Tommy Atkins. All the antioxidant assays TPC, ABTS, DPPH, FRAP and H-ORAC were significantly correlated ($p < 0.05$) to each other indicating that phenolics in flesh, peel and kernel are good sources of antioxidants. In mango peel, only weak-to-moderate correlations were found between the lipophilic antioxidants and hydrophilic antioxidants or antioxidant capacity as measured by TPC, ABTS, DPPH and FRAP. Mango kernel from the less ripe mangoes with low maturity score, TSS, TSS:TA ratio and high TA, firmness and vitamin C content were relatively high in antioxidant capacity relative to those from ripe mangoes. Peel colour was an

unreliable index of the maturity of Tommy Atkins fruit or the antioxidant capacity of their peel, flesh and kernel.

The incorporation of freeze dried mango peel (1% w/w) or kernel (1% w/w) into pork sausages or patties on inhibiting lipid oxidation was investigated over a 10 day chilled storage period. There were small changes in TBARS (2-thiobarbituric acid reactive substances) and myoglobin over the first 4 days whilst off-odours and volatiles increased at day 4 in the control pork sausages and patties without any antioxidants. The volatiles, 1-pentanol (44.0%) and 3-hydroxyl-2-butanone (45.8%) in the sausages and hexanal (36.6%), pentanal (10.8%) and 1-pentanol (14.6%) in the patties were all identified as products of lipid oxidation and were generated by day 4. More volatiles, particularly hexanal, were released in the patties than in the sausages.

The addition of mango kernel (1% w/w) or peel (1% w/w) or synthetic antioxidant BHT (0.01% w/w) to the sausages and patties immediately changed TBARS (only observed in sausages), myoglobin and volatiles at day zero but there was no effect on colour or odour. The redness of sausages was reduced and the discoloration (hue) increased dramatically at day 2 and remained unchanged across the remainder of the storage period whilst the colour of the patties decreased gradually from day 0 to day 10. The addition of mango kernel, peel and BHT stabilised the colour of the sausages and patties especially the redness across the 10 day storage period. The antioxidative effects of the additives were more pronounced after day 4 when the addition of mango kernel or peel or BHT to the sausages and patties inhibited lipid oxidation as shown by the maintenance of stable TBARS, colour (a* and hue) and myoglobin and the reduction in off-odour and volatiles, compared to the controls. The addition of peel or BHT maintained the pleasant odour of pork sausages and patties for up to 4 days of storage and kernel for 6 days.

At 10 days, the mean TBARS values of the low (9.1% fat), medium (22.2%) and high (47.4%) fat control sausages were between 12.1 to 15.0 μmol MDA kg^{-1} DW and sausages with added kernel between 3.1 to 3.8 μmol MDA kg^{-1} DW. For patties, at 10 days, the TBARS values of control low, medium and high fat patties ranged from 12.3 to 29.2 μmol MDA kg^{-1} DW and those with kernel from 6.9 to 7.9 μmol MDA kg^{-1} DW. Mango kernel was more effective than peel or BHT in inhibiting lipid oxidation over the 10 day storage period. There was also an effect of the fat content (9.1 to 47.4% fat) of the product on the effectiveness of the added antioxidants to inhibit lipid oxidation. The TBARS in patties and

the discoloration and loss in redness of the patties and sausages were higher in the high (47.4% fat) compared to low (9.1%) fat products. Furthermore, at 6 days for sausages and 4 days for patties the inhibitory effects on lipid oxidation was more effective with kernel than peel or BHT in the high and medium fat products.

Pork sausages with added kernel under O_2-permeable film at 4ºC were assessed to be microbiologically safe to consume over a 10 day storage period. Pork patties had a high TVC and were contaminated with E.coli and were assessed unsuitable for human consumption. The dried kernel and peel products themselves were judged microbiologically safe to use as food supplements. In addition, mango kernel exhibited antimicrobial effects against E.coli, staphylococci, coliforms, yeasts and moulds and TVC in the pork sausages.

Freeze dried mango kernel or peel contained antioxidants and if added to pork products extended their shelf life by inhibiting lipid oxidation (TBARS) and preventing changes in colour and odour. Kernel showed the highest antioxidant capacity, compared to peel or synthetic BHT. Dried mango kernel extended the shelf life of pork products for an additional 4 (patties) or 6 (sausages) days compared to patties or sausages without mango additives. In summary, dried mango kernel is an excellent natural source of antioxidants and prevents undesirable oxidative damage in pork products.

Key words: mango fraction, pork, antioxidant, ORAC, lipid oxidation, myoglobin, volatiles, TBARS, colour, odour, drying, physicochemical parameters, microbiological characteristics, SPME, GC-MS.

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ABBREVIATIONS

AAPH	2,2'-azobis(2-amidino-propane) dihydrochloride
ABTS	2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid
AUC	area under the curve
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
CV	coefficient of variation
CVA	canonical variates analysis
DPPH	1,1-Diphenyl-2-picrylhydrazyl radical
DW	dry weight
F-C	folin ciocalteu
FRAP	ferric reducing antioxidant power
FW	fresh weight
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectroscopy
GLM	general linear model
H-ORAC	hydrophilic oxygen radical absorbance capacity
HPLC	high performance liquid chromatography
L-ORAC	lipophilic oxygen radical absorbance capacity
LSD	least significant difference
Mb	myoglobin or deoxymyoglobin
MbO_2	oxymyoglobin
MCP	methyl cellulose perceptible
MDA	malondiadehyde
MMb	metmyoglobin
N/S	non-significant
nd	not detected
PCA	principal component analysis
RMCD	randomly methylated *â*-cyclodextrin
SPME	solid phase microextraction
TA	titratable acidity
TBA	thiobarbituric acid
TBARS	2-thiobarbituric acid reactive substances
TBHQ	tertiary butylhydroquinone
TCA	trichloroacetic acid
TE	trolox equivalent
TEAC	trolox equivalent antioxidant capacity
TIC	total ion count
TMP	1,1,3,3 - Tetramethoxypropane
TPC	total phenolic content
Trolox	6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid
TSS	total soluble solids
TVC	total viable count

CHAPTER 1
GENERAL INTRODUCTION

In the past few decades, antioxidants have attracted wide interest from scientists and consumers with regards to their effect on human health because antioxidants may contribute to maintaining the normal physiological functions of the human body (Ou *et al.*, 2002). Antioxidants have also been included as additives in food products to preserve their quality through reducing deterioration initiated by lipid oxidation and microbiological spoilage (Li *et al.*, 2006). The use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) in preserving foods is now banned or under strict regulation in many countries because of their associated toxic (Buxiang and Fukuhara, 1997; Jo *et al.*, 2006) and carcinogenic (Hirose *et al.*, 1998; Jo *et al.*, 2006) side effects. Consequently, there is interest in using naturally occurring antioxidants as food additives. The latter approach aligns with the concerns of consumers about the adverse effects of synthetic food additives on their health. A number of natural antioxidants have been added at the food preparation and processing stage and have increased the shelf life and oxidative stability of stored food products (Chen *et al.*, 2008a).

Recently, it has been suggested that mango kernel and peel from mango fruit processing are good sources of natural antioxidants (Puravankara *et al.*, 2000; Arogba, 2002; Berardini *et al.*, 2005; Soong and Barlow, 2006; Abdalla *et al.*, 2007b). Indeed, mango kernel and peel, can account for 10 to 25% (Abdalla *et al.*, 2007a; Ajila, *et al.*, 2007) of the fruit being discarded as waste with consequential impacts on pollution and environmental risks to communities. Vietnam is one of the ten leading mango producing countries that account for over 85% of the world production and planted 87,500 hectares of mangoes in 2010 (General Statistics Office of Vietnam, 2012). The major cultivars are ‘Cat Hoa Loc’, ‘Cat Chu’, ‘Ghep’ (FAO, 2004, section 2.4) and ‘Nam Dok Mai’; the latter originates from Thailand. Mango wastes such as kernel and peel are available in Vietnam and this research will examine their potential as a natural source of antioxidants for the food industry.

Mango kernel contains hydrophilic (e.g. phenolics) and lipophilic (e.g., tocopherols and carotenoids) antioxidants. Mango peel has a high antioxidant capacity and contains antioxidants such as polyphenols, anthocyanin, carotenoid and vitamin C which can be dissolved in either hydrophilic or lipophilic systems (Berardini *et al.*, 2005; Ajila *et al.*, 2007). The presence of hydrophilic and lipophilic antioxidants together with phospholipids in mango

peel and kernel makes them a good potential source of antioxidants to scavenge free radicals in lipid and aqueous phases of food. In this research, the physicochemical properties, antioxidant capacity and anti-nutrients of mango flesh, peel and kernel from the Vietnamese cultivars and the Tommy Atkins cultivar were determined to characterise the various cultivars and to assess their suitability for specific markets.

Some physicochemical parameters reflect the maturity of mangoes and could possibly be used as indices of the antioxidant capacity of specific mango fractions (Palafox *et al*., 2009). Thus, the relationships between individual physicochemical properties and their relationship with the antioxidant capacity of the specific cultivar, Tommy Atkins, were determined. It is suggested that no one antioxidant assay can reflect the total antioxidant capacity of a fruit. Therefore, six different antioxidant assays TPC, ABTS, DPPH, FRAP, H-ORAC (hydrophilic antioxidants) and L-ORAC (lipophilic antioxidants) were used to measure the antioxidant capacity of flesh, peel and kernel. The correlations between these antioxidant assays were also assessed to ascertain whether one or more methods would be useful to select mango flesh, peel and kernel for their antioxidant activities.

In order to utilise mangoes as a source of antioxidants for the food industry, the mangoes must undergo a drying treatment to produce a suitable dried product that can be incorporated into a food product.

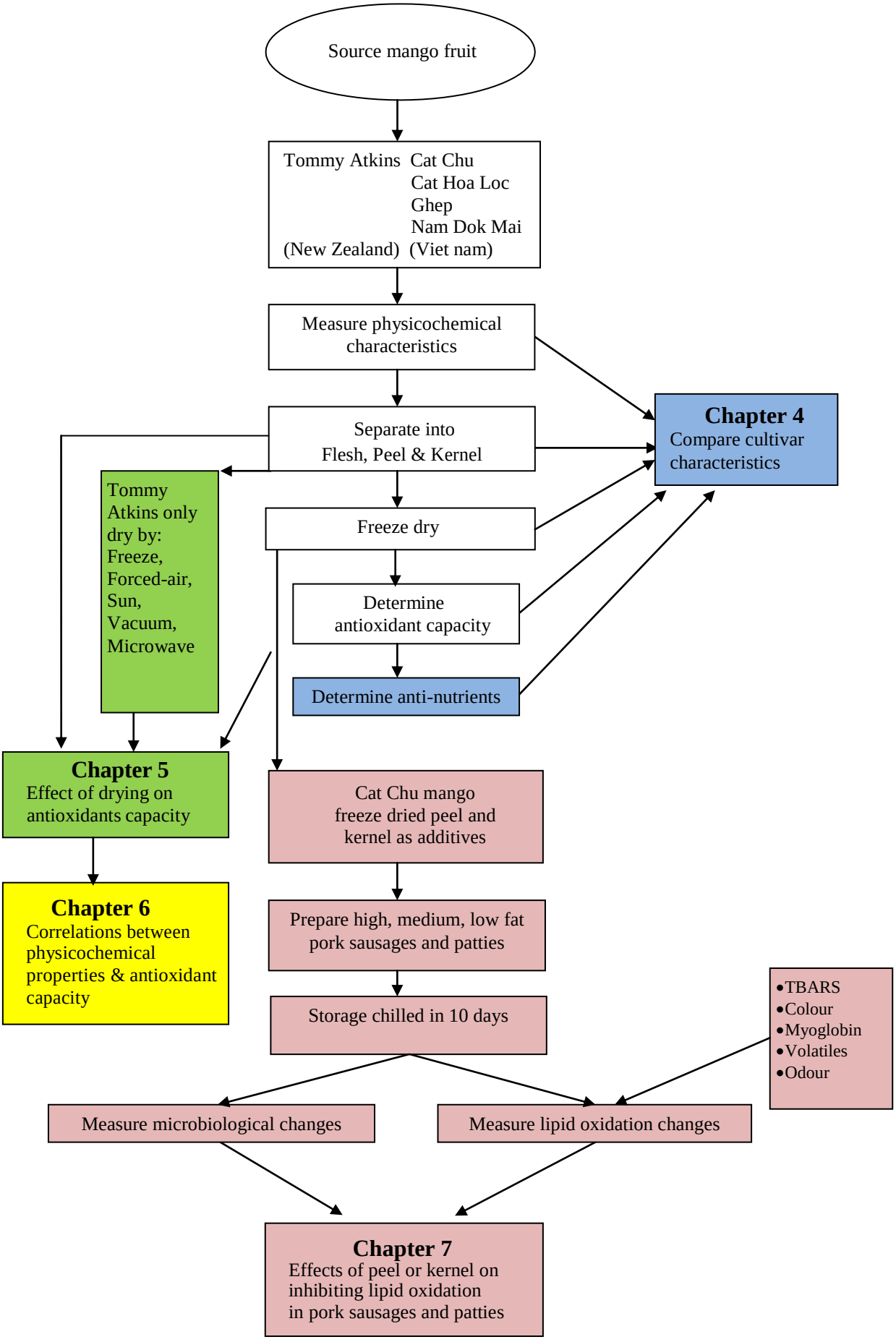
Meat products such as pork sausages and patties are highly perishable and deteriorate rapidly causing potentially dangerous health risks through microbial growth and chemical changes. Lipid oxidation, in particular, is the cause of deterioration causing undesirable changes in flavour (volatiles and odour) and colour (redness and myoglobin) of meat and meat products (Rharjo and Sofos, 1993).

Objectives of research

The objectives of the study were to:

- Compare the physicochemical characteristics, antioxidant capacity and anti-nutrient of mango fractions from Tommy Atkins cultivar imported into New Zealand and the cultivars grown in Vietnam. This is discussed in chapter 4.
- Determine which drying method yields the highest antioxidant capacity in dried mango flesh, peel and kernel. Reported in chapter 5.
- Determine if the physicochemical parameters of mango fruit can be used as indicators of the antioxidant capacity of mango fractions. Reported in chapter 6.
- Evaluate the effects of adding dried mango peel and kernel, as a source of antioxidants, on the shelf life of pork sausages and patties as outlined in chapter 7.

Figure 1. Summary on studying the antioxidant effects of mango wastes on the shelf life of pork products



CHAPTER 2
LITERATURE REVIEW

Antioxidants, which scavenge free radicals and chelate metal ions, play important roles in food and human health. The levels of antioxidant need to be measured and antioxidant assays need to be evaluated for some particular products. However, there are a number of factors such as drying treatments, cultivars and physicochemical characteristics that can affect the antioxidant capacity of a product. Mango is one of the most important fruits in Vietnam in which mango wastes can be a potentially source of antioxidants. Pork and pork products, such as sausages and patties, are easily oxidised and deteriorated. Natural antioxidants extracted from plants are effective in controlling oxidative changes and deterioration of the meat products.

2.1. Antioxidants and their roles in food and human health

2.1.1. What are antioxidants?

Antioxidants are defined as a group of substances that at low concentrations prevent or inhibit the adverse effects that reactive species may have on normal physiological functions (Halliwell and Whitemann, 2004; Moon and Shibamoto, 2009; Karadag *et al*., 2009). Antioxidants protect the key cellular components by neutralising potential damages from free radicals generated as natural by-products of cell metabolism (Badarinath *et al.,* 2010). Free radicals are also formed from the effect of environmental factors such as smoking, pesticides, pollution and radiation on cell metabolism (Bagchi and Puri, 1998).

To study antioxidants, it is important to understand what free radicals are and how they act. A free radical is an atom or group of atoms that have one or more unpaired electrons (Halliwell and Whiteman, 2004). Radicals can have positive, negative or neutral charges. They are often intermediates in a wide variety of biochemical reactions but if generated in excess they can destroy a broad range of macromolecules (Teal and Saggers, 1997). A remarkable feature of radicals is that they have extremely high chemical reactivity which explains why they can cause extensive cellular damage. Free radicals can adversely affect lipids, proteins and DNA and have been implicated in a number of undesirable human diseases.

There are many types of radicals. Some are oxygen derived (ROS, reactive oxygen species) and others nitrogen derived (RNS, reactive nitrogen species) (Valko *et al*., 2007). In

biological systems radicals derived from oxygen are known collectively as the reactive oxygen species (ROS) and include superoxide (O_2^{*-}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^*) and singlet oxygen (O_2*) (Gilbert *et al.,* 1981; Devasagayam, 2004) as illustrated in Figure 2.1.

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Figure 2.1. Reactive oxygen species (ROS) (Held, 2010).
http://www.biotek.com/assets/tech_resources/ROS_White_Paper.pdf

Antioxidants can exercise their protective properties at different stages of the oxidation process and by different mechanisms. Antioxidants are classified into two groups, namely, primary and secondary antioxidants, depending on their mechanism of action. Primary antioxidants are chain breaking or free radical scavengers. Primary antioxidants quench free radicals by two mechanisms: (i) by donating hydrogen or transferring an electron to the free radicals or (ii) by forming complexes with the free radical (Prior *et al*., 2005). The two mechanisms are outlined below:

- *Hydrogen donation or electron transfer to free radical (R)*

- R• + AH → RH + A•
- RO• + AH → ROH + A•
- ROO• + AH → ROOH + A•

- *Acceptor complexing with free radical (R)*

- R• + A• → RA
- RO• + A• → ROA
- ROO• + A• → ROOA

Secondary antioxidants influence the deactivation of metals; inhibit the breakdown of lipid hydroperoxides or the regeneration of primary antioxidants (Gordon, 1990; Koleva e*t al*., 2002). The regeneration of primary antioxidants is outlined below:

- *Regeneration of primary antioxidant*

A• + BH → AH + B•

In the illustrations, AH: antioxidant; ROO•, lipid peroxyl radical; ROOH, hydroperoxide; A•, antioxidant free radical; RH, unsaturated lipid; R•, lipid radical; AH: stable compound (non-radical product); BH: secondary hydrogen donor; B•: secondary antioxidant free radical (Dapkevicius, 2002).

2.1.2. Roles of antioxidants in food and human health

In the past few decades, there has been growing evidence that oxidative stress and specific human diseases can be prevented by including in the diet plant foods that contain large amounts of antioxidants such as vitamins C, E or natural antioxidants such as flavonoids, tannins, coumarins, curcuminoids, xanthons, phenolics and terpenoids (Larson, 1988; Bae and Moon, 1997; Buxiang and Fukuhara, 1997; Yen *et al*., 1997).

Dietary antioxidants can act as free radical scavengers, radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors (Karadag *et al*., 2009). Therefore, there is increasing interest in the use of extending the range of antioxidants that can be used as food ingredients to prevent food oxidation.

Antioxidants such as BHT, BHA and plant extracts have been widely used as additives, preservatives or supplements in food industries. The study of natural antioxidants, functional foods, nutraceuticals and health foods has increased in the past few decades (Andlauer and Furst, 2002; Zulueta, *et al.*, 2007). Ou *et al*. (2002) suggested that increasing the intake of dietary antioxidants may help to maintain the antioxidant status and normal physiological functions of the human body. Although antioxidants are recognised as important phytonutrients, presently, there is no recommended daily "total antioxidant" intake recommended due to the diversity and complexity of antioxidants (Kaliora *et al.*, 2006). Thus, in vitro and in vivo studies on the antioxidant properties of foods such as fruit and vegetables and their antioxidative effects are still required.

Health diseases such as heart disease, macular degeneration, diabetes and cancer are all influenced by cellular oxidative damage. There has been increasing interest in the mechanism of action of antioxidants and whether they specifically intercept or remove free radicals from cells in the human body. Ames *et al*. (1993) reported that antioxidants prevent injury to blood vessel membranes, optimise blood flow to the heart and brain, prevent cancer-causing DNA damage, and lower the risks from cardiovascular and Alzheimer's diseases. Jo *et al*. (2006)

also indicated that antioxidants can prevent or slow the oxidative damage linked to various diseases such as carcinogenesis, atherogenesis and aging.

It is suggested that all these diseases might be retarded or prevented by protective compounds which have the ability to inhibit reactive oxygen species (ROS) formation, scavenge free radicals, or chelate metals (Panteleon *et al.*, 2008). In the body, natural endogenous antioxidant systems have been developed to deal with the production of free radicals and have been divided into enzymatic and non-enzymatic groups. Examples of the enzymatic antioxidants are superoxide dismutase, gluthathione peroxidise and catalase (Rojas and Brewer, 2008) and non-enzymatic antioxidants are beta-carotene, vitamin C, vitamin E and selenium. There are also phytochemical antioxidants, such as polyphenols, lycopene, lutein, lignen and vitamin like antioxidants that can also protect the body from oxidation damage (Moon and Shibamoto, 2009).

However, the roles of antioxidants, particularly those involved with reactive oxygen species (ROS) and oxygen radicals in human diseases (cancer and neurodegenerative) remains unclear (Halliwell, 2012). Therefore, studies on antioxidants and their effects on scavenging free radicals and other ROS are necessary.

2.2. Methods for determination of antioxidant capacity in food

Due to the chemical diversity and complexity of antioxidants in foods, it is necessary to separate individual antioxidants (e.g. flavonoids, resveratrol, lycopene, carotenes, tocopherols and ascorbic acid), however, these techniques require expensive equipments. Furthermore, the level of a single antioxidant in food does not always reflect its total antioxidant capacity (Pellegrini *et al*., 2003). Antioxidants can be either hydrophilic or lipophilic and they can act individually or cooperatively and often, synergistically. For example, Kiokias *et al*. (2008) noted that a mixed antioxidant system can contribute to the inhibition of oxidation by additive or synergistic effects. Several antioxidants such as tocopherols, ascorbic acid, carotenoids and phenolics can exhibit synergistic interactions; consequently, a combination of these compounds has a higher antioxidant activity than the sum of the activities of the individual components (Zuleata *et al.,* 2007). It is suggested that Vitamin C and E can interact directly by vitamin C sparing vitamin E, and that there is an immediate quenching action of vitamin C against aqueous reactive species which prevents the oxidation of vitamin E. In addition, vitamin C may maintain a `redox recycling' effect on oxidised vitamin E within the lipoproteins and membranes lipids (Hamilton *et al.,* 2000). The term total antioxidant capacity

in the present study includes the synergistic protective associations between both the hydrophilic and lipophilic antioxidants (Talegawkar *et al.,* 2009).

To date, many different terms have been used to express the capacity of antioxidants. These include the efficiency, effectiveness, action, power, potential, potency and activity of the antioxidants (Roginsky and Lissi, 2005; Karadag *et al*., 2009). However, all these terms have no specific chemical meaning (Huang *et al.,* 2005). The term ‘‘antioxidant capacity”, used throughout this thesis refers to the antioxidant levels obtained from five different antioxidant assays including TPC (FC assay).

In recent years, a wide range of spectrophotometric assays has been developed to measure the antioxidant capacity of food. The most popular assays are 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) , ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) (Brand-Williams *et al.,* 1995; Re *et al.,* 1999; Van den Berg *et al.,* 1999; Van den Berg *et al.,* 2001; Kim *et al.,* 2002; Ou *et al.,* 2002; Thaipong *et al.,* 2006). ORAC is a fluorescence based assay (Ou *et al*., 2001; Prior *et al*., 2003) has recently been adopted as a standardised method. Both lipophilic and hydrophilic fractions need to be isolated and assayed independently. The lipophilic assays are less effective with water soluble than fat soluble compounds but do provide results which are similar to those existing in biological systems. The hydrophilic assay is superior to other methods in evaluating the antioxidant activity of compounds such as anthocyanins since this method combimnes both inhibition time and degree of inhibition into a single quantity (Hillmann *et al.,* 2011).

Michalak (2006) suggested that all plants produce a diversity of secondary metabolites such as phenolics. However, due to the wide distribution of phenolics and their high antioxidant activity, it is recognised that the total phenolic content is an important index of antioxidant capacity of food. The total phenolic content is usually measured by the Folin Ciocalteu (FC) spectrophotometric based assay.

In general, most of the assays are based on the same principle in which a coloured synthetic radical or redox-active compound is generated. The generation of these radicals is inhibited by antioxidants in the biological sample which scavenges or reduces the radicals or the redox-active compound. Changes in the level of the synthetic coloured radical are monitored by a spectrophotometer or fluorometer. Appropriate standards are used to quantify the antioxidant capacity e.g. Trolox Equivalent Antioxidant Capacity (TEAC) or vitamin C Equivalent

Antioxidant Capacity (VCEAC). Antioxidants may act in various ways such as scavenging the radicals, decomposing the peroxides or chelating metal ions. Consequently the antioxidant capacity values obtained from different assays can be completely different. Furthermore, assays measuring antioxidant capacity are based on two major chemical mechanisms known as hydrogen atom transfer (HAT) and single electron transfer (SET) which have different kinetics and potential for side reactions (Paixao *et al.*, 2007).

HAT methods measure the ability of an antioxidant to quench free radicals by the donation of hydrogen to form stable compounds (Prior *et al*., 2005). In HAT assays, the antioxidant capacity is based on competition kinetics in which the antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds such as in ORAC (Paixao *et al.*, 2007). HAT reactions are solvent and pH independent and usually completed in seconds to minutes.

SET methods measure the ability of a potential antioxidant to transfer one electron to reduce compounds such as radicals, metals and carbonyls by a reduction of a coloured oxidant as in the ABTS, DPPH and FRAP assays (Huang *et al*., 2005; Paixao *et al.*, 2007; Cam *et al.*, 2009). SET assays measure the capacity of an antioxidant to reduce an oxidant which changes colour when reduced. The degree of colour change is correlated with the antioxidant activity. SET reactions are pH dependent, relatively slow and can require a long time to reach completion. Antioxidant capacity is based on the relative percent decrease in product rather than kinetics (Ozgen, 2006; Karadag *et al*., 2009). Compared to HAT, SET is solvent dependent, has the potential to generate new antioxidants through polymerization of phenolic compounds and may underestimate the true antioxidant potential by reactions not reaching their completion.

SET and HAT reactions may occur together in samples and the mechanism finally dominating in a system will be determined by the antioxidants characteristics (Prior *et al*., 2005). The characteristics of the five antioxidant assays ABTS, DPPH, FRAP, ORAC and FC (TPC) are considered below:

2.2.1. Folin Ciocalteu Reagent Assay for Total Phenolic Contents

Principle

The Folin- Ciocalteu assay is used to measure total phenolics by an oxidation/reduction (redox) reaction (Prior *et al*., 2005). The principle is based on the transfer of single electrons

(SET) in alkaline medium from phenolic compounds to molybdenum to form a blue complex that can be monitored spectrophotometrically at 750–765 nm (Magalhaes *et al.,* 2008).

Advantages:

- Convenient, simple, precise and reproducible (Huang *et al*., 2005; Prior *et al*., 2005).
- Excellent linear correlations with other assays (e.g., DPPH, FRAP, TEAC, ORAC etc.) (Gheldof and Engeseth, 2002; De Beer *et al*., 2003; Madhujith *et al.*, 2006; Shahidi *et al*., 2006; Karadag *et al*., 2009).
- Characterising and standardising botanical samples.

Disadvantages:

- Suffers from interference from sugar, aromatic amines, sulphur acids, Fe^{2+}, etc.
- Several nonphenolic organic and some inorganic substances can give false values
- Carried out in aqueous phase and is not applicable for lipophilic antioxidants
- Lack of standardisation of FCR methods which leads to large differences in phenolic levels (Karadag *et al*., 2009).
- Standards with more than one reacting OH group give high absorbance backgrounds.
- There is always a debate as to whether it is a total phenolic content assay or total antioxidant capacity assay.

2.2.2. ABTS radical cation decolourization assay

Principle

The generation of a highly stable chromophoric cation radical of $ABTS^{\bullet+}$ (blue/green) by peroxyl radicals or other oxidants in the presence of H_2O_2 can be reduced by antioxidants. The antioxidant can delay or diminish its absorbance (Miller *et al*., 1996; Paixao, 2007, Floegel *et al*., 2011). ABTS relies on electron transfers.

Advantages:

- An easy and rapid method that produces very reliable results (Paixao, 2007) which has been used in many research laboratories for studying antioxidant capacity.
- $ABTS^{\bullet+}$ can be solubilised in both aqueous and organic media and is not affected by ion strength. It can be used to measure antioxidant activity of hydrophilic and lipophilic antioxidants (Arnao, 2000; Karadag *et al*., 2009).
- Reacts quickly with antioxidants within 30 minutes used over a wide pH range and be automated for microplate use.

Disadvantages:

- TEAC values characterise the ability of a sample to react with $ABTS^{\bullet+}$ rather than to inhibit the oxidative process
- The reaction between ABTS and samples may take a long time to reach an end point. An assay with a fixed short time (4-6 minutes) is too short and may give incorrect antioxidant capacity values because the reaction is incomplete (Huang *et al*., 2005; Karadag *et al*., 2009). TEAC obtained at a fixed end point or measured based on the kinetics behaviour of the samples produce difference results.
- Require special preparation in which the ABTS radical cation ($ABTS^{\bullet+}$) must be generated by enzymes or chemical reaction (Arnao, 2000; Wojdylo *et al.*, 2007).
- The ABTS radical used in TEAC is an artificial radical and not found in a biological system. Consequently, the assay does not reproduce the *in vivo* situation.

2.2.3. DPPH radical scavenging activity assay

Principle

In the presence of a hydrogen/electron donor (free radical scavenging antioxidant) the absorption intensity is decreased and the radical solution (the purple chromogen DPPH radicals) is discoloured to a pale yellow hydrazine according to the number of electrons captured (Locatelli *et al.,* 2009). DPPH works in both electron transfer (SET) and hydrogen transfer (HAT) systems.

Advantages:

- A rapid, simple and inexpensive method for estimating the antiradical activity of foods (Paixao *et al.,* 2007).
- It produces stable organic nitrogen radicals characterised by a deep purple colour in the range 515-520 (Locatelli *et al.,* 2009).

Works in both hydrogen transfer and electron transfer systems and allows the determination of a substance or a complex mixture that donate either hydrogen atoms or electrons in a homogeneous system (Chaillou and Nazareno, 2006; Paixao *et al*., 2007).

- Became a reference assay to evaluate the *in vitro* antioxidant capacity (Gil *et al.,* 2000; Locatelli *et al.,* 2009).

Disadvantages

- Some antioxidants such as carotenoids have spectra that overlap DPPH at 515nm and interfere with the results (Prior *et al.,* 2006; Karadag *et al*., 2009).

- Not a competitive reaction since DPPH is both a radical probe and oxidant.
- DPPH is discoloured from radical reactions (HAT) or reductions (SET) and consequently unrelated reactions giving inaccurate results.
- The DPPH radical can only be dissolved in organic solvents (methanol, ethanol, acetone), which is a limitation when interpreting the role of hydrophilic antioxidants (Arnao, 2000; Karadag *et al*., 2009).
- Several factors may affect the assay such as solvent, pH, sample concentration and reaction time.
- The absorbance of DPPH radical at 515-520 nm after the reaction with an oxidant is reduced by light, oxygen and solvent types (Ozcelik *et al.*, 2003; Apak *et al.*, 2007; Karadag *et al*., 2009).
- Antioxidants that react quickly with peroxyl radicals *in vivo* may react slowly or even be inert to DPPH due to steric effects preventing accessibility.
- DPPH reacts reversibly with eugenol producing false antioxidant levels (Huang *et al.*, 2005; Karadag *et al*., 2009).
- Some researchers have indicated a non-linear relationship exists between the antioxidant concentration and the DPPH radical scavenging activity (Prior *et al.*, 2005; Villano *et al.*, 2005; Eklund *et al.*, 2006; Monica *et al.*, 2009).

DPPH is a stable nitrogen radical but it does not reproduce the *in vivo* situation

2.2.4. Ferric Reducing antioxidant Power (FRAP) assay

Principle

The FRAP assay is based on the ability to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe (II)-TPTZ) by electron-donating antioxidants in acidic medium (Benzie *et al.*, 1999; Wojdylo *et al*., 2007). The FRAP mechanism is totally electron transfer (SET).

Advantages

- Simple, rapid, inexpensive and robust assay requiring no specialised equipment and can be performed manually or automatically.
- It is totally electron transfer rather than a mixed SET and HAT. In combination with other methods it is a very useful assay to distinguish dominant mechanisms of different antioxidants.

- It relies on a redox reaction that can proceed rapidly. All reactions are completed within 4 and 6 minutes.

Disadvantages:

- Requires a longer reaction time to detect some polyphenols that react slowly. The order of reactivity of many different antioxidants can vary considerably.
- Fe^{2+} is a well-known "pro-oxidant" that can react with H_2O_2 to produce a hydroxyl radical (OH^-). The most harmful free radical found *in vivo* (Karadag *et al*., 2009).
- Some antioxidants e.g. ascorbic acid and uric acid can reduce both Fe^{3+} and reactive species in the FRAP assay so their ability to reduce Fe^{3+} may reflect their ability in reducing reactive species.
- Determines the total reducing power of samples but not all the reductants that reduce $Fe^{3+}$are antioxidants (Prior and Cao, 1999; Nilsson *et al.*, 2005; Karadag, 2009).
- Some antioxidants e.g. GSH (glutathione, an important antioxidant *in vivo*) can effectively reduce prooxidants but not reduce Fe^{3+} (Prior and Cao, 1999; Karadag, 2009).

2.2.5. Oxygen Radical Absorbance Capacity (ORAC) assay

Principle

The ORAC method is based on the inhibition of the peroxyl-radical-induced oxidation initiated by the thermal decomposition of azo-compounds such as 2, 2'-azobis (2-amidino-propane) dihydrochloride (AAPH) (Prior *et al*., 2003). The ORAC method uses the fluorescence of B- or R-phycoerythrin (a fluorescent protein) or d FL (a synthetic nonprotein probe) as a fluoresent probe. The loss of fluorescence of the probe is an indication of the damage from the peroxyl radical. ORAC measures antioxidant inhibition of peroxyl radical induced oxidations and reflects the classical radical chain breaking antioxidant activity by H-atom transfer (HAT) (Ou *et al.*, 2001; Karadag, 2009).

Advantages:

- Utilises a biological relevant radical source and it is the only method that combines both inhibition time and degree of inhibition into a single quantity (Prior *et al.*, 2003; Thaipong *et al*., 2006).
- Has recently been adapted to use fluorescein as the fluorescent probe for high-throughput assays.

- Has largely applied as a method of choice to quantify antioxidant capacity usually in combination with a total phenol content assay.
- Has been applied to measure the antioxidant capacity of botanical (Prior and Cao, 2000) and biological samples (Cao and Prior, 1998).
- Can be applied to measure the antioxidant capacity of both lipophilic and hydrophilic components separately using the same peroxyl free radical source and can be used for antioxidants that exhibit distinct lag phases and those that have no lag phases. It is useful for samples that contain multiple ingredients and have complex reaction kinetics.
- Has the ability to use different free radical generators or oxidants, and can measure many different compounds such as antioxidants against peroxyl and hydroxyl radicals.

Disadvantages:

- Can only measure the antioxidant capacity against peroxyl and hydroxyl radicals and not against all reactive oxygen species (e.g. superoxides and singlet oxygen, Apak *et al.,* 2004; Karadag *et al*., 2009).
- The substrate (probe) concentration is often smaller than the antioxidant concentration. However, in food systems, the antioxidant concentration is much smaller than the substrate (e.g. lipid). Therefore, the antioxidant capacity measured in a real food system may be incorrect.
- The use of B-PE as a FL probe has limitations such as large inter batch differences, photo bleaching of B-PE after exposure to the excitation light, and interaction with polyphenols by nonspecific protein binding. All these factors cause inconsistency in the assay results and false low reading values. This limitation can be solved by FL but FL is pH sensitive and must be carefully monitored (MacDonald-Wicks *et al.,* 2006; Karadag *et al*., 2009).
- FL is not sufficiently lipid soluble, and its fluorescence intensity in a non polar organic solvent is low.

In summary, although each antioxidant assay has its own advantages and disadvantages, it is clear that no one antioxidant assay will reflect the total antioxidant capacity of a particular sample. Badarinath *et al*. (2010) suggested that if the antioxidant capacity of a single plant product is determined by different assays, then each assay will give different results. Therefore, it is impossible to use only one assay to evaluate the antioxidant capacity of a plant

product. To evaluate antioxidant capacity of a sample it is, therefore, essential to use several assays. However, the selection of assays should be carefully considered in terms of their reliability and consistency. To achieve this, the antioxidant assays should be applicable to both lipophilic and hydrophilic antioxidants (Karadag *et al*., 2009). Recently, several reports have suggested that despite the similarity and strong correlations between assays (Paixao, 2007; Arcan and Yemenicioğlu, 2009), the results from the different assays and the correlations between these assays have showed inconsistencies (Connor *et al.,* 2002; Ou *et al.,* 2002; Awika *et al.,* 2003; Thaipong *et al*., 2006). In addition, the same antioxidant assay can give widely different results for the same food product when analysed by different laboratories. Therefore, further development and characterisation of the assays and the correlations between the assays for a particular food are needed.

2.3. Effects of drying on antioxidant activity

Fruits are perishable and will deteriorate in their fresh state so the processing and preservation of fruits are important. Among the processing and preservation methods, drying is the most effective process to preserve fruits to maintain their desirable qualities, reduce storage volume and to extend their shelf-life due to the inhibition of microbial growth. In the drying process free water is removed from the fruit, water availability for enzymatic reactions and microbial growth is reduced and the bulk volume and weight is reduced. However, the drying process is complicated by physical, chemical and biochemical changes that might occur during the drying treatment (Baker, 1997). Furthermore, the enzymatic and non-enzymatic changes that occur on drying fresh plant tissues can cause significant changes to phytochemicals (Sthishkumar *et al*., 2009) and produce negative attributes in the final product.

In most cases, drying involves the application of thermal energy which causes water to evaporate into the vapour phase. In dehydration, the degree of thermal damage is directly proportional to the temperature and duration of exposure to a specific temperature (Kwok, 2004). A high temperature and long process time is associated with hot air drying and adversely affects texture, colour, flavour and nutritional value of the final product. Choi (2006) stated that natural nutrients and phytochemicals could be significantly lost due to heat during thermal processing. Therefore, heat processed dried foods are considered to have a lower health promoting capacity than the corresponding fresh foods. Klaudius (2008) suggested that lowering the drying temperature retains higher phenolic levels. However, the degree of thermal damage to antioxidants depends on the heat stability of each antioxidant

such as phenolics, carotenoids and tocopherols. Madrau *et al.* (2009) reported that the high drying temperatures in air dehydration might cause phenolic depletion whilst carotenoids are only degraded on exposure to large amounts of oxygen.

Many reports have indicated that the effects of drying methods on antioxidant capacity vary according to the specific food. Ishiwata *et al*. (2004) reported that dried fruits had lower antioxidant values than fresh. From a study on apricot, Madrau *et al.* (2009) demonstrated that phenol degradation with hot air drying differed according to the variety and that antioxidant capacity increased significantly in Cafona apricots with increasing drying temperature whilst the Pelese apricot showed no change.

In plums, a high processing temperature reduced significantly the polyphenol and ascorbic acid content, whilst in dried prunes the antioxidants increased significantly (Piga, 2003). Sthishkumar *et al*. (2009) also showed the total antioxidant capacity of certain foods increased. Recent studies have shown that thermally processed fruits and vegetables have higher biological activities due to the various chemical changes that occur during the heat treatment (Dewanto *et al.*, 2002; Kim *et al*., 2000, Choi *et al*., 2006). For example, in plants, phenolics are usually covalently bound with insoluble polymers but when subjected to heat treatment the bound phenolics are liberated (Jeong, 2004). Heat also increases the reducing sugar and promotes the formation of Maillard reaction products which also have antioxidant activity in dried fruits (Madrau, 2009).

It should be noted that the effects of heating or drying temperature on the quality of the total phenolics and antioxidants of fruit is still not fully understood. Currently, many drying methods are being used and each method has its advantages and disadvantages. Sun, forced-air, vacuum, microwave and freeze drying are commonly used techniques to preserve fruits.

Traditionally, open air sun drying is used to dry agricultural materials because it is free, renewable, abundant and environmentally friendly (Baker, 1997). However, sun drying requires long drying times and results in a loss of quality due to materials being directly exposed to weather conditions and being contaminated by microbial, insects, bird and rodent products.

To overcome the disadvantages of open sun drying, greenhouses can be utilized to minimise energy cost and microbial spoilage (Ergunes, 2005). Another conventional drying method is hot air. Forced air ensures a continuous supply of air to replace saturated air (Baker, 1997).

However, drying by heated air can damage products by degrading enzymes and reducing the taste, colour and nutritional characteristics. Other drying methods such as vacuum, microwave and freeze drying reduce any thermal effects by applying low temperatures or using short treatment times.

Vacuum drying has advantages over conventional drying because it expands air and water vapour in the food which creates a frothy or puffed structure and a large area-to-volume ratio for enhanced heat and mass transfer (Jaya and Das 2003; Lee and Kim, 2009).This leads to specific vacuum drying characteristics such as high drying rate, low drying temperatures and low oxygen deficient processing. As a result, the quality, nutritive value and antioxidant activity of vacuum dried products increase (Wu *et al.,* 2007). Therefore, vacuum drying is preferred for oxidisable and temperature sensitive products (Nastaj, 1989; Bialobrzewski and Misiak, 1997; Markowski and Bialobrzewski, 1998; Arevalo-Pinedo *et al*., 2006).

Microwave drying has also become popular because it minimizes the drying time and any reductions in quality by providing a rapid and effective distribution of heat throughout the product (Alibas, 2009). Microwave drying is based on the adsorption of microwave radiation by water molecules and converting the microwave energy into heat. At a certain frequency, microwaves cause the molecules of the materials to vibrate and create intermolecular heat that causes water within the material to evaporate. As a result, a large vapour pressure difference between the centre and the surfaces of the material is generated which allows rapid transport of moisture out of the product so preventing structural collapse.

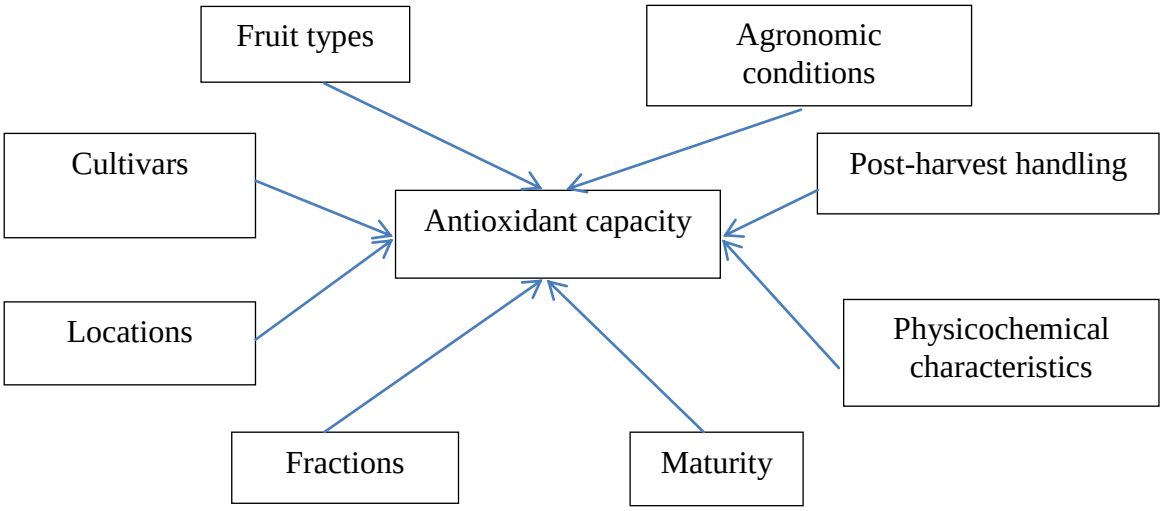
Another drying method is freeze-drying (Barbosa-Canovas and Vega-Mercado, 1996). Freeze-drying or lyophilisation is a drying process in which the water is crystallized at low temperatures and subsequently sublimed from the solid state into the vapour phase under vacuum (George *et al.,* 2004). Freeze-drying produces the highest quality dried foods because it retains features that are close to the fresh product. The major problems with freeze-drying are the long drying time, high energy consumption and high capital costs (Wang *et al.*, 2010).

There is little data on comparing the effects of sun, forced-air, vacuum and microwave drying on the antioxidant activity of fruits. In addition, the effect of the drying treatments on the quality of fruits is also unclear due to conflicting data from previous researches. Therefore, studies on the effects of the various drying treatments on the antioxidant capacity of mango fruit and its fractions are required.

2.4. Relationships between cultivars, physicochemical characteristics and antioxidant properties of fruits

There is a wide range of antioxidants in fruits, such as vitamin A, vitamin C, vitamin E, carotenoids, polyphenolics, anthocyanins and flavonoids (Miladi and Damak, 2008). The antioxidant capacity of fruits is influenced by several factors, such as: cultivar, agronomic conditions, post-harvest manipulation and stage of ripeness (Scalzo *et al.*, 2005; Kevers *et al.*, 2007; Koca and Karadeniz, 2009).

Figure 2.2. Factors affecting antioxidant capacity of fruits



Siriwoharn *et al.* (2004) concluded that cultivar, maturity, UV light exposure, and harvesting method are significant factors that influence the antioxidants of berries. Other studies with blackberries have also indicated that the levels of antioxidants are influenced by maturity and cultivar (Wang and Lin, 2000; Siriwoharn *et al.*, 2004). Wang and Stretch (2001) also suggested that ORAC, anthocyanin, and total phenolics were significantly different in 10 different cranberry cultivars. Other reports have indicated that changes in antioxidants in fruits are often associated with their ripening (Burda *et al.*, 1990; Amiot *et al.*, 1995; Lima *et al.*, 2005; Kobayashi *et al.*, 2008). Prior *et al.* (1998) found there were different levels of antioxidants in various cultivars of blueberries and there was a positive correlation between maturity with the antioxidants, anthocyanin and total phenolic levels.

Serrano *et al.* (2009) investigated several cultivars of sweet cherries at different maturity stages and suggested that irrespective of the differences between the cultivars, antioxidant levels varied during the ripening process and postharvest storage. It should be noted that there are correlations between the stage of maturity and antioxidant activity in fruits. However, it is

unclear as to whether the correlations are positive or negative. Siriwoharn *et al.* (2004) reported that an increase in colour intensity and decrease in acidity are correlated with increases in phenolics, anthocyanins, and total antioxidant activity. Also, in blackberries antioxidant activity increases as the fruit ripens and reaches a maximum at the overripe stage (Siriwoharn *et al.,* 2004). Stoner *et al.* (2010) indicated that the increased maturity of blueberries at harvest enhanced the anthocyanin and antoxidant capacity (Prior *et al*., 1998; Kalt *et al.,* 2003). Meanwhile, Kobayashi *et al*. (2008) indicated that the antioxidant capacity of two pawpaw cultivars tended to decrease on ripening.

Interestingly, Ribera (2010) found that the phenolics and total antioxidant activity was high in unripe and ripe fruits and low in intermediate ripe fruits. A study on blackberries also found that the total anthocyanin levels increased during ripening whilst phenolics and antioxidant capacity showed no change (Siriwoharn *et al.,* 2004).

It should be noted that in some fruits, antioxidant capacity increases over the storage time whilst in others it decreases. This could be attributed to the higher concentrations of phenolic acids (mainly chlorogenic acid) and flavonols (mainly rutin) at the immature fruit stage whilst the total antioxidant activity could increase in mature fruits due to the elevated amounts of anthocyanin.

The changes in total phenolics and antioxidant activity are inconsistent and could be attributed to fruit type. Wang and Lin (2000) investigated the antioxidant activity and maturity of berries and suggested that blackberries and strawberries show the highest antioxidant activity as measured by ORAC, during the green stage whilst red raspberries exhibit the highest antioxidant activity at the ripe stage. Total anthocyanin content increased with maturity for all the three fruit species. Thus further studies on the relationships between antioxidant activity and maturity of a specific fruit such as the mango are required.

Many recent reports have found however that different part of fruits can yield different levels of phenolics and antioxidants. Investigations on the antioxidant activity of fruits and leaves of blackberry, raspberry and strawberry showed that the antioxidant capacity varied according to the cultivar, stage of maturity and plant component (Wang and Lin, 2000).

Lee and Talcott (2004) also stated that antioxidant capacity was influenced by cultivar, maturity and location in the fruit. Their study showed that polyphenolics including ellagic acid, anthocyanins and total antioxidant capacity in eight muscadine grape (*Vitis rotundifolia*)

cultivars were influenced by the ripening stage and their location within the fruit (skin, pulp, and juice).

Another report suggested that in apples the polyphenol profile is characteristic of the cultivar, maturity stage and component part of the fruit (Duda *et al*., 2011). The findings are in agreement with the study by Vieira *et al*. (2009) who suggested that in apples, the concentration of polyphenolics such as flavanols and anthocyanins, and the antioxidant activity differ with cultivar, maturity stage, environmental conditions and fruit component. According to many authors, the content of total phenolics and antioxidants in apples is high in the peel compared to flesh and whole fruit. These facts suggest that apple peel possess more bioactivity than flesh. Caro and Piga (2007) showed seeds and peels of Italian fresh fig cultivars contained higher antioxidant capacity and phenolic content than the edible portions. In dragon fruits, it was also found that the highest TPC and radical scavenging activity were in peel compared to the pulp (Ruzlan, 2010). Guo *et al.* (2003) also reported that peel and seeds of fruits, such as pomegranate, grape, hawthorn, longan and lychee possessed high antioxidant activities, as measured by FRAP, and are rich sources of natural antioxidants.

Although some data has indicated the importance of cultivars and maturity in determining TAC in fruits, the effects of genotype on TAC in fruit components such as peel and kernel have not been investigated. It is therefore important to evaluate the effects of genotypes (cultivars) and physicochemical properties (maturity) on the total phenolic and antioxidant capacity of peel and kernel in fruits (Girish, 2011).

Several reports found that the phenolics and antioxidants of fruits depend on the environment, harvest season and production area (Wang and Lin, 2000; Siriwoham *et al.,* 2004; Cornor *et al.,* 2005). For example, the antioxidant levels of blackberries grown in New Zealand ranged from 56.6 to 66.3 µmol Fe/g fruit and those grown in the United States from 65.5 to 71.8 µmol Fe/g fruit (Connor *et al.,* 2005). Furthermore, blueberry which is well known for its antioxidant capacity, can vary its antioxidant activity depending on cultivar, storage time (Connor *et al.,* 2002), and place of production (Taruscio *et al*., 2004).

Because of the influence of different factors such as cultivars, maturity, parts of fruits and production area it is often difficult to identify which of the factors is the most important influencing antioxidant activity. Further studies are therefore required to determine the effects of cultivars grown in different areas and at different stages of ripening on the phenolics and antioxidant capacity of fruits and their fractions.

2.5. Antioxidant potential of mango fruit

2.5.1. Production of mangoes in the world and in Vietnam

Mango (*Mangifera indica* L) is often known as the king of fruits and is one of the most important tropical fruits in the world (Shahnawz *et al.*, 2012). It is currently ranked 5th in the total world production of fruit crops (Sauco, 1997). Mango is a popular and economically important tropical fruit due to its excellent eating quality (bright colour, sweet taste and luscious flavour) and high nutritive content (vitamins, minerals, fibre, and phytochemicals) (Kim *et al.*, 2009; Parafox-Carlos *et al*., 2012). According to the Tropical fruits (2009), mango dominated global global output of tropical fruits with a share of nearly 40 percent, followed by pineapple, papaya and avocado. Asia was the largest producer of mangoes, accounting for 74 percent of world production by volume, with Latin America and the Caribbean at 16 percent and Africa 10 percent (Tropical fruits, 2009). The leading mango producing countries are India, China, Thailand, Mexico, Pakistan, Brazil, Philippines, Indonesia, Nigeria and Vietnam (Naidu, 2009) with the ten countries accounting for over 85% percent of world production. Meanwhile, Mexico is the leading mango-exporting country (41% of the world market). The United States and European Union together account for 75 percent of world mango imports (Tropical fruits, 2009).

Vietnam is in a tropical region and has a wide range of fruits that can be viable sources of antioxidants. The fruit industry in Vietnam has the potential to expand and plays an important role in agricultural production. The area under fruit tree cultivation of Vietnam has increased rapidly and the production reached 5.721 million metric tons in 2009 (Codex Alimentarius Commission, 2011).

According to FAO, the production of mangoes, mangosteens and guavas in Vietnam ranked 11th and reached 554,000 MT in 2009. The planted area of mangoes was 76,700 hectares in 2010 (General Statistics Office of Vietnam, 2012). Mangoes have traditionally been cultivated in the central and southern parts of Vietnam and their main production areas are Tien Giang, Dong Thap, Can Tho, Vinh Long and Khanh Hoa. Currently, the major cultivars are ‘Cat Hoa Loc’, ‘Cat Chu’, ‘Hon’, ‘Xiem Num’, and ‘Ghep’ (FAO, 2004, section 2.4). The Vietnamese government aims to increase fruit production to 10 million tonnes per year and export fruit and vegetables to a value of 1 billion US by 2015 (Fruit in Vietnam, 2008). Although fresh fruit exports will increase, processed fruits such as juices, condensed juices, jams, starches as well as sugar-infused, dried fruits and pickled fruits are attracting more attention from Vietnamese producers, especially hygienic and safe processed products. As a key fruit in

Vietnam, mango production areas are expected to continuously increase in the future, therefore, the production, processing and utilisation of mango and its products have a promising future.

2.5.2. Utilisation of fruit wastes including mango peel and kernel as a source of antioxidants

Environmental issues such as the costs of discharging waste have forced food processors and food manufacturers to examine alternative ways to treat and utilise their wastes and by-products from fruit processing plants. Environment friendly food processes that minimize waste disposal whilst producing marketable value-added products are required (Stachowski, 1999). Such an approach will resolve the environmental issues whilst increasing the economic efficiency of the processing plant (Zwetsloot, 1995). Processing of fruits produces two types of waste: a solid waste of peel or skin, seeds, stones, etc. and the liquid waste of juice and wash-waters. For some fruits, the discarded portion is very high, e.g. mango 35-60% (Berardini *et al*., 2004), banana 33%, pineapple 35% (Bardiya *et al*., 1996) and orange 30-50% so there is a serious waste disposal problem which needs to be managed (Practical action, 2002). There are six major products that can be produced from solid fruit wastes; they are pectin, reformed fruit pieces, enzymes, wine/vinegar, candied peel and oils.

Commercially, pectin is extracted from citrus peel, apple pomace and some other tropical fruits containing high levels of pectin, e.g. passion fruit. The three most important enzymes that can be extracted from fruit wastes are papain (from papaya), bromelain (from pineapple) and ficin (from figs).

Fruit pulp can be recovered and converted into synthetic fruit pieces. It is technically feasible to produce wine or vinegar from solid and liquid fruit wastes. Peel from citrus fruits can be used in baked goods or as a snack food. The stones of some fruits (e.g. mango, apricot and peach) contain considerable quantities of oil or fat and are used in culinary or perfumery applications. Some seeds (e.g. grape, papaya and passion fruit) yield oil, which are marketed as health promoting products (Practical action, 2002).

Recent studies have shown that fruit wastes can also be good sources of natural antioxidants for preserving food. Apple pomace is a good source of polyphenols and exhibits strong antioxidant and anti-proliferative characteristics (Wolfe *et al.*, 2003). Citrus peel is a rich source of flavanoids. Banana and tomato peels are good sources of carotenoids. Grape pomace, which is a wine industry by-product, is an excellent source of anthocyanins,

catechins, flavanoids, phenolic acids and dietary fibre (Laurrauri *et al.,* 1996; Mazza and Miniati, 1995, cited in Ajila *et al.,* 2007). Grape seed extract is a natural plant substance that is rich in polyphenols and a concentrated source of oligometric proanthocyanidins exhibiting more powerful antioxidant characteristics than vitamin C, E, and beta-carotene. Grape seed extract has the potential as a processed meat additive to reduce lipid oxidation and the formation of off-odours. Mango seed kernels and peel discarded from processing could be recycled into potentially valuable sources of antioxidants that could be used as food ingredients (Ajila, *et al.*, 2007a). Discarded waste mango seeds and peel are currently a source of pollution (Ajila *et al*., 2007a). Previous studies have shown that peel and kernel contribute to 7-24% and 9-40% of mango fruit, respectively (Wu *et al.*, 1993; Berardini *et al*., 2004). Other reports have noted that the waste products of industrial processing of mango such as seed kernels and peels could be potential sources of natural antioxidants and be used as ingredients by the food industry (Laurrauri *et al.*, 1996; Berardini *et al.*, 2004).

2.6. Deterioration in pork and pork products

2.6.1. Pork and pork products

In recent years, health concerns have been raised about the consumption of meat (Hui, 2006) in which pork contains much higher proportions of the major polyunsaturated fatty acid (PUFA) such as linoleic acid than cattle and sheep (Wood *et al.,* 2008). The consumers' decision to purchase pork is based on factors such as colour, fat level, marbling as well as food safety, price, nutritive value and meal convenience. Other important factors are the tenderness, juiciness, flavour and aroma of pork (Taylor-Pickard and Spring, 2007).

The main components of carcasses are muscle, fat, bone and skin. Muscle is converted to meat post-mortem. The average percentage of muscle in relation to live weight varies according to the species, degree of fatness and dressing methods. Typical muscle yields are 39% for chicken, 35% for beef, 32% for veal, 25% for lamb and 36% for pork (Hui, 2006). Lean pork contains 74.5% moisture, 21.4% protein, 2.7% fat, 0.9% ash and 0.5% of carbohydrate (Hui, 2006). These proportions are variable, particularly the lipid content.

Pork is used as a raw material for processing to a number of products such as fermented and dry-cured meats like bacon, cooked ham, dry-cured ham, uncooked, cooked and fermented sausages (Flores and Toldra, 1993; Toldra, 2002; Hui, 2006). Patties and sausages are two common ground pork products which are usually made by mixing minced pork and ingredients to promote taste and shelf life. Sausages are defined as comminuted seasoned meats, stuffed into casings and either smoked, cured, fermented or heated depending on the

type of sausage. Meat for making patties or sausages is from the edible part of a slaughtered animal. High quality patties or sausages cannot be made from inferior raw materials. Formulations are critical for a quality finished product (Savic, 1985). The production of a wide variety of sausages is related to variations in meat formulation, processing temperature, type of casing and particle size. By manipulating the variables, changes occur in the texture, flavour, moisture, and shelf life properties of the sausages (Savic, 1985).

2.6.2. Deterioration of pork and processed pork products during storage and processing

Technological developments in food processing, preservation and handling have provided a variety of meat products. However, meat products are highly perishable and deteriorate rapidly causing potential dangerous health risks through microbial growth and chemical changes. The microbial and enzymatic deterioration can be controlled to a great extent by storing foods at low temperature but such storage cannot prevent lipid oxidation (Dave and Ghaly, 2011). Lipid oxidation is the major cause of deterioration in the quality of meat and meat products. Therefore, more studies are required in this area particularly in lipid oxidation to improve product quality. In raw meat and meat products, the primary factors that influence lipid oxidation are fatty acid composition, endogenous prooxidation and antioxidative constituents and other non-meat additives (Gheisari *et al.,* 2010). Lipids of meat are particularly susceptible to oxidative deterioration (Raghavan and Hultin, 2004). Indeed, one of the major effects of oxidative change is the damage initiated by lipid peroxidation. A common target for peroxidation is the unsaturated fatty acids in membrane phospholipids. The peroxidation reaction involving a fatty acid is illustrated in Figure 2.3.

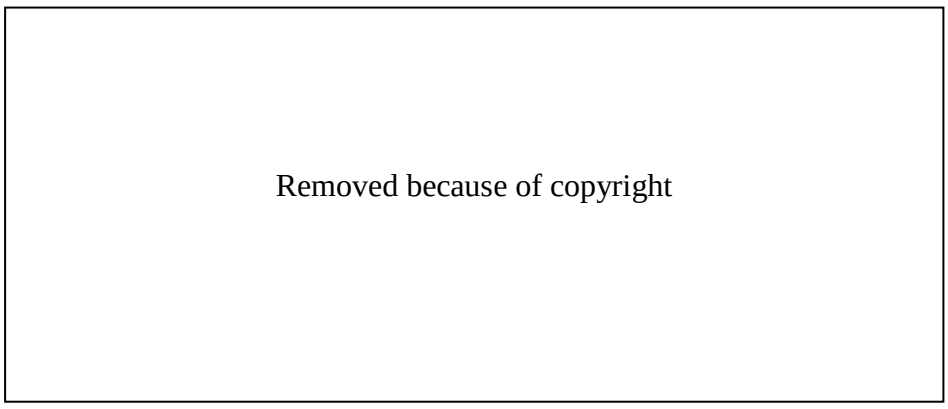


Figure 2.3: Lipid peroxidation reaction (Held, 2010).
http://www.biotek.com/assets/tech_resources/ROS_White_Paper.pdf

The peroxidation reaction of polyunsaturated fatty acids (PUFA) in meat can be initiated by free radicals which are always present in metabolic active animal cells. In addition, meat post-slaughter is often exposed to oxygen that promotes the lipid oxidation. Oxygen cannot interact directly with the PUFA but it can be converted to reactive species (ROS) such as hydroxyl radical (•OH), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2•), hydroperoxyl radical (HO_2•), lipid peroxyl radical (LOO•), alkokyl radical (LO•), iron-oxygen complexes (ferryl- and perferryl radical)) and singlet oxygen ($^1O_2$). All of these radicals are highly reactive and can initiate lipid oxidation (Min and Ahn, 2005). Pork products, due to their relatively high content of unsaturated fatty acids, oxidise more rapidly than beef or lamb. Pork products manufactured from minced meat, such as pork patties and sausages are particularly prone to lipid oxidative changes due to the disintegration of the tissue and cellular structures during the grinding process. The manufacturing process also exposes any unsaturated fat or proteins to molecular oxygen (Chen *et al.,* 2008b) and any oxidation can ultimately result in the pork products turning brown and producing off-odours.In short, lipid oxidation produces undesirable changes in flavour, colour, texture, tenderness and nutritive value of meat products during the processing and storage stages (Lee *et al.,* 2006).

The colour of meat is important because consumers use discoloration as a visual indicator of the freshness and wholesomeness of the meat when making a decision to purchase (Macini and Hunt, 2005). Fernandez-Lopez *et al*. (2003) indicated that colour stability is affected by factors such as packaging, oxygen, bacteria, pH, temperature and humidity. In fresh meat products, colour is affected by three factors: the concentration of muscle haem pigments, the chemical state of pigments on exposed muscle surfaces, and the physical light-scattering properties of the meat structure (MacDougall, 1983; Fernandez-Lopez *et al.,* 2003). The pigment myoglobin (Figure 2.4) is a complex protein that binds with oxygen, and is required for metabolic activity. It plays an essential role in storing oxygen within muscles. Myoglobin is primarily responsible for the different levels of redness in meat such as poultry, pork, and beef (Brewer, 2004). Meat with high colour stability is characterised by low oxygen consumption and reduced lipid oxidation. In practice, controlling and monitoring lipid oxidation during processing and storage is difficult. For example, as meat is exposed to oxygen, oxygen is absorbed and combines with myoglobin, turning meat bright red due to the formation of oxymyoglobin. Both the myoglobin and oxymyoglobin are prone to losing an electron (oxidise) which turns the pigment to metmyoglobin which is a brown colour. Myoglobin, oxymyoglobin and metmyoglobin can all be inter-converted (Figure 2.5). The state of myoglobin and, therefore, meat colour clearly depends on the oxidation state of the

iron atom in the haem group (ferrous (Fe^{2+}) or ferric (Fe^{3+})). When ferrous haem lacks a sixth ligand it has a purple red colour and is called deoxymyoglobin. If oxygen is the sixth ligand, then the molecule appears cherry red (fresh meat colour) and is called oxymyoglobin (Faustman and Cassens, 1990; Yin and Cheng, 1997). Ferric haem with water as the sixth ligand is metmyoglobin with a brown colour which is the dominant form in brown meat (Faustman and Cassens, 1990).

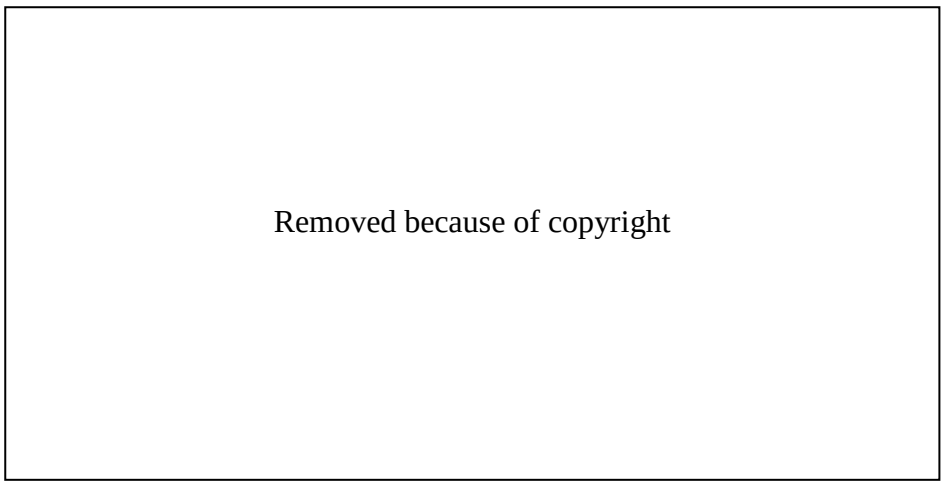


Figure 2.4. Chemical structure of part of Myoglobin
(Pearson and Young, 1989).

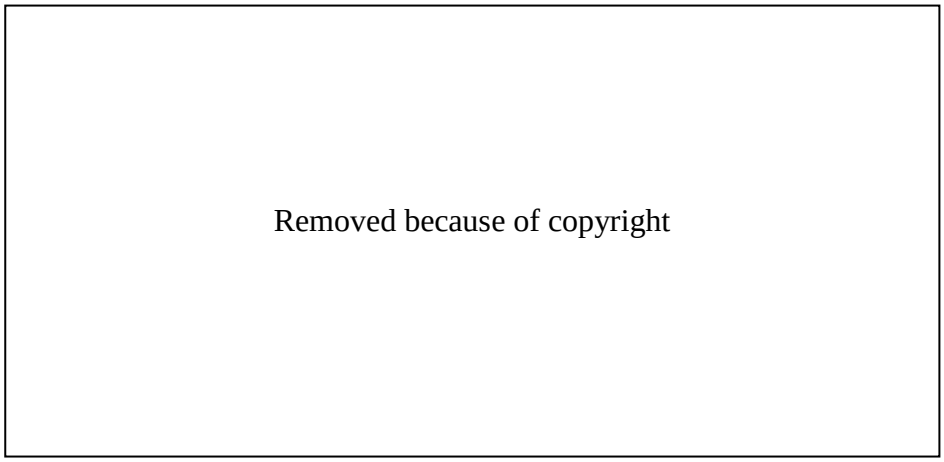


Figure 2.5. Conversion of Myoglobin, Oxymyoglobin and Metmyoglobin
(Aberle *et al*., 2001).

Along with colour, odour is another meat quality characteristic important to consumers. The development of oxidative off-flavours (rancidity) is a particularly serious problem during the storage of meat products (Gray and Pearson, 1987; Ladikos and Lougovois, 1990; Gray *et al*., 1996; Langourieux and Escher, 1998). The amount of perceived odour is influenced by odour intensity and the composition of volatiles in the odour (Bailey *et al.,* 1994; Specht and Baltes, 1994; Ahn *et al*., 1999). In meat, lipids are probably the most important precursor of volatiles from the breakdown of fatty acids (Mottram, 1987). The autoxidation of lipids can occur in raw meat at room temperature or under refrigeration, the net result is a rancid flavour (Farmer, 1994).

There is a concern about aerobically stored meat producing oxidation products such as aldehydes which produce rancid off-odours. Warmed over flavour (WOF) often refers to the characteristic off-flavour that develops in cooked or raw meat (Sato and Hegarty, 1971; Pearson *et al.,* 1977; Pearson and Dutson, 1994).

Thus, the production of volatile compounds in meat and meat products has been increasingly studied due to the role of odour and flavour in the overall acceptability of meat to consumers. However, the development of aroma and odour in meat is a complex process in which different volatiles interact to produce intermediary compounds that also contribute to the odour. Unfortunately, the correlation between the analytical profile of volatile compounds and odour perception by consumers is not always strong. It is therefore essential that any aroma assessment of volatile compounds by analytical techniques is accompanied with human sensory evaluation (Garcia-Gonzalez *et al*., 2008).

2.7. Natural antioxidants in preservation of meat products

For many years, synthetic antioxidants such as tert-butyl-4-hydroxyanisol (BHA) and tert-butyl-4-hydroxytoluene (BHT) have been considered practical and effective inhibitors of lipid oxidation but recently there has been a trend to replace them with natural antioxidants due to the possible toxicity of synthetic antioxidants to consumers (McBride *et al.,* 2007). These synthetic antioxidants have been restricted by legislation because they are suspected of having some possible carcinogenic effects (Imaida *et al.,* 1983; Madhavi *et al*., 1996; Hirose *et al.,* 1998). Consequently, research has concentrated on the efficacy of including natural antioxidants in foods such as vitamin E, vitamin C, ß-carotene (Morrissey *et al.,* 1998) and a wide range of plant extracts (Namiki, 1990; Madsen and Bertelsen, 1995; McBride *et al.,* 2007).

It is should be noted that the ability of antioxidants to intercept or remove free radicals can contribute to preventing the deterioration of meat. Ideally, antioxidants should not affect the organoleptic properties of meat and should be effective at low concentrations. However, in the past, a major obstacle to the use of plant extracts as ingredients has been the undesirable flavours and odours associated with the extracts. Rosemary has been shown to have high antioxidant capacity activity but some of the compounds in rosemary such as verbenone, borneol and camphor can impart an undesirable rosemary odour to food even at low concentrations (Brewer, 2011). Red cabbage and radish extracts containing anthocyanins can only be successfully applied as additives after removing or reducing the concentration of aroma and flavour compounds that give strong vegetative note to these extracts (Giusti and Wrolstad, 2003). Grape pomace containing anthocyanin provides good sources of antioxidants can impart undesirable odour or colour to the food (Bhowmik *et al.,* 2009). However, technological developments have resolved this problem by producing antioxidant extracts without any sensory characteristics but with antioxidant properties (McBride *et al.,* 2007). Such technologies include solvent extraction, hydro-distillation, spray-drying, freeze-drying and supercritical fluid extraction (Dorman *et al.,* 2003; Hadolin *et al.,* 2004; McBride *et al.,* 2007).

In addition, several reports have stated that the antioxidant activities of natural antioxidants are lower than that of synthetic antioxidants (Miladi and Damak, 2008) and the manufacturing costs of natural extracts are too high (Addis and Hassel, 1992; Lee *et al*., 2003). There is, therefore, considerable interest in finding new, safe and inexpensive antioxidants from natural sources (Namiki, 1990; Gazzani, 1998).

A number of researchers have attempted to improve the quality of meat by using natural antioxidants such as vitamin C, vitamin E and extracts from rosemary, oregano, sage, onion and cranberry, etc. Studies have shown that the natural plant antioxidants can scavenge free radicals, inhibit lipid oxidation and extend the shelf life of meat products. (Mitsumoto *et al.,* 1991; Sahoo and Anjaneyulu, 1997; Karastogiannidou, 1999; Lee *et al*., 2006; Hernandez-Hernandez *et al.,* 2009; Mariutti *et al.,* 2011).

Table 2.1. Summary of some previous reports on effects of natural antioxidants on extending shelf life of meat products.

Antioxidant	Concentration (w/w)	Meat	Effects on shelf life	References
Vitamin C	1%	Beef	Maintained colours and lipid	Mitsumoto *et al.*, 1991
Vitamin E acetate	10ppm	Ground buffalo meat	Lengthened desired visual colour, odour and lowered MetMb and TBARS	Sahoo and Anjaneyulu, 1997
Rosemary and oregano extracts	0.01%	Raw pork batters	Reduced TBARS and maintained colour	Hernandez-Hernandez *et al.*, 2009
Sage	0.1%	Minced chicken breast	Reduced lipid oxidation	Mariutti *et al.*, 2011
Dried onion flesh	1.6%	Cooked chicken	Reduced TBARS	Karastogiannidou, 1999
Cranberry juice powder	0.32%	Cooked ground pork and turkey	Inhibited lipid oxidation, TBARS and rancidity	Lee *et al.*, 2006
Mango kernel and peel	1%	Pork sausages and patties	Proposed that mango peel and kernel will reduce lipid oxidation	Present study

However, several of the reports have indicated that the effectiveness of the various antioxidants in a food system depends on factors such as the physical state of the substrate and the solubility, phase partitioning and concentration of the antioxidants (Lin and Liang, 2002; McBride *et al*., 2007). It is now recognised that suitable carrier systems may be required to direct and aid the incorporation of antioxidants to a specific location in prepared meat products. Furthermore, the synergistic interactions between hydrophilic and lipophilic antioxidants can also have an effect on the efficiency of incorporating antioxidants into meat products.

Therefore, although natural antioxidants are effective in controlling oxidative changes in meat, the precise evaluation and prediction of antioxidant activity in a complex system such as meat is extremely difficult and always requires support from practical experiments such as shelf-life stability trials (McBride *et al.,* 2007). Although there have been many studies on the effectiveness of natural antioxidants from plants in preserving meat and meat products, to date the results have been variable.

2.8. Summary

- Antioxidants have the ability to inhibit ROS formation, scavenge free radicals or chelate metals. Natural antioxidants have attracted wide interest from scientists and consumers in regards to whether dietary antioxidants have beneficial effects on human health.

- There is however chemical diversity and complexity in antioxidants which can act individually, cooperatively or synergistically in foods so it is important to determine the antioxidant capacity. It is clear that no one assay will reflect the total antioxidant capacity of a particular antioxidant source and that several assays are required for their evaluation. They are TPC (or F-C), ABTS, DPPH, FRAP and ORAC.

- The use of synthetic antioxidants has raised many serious problems about their continued use particularly in regards to safety and toxicity problems connected with human body organs and tissues. Mango is one of the most important tropical fruits in the world and Vietnam. Mango seed kernels and peel have been suggested as good sources of natural antioxidants.

- To be utilised as food ingredients, mango peel and kernel may require drying. However, the drying of food is complicated by physical, chemical and biochemical changes that may produce either positive or negative attributes in the final product. Therefore, studies on the effects of the various drying treatments on the antioxidant capacity of mango fruit and its fractions are required.

- The antioxidant capacity of fruits is influenced by different factors such as cultivars, maturity and production area. However, the effects of genotype and maturity on antioxidants in fruit components such as peel and kernel have not been investigated. It is therefore important to evaluate the effects of genotypes (cultivars) and physicochemical properties (maturity) on the total phenolic and antioxidant capacity of peel and kernel in fruits.

- Lipid oxidation produces undesirable changes in flavour, colour, texture, tenderness and nutritive value of meat products during the processing and storage stages. Pork products, due to their relatively high content of unsaturated fatty acids, oxidise more rapidly than beef or lamb.

- A number of researchers have attempted to improve the quality of meat by using natural antioxidants to scavenge free radicals, inhibit lipid oxidation and extend the shelf life of meat products. However, the results have been variable. There is still a need to study the characteristics of natural antioxidant from plants such as mango peel and kernel and their effects on the shelf-life of meat products such as pork sausages and patties.

CHAPTER 3
MATERIALS AND METHODS

3.1. Source of mangoes

Tommy Atkins mangoes (n = 72, average weight 400 g) imported into New Zealand, from Mexico, were obtained from a local New Zealand supermarket. “Ghep”, “Cat Hoa Loc”, “Cat Chu” and “Nam Dok Mai” mangoes (n = 3 per cultivar) were collected from retail markets in Vietnam and were at the ripe “ready to eat” stage. The physicochemical characteristics of the mangoes collected in Vietnam were evaluated at the laboratory of the Southern-Sub Institute of Agricultural Engineering and Postharvest Technology, Vietnam and those of Tommy Atkins at the Lincoln University laboratory, New Zealand. Mango peel and kernel destined for incorporation into pork products were collected from a mango processing factory (Cofidec Ltd.,) in Vietnam. The Vietnamese mango peel and kernel were freeze dried and finely ground into powder and vacuum sealed prior to transport to Lincoln University, New Zealand for further analysis.

3.2. Preparation of mango fractions for analysis

Mango flesh, peel and kernel were separated from each mango (Appendix A.1.1). The peel (skin) with the thickeness of about 1.0 mm was removed with a scalpel followed by the flesh and seed. A careful peeling was applied to avoid any flesh left in the peel. The flesh was sliced into pieces (10cm length x 3cm width x 0.2cm thickness). The kernel inside the seed was removed manually using a hammer or knife. The methods of manually separating mango flesh, peel and kernel fractions were used similarly to those applied in the Vietnamese mango factories. The whole mango, peel, flesh and kernel were weighed separately. Fresh Tommy Atkins mango flesh (n = 12) was homogenised in a Grindomix GM200 homogenizer (Retsch GmbH, Haan, Dusseldorf, Germany) at 10,000 rpm and kept at 4°C prior to solvent extraction. Peel was cut into small pieces (1 cm length x 1 cm width) with a scalpel, immediately frozen in liquid nitrogen and then ground in a grinder for 20 seconds. The frozen finely ground peel was kept at -40°C before solvent extraction. Mango kernel was finely ground under the same conditions as the peel. In the case of the Tommy Atkins mangoes (n = 60) destined for drying treatments, all the separated flesh, peel and kernel fractions were dried by one of five drying treatments prior to analysis. Once, the drying process was complete, the dry weights were recorded to calculate the percentage of water loss. The dried products were then ground into a powder using a laboratory blender (Sunbeam, model:

EM0415). The powder was vacuum-packed and stored at -40ºC until analysed. The seeds were not used in this study.

3.3. Determination of the physiochemical characteristics of mango

3.3.1. Maturity score

Maturity score is usually measured by the taste, flavour, firmness, peel colour, and shape of the mango fruits (Mitcham and McDonald, 1992; Baez-Sanudo *et al.*, 1999; Kader, 2008) as described in Table 3.1. In this thesis, the maturity score was assessed by the same person and each individual fruit was allocated a maturity score (from 1 to 5) based on their odour, firmness, peel colour and fruit shape. The scores from seventy mangoes were averaged to give the overall maturity score.

Table 3.1. Parameters to score the maturity of mangoes (Kader, 2008).

Maturity score	Odour	Firmness	Peel colour	Fruit shape
1	Weak	Very firm	Green	Not full cheek
2	Relatively strong	Firm	Green to yellow	Less full cheek
3	Desirable	Relatively soft	Yellow	Relatively full cheek
4	Strong	Soft	Yellow to orange	Full cheek
5	Too strong	Very soft	Orange	Very full cheek

3.3.2. Colour

Colour parameters were determined using a Hunter Lab Colorimeter (HunterLAB Miniscan XE Plus, model 4500L, USA) with 25-mm aperture, CIE D65 illuminant, and 10^{o} standard observer. The colorimeter was calibrated using white and black tiles prior to the measurement of L*, a*, and b* values of the samples, where L* represents the brightness, a* the redness and b* the yellowness coordinates. The hue angles (H°) and chroma (C*) were calculated (Voss, 1992; McGuire, 1992) with hue angle (H°) = arctan (b*/a*) and Chroma (C*) = $(a^{*2} + b^{*2})^{0.5}$. Peel colour was measured by placing the colorimeter measuring head at the middle of the large surface on 2 opposite sides of the fruit.

3.3.3. Firmness

A portion of mango peel (about 1–2mm thick) was removed from the same area of each mango fruit. The firmness of the exposed flesh was measured from the peeled area using a Fruit Pressure Tester (FT 327, Italy) or penetrometer equipped with a 6 or 8 or 11mm-diameter plunger tip depending on the degree of firmness. The firmness value was the calculated by dividing the measured force by the surface area of the penetrometer tip. The penetrometer was bench-mounted on a fixed rigid drill stand to ensure the pressure is applied at a steady controlled rate and at a constant angle to the fruit. The penetrometer was located at the end of a pivoting arm and a measurement was done by pulling down the arm until the probe enters the fruit to a depth of 8 mm. Firmnesswas reported as the pressure to push the plunger of a specific size into the flesh of the fruit to a specific depth. The firmness or the resistance of mangoes was measured in load units (depends on the distance the crosshead travels) expressed as kg cm^{-2}.

3.3.4. Total acidity

Titratable acidity (TA) was determined by the AOAC method 942.15 (AOAC, 2000) using a 670 Titroprocessor with sample changer and a Pt Titrode (Metrohm, Switzerland) (Kerkhofs, *et al.*, 2005). Fresh mango flesh (5 g) was weighed into a beaker followed by 50 mL distilled water (20°C). The sample was stirred for approximately 30 seconds and then titrated with 0.1N NaOH to pH 8.1. The calculation of titratable acidity was based on the following equation as follows:

$$\%\ acid\ (wt/wt) = \frac{N\ x\ V\ x\ Eq\ wt}{W\ x\ 1000} x\ 100$$

Where: N = normality of NaOH (mEq/ml)

V = volume of NaOH

Eq.wt. = equivalent weight of predominant acid (mg/mEq) which was citric acid (molecular weight = 192; equivalent weight = 64)

W = mass of sample (g)

1000 = factor relating mg to grams

Titratable acidity is expressed as % citric acid (g 100 g^{-1}).

3.3.5. Total soluble solids

Total soluble solids (TSS) were determined using a hand-held refractometer (model WZ103). The peeled mango flesh was homogenised in the blender and a few unfiltered drops applied to

the prism of the refractometer. Direct readings were taken from the scale on the meter as described by AOAC (2000) and the results expressed as ^{0}Brix.

3.3.6. TSS/TA ratio

TSS and TA were used to calculate the TSS:TA ratio which is the best parameter to predict sweetness, sourness and stage of maturity.

3.3.7. Vitamin C

Vitamin C (ascorbic acid) content of samples was determined according to the AOAC method 967.21 (2000) using an automated titrimetric technique, based on a 670 Titroprocessor with sample changer and Pt Titrode (Metrohm, Switzerland) (Toor *et al.*, 2006). Two grams of fresh mango flesh were homogenised in a blender and then mixed with 40 mL of aqueous buffer solution (3.85 kg L^{-1} sodium acetate and 0.808 g oxalic acid; pH 4.2) in a 50 mL beaker. The homogenate was titrated against a dye solution containing 2, 6-dichlorophenol indophenols (295 mg L^{-1}) mixed with 100mg sodium bicarbonate. The titration end point was determined potentiometrically by reading the titre volume at the "point of inflection" of the titration curve automatically generated by the 670 Titroprocessor. The standard (L-ascorbic acid) curve was generated by the titration with the dye solution. The ascorbic acid content in the samples was determined from the standard curve and the results were expressed as as mg vitamin C 100 g^{-1} FW.

3.3.8. Moisture content

The moisture content of mango flesh was determined gravimetrically by oven drying mango samples at 105^{o}C for 24 hours (AOAC, 1995).

3.4. Drying procedures for mango fractions

Mango flesh, peel and kernel fractions sourced individually in Vietnam were dried by freeze-drying using a Cole-Parmer freeze dryer and the individual dried weights recorded. The dried samples were ground into a powder using a laboratory blender and vacuum sealed in Polyethylene (PE) bags. These freeze-dried mango powders were subsequently transported to Lincoln University, New Zealand for analyses (MAF permit No: #489). Tommy Atkins mango fruit sourced in New Zealand were randomly separated into six groups (n = 12 per group). Group one (fresh) was the control group. The other five groups were the, forced-air, freeze, vacuum and microwave drying treatments. These drying methods with specific conditions were selected based on the two important factors which are time and temperature of drying. They were long time and low temperature (sun, freeze, forced-air and vacuum) or

short time and low temperarture (microwave drying). The moisture content of the dried samples ranged from 6 to 13%. Before drying each individual mango was separated into flesh, peel and kernel components as described in 3.2. The whole mango fruit as well as the flesh, peel and kernel fractions were weighed before and after drying (Appendix A.1.2). The drying treatments were as follows:

3.4.1. Sun drying

Mango fractions were placed on trays in a greenhouse for 72 hours at a temperature of 25-28˚C and humidity 52-64%. At night, the temperature was 16˚C and a UV lamp was applied.

3.4.2. Forced-air drying

Mango materials were spread uniformly on trays and placed in a forced-air dryer (Unitherm drier, Birmingham & Blackburn Construction Co. Ltd, England) set at 65˚C for 65 hours. The blower fan was set for forced air drying.

3.4.3. Freeze drying

Mango materials were placed in PE bags and freeze-dried in a freeze-drier (Cuddon, Model E.D. 5.3., New Zealand) at -50˚C for 72 hours for flesh and 48 hours for peel and kernel.

3.4.4. Vacuum drying

Mango materials were placed in the Thermostat vacuum oven (Croydon, Townson & Mercer Ltd, England) with the vacuum set at 300mmHg and the temperature at 60˚C. The drying process time for flesh, peel and kernel were 18, 8 and 12 hours, respectively.

3.4.5. Microwave drying

Mango materials were placed in the middle of the turntable of a kitchen microwave (Samsung Model M1733CE, Timesaver 800W) with the power setting at 180W. The processing time to produce dried mango flesh, peel and kernel was 40, 28 and 20 minutes, respectively.

3.5. Extraction of mango fractions for antioxidant assays

The mango fractions were extracted as described by Zhao *et al.* (2006) with some modifications. Approximately 2 g of fresh mango flesh, peel or kernel powder and 0.5 g of dried samples were weighed into 50 mL volumetric flasks followed by 20 mL of 80% (v/v) acetone (with distilled water) and the flasks were rotated on a rotary shaker in a dark cold room (4^{o}C) for 2 hours. The volume of the mixture was then adjusted to 50 mL (with acetone) and the contents transferred to 50 mL acetone resistant centrifuge tubes and centrifuged

(Kendro manufactured by Sorvall Evolution) at 3,500 rpm for 10 minutes. The resulting supernatant was transferred to glass test tubes with caps and kept at -40°C prior to analysis. These extracts were used for the determination of TPC, ABTS, DPPH and FRAP.

For hydrophilic ORAC (H-ORAC) and lipophilic ORAC (L-ORAC) assays, the mango fractions were extracted as described by Prior *et al.* (2003). Fresh (1 g) and dried (0.5 g) mango samples were extracted in 50 mL centrifuge tubes with 10 mL of hexane, vortexed for 10 minutes, centrifuged and the hexane layer collected. This extraction procedure was repeated twice and the combined hexane fractions evaporated under a nitrogen flow in a 30°C water bath. The residue was reconstituted with 10 mL of 80% acetone. After centrifugation, the supernatant was used to measure lipophilic antioxidant capacity, with the buffer used for any further dilutions. The residue (after hexane extraction) was extracted with 10 mL of acetone/water/acetic acid (AWA) (70:29.5:0.5 v/v/v). After adding solvent, the tube was vortexed for 30 seconds, followed by sonication at 37°C for 5 minutes. The tube was inverted once in the middle of the sonication step to suspend the samples. Then, the tube remained at room temperature for 10 minutes with occasional shaking. The tube was centrifuged at 2889 g for 15 minutes. The supernatant was removed and transferred to a volumetric flask and diluted to 25 mL with AWA. Any further dilutions were with AWA.

3.6. Antioxidant assays

3.6.1. Total phenolic content using Folin-Ciocalteu assay

TPC of fresh flesh, peel and kernel were estimated using the Folin-Ciocalteu method as described by Singleton (1999) with some minor modifications. Acetone extracts of peel and kernel were diluted ten and twenty times, respectively, with 80% acetone prior to the phenolic assays. Flesh extracts required no dilutions. An aliquot (0.5 mL) of extracts was mixed with 2.5 mL of the Folin–Ciocalteu reagent (0.2N) and after 8 minutes at room temperature, 2 mL of 7.5% (v/v) sodium carbonate was added. The mixture was vortexed, incubated at 50^{o}C in a water bath for 5 minutes and then cooled in an ice bath. The absorbance was measured at 765nm with a Unicam UV/Visible spectrophotometer. Results were expressed as mg of gallic acid (GAE) 100g^{-1} dry weight sample. Gallic acid standard (75 µg mL^{-1}) was prepared and a standard curve established by aliquoting 0-0.5 mL of the gallic acid standard and diluting to 0.5 mL with 80% acetone. The same procedure was used to determine the TPC of dried peel, flesh and kernel extracts. However, due to the higher amount of phenolic content and antioxidants in the dried products, the dried flesh, peel and kernel extracts were diluted two, twenty and forty times, respectively.

3.6.2. ABTS radical cation decolourization assay (Trolox Equivalent Antioxidant Capacity)

The free radical scavenging activity of the mango products was determined by the ABTS radical cation decolourization assay according to Re *et al.* (1999) and Ozgen *et al.* (2006). $ABTS^{\bullet+}$ was prepared by reacting colourless ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate and allowing the reaction to stand for 12-16 hours in the dark at room temperature until a stable oxidative state is reached. This ABTS reagent was then stable for several weeks when stored in the dark (Ozgen *et al.*, 2006). On the day of analysis the $ABTS^{\bullet+}$ solution was diluted with PBS to an absorbance of 0.70 (± 0.02) at 734 nm and 1 mL transferred to a cuvette. After the addition of 100µl standard Trolox (see 3.16) or mango extract, the mixture was well mixed, allowed to stand for 6 minutes and the absorbance read at 734 nm. All samples were assayed in triplicate and the concentrations of antioxidants calculated by reference to a 0-200 µmol Trolox standard curve. The results are expressed as Trolox equivalents (TE), which is defined as µmol Trolox activity equivalent per g DW.

3.6.3. DPPH radical scavenging activity assay

The DPPH radical scavenging activity of mango samples was determined according to the method of Blois (1958) with modifications described by Brand-Williams *et al.* (1995), Fan *et al.* (2009) and Wojdylo *et al.* (2009). The acetone extracts of mango were diluted then 100µl of the diluted extracts or Trolox standards were added to 900µl of 0.1M DPPH solution. The mixture was vortexed and incubated at room temperature for 20 minutes. The decrease in absorbance at 515nm was measured using the UV-Vis Spectrophotometer (Shimadzu UV-Vis). All the determinations were performed in triplicate. A Trolox calibration curve was established and the results expressed as µmol Trolox equivalents per g DW.

3.6.4. Ferric Reducing/Antioxidant Power (FRAP) assay

FRAP was assessed according to Benzie and Strain (1999) with slight modifications. A fresh working solution of FRAP reagent was prepared each time by mixing acetate buffer (300 µM, pH 3.6), a solution of 10mM TPTZ in 40mM HCL, and 20mM $FeCl_3.6H_2O$ at 10:1:1 (v/v/v). 100 µL of standards of iron (II) sulphate ($FeSO4.6H_2O$) or appropriately diluted extracts were added to 900 µL of the FRAP reagent and the absorbance at 593 nm recorded immediately after the addition of the sample and after a 2 hours incubation at 37°C. A standard curve from 0 to 300 µM was prepared using $FeSO4.6H_2O$ solution. The results were expressed as µmol Fe^{2+} $100g^{-1}$ DW. Each sample was analysed in triplicate.

3.6.5. Oxygen Radical Absorbance Capacity (ORAC) assay

ORAC (H-ORAC or L-ORAC) was determined according to Prior *et al.* (2003) with some modifications. Using a 96-well blank plate, 150 µL buffer (75mM, pH 7.0) was added to the outer wells to act as a thermal buffer. 25 µL of sample or standard and 150 µL Fluorescein were added to the wells using a multi-channel pipette. The plate was covered with parafilm to stop evaporation and incubated in a plate reader (Fluostar Omega, BMG LabTech, Alphatech Systems Ltd, New Zealand) at 37°C for at least 30 minutes. Following pre-incubation, 25 µl of AAPH solution was added using the microplate reader's injector. The AAPH (2-2'-azobis (2-amidino propane) dihydrochloride or 2,2-azobis (2-methyl-propionamidine) dihydrocholride) was made by adding 0.6456 g AAPH to 10 mL phosphate buffer (pH 7.0) at 37°C. AAPH was prepared immediately before use. The Fluorescent microplate reader was set at 37°C with an excitation wavelength of 480 nm and an emission wavelength of 520 nm. The syringes were primed with freshly prepared AAPH before starting the run. The fluorescence was monitored kinetically with data taken every minute for 90 minute. ORAC values were calculated using the area under the curve (AUC). The net AUC was obtained by subtracting the AUC of the blank from that of the sample and then compared to a Trolox standard curve, which plots net AUC versus Trolox concentration (Appendix A.1.3.). The results of ORAC assay are expressed as µmol Trolox equivalents (TE) per g DW. Hydrophilic and lipophilic results were obtained by H-ORAc and L-ORAC assays, respectively.

3.7. Anti-nutritive factors

3.7.1. Determination of Tannin

Tannins were determined using MCP (Methyl Cellulose Precipitable) according to the standard method described by Sarneckis *et al.* (2006). The assay is based on the formation of an insoluble polymer tannin complex. The absorbance of tannin extracts were measured at 280nm, before (total phenolics) and after precipitation (phenolic compounds remained after precipitation). A standard (epicatechin) curve was prepared and the MCP tannin in solution was determined and calculated as epicatechin equivalent.

3.7.2. Determination of oxalate

Oxalate was determined according to the method described by Savage *et al.* (2009). Total oxalate was extracted with 0.2 M HCl and soluble oxalate was extracted with water. Both extracts were analysed by HPLC.

Specifically, total oxalate was extracted from 1 gram of freeze dried mango powder (flesh, peel or kernel) with 40 mL of 0.2 M HCl in a water bath at 80°C for 15 minutes then cooled.

The extract was centrifuged at 2889 g for 15 minutes then the supernatant was filtered through a 0.45 μm cellulose nitrate filter. The supernatant (20 μL) was injected onto a HPLC with a 300 mm x 7.8 mm Rezex ion exclusion column attached to a cation Hþ guard column and eluted with a flow rate of 0.6 mL min^{-1}. The HPLC system consisted of an autosampler, a UV/vis detector Spectra-Physics SP8450 set at 210 nm and a ternary Spectra-Physics, SP 8800 HPLC pump supplied with 25 mM H_2SO_4 as the mobile phase. Data was captured via a PeakSimple chromatography data system (SRI model 203, SRI Instruments, CA) and processed using PeakSimple version 3.54 (SRI Instruments, Torrance, CA).

Soluble oxalate was extracted using the same procedure but with Nanopure II water instead of 0.2 M HCl. Insoluble oxalate was calculated by subtracting the soluble oxalate from the total oxalate (Holloway *et al.*, 1989). Standard curves containing 0.1 – 20 mg/100 mL of oxalic acid (Sigma-Aldrich Co., St Louis, MO) were prepared in 0.2 M HCL and Nanopure II water to quantify total and the soluble oxalates in the samples respectively. The extraction and analysis were conducted in triplicate

3.8. Preparation of pork patties and sausages

Fresh (24 hours post- slaughter) pork shoulders from female pigs were purchased from a New Zealand local supermarket, and minced to three different fat specifications (fat, protein and moisture content were determined by Lincoln University Analytical Laboratory). Mince A had a low fat, B a medium fat and C a high fat content. Minces A, B and C were individually divided into 2 separate batches to prepare pork patties and sausages respectively. Each batch was then divided into four equal portions for four different treatments. Treatment 1 contained mince with no antioxidant (control); treatment 2 contained mince with 1% w/w freeze dried mango peel (from Vietnamese mangoes, cv. Cat Chu), treatment 3 contained mince with 1% w/w freeze dried mango kernel (from Vietnamese mangoes) and treatment 4 contained mince with 0.01% w/w BHT (Butylated Hydroxy Toluene). One percent of rice bran oil was pre-dispersed with mince before mixing peel, kernel and BHT into the mince by manually blending for 3 minutes. Similarly, 1% rice bran oil was included in the control mince. In the case of pork patties, the final mince mixture (900 g) from each treatment was formed into 12-mm thick patties (40 g each) and individually placed into plastic Petri dishes and covered with a lid. For the pork sausages, the mince mixture for each treatment (900 g) was supplemented with salt (4 g) and pepper (2.4 g) and the mixture was then extruded into 10 cm natural sheep intestinal casings and air dried at 10^0C for 6 hours.

The pork patties (still in the petri dishes) and sausages (n =18 per treatment) were placed in polystyrene trays which were covered with high oxygen permeable polyvinyl chloride film (O_2 permeability > 2000 mL m^{-2} atm^{-1} 24 $h.^{-1}$ at 25°C, AEP FilmPac (Ltd), Auckland, New Zealand) and then stored at 4^0C in a fluorescent illuminated display cabinet to reflect retail conditions for 0, 2, 4, 6, 8 and 10 days.

3.9. Proximate analysis

Moisture content was determined as as described in 3.3.8 (AOAC, 1995). Crude fat, crude protein and dry matter of fresh pork mince was determined as outlined by AOAC (1995). Dry matter was determined as as described in 3.3.8 (AOAC, 1995). Crude fat content was determined by using Soxhlet extraction (Tecator Soxtec HT6) (AOAC 976.21). Crude protein was measured by determining nitrogen using the Kjeldahl method (Block Digestion) (AOAC 981.10). Nitrogen was converted to crude protein content by multiplying with the factor 6.25. The results are reported on a percentage wet and dry basis.

3.10.TBARS

Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS) in pork and pork products according to Maraschiello *et al*. (1999). Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS) in pork and pork products. Sample (2.0 g) from pork sausages or patties was placed in 50-mL test tubes and homogenised with 20 mL of deonised distilled water by Ultra-Turrax using a T25 head at 13,500 rpm for 20 seconds. Five millilitres of 25% TCA was added to precipitate the protein then the mixture was centrifuged at 13,000 g for 15 minutes. A 3.5 mL aliquot of supernatant was transferred to screw cap test tubes (13 x 1002 mm) and 1.5 mL of 0.6% aqueous TBA (see 3.16) was added. The mixture was vortexed and then incubated in a water bath at 90^oC for 1 hour to develop colour. At the end of the incubation, samples were cooled in cold water and the absorbance read at 532nm. TMP (1,1,3,3–Tetramethoxypropane) was used to prepare a malondiadehyde (MDA) standard. A volume of 17µl of TMP was diluted in 0.1N HCL into a 10 mL volumetric flask and incubated at 40^oC for 60 minutes to hydrolyse TMP into MDA (final concentration ca, 10mM). The stock solution (#1) was stored at 4^oC and freshly prepared on a weekly basis. A calibration curve of MDA standards were prepared on the day of use by further dilution of stock solution #1 with 0.1N HCL to make a series of standards ranging from 0-50µM. Standards (0.2 mL) were added to 2.3 mL water plus 1 mL 25% TCA and 1.5 mL 0.6% TBA. The results were calculated based on the standard curve and expressed as TBARS (mg MDA kg^{-1} sample).

3.11. Colour

The meat samples were taken out of the fridge and placed on the table for 5 minutes at room temperature (22^{o}C) before colour measurement. Colour measurements were made on the surface of raw pork patties with a Hunter colorimeter (Hunter Associated Labs., Inc. Reston, VA) as described in 3.3.2.

3.12. Myoglobin

Myoglobin (deoxymyoglobin [Mb]), oxymyoglobin (MbO_2) and metmyoglobin (MMb) in stored raw pork patties and sausages were determined using the procedure of Krzywicki (1982). Triplicate pork samples weighing 2.0 g were mixed into 18 mL of 40 mM phosphate buffer (pH 6.8) and homogenised at high speed by an Ultra-Turrax using T25 head at 13,500 rpm for 20 seconds. The homogenates were poured into 50 mL screw-top centrifuge tubes and kept on ice for 2 hours to extract the pigments. The tubes were centrifuged at 4^{o}C for 60 minutes at 30,000 g. The supernatants were filtered through Whatman no. 1 filter paper and kept on ice. The absorbance of each supernatant was read at 572, 565, 545 and 525 nm in a UV-visible spectrophotometer (model UV-160, Shimadzu Co. Ltd., Columbia, MD), and three determinations were conducted for each sample solution. Exposure to light was kept minimal throughout the procedure by placing sample solutions in the dark to minimize any possible oxidation of the pigment. Total myoglobin and percent Mb, MbO_2 and MMb in the pigment extracts were calculated based on the following equations (Krzywicki, 1982):

$$\text{Total myoglobin (mmol/L)} = (-0.166R_1 + 0.086R_2 + 0.088R_3 + 0.099) \times A_{525}$$

$$\text{Mb (\%)} = (0.369R_1 + 1.140R_2 - 0.941R_3 + 0.015) \times 100\%$$

$$MbO_2\ (\%) = (0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361) \times 100\%$$

$$\text{MMb (\%)} = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100\%$$

where R1, R2 and R3 are absorbance ratios of A_{572}/A_{525}, A_{565}/A_{525} and A_{545}/A_{525}, respectively. For the total myoglobin content, the values were converted to mg/kg meat based on the dilution and the molecular weight (16,950 Da) of myoglobin (Feng *et al.*, 1991; Dobberstein and Schroeder 1993; Chen *et al.*, 2008b).

3.13. Volatiles

The volatile compounds in minced pork products were determined using a HS-SPME-GC-MS technique as described by Ramirez *et al.* (2004) with some modifications as follows. One gram of pork product was placed into a 20 mL amber headspace glass vial (SUPELCO, USA),

followed by 3g of crystalline sodium chloride plus 10 mL of distilled water, and then quickly capped with a polytetrafluoroethylene/silicone liner tin plate seal (SUPELCO, USA). The samples were equilibrated for 2 hours at room temperature (20ºC). The volatile compounds were extracted by solid phase microextraction (SPME) using a 2 cm long DVB/CAR/PDMS combination SPME fibre (p/n 57348-U, Supelco Bellefonte, PA, USA, through Sigma-Aldrich, Australia) that was exposed to the headspace for 45 minutes at 30ºC during which time the headspace volatiles were adsorbed onto the fibre. Desorption of these volatiles occurred when the fibre was inserted in the injection port (270ºC for 5 minutes in the splitless mode) of a Shimadzu gas chromatograph-mass spectrometer (GCMS-QP2010). The GC-MS was equipped with a CTC-Combi PAL autosampler (Shimadzu AOC-5000) which automated the extraction and desorption of the volatiles.

Two capillary columns in series, namely a Rtx-Wax 30.0m x 0.25mm ID x 0.5µm film thickness (Polyethylene Glycol - Restek, Bellefonte, PA, USA) and a Rxi-1MS 15m x 0.25mm ID x 0.50µm (100% dimethyl polysiloxane - Restek, Bellefonte, PA, USA) were used to separate the volatile compounds. Helium was the carrier gas for the GCMS and was set to a constant linear velocity of 32.3cm/seconds. The column oven was held at 40°C for 3 minutes during desorption of the SPME fibre, then heated to 250°C at 5°C min^{-1} and held at this temperature for 7 minutes. The mass spectrometer (MS) was operated in electron impact ionization mode with 70eV and mass range of 33 to 350 m z^{-1}. The temperature of the capillary interface was 250°C, with the ion source temperature set at 200°C.

The purpose of the analysis of volatiles in the present study was to determine whether particular volatile compounds were produced by lipid oxidation during storage of the pork products. To manage the evaluation of the extensive number of volatiles produced, only the main volatile compounds are reported in this section. The volatile compounds were identified by matching the retention indices for wax columns and EI mass spectra against NIST 05 (NIST EPA/NIH Mass Spectral Library database) as well as the regression line between retention time and Kovats indices (Appendix A.1.4.). Relative quantification of the main peaks by peak area (Total Ion Count-TIC x 10^5) was used to compare the identified volatiles in the different chromatographic samples. The results in the text are expressed as means with standard errors. The percentages of individual volatile compounds relative to the total area of the major peaks were also calculated.

3.14. Odour

Odour acceptability (n = 3) was assessed by the same person at room temperature ($22^{o}C$) using a 5-point hedonic scale where 1 = very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4 = pleasant and 5 = very pleasant (Das *et al.*, 2011).

3.15. Microbiology

Microbiological characteristics (TPC, E.coli, staphylococci, coliforms yeasts, moulds and Salmonellae) of pork sausages and patties stored chilled for 0, 4 and 10 days were determined by Hills Laboratories, New Zealand. The freeze dried mango peel and kernel samples were also analysed for TPC, E.coli, Bacillus cereus, yeasts and moulds.

3.16. Chemicals and reagents

Folin–Ciocalteu reagent, gallic acid, sodium acetate anhydrous (CH_3COONa), sodium carbonate (Na_2CO_3), ferric chloride hexahydrate ($FeCl_3.6H_2O$), 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), 6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), oxalic acid, potassium persulfate (K_2SO_4), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), the 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH), Randomly Methylated *â*-Cyclodextrin (RMCD), fluorescein were purchased from Sigma-Aldrich (Steinheim, Germany). Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), 1,1,3,3-Tetramethoxypropane (TMP), disodium phosphate and monosodium phosphate, acetone, methanol, hexane, sulphuric acid, chloride acid, acetic acid, ascorbic acid, citric acid were obtained from BioLab, New Zealand.

3.17. Statistical analysis

3.17.1. One way-ANOVA

Differences between samples across cultivars or fractions or drying treatments were determined using a one-way analysis of variance (ANOVA) at $p < 0.05$. Mean values and standard errors of the mean (SEM) are reported in the text. Significant differences between means were determined using the Fisher's LSD multiple comparison tests. The analysis was performed in MINITAB 16.

3.17.2. Two-way ANOVA

A two-way ANOVA was used to analyse the effects of factors (antioxidant treatments, fat contents, storage time) and the interaction between them. Main effects and interactions were

considered significant at $p < 0.05$ and significant differences between means were determined using the Fisher's LSD multiple comparison tests. The analysis was performed in MINITAB 16.

3.17.3. Three-way ANOVA

The effects of three different factors (antioxidant treatments, fat contents, storage time) on the values of samples were evaluated by using the General Linear Model. Interaction plots which differentiated the results of samples exhibited interactions with other factors. Main effects and interactions were considered significant at $p < 0.05$ and significant differences between means were determined using the Fisher's LSD multiple comparison tests. Mean values and standard errors of the mean (SEM) are reported. The analysis was performed in MINITAB 16.

3.17.4. Interaction effects

Interaction effects were tested to evaluate the impact of one factor on the level of another factor. In the cases where the interactions were significant, the simple effects test was used to break the interaction effects into component parts and then test the separate parts for significance. The simple effects tests were implemented in MINITAB 16 by the ANOVA procedure using the General Linear Model (GLM) (Aiken and West, 1991; Pedhazur and Schmelkin, 1991) and with any of the simple effects tests with $p < 0.05$ being considered significant.

3.17.5. Principal component analysis (PCA)

PCA was used as a means of simplifying data by reducing the number of variables. Linear combinations of the original variables, called principal components, were derived which explained the maximum amount of variation in the data set and which were orthogonal to each other. PCA was used to (1) establish whether there are any correlations between physicochemical characteristics, between antioxidant assays and between physicochemical chracteristics and antioxidant assays and (2) to gain an overview of similarities and differences between fresh and dried mango products or pork products with different antioxidant treatments. PCA was carried out using MINITAB 16.

3.17.6. Canonical variates analysis (CVA)

CVA was used as a multivariate statistical technique to separate objects or samples into groups or classes by maximizing the ratio of between-group to within-group variations,

thereby giving functions that can be used to maximise the discrimination between the groups. CVA was performed using GENSTAT 13.

3.17.7. Pearson's correlation analysis

The Pearson correlation coefficients were calculated to evaluate the relationships between different factors at $p < 0.001$ or $p < 0.01$ or $p < 0.05$ using MINITAB 16.

3.17.8. Repeatability of assays

Samples were analysed for their antioxidant capacity using six different assays. Each measurement was performed in triplicate. The repeatability of each assay was assessed using triplicate measurements made on the same and was expressed as a Coefficient of Variation (CV). The CV for each sample was calculated as the standard deviation of the data, divided by the mean of the triplicate values and multiplied by 100 to give a percentage score. This expressed the standard deviation as a proportion of the mean. The higher the CV, the more variability was shown in the results from a given sample.

CHAPTER 4
PHYSICOCHEMICAL CHARACTERISTICS, ANTIOXIDANT CAPACITY AND ANTI-NUTRIENTS OF MANGO CULTIVARS

4.1. Introduction

Mango (*Mangifera indica L.*) is an important tropical fruit and has widespread consumer appeal due to its excellent eating qualities (bright colour, sweet taste and luscious flavour) and nutritional attributes. Mango seed including kernel constitutes 10 to 25% (Hemavathy *et al.*, 1988; Abdalla *et al.*, 2007a) and mango peel 15–20% (Ajila *et al.*, 2007a) of the whole fruit depending on the variety. Recent reports have shown that flesh, peel and kernel of mangoes are potential sources of natural antioxidants, such as carotenoids, tocopherol, vitamin C and phenolic compounds for use as food ingredients (Puravankara *et al.*, 2000; Arogba, 2002; Berardini *et al.*, 2004; Soong and Barlow, 2006; Abdalla *et al.*, 2007; Ribeiro *et al.*, 2007; Maisuthisakul and Gordon, 2009).

Kim *et al.* (2007) examined the relationships between the antioxidant activity of mangoes and their physicochemical characteristics particularly with Tommy Atkins which is the most important cultivar in the global trade. There is, however, no information on the antioxidant activity and physicochemical characteristics of Tommy Atkins mangoes imported into New Zealand or on mango cultivars grown in Vietnam. Mangoes are considered the most important fruit in Vietnam with the four main cultivars being "Cat Hoa Loc", "Cat Chu," "Ghep" and "Nam Dock Mai" which originate from Thailand.

Mango peel and kernel are usually considered as waste products of the industry but may be a commercial source of antioxidants. Several studies have already reported that there are no adverse toxicants in mango kernels and that they are safe as food ingredient for humans and animals (Arogba, 1997; Kabuki *et al.*, 2000; Fayeye and Joseph, 2004). However, Ravindran and Sivakanesanb (1996) identified that raw mango kernels do contain some potential anti-nutrients such as tannins (56.5 g kg^{-1} DW) and oxalates (42 mg kg^{-1} DW) which may cause adverse health effects in human. These anti-nutritive factors could, however, be reduced by soaking and boiling the mango products in water. There is limited literature available on the anti-nutrients in peel.

The present study investigated the physicochemical characteristics, antioxidant capacity and anti-nutritional factors in the five mango cultivars, Tommy Atkins, Nam Dok Mai, Cat Hoa Loc, Cat Chu and Ghep.

4.2. Materials and methods

4.2.1. Chemicals and reagents

Chemicals and reagents used for this chapter are in chapter 3.16.

4.2.2. Preparation of mango fractions

Flesh, peel and kernel from the Tommy Atkins mangoes (n=12) and "Ghep", "Cat Hoa Loc", "Cat Chu" and "Nam Dok Mai" mangoes (n=3 per cultivar) collected from Vietnam were prepared as described in 3.1 and 3.2.

4.2.3. Mango physicochemical characteristics

All mango cultivars were assessed for their physicochemical characteristics colour, firmness, total soluble solid (TSS), titratable acidity (TA), vitamin C, moisture content and weights of fruit, flesh, peel and kernel as described in 3.3.

4.2.4. Extraction of mango fractions

The extraction processes of mango fractions for analyses were described in 3.5.

4.2.5. Determination of total phenolic content and antioxidant capacity

TPC, ABTS and DPPH assays were performed as described in 3.6.1, 3.6.2 and 3.6.3.

4.2.6. Anti-nutritive factors

4.2.6.1. Determination of tannin

Tannins were determined using MCP (Methyl Cellulose Precipitable) according to the standard method described by Sarneckis *et al.* (2006) as outlined in 3.7.1.

4.2.6.2. Determination of oxalate

Oxalate was determined according to the method described by Savage *et al.* (2009) as outlined in 3.7.2.

4.2.7. Statistical analysis

One-way ANOVA was used to compare the characteristics of each fraction (flesh, peel and kernel) between five cultivars and to compare characteristics of three mango fractions from

each cultivar with a significance of $p < 0.05$. Significant differences between means were determined using the Fisher's LSD multiple comparison tests.

4.3. Results

4.3.1. Physicochemical characteristics of mango cultivars

Fruit weight, firmness, total soluble solid, titratable acidity, ascorbic acid and colour used to evaluate the physicochemical characteristics of the five mango cultivars are given in Table 4.1. 4.1. The weight, percentage and moisture content of flesh, peel and kernel from the five mango cultivars were also determined (Table 4.1).

Table 4.1. Physicochemical characteristics of mangoes (mean ± SE) sourced from New Zealand and Vietnam.

Parameters	Mango cultivars				
	Ghep (n=3)	Cat Hoa Loc (n=3)	Cat Chu (n=3)	Nam Dok Mai (n=3)	Tommy Atkins (n=12)
Total weight (g)	354.8 ± 21.9	365.3 ± 11.9	335.6 ± 18.9	222.5 ± 5.5^{a}	333.4 ± 8.5
Flesh (g)	274.3 ± 19.0	277.0 ± 10.1	257.4 ± 16.3	175.5 ± 4.0^{a}	276.8 ± 7.8
Peel (g)	25.5 ± 1.0^{ab}	25.4 ± 1.5^{ab}	26.3 ± 0.9^{a}	17.1 ± 0.8^{c}	20.5 ± 1.4^{bc}
Kernel (g)	38.1 ± 1.0^{a}	33.0 ± 1.1^{b}	29.2 ± 0.5^{b}	14.9 ± 1.7^{c}	16.2 ± 1.0^{c}
Flesh (%)	77.3 ± 0.7^{bc}	75.8 ± 0.8^{c}	76.6 ± 0.6^{bc}	78.9 ± 0.4^{b}	83.1 ± 0.6^{a}
Peel (%)	7.2 ± 0.2^{ab}	7.0 ± 0.2^{ab}	7.9 ± 0.2^{a}	7.7 ± 0.2^{ab}	6.2 ± 0.4^{b}
Kernel (%)	10.8 ± 0.9^{a}	9.1 ± 0.5^{ab}	8.7 ± 0.3^{b}	6.7 ± 0.6c	4.9 ± 0.3^{d}
Flesh:peel	10.7 ± 0.3^{ab}	10.9 ± 0.3^{ab}	9.8 ± 0.4^{b}	10.3 ± 0.4^{b}	14.2 ± 1.0^{a}
Flesh:kernel	7.3 ± 0.7	8.4 ± 0.5	8.8 ± 0.4	12.1 ± 1.1	17.7 ± 1.2^{a}
Peel:kernel	0.7 ± 0.04^{b}	0.8 ± 0.05^{b}	0.9 ± 0.01^{ab}	1.2 ± 0.08^{ab}	1.3 ± 0.1^{a}
Moisture of flesh (%)	81.5 ± 0.4^{b}	78.4 ± 0.8^{c}	80.4 ± 0.8^{bc}	74.8 ± 1.2^{d}	84.1 ± 0.7^{a}
Moisture of peel (%)	74.8 ± 0.6	72.8 ± 0.5	74.2 ± 1.1	67.5 ± 0.5^{a}	75.9 ± 0.9
Moisture of kernel (%)	43.7 ± 0.9	42.3 ± 0.6	47.0 ± 0.5	41.3 ± 0.8	59.4 ± 3.2^{a}
TSS (°Brix)	14.0 ± 0.6^{b}	17.2 ± 0.3^{a}	16.5 ± 0.2^{a}	12.0 ± 0.1^{c}	6.5 ± 0.8^{d}
Firmness (kg cm^{-2})	6.9 ± 0.4^{b}	4.4 ± 0.2^{c}	3.6 ± 0.2^{d}	14.3 ± 1.0^{a}	30.6 ± 7.4^{a}
TA as citric acid (%), FW	0.1 ± 0.0^{d}	0.2 ± 0.02^{c}	0.1 ± 0.0^{cd}	0.4 ± 0.08^{b}	0.9 ± 0.1^{a}
TSS:TA	132.9 ± 11.5^{a}	113.3 ± 7.5^{a}	118.1 ± 5.3^{a}	28.9 ± 4.7^{b}	7.7 ± 1.1^{c}
Vitamin C (mg 100 g^{-1}), FW	33.2 ± 0.8^{b}	46.0 ± 3.2^{a}	31.8 ± 1.2^{bc}	28.5 ± 1.5^{c}	31.4 ± 0.6^{bc}
L*	43.4 ± 0.5^{cd}	78.5 ± 0.2^{a}	58.7 ± 2.6^{c}	49.6 ± 0.8^{b}	41.0 ± 1.9^{d}
a*	-1.4 ± 0.9^{b}	-5.6 ± 0.1^{c}	-7.3 ± 0.3^{d}	-10.9 ± 0.2^{e}	20.2 ± 3.9^{a}
b*	6.6 ± 1.3^{d}	52.2 ± 0.2^{a}	20.7 ± 1.2^{c}	19.6 ± 0.5^{bc}	24.8 ± 2.4^{b}
[1]Hue (°)	101.0 ± 7.4^{cd}	96.1 ± 0.1^{d}	117.8 ± 1.9^{bc}	110.6 ± 1.1^{b}	233.1 ± 6.8^{a}
Chroma (C*)	6.8 ± 1.4^{d}	52.5 ± 0.2^{a}	23.4 ± 0.9^{c}	20.9 ± 0.5^{c}	34.5 ± 2.4^{b}

Means in the same row with the different superscripts (a-e) are significantly different (p <0.05). [1] Hue (°) = Hue value measured + 180^{o}
Seed is not included in the calculation in this table. Weight and percentage of seed can be obtained by substraction.

4.3.1.1. Weight of fruit and fruit fractions

The average weight of fresh fruit from the five studied cultivars ranged from 222.5 to 365.3g. The Nam Dok Mai cultivar had the lowest weight (222.46 g) and the remaining four cultivars were of similar weight.

The flesh: peel ratio in the mangoes ranged from 9.8 to 14.2, flesh: kernel ratio from 7.3 to 17.7; and the peel: kernel ratio from 0.7 to 1.3. The flesh: peel ratio was lowest for Cat Chu (9.8%) and Nam Dok Mai (10.3%) and highest for Tommy Atkins (14.2%). The flesh: kernel ratio was highest for Tommy Atkins (17.7%) and those in the remaining four cultivars were similar. The percentage of flesh from the mangoes ranged from 75.8 to 83.1%. The lowest yields were from Cat Hoa Loc and the highest from Tommy Atkins. The percentage of peel ranged from 6.2 to 7.9% with Tommy Atkins yielding the lowest and Cat Chu the highest peel yields. The percentage yield of kernel ranged from 4.9 to 10.8% with Tommy Atkins producing the lowest and Ghep the highest kernel yields.

4.3.1.2. Moisture content

The highest moisture content of flesh was in Tommy Atkins (84.1%) and the lowest (74.8%) in Nam Dok Mai. There were no significant differences in the moisture content of peel between the cultivars, except that Nam Dok Mai peel had the lowest moisture content of the five cultivars. There were no significant differences in the moisture content of kernel between the studied cultivars, except for Tommy Atkins which had the lowest moisture content.

4.3.1.3. Firmness

Firmness of the mango cultivars ranged from 3.56 to 30.06 kg cm^{-2}. Tommy Atkins and Nam Dok Mai were significantly firmer than the other cultivar followed by Ghep, Cat Hoa Loc and Cat Chu. There was no significant difference in firmness between Tommy Atkins and Nam Dok Mai.

4.3.1.4. Total soluble solid (TSS)

The TSS (^{o}Brix) of four of the five cultivars was significantly different ($p < 0.05$). Cat Hoa Loc and Cat Chu contained the highest TSS% followed by Ghep, Nam Dok Mai and Tommy Atkins. There were no significant differences in the TSS contents of Cat Hoa Loc and Cat Chu.

4.3.1.5. Titratable acidity (TA)

The titratable acidity values ranged from 0.1 to 0.9 mg 100 g^{-1} FW in the five cultivars with significant differences ($p < 0.05$) occurring between four of the cultivars. The highest and the lowest acidity were recorded in Tommy Atkins and Ghep, respectively (Table 4.1). The second highest TA was in Nam Dok Mai, followed by Cat Hoa Loc and Cat Chu. There were no significant differences in the titratable acidity between Cat Hoa Loc and Cat Chu.

4.3.1.6. TSS:TA

The sugar-acid ratio has been reported to be the best parameter to use as a predictor of the stage of maturity in mangoes (Mahayothee, 2004). Nam Dok Mai and Tommy Atkins mangoes had significantly lower TSS: TA ratios than the other Vietnamese cultivars Cat Chu, Cat Hoa Loc and Ghep.

4.3.1.7. Vitamin C

The mean ascorbic acid levels were 46.04, 33.79, 31.84, 31.36 and 28.53 mg 100g^{-1} FW in the Cat Hoa Loc, Ghep, Cat Chu, Tommy Atkins and Nam Dok Mai cultivars, respectively.

4.3.1.8. Colour

Large significant differences ($p < 0.05$) were observed in the lightness (L* value), redness (a* value), yellowness (b* value), hue angle (h^o) and chroma (C*) of the mangoes (Table 4.1). Particularly, the hue angles of the mango cultivars in Vietnam were negative so the values of hue from all the cultivars were adjusted by adding to 180^o. The L, a*, b*, hue angles and chroma of the mangoes from the five cultivars ranged from 52.5 to 78.0, -10.9 to 20.2, 6.6 to 24.8, 96.1 to 233.1° and 6.8 to 41.0, respectively. The peel of Tommy Atkins showed a significantly higher a* and hue angle and was darker than any other cultivar.

The peel of Cat Hoa Loc had the highest b* value (indicating a more yellow colour) and was significantly lighter (indicated by the higher *L* value) than any of the other cultivars. Cat Hoa Loc mangoes were significantly more vivid in colour (indicated by the higher chroma value) than any of other cultivar. The peel of Nam Dok Mai cultivar was less red-coloured (indicated by the low a*) (Table 4.1).

4.3.2. Total phenolic content and antioxidant capacity of mango fractions from five cultivars

The total phenol content and antioxidant capacities of flesh, peel and kernel of mangoes from the five cultivars; Ghep, Cat Hoa Loc, Cat Chu Nam Dok Mai and Tommy Atkins, were investigated.

4.3.2.1. Total phenolic content in peel, flesh and kernel

Phenolics, commonly found in fruits, have been reported to exhibit antioxidant activity and to scavenge free radicals. Phenolics that possess antioxidant activity are phenolic acids and flavonoids. Many individual phenolic compounds that possess antioxidant activity in fruits have not yet been identified or measured individually by HPLC consequently total phenolic content (TPC) determined by the Folin Ciocalteu's phenol reagent is usually used as an indicator of antioxidant capacity. The total phenolic content of flesh, peel and kernel from the five mango cultivars analysed by one-way ANOVA are shown in Table 4.2.

Table 4.2. Total phenolic content (mean ± SE) of flesh, peel and kernel of mangoes sourced from New Zealand and Vietnam.

Cultivars	Total phenolic content (mg GAE $100g^{-1}$ DW)		
	Flesh	Peel	Kernel
Ghep (n = 3)	$252.6 \pm 54.6^{a\text{-}x}$	$822.3 \pm 94.5^{c\text{-}y}$	$6286.0 \pm 28.7^{c\text{-}z}$
Nam Dok Mai (n = 3)	$378.5 \pm 83.4^{a\text{-}x}$	$8089.4 \pm 1024.6^{a\text{-}y}$	$6987.7 \pm 333.3^{bc\text{-}y}$
Cat Hoa Loc (n = 3)	$396.9 \pm 68^{a\text{-}x}$	$2885.9 \pm 267^{b\text{-}y}$	$7973.3 \pm 441.0^{bc\text{-}z}$
Cat Chu (n = 3)	$699.4 \pm 119.0^{b\text{-}x}$	$4120.0 \pm 677.2^{b\text{-}y}$	$10664.1 \pm 1199.3^{ab\text{-}z}$
Tommy Atkins (n = 12)	$754.4 \pm 48.0^{b\text{-}x}$	$6935.6 \pm 394.7^{a\text{-}y}$	$13888.8 \pm 1313.8^{a\text{-}z}$

Different superscripts (a-c) within the same column mean significantly different and (x-z) within the same row are significantly different ($p < 0.05$).

The flesh of Tommy Atkins and Cat Chu had significantly higher ($p < 0.05$) total phenolic content than Cat Hoa Loc, Nam Dok Mai and Ghep which had similar concentrations. The peel of Nam Dok Mai and Tommy Atkins had significantly higher concentrations of phenolics than Cat Chu, Cat Hoa Loc and Ghep. The total phenolic content of Tommy Atkins kernels was significantly higher ($p < 0.05$) than Cat Hoa Loc, Nam Dok Mai and Ghep. There was no significant difference between TPC of Tommy Atkins and Cat Chu kernels.

In all the five cultivars, the total phenolic content was significantly higher in kernels than peel or flesh except for Nam Dok Mai, which had similar levels of total phenolic in peel and kernel.

4.3.2.2. The antioxidant capacity of peel, flesh and kernels

The ABTS and DPPH assays are often used to evaluate the free radical-scavenging properties or antioxidant capacity of fruits (Wojdylo *et al.*, 2007).

a. ABTS assay

The results of the ABTS assay from the five mango cultivars are shown in Table 4.3. There were significant differences (analysed by one-way ANOVA) in the antioxidant capacities between the cultivars.

Table 4.3. ABTS scavenging activity (mean ± SE) of flesh, peel and kernel of mangoes sourced from New Zealand and Vietnam.

Cultivars	ABTS scavenging activity (µmol TE g^{-1} DW)		
	Flesh	Peel	Kernel
Ghep (n = 3)	$43.6 \pm 0.8^{b\text{-}x}$	$406.3 \pm 74.2^{d\text{-}y}$	$1066.3 \pm 93.2^{b\text{-}z}$
Nam Dok Mai (n = 3)	$49.7 \pm 2.4^{b\text{-}x}$	$1188.3 \pm 112.7^{a\text{-}y}$	$1226.8 \pm 32.0^{b\text{-}y}$
Cat Hoa Loc (n = 3)	$53.3 \pm 4.7^{b\text{-}x}$	$641.7 \pm 92.6^{bc\text{-}y}$	$1330.3 \pm 11.8^{b\text{-}z}$
Cat Chu (n = 3)	$70.0 \pm 3.7^{a\text{-}x}$	$540.3 \pm 46.7^{cd\text{-}y}$	$1527.6 \pm 46.7^{b\text{-}z}$
Tommy Atkins (n = 12)	$54.1 \pm 5.7^{b\text{-}x}$	$787.2 \pm 40.2^{b\text{-}y}$	$2227.6 \pm 197.8^{a\text{-}z}$

Different superscripts (a-d) within the same column mean significantly different and (x-z) within the same row are significantly different ($p < 0.05$).

The antioxidant capacity of flesh, peel and kernel, as measured by ABTS, ranged from 43.5 to 70.0; from 406.3 to 1188.3 and from 1066.3 to 2227.8 µmol Trolox equivalents per g dry weight (TE g^{-1} DW), respectively. The flesh from Cat Chu exhibited significantly ($p < 0.05$) higher antioxidant activity than any other cultivar. There were no significant differences in ABTS between flesh samples from the other four cultivars. There were larger variations in the antioxidant activities of peel, compared to flesh, between the five mango cultivars. Peel extracts from Nam Dok Mai showed the highest antioxidant activity (1188.3 µmol TE g^{-1} DW), followed by Tommy Atkins, Cat Hoa Loc, Cat Chu and Ghep.

Kernel from Tommy Atkins showed the highest antioxidant activity (2227.6 µmol TE g^{-1} DW). Of the other four cultivars, Cat Chu had the highest TEAC values, as measured by

ABTS, but there were no significant differences between the four cultivars. In all the cultivars, the TEAC values measured by ABTS were significantly higher in kernel than peel or flesh, except for Nam Dok Mai, which had similar TEAC levels in peel and kernel.

b. DPPH assay

Table 4.4. DPPH scavenging activity (mean ± SE) of flesh, peel and kernel of mangoes sourced from New Zealand and Vietnam.

Cultivars	DPPH scavenging activity (μmol TE g^{-1} DW)		
	Flesh	Peel	Kernel
Ghep (n = 3)	$30.0 \pm 0.4^{a\text{-}x}$	$210.6 \pm 3.1^{c\text{-}y}$	$667.4 \pm 38.8^{a\text{-}z}$
Nam Dok Mai (n = 3)	$45.2 \pm 11.3^{a\text{-}x}$	$724.5 \pm 76.7^{a\text{-}y}$	$836.1 \pm 9.1^{a\text{-}y}$
Cat Hoa Loc (n = 3)	$39.8 \pm 6.8^{a\text{-}x}$	$445.9 \pm 100.9^{b\text{-}y}$	$932.6 \pm 32.0^{a\text{-}z}$
Cat Chu (n = 3)	$51.6 \pm 2.8^{a\text{-}x}$	$379.8 \pm 37.6^{b\text{-}y}$	$950.7 \pm 57.1^{a\text{-}z}$
Tommy Atkins (n = 12)	$45.4 \pm 4.7^{a\text{-}x}$	$735.4 \pm 43.3^{a\text{-}y}$	$2205.7 \pm 205.7^{b\text{-}z}$

Different superscripts (a-c) within the same column mean significantly different and (x-z) within the same row are significantly different ($p < 0.05$).

The relatively stable organic radical, DPPH, has been widely used for determining the antioxidant activity of single compounds and plant extracts (Katalinic *et al.*, 2006; Wojdylo *et al.*, 2007). The antioxidant activity of flesh, peel and kernel as measured by DPPH, ranged from 30.0 to 51.6; 210.6 to 735.4 and 667.4 to 2205.7 μmol TE g^{-1} DW, respectively. The antioxidant activity in mangoes measured by DPPH showed a similar ranking of cultivars to the ABTS method. The DPPH values were slightly lower than ABTS on a TE basis. Although the flesh of Cat Chu showed the highest DPPH values, there were no significant differences between the five cultivars.

The peel extracts from the five mango cultivars showed significant differences in their DPPH activity (Table 4.4). Tommy Atkins and Nam Dok Mai scavenged more DPPH radicals than any of the other three cultivars.

The DPPH radical-scavenging capacity of kernel ranged from 667.4 to 2205.7 μmol TE 100 g^{-1} DW. Tommy Atkins exhibited the highest DPPH scavenging activity which was 2.3, 2.4, 2.6 and 3.3 times higher than kernel from Cat Chu, Hoa Loc, Nam Dok Mai and Ghep respectively. There were no significant differences in the DPPH scavenging activity between the four mango cultivars cultivated in Vietnam.

For all the cultivars, DPPH scavenging activity was significantly higher in kernel than peel and flesh, except for Nam Dok Mai, which had similar antioxidant capacity in the peel and kernel.

4.3.3. Anti-nutrients of mango fractions from five cultivars

The results in sections 4.3.1 and 4.3.2 indicated that mango peel and kernel were promising sources of antioxidant for inclusion in the diets of domesticated animals and humans. However, like many natural plants, mango peel and kernels may also contain low levels of toxicants. Tannins and oxalates have been previously implicated as dietary anti-nutritive factors in plants (Cheeke, 1998). The presence of these anti-nutritional factors in mango peel and kernels was therefore assessed. The total condensed tannins were extracted using a methanol-water mixture and then precipitated as an insoluble polymer tannin complex. The tannin content is calculated as the difference in absorbance at 280 nm of tannin extracts before and after precipitation.

4.3.3.1. Tannins

Table 4.5. Tannin content as epicatechin (mean ± SE) of mango fractions from five different cultivars

Cultivars	Tannin content (g EE kg^{-1} DW)		
	Flesh	Peel	Kernel
Ghep (n = 3)	$1.0 \pm 0.1^{a\text{-}x}$	$19.8 \pm 0.7^{b\text{-}y}$	$21.3 \pm 0.4^{d\text{-}z}$
Nam Dok Mai (n =3)	$1.1 \pm 0.0^{a\text{-}x}$	$22.9 \pm 0.1^{a\text{-}y}$	$51.7 \pm 0.3^{a\text{-}z}$
Cat Hoa Loc (n = 3)	$1.1 \pm 0.0^{a\text{-}x}$	$7.9 \pm 0.2^{e\text{-}y}$	$34.0 \pm 0.3^{c\text{-}z}$
Cat Chu (n =3)	$1.1 \pm 0.1^{a\text{-}x}$	$9.6 \pm 0.1^{d\text{-}y}$	$43.5 \pm 0.1^{b\text{-}z}$
Tommy Atkins (n =12)	$0.9 \pm 0.1^{a\text{-}x}$	$12.7 \pm 1.1^{c\text{-}y}$	$48.3 \pm 2.5^{ab\text{-}z}$

Different superscripts (a-e) within the same column mean significantly different and (x-z) within the same row are significantly different ($p < 0.05$).

The tannin content of flesh from the five cultivars as shown in Table 4.5 was similar and ranged from 0.9 to 1.1g epicatechin equivalent kg^{-1} DW. The tannin content of peel from the five cultivars varied more widely from 7.9 to 22.9g epicatechin equivalent kg^{-1} DW (Table 4.5). Nam Dok Mai peel contained the highest tannin content followed by Ghep, Tommy Atkins, Cat Chu and Cat Hoa Loc.

The tannin content of kernel of the five cultivars varied markedly from 21.3 to 51.7 g kg^{-1} DW as epicatechin equivalent (EE) (Table 4.5). Nam Dok Mai kernels contained the highest tannin content followed by Tommy Atkins, Cat Chu, Cat Hoa Loc and Ghep. There were no significant differences in the tannin content of Tommy Atkins and Nam Dok Mai kernels. The tannin content was the highest in kernel then peel then flesh in all the mango cultivars.

4.3.3.2. Oxalates

No oxalates were detected in mango flesh of any of the five cultivars or in the kernel of Ghep, Cat Hoa Loc and Cat Chu (Table 4.6). The oxalate content of kernels in Nam Dok Mai (36.3 mg 100 g^{-1}) was 10 times higher than in Tommy Atkins (3.6 mg 100 g^{-1}). Oxalates were found in peel from all the cultivars. Nam Dok Mai, Cat Hoa Loc and Ghep peel contained approximately 4 times more oxalate than the peel of Tommy Atkins and Cat Chu.

Table 4.6. Total oxalate content (mean ± SE) as oxalic acid of mango fractions from five different mango cultivars.

Cultivars	Total oxalate content (mg oxalic acid equivalent 100 g^{-1}DW)		
	Flesh	Peel	Kernel
Ghep (n =3)	nd	108.9 ± 5.2^{a}	nd
Nam Dok Mai (n = 3)	nd	$126.2 \pm 3.0^{a\text{-}x}$	$36.3 \pm 2.2^{a\text{-}y}$
Cat Hoa Loc (n = 3)	nd	123.0 ± 5.3^{a}	nd
Cat Chu (n = 3)	nd	27.2 ± 2.2^{b}	nd
Tommy Atkins (n 12)	nd	$33.2 \pm 2.0^{b\text{-}x}$	$3.6 \pm 0.0^{b\text{-}y}$

Different superscripts (a-c) within the same column mean significantly different and (x-z) within the same row are significantly different ($p < 0.05$); nd: not detected.

4.4. Discussion

4.4.1. Physicochemical characteristics of mango cultivars

Differences in any of the physicochemical characteristics can be due to genetic variations between varieties, the growing and environmental conditions or method of storage (Randhawa, 2002; Muhammad, 2010).

4.4.1.1. Fruit weight, fraction weight, percentage and ratio

The weight of Tommy Atkins was slightly lower than those reported by Sosa-Morales *et al.* (2009) (374.4 g) and Rocha-Ribeiro *et al*. (2007) (477.2 g). Cat Hoa Loc, had the highest fruit weight (365.3g) of the Vietnamese cultivars. Nam Dok Mai had the lowest weight (222.46 g)

which was lower than the Nam Dok Mai mango weights reported by Sriwimon and Boonsupthip (2011).

Cultivars that provide a high fruit weight or a high flesh percentage in combination with a low percentage yield of peel or kernel, as found with Tommy Atkins, are ideal mangoes for the processing industry due to their ability to provide high yields of useable flesh. The high percentage of peel in Cat Chu and kernel in Ghep suggests that these cultivars may yield components that are viable sources of antioxidants that can be incorporated into dietary supplements (Zatylny *et al.,* 2004).

4.4.1.2. Moisture content

The moisture content of flesh from all the mangoes was similar to the values of 84% as reported by Laohaprasit *et al.* (2011). There were no significant differences between the moisture content of the kernels from the four Vietnamese cultivars.

Ueda (2001) reported that the moisture content of mangoes decreased gradually as they ripen. Tandon and Kalra (1983) also stated the moisture content decreased during maturation and was linked to the cultivar and cultivation conditions (Tandon and Kalra, 1983; Ueda, 2001). Al-Hooti *et al.* (1997) stated that the moisture content of flesh from different cultivars decreased as the fruit reached maturity and ascribed the changes to the accumulation of total soluble solids such as fructose, glucose and sucrose. The reported changes should impact on the taste, physicochemical characteristics and antioxidant capacity of the fruits.

The moisture content of peel from all the five cultivars studied were similar, except for Nam Dok Mai which was significantly lower ($p < 0.05$). The moisture content of Nam Dok Mai peel (67.5%) was similar to that of "Raspuri" (66 - 72.5%) whilst the other four cultivars (72.8 - 75.9%) were similar to "Badami" (70.3-75.3%) reported by Ajila *et al.* (2007a). The moisture contents of kernels from all the five cultivars were similar except for Tommy Atkins which was significantly higher ($p < 0.05$). The moisture content of Tommy Atkins kernel (59.4%) was similar to that of kernels (50.7%) reported by Abdalla *et al.* (2007).

There is no data on the relationship between the moisture content of peel or kernel to the maturity of mango fruits. Sinclair (1972) suggested that the moisture content of grape-fruit peel was not correlated with peel firmness, and that firmness and moisture content were two unrelated factors which contributed to the low texture resistance of peel. Such data infers that peel texture is, in some instances, influenced by factors that are independent of moisture

content (Sinclair, 1972). It is generally accepted that the moisture content of mango flesh, peel and kernel are dependent on the genetic makeup and agronomic and climactic backgrounds of the cultivars (Sobeih and El-Helaly, 2002; Ahmad, 2010; Abdelazim, 2011).

4.4.1.3. Firmness

The firmness readings in the present study (30.6 kg cm^{-2}) were higher than the 1.74 kg cm^{-2} reported by Sabato *et al.* (2009) for Tommy Atkins stored for 6 days or the values (7.6 kg cm^{-2}) reported by Rocha Ribeiro *et al.* (2007). Thus, the Tommy Atkins mangoes used in this study were at the green or unripe stage of maturity.

The firmness readings of Nam Dok Mai cultivated in Vietnam were higher than those reported by Chonhenchob *et al.* (2010) but similar to "Dashehari" reported by Jha *et al.* (2006) and "Dodo" by Msgoya and Kimaro (2011). In general, any difference in firmness is usually a reflection of the stage of maturity of the mango and the rate of maturity development is usually dependent on the cultivar. In the initial stages of fruit development, firmness remains almost unchanged but on reaching maturity, firmness decreases due to possible changes in the structure of the pectin polymers in the cell wall (Kalra *et al.,* 1995). All the mango cultivars used in the present study were collected from retail markets and at the ready-to-eat stage; however, their actual stages of maturity were all different.

4.4.1.4. Total soluble solids (TSS)

All the cultivars, except for Tommy Atkins (6.5 ^{o}Brix or %) had TSS levels above the recommended minimum for consumption (12.0 ^{o}Brix). The TSS level in Tommy Atkins was similar to the 6.5 ^{o}Brix reported for Tommy Atkins grown in Jamaica (Medlicott *et al.,* 1986) or Cogshall in Florida (Lebrun *et al.,* 2008). Although the TSS of Tommy Atkins was low, it does satisfy the 6.8 ^{o}Brix requirement by Mexico for mangoes to be exported (Baez-Sanudo and Bringas-Taddei, 1996). TSS of Nam Dok Mai was lower than that reported by Laohaprasit *et al.* (2011) indicating that the 'Nam Dok Mai' mangoes are quite green. Nevertheless, the TSS levels (Table 4.1) are similar to the range (6.85 to 14.67 ^{o}Brix) reported by Lebrun *et al.* (2008) for "Cogshall" mangoes which varied from green to the ripe stages of maturity. The TSS levels are higher than those reported by Sosa-Morales *et al.* (2009) and Sabato *et al.* (2009) who found 10.2 ^{o}Brix at day 1 and 11.9 ^{o}Brix at day 6 of storage.

The TSS levels of the four Vietnamese cultivars are comparable to the levels reported for "Dodo" in Tanzania (Theodosy and Elde, 2011) and "Nam Dok Mai" in Thailand (Po, 2006; Laohaprasit *et al.,* 2011) which ranged from 14 to 23 ^{o}Brix. The results imply that

Vietnamese growers are selling mangoes at the stage that yield desirable TSS levels (12.0 ^{o}Brix) required by consumers.

In general, the TSS content of stored mangoes increased to 19.73 ^{o}Brix on ripening and then declined to 15 ^{o}Brix on further storage (Jha *et al.*, 2006). The observed decrease in TSS may be due to excessive ripening or rotting of the mango during storage. Thus, like firmness, TSS levels can be used to monitor the stage of mango maturity.

4.4.1.5. Titratable acidity (TA)

The titratable acidity of Nam Dok Mai is similar to that reported for Cogshall sourced from Florida (Lebrun *et al.,* 2008). The total acidity of 0.1-0.4% FW (Table 4.1) of the four cultivars from Vietnam was similar to the 0.11-0.48% range reported by Laohaprasit *et al.* (2011). However, the TA of 0.17% reported by Laohaprasit *et al.* (2011) for Nam Dok Mai is lower than 0.4% found in the current study. The TA of Tommy Atkins is similar to the values of 1.1% reported by Sosa-Morales (2009) but higher than the values of 0.21 and 0.68%, reported by Sabato *et al.* (2009) and Jose *et al.* (2004) respectively for the same cultivar. It is now recognised that a reduction in TA occurs over the storage period due to the utilisation of organic acids by fruit respiration (Anthon *et al*., 2011). The level of TA is therefore another indicator of the stage of ripening. Thus, the high levels of TA found in Tommy Atkins and Nam Dok Mai indicate that both cultivars were quite green when they were analysed in this study. The latter conclusion is supported by the firmness and TSS results.

4.4.1.6. TSS:TA

In this study the TSS: TA ratio of the Vietnamese mangoes Ghep, Cat Chu and Cat Hoa Loc were significantly higher than Nam Dok Mai and Tommy Atkins. This could reflect that in Vietnam, mangoes only reach the retail markets at the ripe stage because they are harvested late when the fruit have attained maturity. In contrast Tommy Atkins cultivars are usually harvested earlier at an undefined "mature green" stage for the export markets. Nevertheless, both Tommy Atkins and Nam Dok Mai mangoes in the present study were purchased in retail markets and were supposed to be sufficiently mature for consumption but the mangoes were still relatively green. Some of the observed differences in TSS: TA ratios could also be attributed to differences in the mango cultivars.

4.4.1.7. Vitamin C

The vitamin C content decreases as the fruit ripened and the storage time increases (Kalra and Tandon, 1995). The mean vitamin C content, which ranged from 28.5 to 46.0 mg $100g^{-1}$ FW was higher than that reported for Tommy Atkins by Manthey and Perkins-Veazie (2009) (19.3mg $100g^{-1}$ FW), (Rocha Ribeiro *et al.,* 2007) and Gonzalez-Aguilar, (2007) (≈ 6.9-16.0 mg 100 g^{-1} FW). The range of vitamin C concentrations in the five cultivars was similar to that previously reported by Carvalho (2004). The values obtained for Tommy Atkins (31.4 mg 100g $^{-1}$ FW) were similar to the values reported for other cultivars such as Keith, Kent and Haden.

The differences in vitamin C between the five cultivars and those previously reported can be attributed to factors such as differences in the individual cultivar, climatic conditions, cultural practices, stage of maturity and post-harvest factors (Lee and Kader, 2000). For example, Ribeiro *et al.* (1995) reported a range of 25.3 to 182.55 mg ascorbic acid $100g^{-1}$ FW mango which varied according to the cultivar and assay method. Predictions of the actual concentration of vitamin C in cultivars should therefore take into account that their physicochemical characteristics are linked to the maturity of the mangoes (Rocha-Ribeiro *et al.,* 2007).

4.4.1.8. Colour

The colour of fruits directly affects their appearance and consumer acceptability. All the cultivars had different colours as measured by the Hunter colorimeter. High a*, Hue and b* values and low L* values of Tommy Atkins mangoes indicated that these mangoes were darker, redder and more yellow (only less yellow than Cat Hoa Loc) than any of the cultivars grown in Vietnam. Indeed they exhibited more strong and distinctive colour characteristics than any other cultivar. Cat Hoa Loc mangoes were lighter and had more vivid colour than any other cultivar. Nam Dok Mai mangoes were green and tended to turn gradually to yellow. Of the cultivars tested, Nam Dok Mai cultivar was the greenest.

When ripe, the colour of mango fruit can dramatically change from green to yellow to red. Cat Hoa Loc and Cat Chu mangoes were yellow when ripe, Tommy Atkins red, and Nam Dok Mai and Ghep green or yellow-green. The significant differences in peel colour of other fruits (e.g. apple) have been reported by other researchers (Drogoudi *et al.*, 2008; Iglesias *et al.*, 2008).

4.4.2. Total phenolic content and antioxidant capacty of mango fractions from five cultivars

In the literature, there have only been reports on TPC and the antioxidant capacity of mango fractions from Tommy Atkins and Nam Dok Mai cultivars. The total phenolic content of Tommy Atkins mango flesh in the present work was higher than reported by previous researchers (Gonzalez Aguilar *et al.,* 2007; Rocha-Ribeiro *et al.,* 2007; Manthey and Perkins-Veazie, 2009). The total phenolic content of Tommy Atkins peel and kernel were lower than reported by Ashoush and Gadallah (2011) but were similar to the values reported by other researchers (Rocha-Ribeiro *et al.,* 2007; Rocha-Ribeiro *et al.,* 2008; Manthey and Perkins-Veazie, 2009).

On the other hand, Nam Dok Mai mango kernels in the present study showed similar TEAC values when measured by ABTS and lower TPC values than reported by Khammuang and Sarnthima (2011)**.** The different results obtained with Tommy Atkins could be due to differences in the growing and storage conditions or the maturity of the fruit. Variations in the experimental procedures such as the extraction technique, extraction solvent or assay protocols could also be contributing factors.

There has been no published data on the antioxidant capacity of Vietnamese mango cultivars. However, compared to those reported previously for different mango cultivars (Soong and Barlow, 2004; Matsusaka and Jun Kawabata, 2010), the three Vietnamese mango cultivars had similar ranges of TPC, ABTS and DPPH levels in flesh, peel and kernel.

The results from flesh, peel and kernel in Tables 4.2, 4.3 & 4.4 show that the rank order of the cultivars is the same whether the antioxidant capacity is measured by TPC or ABTS or DPPH. In regards to flesh, Tommy Atkins and Cat Chu cultivars showed significantly ($p < 0.05$) higher TPC than any of the other cultivars. The Cat Chu cultivar also exhibited the highest antioxidant capacity as measured by ABTS. Although Cat Chu and Tommy Atkins showed the highest DPPH values than any other cultivars, the differences were not significant.

The peel of Nam Dok Mai and Tommy Atkins showed the highest TPC, ABTS and DPPH scavenging activity followed by Hoa Loc, Cat Chu and Ghep cultivars. The kernel of Tommy Atkins showed the highest TPC, ABTS and DPPH values, followed by Cat Chu, Hoa Loc, Nam Dok Mai and Ghep but only Tommy Atkins kernel exhibited a significantly higher ($p < 0.05$) activity than any of the other cultivar. Phenolic compounds in peel and kernel contributed to antioxidant activity as measured by the ABTS and DPPH assays.

For all the selected cultivars, total phenolic content, ABTS and DPPH scavenging activities were significantly higher in the kernel than peel and flesh, except for Nam Dok Mai, which had similar levels of phenolics and antioxidants in the peel and kernel. This finding is in agreement with previous studies (Khammuang and Sarnthima, 2001; Rocha-Ribeiro *et al.*, 2007)**.**

4.4.3. **Anti-nutrients** in **mango fractions from five cultivars**

4.4.3.1. Tannins

Tannins are phenolic compounds that precipitate proteins which exhibit their toxicity effects mainly in the gastrointestinal tract (Bressani *et al.*, 1983). Mango flesh from the five cultivars contained low and similar tannin contents (0.9 to 1.1 g kg^{-1} DW) to each other. The tannin content of flesh from all the cultivars were considerably lower than those reported (31 g kg^{-1} DW, respectively) by Joseph and Aworh (1991). Tannins, due to their phenolic characteristics, if used or consumed in a small amount, can be a good antioxidant source. Therefore, mango flesh from all the studied mango cultivars is safe for consumption.

Nam Dok Mai peel contained the highest tannin content followed by Ghep, Tommy Atkins, Cat Chu and Cat Hoa Loc. The tannin content of peel from all the cultivars (7.9 - 22.9 g kg^{-1} DW) is lower than reported (37.3 g kg^{-1} DW) by Joseph and Aworh (1991). The tannin content of peel from Nam Dok Mai, Ghep and Tomy Atkins were within the values reported by Dorta *et al.* (2011) who found that the peel tannin content of 14 to 130 g kg^{-1} DW depended on the solvent extraction procedure.

The tannin content of kernel of Nam Dok Mai (51.67 g kg^{-1} DW) was similar to the levels reported (56.5 g kg^{-1} DW) by Ravindran and Sivakanesan (1996) and higher than 44.5 g kg^{-1} DW reported for Indian mangoes by Patil *et al.* (1982) or 44.8 g kg^{-1} DW reported for the Ikanekpo variety (Arogba, 1997). Tommy Atkins, Nam Dok Mai and Cat Chu mango kernels have similar tannin levels whilst Cat Hoa Loc and Ghep cultivars have lower tannin levels than previously reported. For all the five cultivars, the tannin levels were significantly higher in kernel than peel or flesh (Table 4.5).

4.4.3.2. Oxalates

The absence of oxalates in the mango flesh of all the cultivars and in kernel of Vietnamese mango cultivars shows that these particular fractions will have no negative effects on the bioavailability of minerals to animals if used as a dietary ingredient. It is recognised that a large amount of dietary oxalates can be undesirable since the oxalates will complex with

calcium and reduce the availability calcium and, as a consequence, increases the risk of renal calcium absorption (Noonan and Savage, 1999). Dietary oxalate also promotes a calcium, magnesium and iron complex which is the basis of oxalate kidney stones (Jaeger and Robertson, 2004).

Only Nam Dok Mai and Tommy Atkins kernels contained oxalates. Furthermore, the oxalate levels in Nam Dok Mai were 10 times higher than in Tommy Atkins and were also higher than 4.2 mg $100g^{-1}$ DW reported by Ravindran and Sivakanesan (1996) and 1.49 mg $100g^{-1}$ DW reported by Fowomola (2010). The levels in Tommy Atkins kernel are consistent with previous reports.

In the present study, the Nam Dok Mai peel had the highest oxalate levels followed by Cat Hoa Loc, Ghep, Tommy Atkins and Cat Chu. The oxalate content of peel ranged from 27.17 to 126.23 mg kg^{-1} DW. The oxalate content of peel was significantly ($p < 0.05$) higher than in Nam Dok Mai and Tommy Atkins kernels (Table 4.6). There is little published data on the oxalate content of mango peel and flesh.

Overall, mango flesh from all the cultivars contained low levels of tannins and no oxalates and are, therefore, within acceptable levels to consume. In order for mango peel and kernel to be utilized as food ingredients, they require further processing to reduce tannins and oxalates to acceptable levels. For several years, some mangoes have been processed to remove the majority of the tannins and oxalates. However, Ravindran and Sivakanesan (1996) reported that processing with boiling water only removes 67% of the tannin which means that tannins cannot be completely removed from kernels. Tannins, however, can cause bitter or astringent taste problems and inhibit protein digestion. A recommendation of Patil *et al.* (1982) was that the substitution of 14.1% of dietary maize in poultry diets with the equivalent level of mango kernel was safe and had no effect on the performance of the animals. There is no report on the the limited concentration of oxalate in human or animal dietary but the previous studies have suggested that intakes of oxalate exceeding 180mg day^{-1} result in a marked increase in the amount excreted (Noonan and Savage, 1999). The study concluded that the kernel supplements should be limited to a maximum level of 14.1% in diets. Morrison and Savage (2003) suggested that the amount of oxalates in diets could be reduced by soaking, blanching or cooking. Mango kernels are often dried and only used in small quantities in formulated animal foods and should therefore be of little risk as a dietary ingredient.

4.5. Conclusions

- The physicochemical characteristics of mangoes are important factors since they influence the selection of cultivars for specific markets. The size and weight of a fruit is a varietal property, which fluctuates depending on climatic and agricultural conditions as well as the number and position of the fruit on a tree. Most of the mango cultivars in this study were of equal weight except for Nam Dok Mai which was smaller. Peel colour directly affects the appearance and the acceptability of the fruit to consumers. Tommy Atkins was red, Hoa Loc and Cat Chu yellow, and Nam Dok Mai and Ghep green. Firmness is widely used as a test for fruit ripeness. Flesh firmness decreases as fruit ripen which is associated with a decrease in pectin and an increase in soluble solids. The results illustrate that maturity, based on firmness, was relatively uniform for the cultivars grown in Vietnam and variable for Tommy Atkins imported in to New Zealand. The acid, sugar and moisture content of mangoes are important components contributing to the taste and flavour characteristics of the fruits. The vitamin C content of mangoes is an essential contributor to their nutritional value. All the mango cultivars grown in Vietnam showed high levels of soluble solids and TSS: TA ratios and the low levels of firmness, TA and moisture content indicating that the mangoes were softer than Tommy Atkins and more suitable for the short term fresh market. Tommy Atkins had the highest mean acidity value than any other cultivar and is ideally suited for processing which requires acidity in fruits. Moreover, Tommy Atkins mangoes had the highest flesh percentage and lowest peel and kernel (waste) percentage than the other cultivars grown in Vietnam, suggesting that they are ideal fruits for processing. There were only slight differences in vitamin C between the cultivars which could be attributed to variations in climatic conditions, cultivation and stage of maturity or post-harvest treatment.

- Overall, the physicochemical characteristics of Tommy Atkins were different from those of Nam Dok Mai and the Vietnamese cultivars. Although, the measured physicochemical characteristics can be used as indicators of the stage of maturity of the mangoes, some of the differences could be attributed to genetics, growing, environmental and storage differences. The main value of the physicochemical parameters is to provide reference criteria for characterising the individual mango cultivars rather than determining their exact stage of maturity.

- The results show that flesh, peel and kernel from Tommy Atkins mangoes imported from Mexico into New Zealand and flesh from Cat Chu mangoes contained the highest antioxidant capacity among the cultivars investigated to date. Of the cultivars grown in Vietnam, Cat Chu flesh and kernel and Nam Dok Mai peel are good sources of phenolics and antioxidants. On the other hand, from all the cultivars examined, kernels contain the highest concentration of total phenolics and antioxidants, followed by peel and flesh. It is apparent that, irrespective of the country of origin, there is an opportunity to use mangoes as a source of phenols, which are powerful and effective antioxidants. This study also shows that mango kernel and peel, which are often discarded as waste products, could be considered as dietary ingredients to protect food from oxidative damage.

- In all the selected cultivars, the tannin contents were significantly higher in the kernel than peel than flesh. The oxalate contents were highest in peel. Oxalates were only found in the kernel of Nam Dok Mai and Tommy Atkins mangoes and were not present in the flesh of any other cultivar. Mango flesh from all the cultivars studied contained low levels of tannins and no oxalates. Peel and kernel of Nam Dok Mai mango cultivars had the highest content of tannins and oxalates among the cultivars studied. The oxalates in kernel of Tommy Atkins were also higher than those in the Vietnamese cultivars. Although tannins and oxalates can be reduced by various processing treatments, mango peel and kernel when used as a food ingredient should be consumed in moderation (less than 14.1% of the total diet) to ensure there are no adverse effects on protein or mineral availability.

CHAPTER 5
DRYING EFFECTS ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF MANGO FRACTIONS

5.1. Introduction

Like other fruits, mangoes are perishable and deteriorate in their fresh state. Consequently the processing and preservation of mangoes is important to the industry with drying being the most effective process to preserve fruits or their products. The objective of drying is to maintain fruit quality, reduce storage volume and to extend the shelf-life of the product. In the drying process, free water is removed from a product and, simultaneously, the availability of water for undesirable enzymatic reactions and microbial growth is reduced. As food additives and preservatives, dried mango products canpresent several advantages including greater chemical, physicochemical and microbiological stabilities, easier standardization, higher concentration of active compounds (Silva *et al*., 2012). However, the drying of food is difficult because of the various physical, chemical and biochemical transformations that may occur during the drying process and, as a consequence, the undesirable end products that can be produced (Baker, 1997). In particular, the enzymatic and non-enzymatic changes that occur on drying fresh plant tissues can affect phytochemicals (Sathishkumar *et al*., 2009) and create negative attributes to the final products.

In most cases, drying involves the application of thermal energy which causes water to evaporate into the vapour phase. The degree of thermal damage is directly proportional to the temperature and time that products are exposed to a specific temperature (Kwok *et al*., 2004). A high temperature and long-time exposure to hot air drying, for example, adversely affects the texture, colour, flavour and nutritional values of food products. Consequently, heat processed dried foods are considered to have lower health promoting characteristics than fresh products (Choi *et al*., 2006). However, the degree of thermal damage to antioxidants depends on the response of individual antioxidants to the drying conditions.

Many reports have also indicated that the effect of the drying method on antioxidant capacity depends on the source of the antioxidants. Furthermore, studies have shown that the total antioxidant content of certain foods may actually increase due to specific reactions being elicited by the heat treatment (Kim *et al.*, 2000; Dewanto *et al*., 2002; Sathishkumar *et al*., 2009). For example, many plant antioxidative phenolic compounds occur in a covalent bound

form and the heat treatment, in some cases, releases the antioxidants from their bound status (Jeong, 2004). Heat also promotes the formation of reducing sugars and Maillard reaction products. Both are known to have antioxidant activity (Madrau *et al*., 2009).

The effects of heating or drying temperature on the total phenolic and antioxidant content of fruits are still unclear. Therefore, determining the impact of applying specific drying methods to particular fruits or fruit fractions is an important area of research particularly in regards to the effect on antioxidant properties. Currently, many drying methods are used to produce plant products and each method has its advantages and disadvantages. The major dehydration techniques to preserve fruits include sun, forced, vacuum, microwave and freeze drying. Although freeze drying is often used due to its low heat requirements, information on the changes in total phenolics and antioxidant capacity in fruits such as mango is limited. The effects of heat or drying treatment on the quality of bioactive compounds in plant material are also unclear.

Mango flesh, peel and kernel can be dried and ground (Baker, 1997) to produce powders suitable as food ingredients. Information is required, however, on the effects of the various drying methods on the total phenolic compounds and antioxidative characteristics of flesh, peel and kernel products.

The present study has investigated the effects of sun, forced-air, freeze, vacuum and microwave drying on the total phenolic content and antioxidant capacity of mango flesh, peel and kernel from the Tommy Atkins cultivar as measured by Folin-Ciocalteu, ABTS, DPPH, FRAP and ORAC assays. The corresponding fresh samples were used as controls for comparison.

5.2. Materials and methods

5.2.1. Chemicals and reagents

Chemicals and reagents were used as outlined in 3.16.

5.2.2. Methods

Flesh, peel and kernel from the Tommy Atkins mangoes (n = 72) were prepared as described in 3.1 and 3.2). Mango fractions were dried by sun, forced-air, freeze, vacuum and microwave dried as described in 3.4. Flesh, peel and kernel from fresh (n = 12) and dried mangoes (12 samples per treatment) were extracted for antioxidant assays as described in 3.5. Six

antioxidant assays were applied to mango flesh, peel and kernel as described in 3.6. They were TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC.

5.2.3. Statistical analysis

Each analysis was performed in triplicate. The differences between antioxidant capacity measured by each assay (TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC) of fresh mango samples and those dried by the five different drying methods were determined using a one-way analysis of variance (ANOVA) at $p < 0.05$. Significant differences between means were determined using the Fisher's LSD multiple comparison tests. Two types of multivariate analysis (Principal Components Analysis and Canonical Variate Analysis) were carried out to investigate patterns among the fresh and dried mango samples and patterns among the assays. Graphical displays (biplots) were produced following Principal Components Analysis to show the association between the samples and the association beween the variables (assays) on the same plot. Minitab 16 was used to analyse the data using one-way ANOVA and Principal Component Analysis (PCA). Genstat 13 was used to analyse the data using Canonical Variates Analysis (CVA). These statistical analyses techniques are described in 3.17.

5.3. Results

5.3.1 Effects of drying treatments on the total phenolic content (TPC) of mango flesh, peel and kernel

a. Flesh

The total phenolic content of fresh, sun, forced-air, freeze, vacuum and microwave dried mango flesh were 531.5, 504.1, 825.5, 754.4, 527.2 and 701.1 mg GAE $100g^{-1}$ DW, respectively (Figure 5.1a). The results show that forced-air, freeze and microwave drying of mango flesh significantly increased ($p < 0.05$) the total phenolic content by 55.3, 41.9 and 31.9%, respectively compared to fresh flesh. Sun and vacuum drying had no significant effect on the TPC compared to fresh flesh. Of the drying treatments, forced-air drying produced a significantly higher TPC than any other drying treatment except for freeze drying.

b. Peel

The phenolic content of fresh mango peel was 4858.0 mg GAE $100g^{-1}$ DW compared to 5825.2, 6041.0, 6935.6, 5533.6 and 6385.9 mg GAE $100g^{-1}$ obtained with sun, air, freeze, vacuum and microwave dried mango peel, respectively (Figure 5.1b). The TPC of freeze, forced-air and microwave dried peel were increased significantly by 43, 31 and 24%, respectively compared to fresh peel. Of the drying treatments, freeze drying produced a

significantly higher TPC than sun or vacuum drying. There were no significant differences in the TPC of dried peel produced by freeze, forced-air or microwave drying.

c. Kernel

The total phenolic content of fresh, sun, air, freeze, vacuum and microwave dried mango kernel was 10749, 12696, 9129, 13888, 13655 and 8113 mg GAE $100g^{-1}$ DW respectively (Figure 5.1c). There were no significant ($p \geq 0.05$) differences between the TPC of fresh kernel and dried kernel produced by any of the five drying processes. Of the drying treatments, sun, freeze and vacuum drying produced significantly higher TPC in the dried kernel products than forced-air or microwave drying.

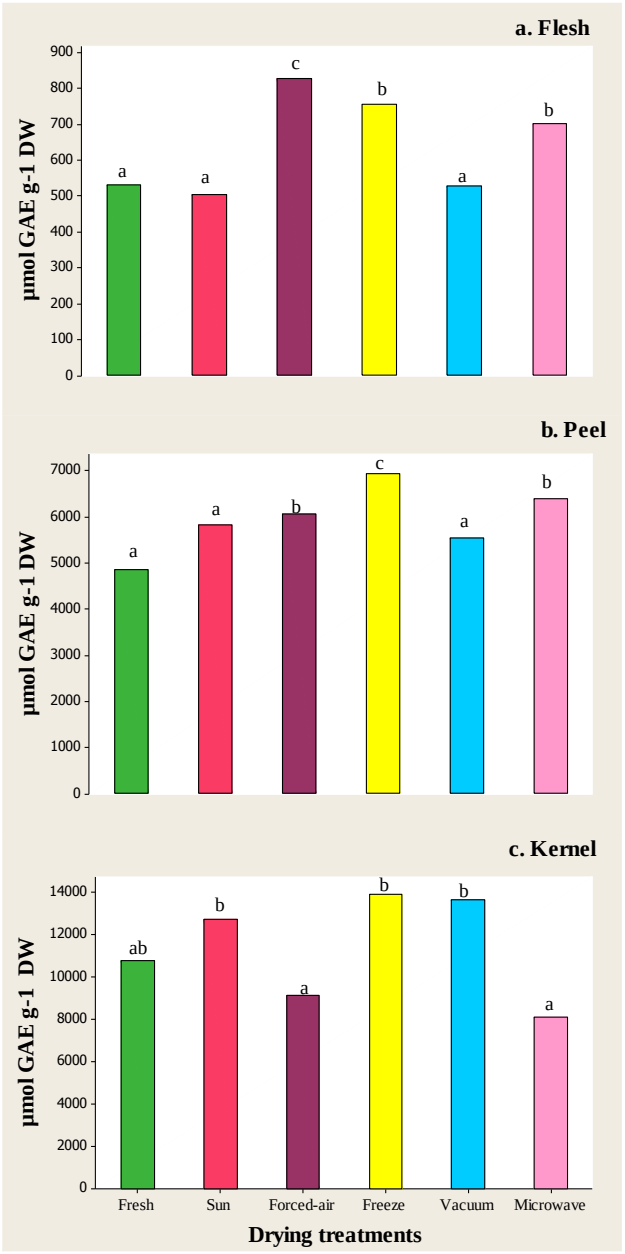


Figure 5.1. Effects of drying on TPC of mango flesh, peel and kernel.

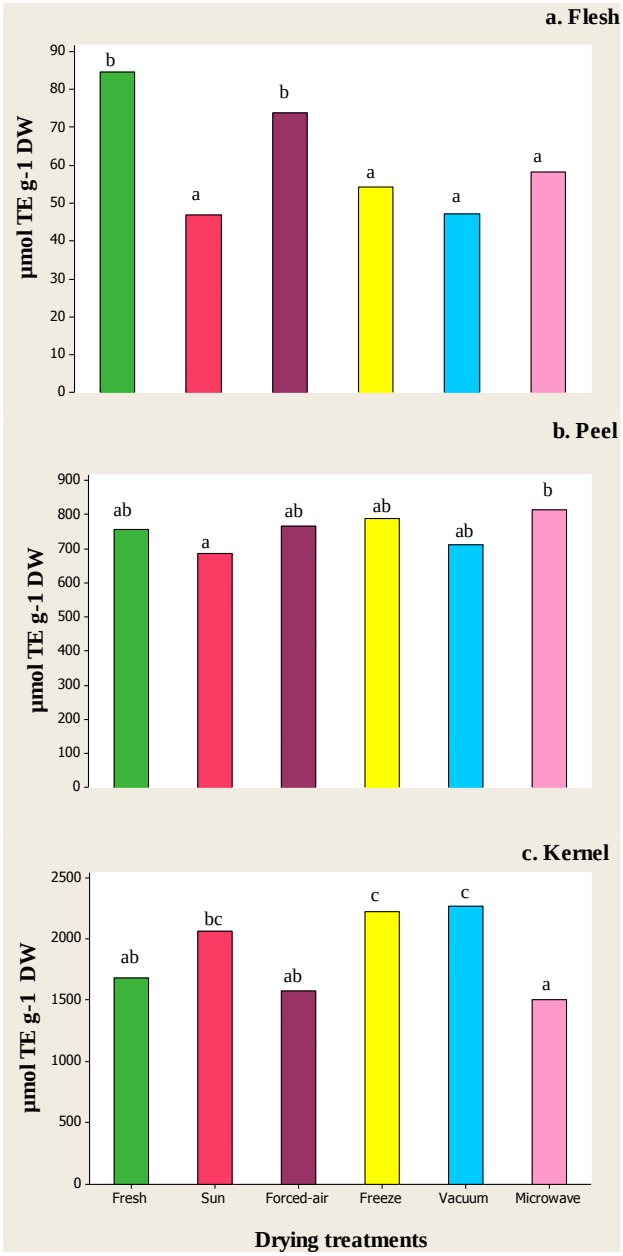


Figure 5.2. Effects of drying on ABTS values of mango flesh, peel and kernel.

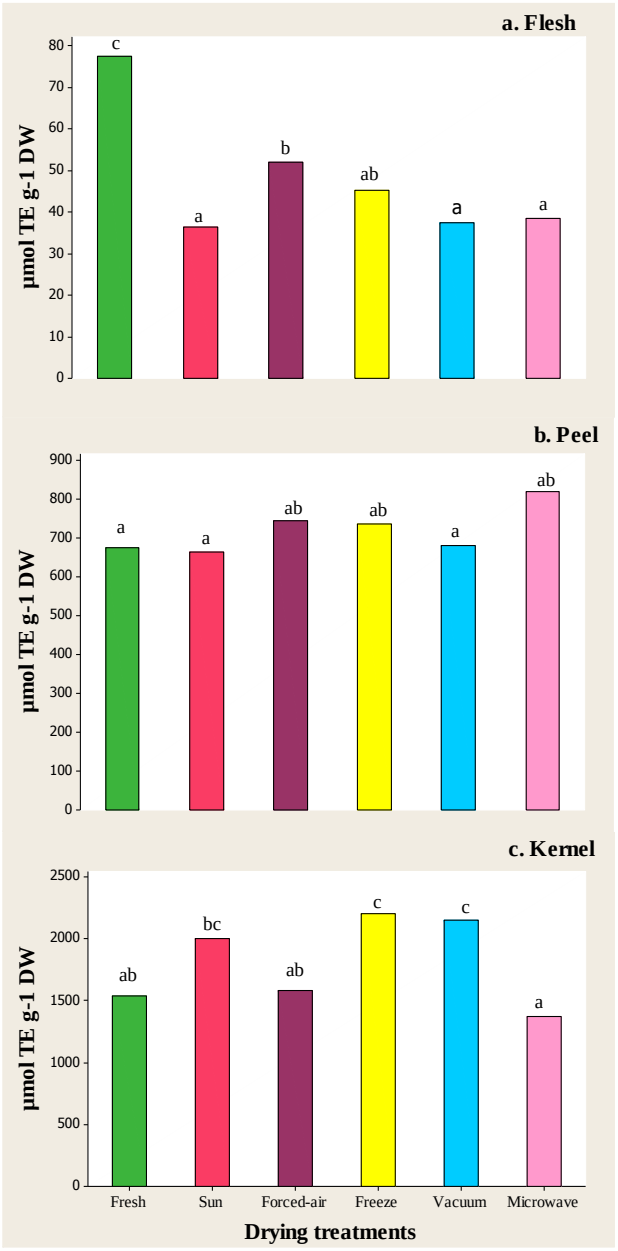


Figure 5.3. Effects of drying on DPPH values of mango flesh, peel and kernel.

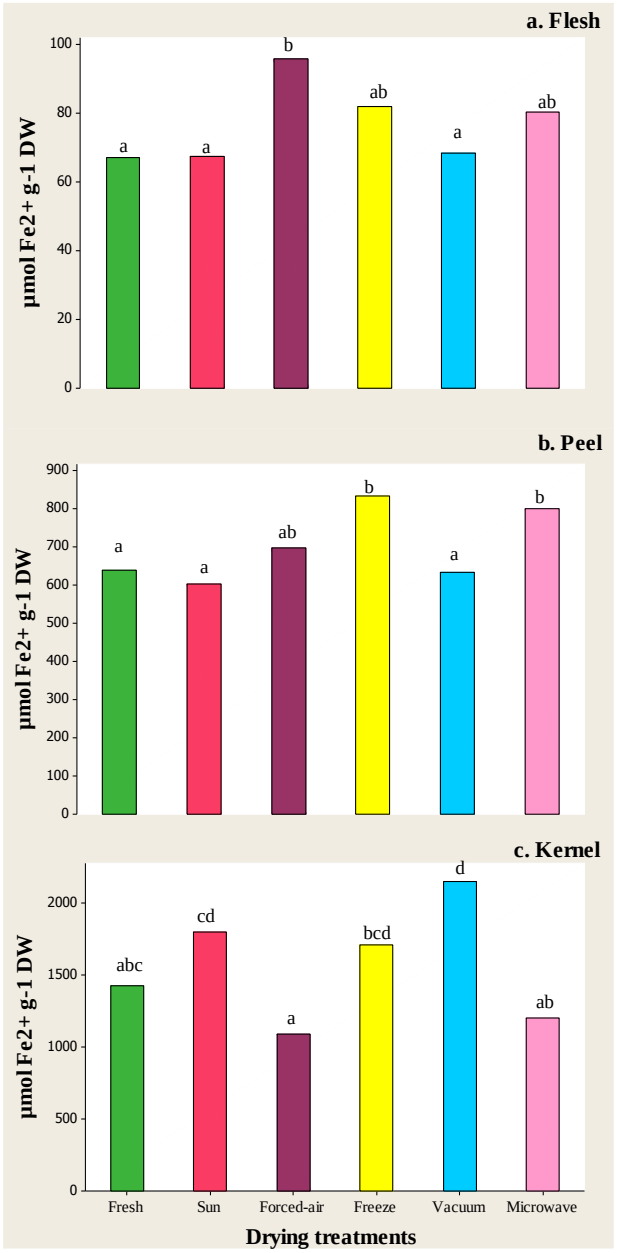


Figure 5.4. Effects of drying on FRAP values of mango flesh, peel and kernel.

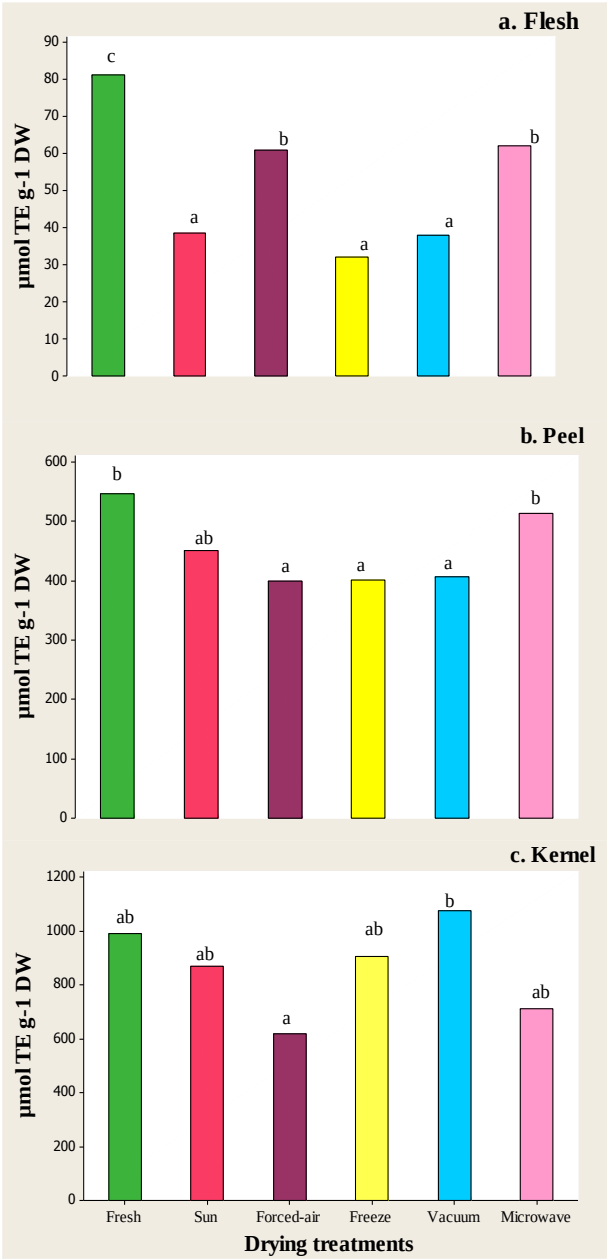


Figure 5.5. Effects of drying on H-ORAC values of mango flesh, peel and kernel.

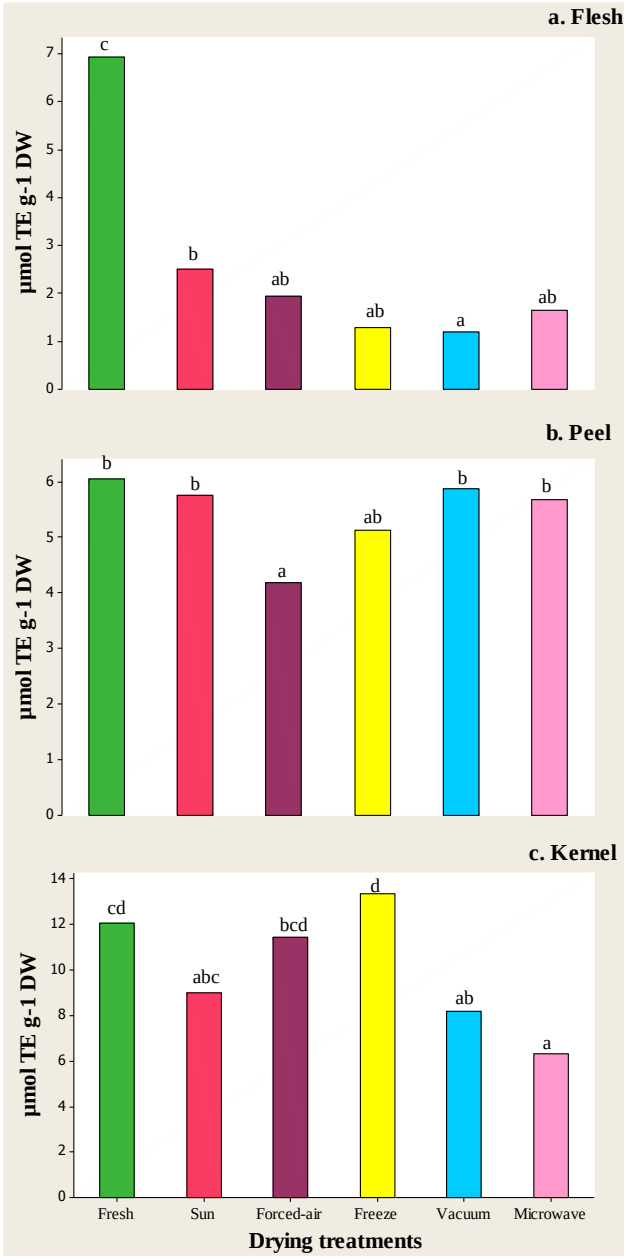


Figure 5.6. Effects of drying on L-ORAC values of mango flesh, peel and kernel.

Values are the mean (n=12). Different letters (a-d) show significant differences at $p < 0.05$ using the Fisher's LSD multiple comparison tests.

5.3.2. Effect of various drying treatments on the antioxidant capacity of mango fractions as measured by ABTS, DPPH, FRAP, H-ORAC and L-ORAC methods.

Antioxidants prevent or repair free radical damage by donating electrons to free radicals and converting free radicals to harmless molecules (Frankel and Meyer, 2000; Koleva *et al*., 2002). Plants contain many different types of natural antioxidants such as vitamins A, C and E, carotenoids, flavonoids and simple phenolic compounds. Determination of the antioxidant capacity TAC of a food component is an accepted indicator of the capacity of the food to scavenge free radicals (Mackerras, 1995; Duell, 1996; Cano *et al*., 1998). The ABTS, DPPH, FRAP and ORAC are four techniques commonly reported in the literature that are used to determine the antioxidant capacity of food components.

5.3.2.1. ABTS radical cation decolourization assay

a. Flesh

ABTS scavenging activities of fresh mango flesh was 84.6 µmol TE g^{-1} DW and those of sun, forced-air, freeze, vacuum and microwave dried mango flesh samples were 46.7, 73.8, 54.1, 47.1 and 58.2 µM TE g^{-1} DW, respectively (Figure 5.2a). The drying treatments except for the forced-air drying resulted in a reduction in the ABTS radical scavenging activity in the dried mango flesh products ($p < 0.05$). Forced-air drying was the most effective of the five drying treatments evaluated.

b. Peel

ABTS scavenging activities of fresh, sun, forced-air, freeze, vacuum and microwave dried mango kernel samples were 754.6, 684.8, 765.8, 787.2, 711.0 and 812.4 µmol TE g^{-1} DW, respectively (Figure 5.2b). There was no significant difference in the ABTS scavenging activities between fresh peel and dried peel produced by any of the five drying treatments. The only significant observation was that the ABTS scavenging activity was higher in microwave dried peel than sun dried peel.

c. Kernel

ABTS scavenging activities of fresh, sun, forced-air, freeze, vacuum and microwave dried mango kernel samples were 1683.6, 2067.3, 1577.6, 2227.6, 2268.5 and 1510.0 µmol TE g^{-1} DW, respectively (Figure 5.2c). There were no significant differences between fresh kernel and the dried kernel products produced by sun, forced-air or microwave drying. In contrast, the vacuum and freeze dried kernel products had significantly higher ABTS scavenging activity (34.7% and 32.3%, respectively) than fresh kernel ($p < 0.05$).

5.3.2.2. DPPH radical scavenging activity assay

a. Flesh

The antioxidant capacity of fresh mango flesh was 77.6 µmol TE g^{-1} DW and that of sun, forced-air, freeze, vacuum and microwave dried mango flesh products were 36.5, 52.0, 45.4, 37.4 and 38.6 µmol TE g^{-1} DW, respectively (Figure 5.3a). Drying produced a significant ($p < 0.05$) and dramatic decrease in DPPH radical scavenging activity compared to fresh mango flesh. The forced-air dried samples had significantly higher ($p < 0.05$) DPPH scavenging activity than microwave, vacuum and sun dried products. However, forced-air drying decreased the DPPH levels by 53.0% relative to non-drying (Figure 5.3a). The sun dried flesh samples had the lowest DPPH levels (36.5 µmol TE g^{-1}).

b. Peel

The total antioxidant capacities of fresh, sun, forced-air, freeze, vacuum and microwave dried peel samples were 675.7, 664.9, 743.7, 735.4, 679.8 and 817.8 µmol TE g^{-1}, respectively. There were no significant differences between fresh and dried mango peel samples except those dried by microwave (Figure 5.3b). The DPPH scavenging ability of microwave dried peel samples was 21.0, 23 and 20.3% significantly ($p < 0.05$) higher than fresh, sun or vacuum dried peel products, respectively.

c. Kernel

The DPPH scavenging activities of fresh, sun, forced-air, freeze, vacuum and microwave dried kernel samples were 1540.0, 1997.6, 1585.8, 2205.7, 2150.7 and 1376.1µmol TE g^{-1}, respectively. The DPPH scavenging ability was significantly higher in the freeze (43.3%) and vacuum dried (39.7%) kernel compared to fresh kernel. The freeze or vacuum dried kernels had significantly higher antioxidant capacity than those dried by microwave or forced-air drying. There were no significant differences between the DPPH scavenging ability of fresh and the sun, air or microwave dried kernel samples (Figure 5.3c).

5.3.2.3. Ferric Reducing Antioxidant Power (FRAP) assay

In the FRAP assay, mango samples are assessed by their ability to reduce TPTZ-Fe^{3+} to TPTZ-Fe^{2+} as a measure of their antioxidant activity. Thus, the FRAP assay is a method for measuring the total reducing power of electron donating substances which are not directly related to free radical reactions.

a. Flesh

The FRAP levels of fresh, sun, forced-air, freeze, vacuum and microwave dried flesh samples were 67.1, 67.4, 95.8 , 82.1, 68.3 and 80.4 µmol $Fe^{2+}g^{-1}$ respectively (Figure 5.4a.). Unlike the ABTS and DPPH results, there was no significant difference in FRAP activity between the fresh and dried flesh products except for the forced-air-dried flesh which showed a significant increase compared to fresh flesh. The increase of 22.4% indicated that the reducing power in mango flesh was increased by the forced-air drying process.

b. Peel

The FRAP values of fresh, sun, forced-air, freeze, vacuum and microwave dried peel samples were 639.2, 601.5, 697, 834.2, 634.1 and 799.0 µmol $Fe^{2+}g^{-1}$ DW, respectively. The FRAP values of freeze and microwave dried mango peel samples were 30.5 and 25.2%, respectively and significantly higher than the fresh product. However, there were no significant differences in FRAP between fresh and sun dried or vacuum dried or forced-air dried peel products (Figure 5.4b).

c. Kernel

The FRAP values of fresh, sun, forced-air, freeze, vacuum and microwave dried kernel samples were 1430.4, 1801.8, 1094.2, 1710.9, 2150.4 and 1206.0 µmol $Fe^{2+}g^{-1}$, DW respectively. There were no significant differences in the FRAP levels between the fresh and any dried kernel products except those dried by vacuum drying (Figure 5.4c). The FRAP level of the vacuum dried product was significantly higher (50.4%) than fresh kernels. The vacuum dried kernels contained significantly ($p < 0.05$) 78.3 and 96.5% more FRAP than microwave or forced-air dried products, respectively.

5.3.2.4. Hydrophilic Oxygen Radical Absorbance Capacity (H-ORAC) assay

a. Flesh

The H-ORAC of fresh mango flesh was 81.3 µmol TEg^{-1} DW and for sun, forced-air, freeze, vacuum and microwave dried kernel products were 38.7, 61.1, 32.2, 38.0 and 62.1 µmol TE g^{-1} DW, respectively (Figure 5.5a). All the drying treatments significantly ($p < 0.05$) reduced H-ORAC levels compared to fresh mangoes. The fresh flesh contained nearly 151.8% more H-ORAC than freeze dried and 110 to 114% more than vacuum or sun dried flesh products. Although microwave and forced-air drying were more effective than any other drying treatment, the H-ORAC values in the microwave and forced-air dried flesh products were still 31.0 and 33.2%, respectively lower than in fresh flesh.

b. Peel

The H-ORAC of fresh mango peel was 545.6 µmol TE g^{-1} DW. The hydrophilic antioxidant capacities of sun, forced-air, freeze, vacuum and microwave dried peel samples were 450.3, 398.5, 401.5, 407.5 and 513.3 µM TE g^{-1} DW, respectively. Fresh peel had significantly higher H-ORAC levels than forced-air, freeze or vacuum dried peel products (Figure 5.5b). There was no significant difference between fresh peel and sun or microwave dried peel products.

c. Kernel

The H-ORAC of fresh, sun, forced-air, freeze, vacuum or microwave dried kernel samples were 989.5, 869.8, 619.2, 907.3, 1076.1 and 712.8 µmol TE g^{-1} DW, respectively. There was no significant difference between fresh kernel and dried kernel products produced by the five drying treatments (Figure 5.5c).

5.3.2.4. Lipophilic Oxygen Radical Absorbance Capacity (L-ORAC) assay

a. Flesh

The L-ORAC of fresh mango flesh was 6.9 µmol TE^{-1}g sample and for sun, air, freeze, vacuum or microwave dried flesh products were 2.5, 2.0, 1.3, 1.2 and 1.7 µmol TE g^{-1} DW, respectively (Figure 5.6a). All of the drying treatments produced a significant reduction in the lipophilic antioxidant capacity of the dried products compared to fresh mango flesh.

b. Peel

L-ORAC values of fresh, sun, forced-air, freeze, vacuum or microwave dried peel samples were 6.0, 5.8, 4.2, 5.1, 5.9 and 5.7 µmol TE g^{-1} DW, respectively. The forced-air dried peel samples had significantly lower L-ORAC values. None of the drying treatments affected the L-ORAC value (Figure 5.6b). The results suggest that lipophilic antioxidants are retained during the drying treatments except that the high temperatures (65^{o}C) and long exposure time (72 hours) during the forced-air drying treatment can reduce L-ORAC.

c. Kernel

The lipophilic antioxidant capacity of fresh, sun, forced-air, freeze, vacuum and microwave dried kernel samples were 12.1, 9.0, 11.4, 13.4, 8.2 and 6.4 µmol TE g^{-1} DW, respectively. The microwave, vacuum and sun drying treatments significantly reduced the L-ORAC levels in the dried products compared to fresh kernels ($p < 0.05$). There were no significant differences between fresh kernel and the dried kernel products produced by freeze or forced-air drying (Figure 5.6c).

5.3.3. Analysis of the effects of drying on total phenolic content and antioxidant capacity using PCA

In order to gain a better insight into the effects of the various drying treatments on TPC and antioxidant capacity of mango flesh, peel and kernel, PCA was used as a tool to determine the multivariate dependence among the variables (Brenna, 2009). In PCA, linear combinations of the original variables are derived which explain the maximum amount of variation in the data set with a minimum loss of information. The linear combinations of the original variables are called principal components. In this study, the variables are the assays.

Since the variables (antioxidant values from different assays) had substantially different numerical ranges, the correlation matrix was used for the principal component analysis. Using the correlation matrix means that each variable is standardised by subtracting its mean and dividing by its standard deviation.

With the six variables (assays) it is possible to generate a maximum of six principal components (Appendix A.2.1). Each principal component accounts for a portion of the variation in the original variables. In this study, the first two principal components were found to account for much of the variation. The values listed for each principal component are used to calculate the principal component value (or score) for each sample (Appendix A.2.1). A biplot can be produced by PCA. The biplot is a graphical display showing both the relationships between the samples and their characteristics with respect to the assays. The first two principal components are represented by the horizontal and vertical axes of the biplot and the principal component scores for each sample define the location of the sample on the biplot.

Information about the variables appears in the biplot as lines (vectors) labelled with the variable name. The angle between two vectors represents the correlation between the variables represented by the vectors (the smaller the angle, the greater the correlation).

The original data comprised 12 samples from each of 6 treatment groups, with 6 different assays being measured for each sample. These data were analysed by one-way ANOVA and presented in bar graphs (Figure 5.1 – Figure 5.6). For PCA, only the mean of the 12 samples for each treatment group was used (Hossain *et al.*, 2011).

Loading plots and score plots are presented in Appendix A.2.1. The scale for the score plot reflects the range of scores for the samples (fresh and dried samples). The scale for the loading plot reflects the range of values for the coefficients of PC1 and PC2. When these 2

plots are combined to form the biplot, the horizontal and vertical axes use the same scale as for the score plot and the vectors from the loading plot may be lengthened (scaled) to improve the visualisation. PC scores and loading coefficients for flesh, peel and kernel are in Appendix A.2.1.

5.3.3.1. Mango flesh

The results reflect the mean TPC and antioxidant capacity of all fresh flesh samples and samples of flesh dried by sun, forced-air, freeze, vacuum and microwave drying. The two principal components (PC1 and PC2) explain 95.5% (Appendix A.2.1.1a.) of the total data variance and were chosen on the basis of their Eigenvalues (> 1). PC1 explained 59.1% of the total variance and in PC1, fresh flesh samples were able to be separated from dried samples. Dried samples were separated along PC2 (36.4% of the total variance explained).

Figure 5.7. Biplot of first two principal components obtained from PCA showing the relation between antioxidant assays and the relation between the fresh and dried flesh samples.

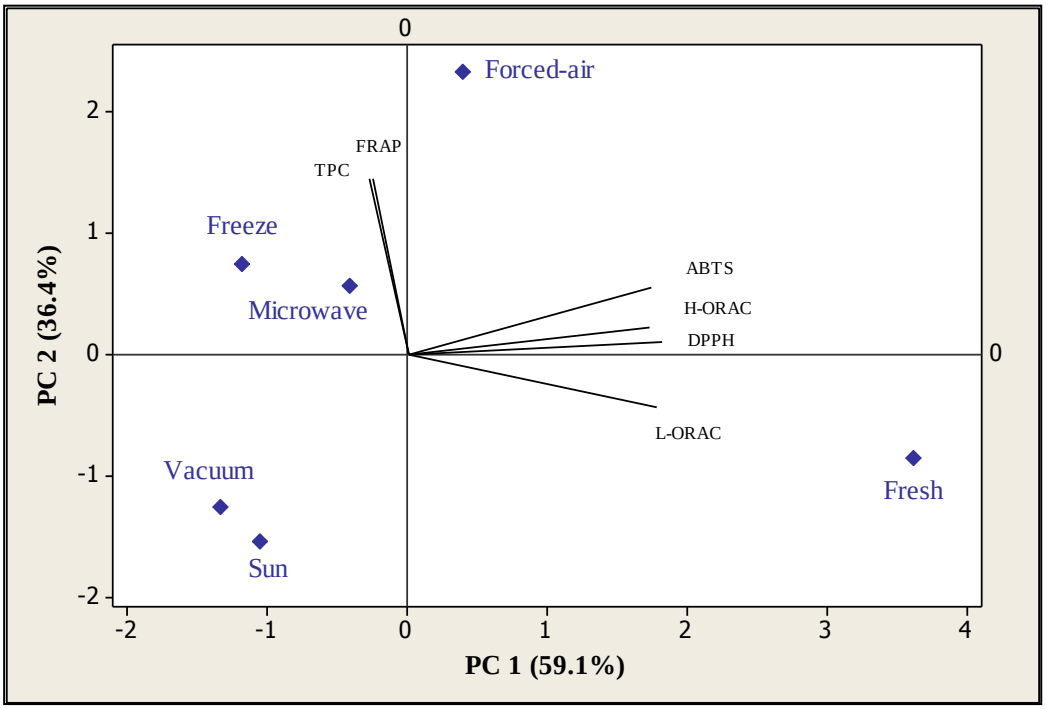


Figure 5.7 shows the projection of the samples and antioxidant values in the plane as defined by the two principal components. The antioxidant capacity measured by ABTS, DPPH, H-ORAC and L-ORAC are clustered together on the right hand side of plot (positive values) and these assay variables show higher loading coefficients in PC1 than TPC and FRAP (Appendix A.2.1.1b). The distance between the locations of any two treatments on the plot is directly proportional to the degree of differences or similarities between the treatments. Figure

5.7 displayed that all the variables relating to ABTS, DPPH, H-ORAC and L-ORAC separated fresh flesh samples from all the dried flesh products along PC1. The location of fresh flesh was well separated from the dried flesh products and had higher score on PC1 (Appendix A.2.1.1c).

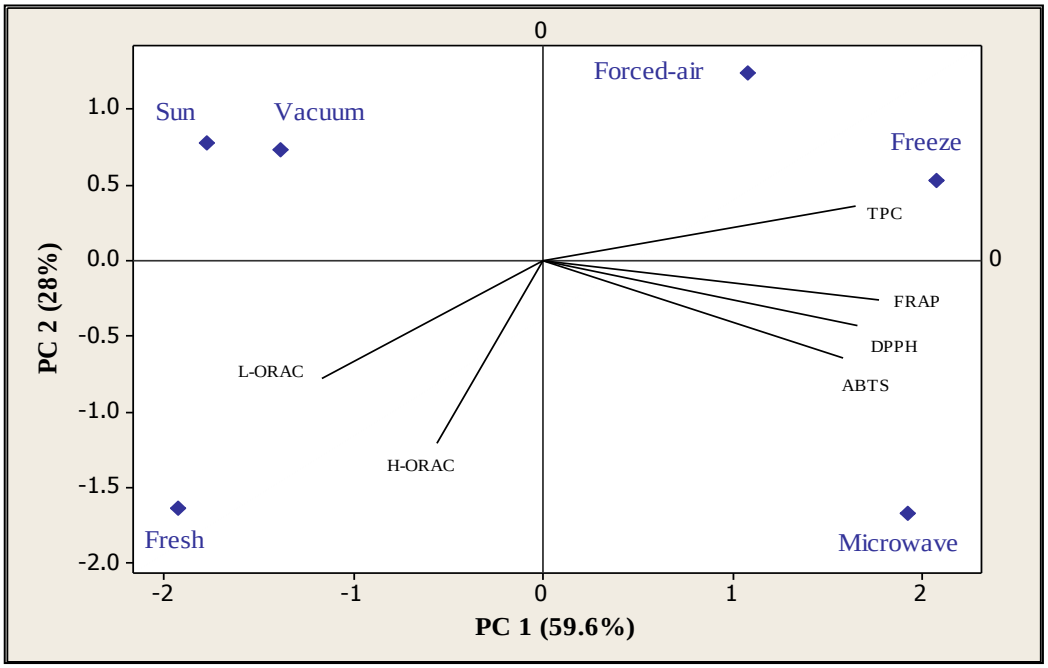
With the exception of forced-air dried flesh, all the flesh samples dried by the other four drying treatments were in the negative PC1 plane and were characterised by low antioxidant capacity values as measured by ABTS, DPPH, H-ORAC and L-ORAC. The drying methods are discriminated from each other along PC2 due to the differences in TPC and FRAP values (TPC and FRAP had higher loading coefficients than other assay variables in PC2 as indicated in Appendix A.2.1.1b). In PC2, forced-air dried flesh showed the highest score in PC1 whilst fresh, vacuum dried and sun dried flesh samples were located in the lower part and showed the lowest values for TPC and FRAP (Appendix A.2.1.1c). Vacuum and sun dried flesh samples were grouped in the opposite quadrant from all the other drying treatments and showed the lowest values.

5.3.3.2. Mango peel

The results reflect the mean TPC and antioxidant capacity of all fresh peel samples and samples of peel dried by sun, forced-air, freeze, vacuum and microwave drying. Two principal components explained 87.6% (Appendix A.2.1.2a) of the total data variance and were chosen on the basis of their Eigenvalues (>1).

PC1 explained 59.6% of the total variance which had positive loading coefficients of TPC, FRAP, DPPH and ABTS. In contrast, L-ORAC and H-ORAC were negative in PC1 (Appendix A.2.1.2b). PC1 showed that freeze and microwave dried peel samples had higher positive scores (Appendix A.2.1.2c) compared to fresh and other dried peel products which reflect the higher antioxidant activity values obtained with the TPC, FRAP, DPPH and ABTS assays (Figure 5.8).

Figure 5.8. Biplot of first two principal components obtained from PCA showing the relation between antioxidant assays and the relation between the fresh and dried peel samples.



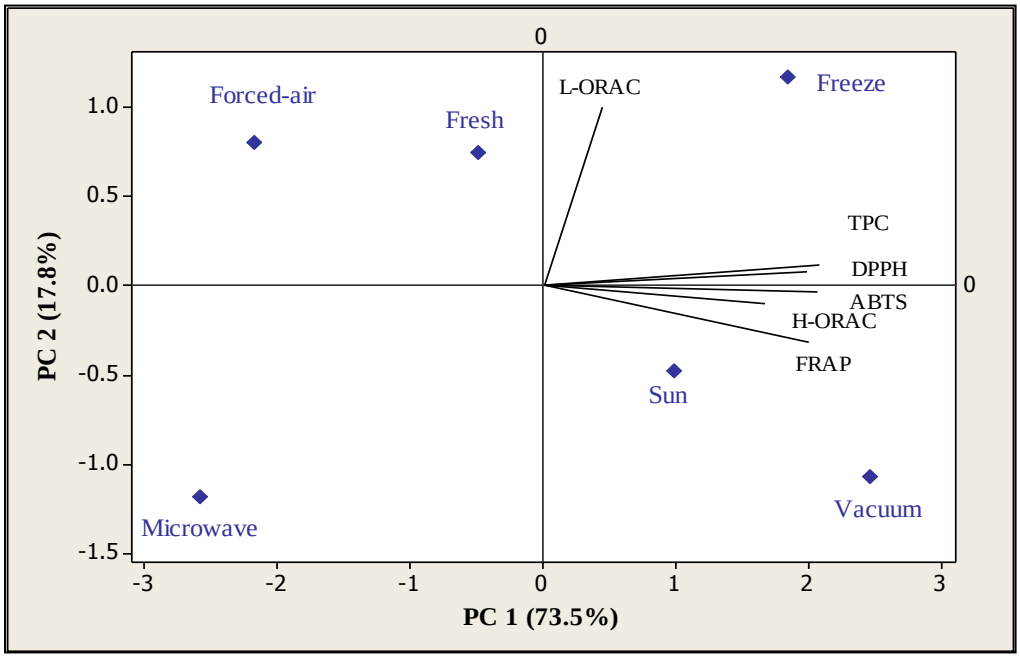
PC2 explained 28% of the total variance and had negative loading coefficients of H-ORAC and L-ORAC (Appendix A.2.1.2b). Particularly, H-ORAC had lowest loading coefficient in PC2, therefore, fresh and microwave dried peel products which were in the lower quadrant showed higher H-ORAC than the other dried peel samples which were located in the upper part of the coordinates. No difference in L-ORAC between fresh and dried peel products, except for forced-air dried peel which had the highest loading coefficient in PC2.

5.3.3.3. Mango kernel

The results reflect the mean TPC and antioxidant capacity of all fresh kernel samples and dried kernel products obtained from sun, forced-air, freeze, vacuum and microwave drying.

From Figure 5.9, the first principal component (PC1) accounted for 73.5% of the variability and the second principal component (PC2) accounted for 17.8%. Together PC1 and PC2 accounted for 91.3% of the total variance (Appendix A.2.1.3a).

Figure 5.9. Biplot of first two principal components obtained from PCA showing the relation between antioxidant assays and the relation between the fresh and dried kernel samples.



The TPC and antioxidant capacity as measured by ABTS, DPPH, FRAP and H-ORAC had higher loading coefficients (Appendix A.2.1.3b) than L-ORAC in PC1, which explains 73.5% of the total variance.In PC1, vacuum and freeze dried kernel had the largest positive scores (Appendix A.2.1.3c) which reflects the higher antioxidant capacities, except for L-ORAC, compared to fresh and other dried kernel products.

PC2, which explains a 17.8% of the total variance, had a very high loading coefficient of L-ORAC. There were no differences in L-ORAC between fresh and dried peel, except for the fact that freeze dried kernel had the highest score (Appendix A.2.1.3c) whilst microwave and vacuum dried kernel had the lowest scores (Appendix A.2.1.3c) in PC2. Therefore, freeze dried kernel showed higher L-ORAC than microwave and vacuum dried kernel.

5.3.4. Effects of drying on total phenolic content and antioxidant capacity using CVA

In general, the principle of Canonical Variates Analysis (CVA), also referred to as Discriminant Function Analysis (DFA), is similar to that of principal component analysis (PCA). The major difference between PCA and CVA is the following. PCA aims to find linear combinations of the original variables (the six antioxidant assays) which explain the maximum amount of variation in the data set with minimum loss of information. CVA is appropriate when the samples are in groups. The aim is to find linear combinations (called canonical variates) of the original variables that maximise the ratio of between groups to

within group variation thereby giving functions that can be used to discriminate between the groups (Alsberg *et al*., 1997).

The first canonical variate from CVA is the linear combination of the original variables that accounts for most of the variation and best discriminates between the six treatments (the fresh or non-drying and the five drying treatments). The second canonical variate is the linear combination of the original variables which is second best in discriminating between the six drying treatments (Finlayson *et al*., 1985).

Canonical variate analysis (CVA) was performed to evaluate the differences or similarities of fresh (n = 12) and dried mango samples produced by the five different drying treatments (n=12 for each treatment) using the values from the 6 antioxidant assays. Each mango fraction, namely flesh, peel and kernel was analysed separately. The original data (6 treatment groups x x 12 samples) as shown in Figure 5.1 to 5.6 was used for CVA. The CVA plot is a graphical display showing the separation of sample groups. In CVA plot, the circles are the confidence regions about the mean for the group and when 2 circles overlap, they are not statistically different at the 95% confidence level. To statistically evaluate the discrimination between circles, the different letters a-d indicated the statistically significant differences between circles (treatments) and is presented in Appendices A.2.2.1b, A.2.2.2b and A.2.2.3b).

5.3.4.1. Mango flesh

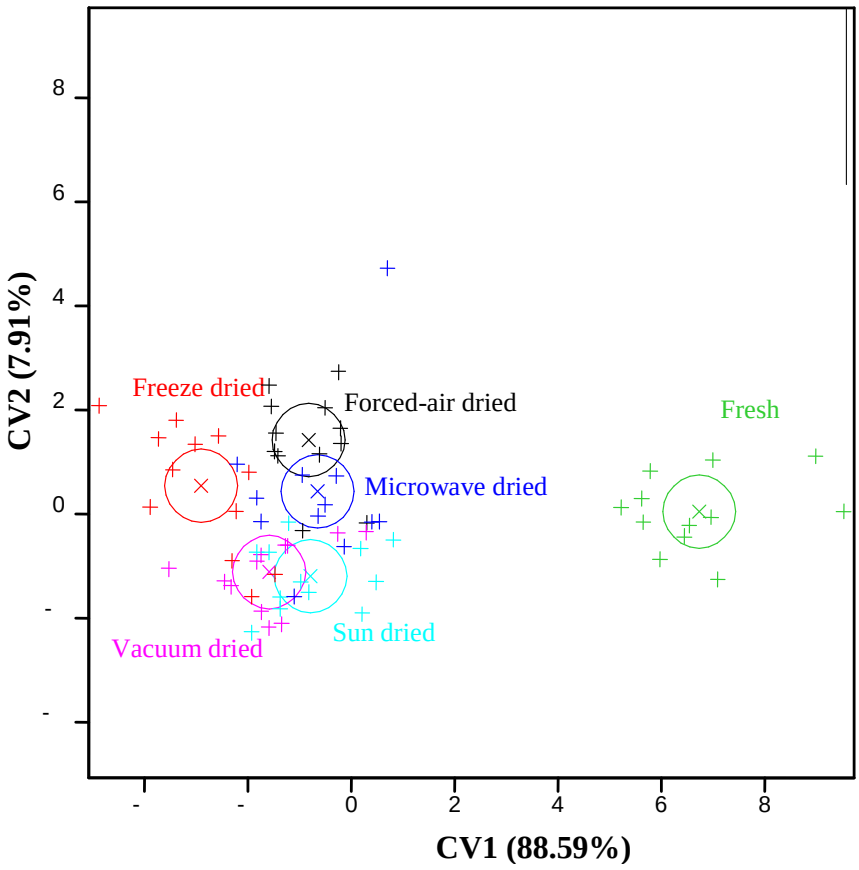
The canonical variate (CV) plot (Fig 5.10) show that the first canonical variate dimension explains 88.59% of data and the second dimension explains 7.91% of the variables together they account for 96.50% of the total variance. CV2 accounts for only 7.91% of the variation so separation of the groups was mainly presented in CV1. The differences in antioxidant capacity (measured by assays) between flesh from different groups therefore, are reported based on CV1.

CV1 was primarily driven by L-ORAC which had the highest loading in CV1 followed by H-ORAC, DPPH and ABTS (Appendix A.2.2.1a).

Figure 5.10 shows the samples plotted using the canonical variate scores. The CV1 (on the horizontal axis) separates the fresh and dried products. The wide distance between the circles indicate there are clear differences between the antioxidant capacities of fresh and the dried products. The fresh flesh had significantly ($p < 0.05$) higher L-ORAC values than dried flesh (Appendix 2.2.1b). Of the dried flesh products, the plot showed that there was a visible

distance difference between the circle of freeze dried and the other dried flesh. Freeze dried flesh samples showed significantly ($p < 0.05$) lower L-ORAC, than the other dried flesh products (Appendix 2.2.1b).

Figure 5.10. Canonical variates analysis of fresh and dried flesh produced by five different drying treatments

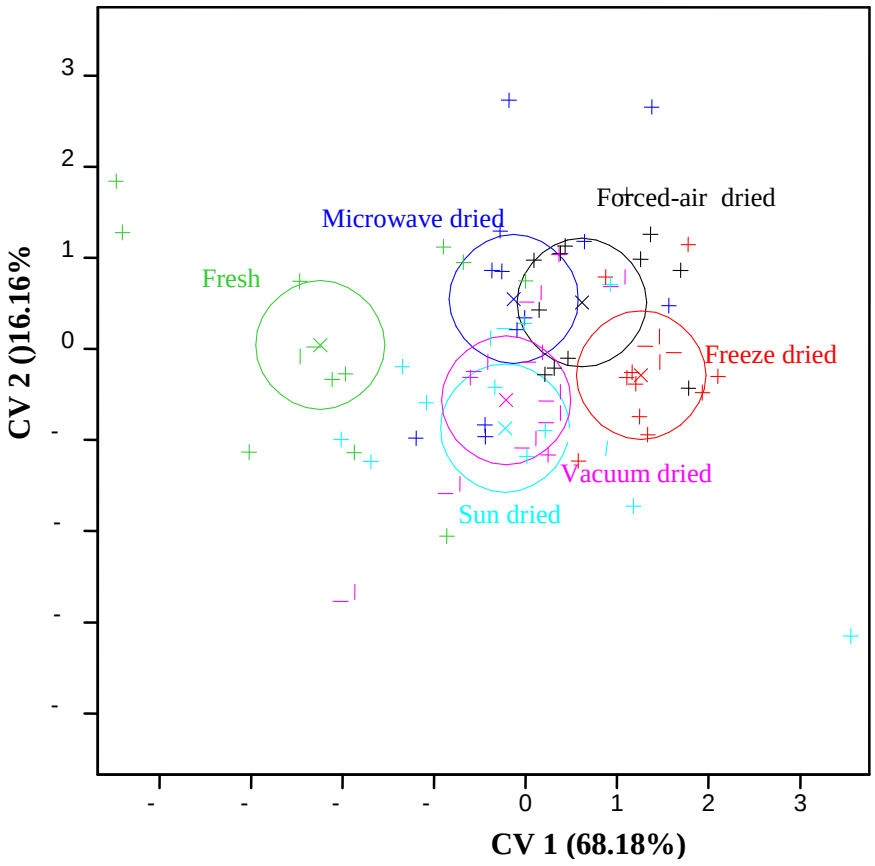


Note: Explanation of circles in the text in 5.3.4.

5.3.4.2. Mango peel

The canonical variate (CV) plot (Fig 5.11) show he canonical variate analysis in peel of 6 antioxidant assays for the first two eigenvalues. The first canonical variate dimension explains 68.18% of data and the second dimension explained 16.16% of the variables. Both CV1 and CV2 account for 84.35 % of the total variance ratio. TPC, DPPH and FRAP had positive loadings whilst H-ORAC and L-ORAc had negative loadings in CV1 (Appendix A.2.2.2b).

Figure 5.11. Canonical variates analysis of fresh and dried peel produced by five different drying treatments.



Dried peel, had significantly ($p < 0.05$) increased TPC, DPPH and FRAP values but decreased H-ORAC and L-ORAC values compared to fresh peel.

Of the dried peel products, freeze and forced-air dried peel had significantly higher TPC, DPPH and FRAP values than any other dried peel sample. The microwave and forced-air products showed significantly higher ($p < 0.05$) ABTS and DPPH than sun and vacuum dried peel (Appendix A.2.2.2b).

With CV2, most of the circles of dried and fresh peel overlapped indicating there were no significant differences ($p < 0.05$) in antioxidant capacity in fresh and dried group. Of the dried peel products, freeze and forced-air dried peel had significantly higher TPC, DPPH and FRAP values than any other dried peel sample (Appendix A.2.2.2b).

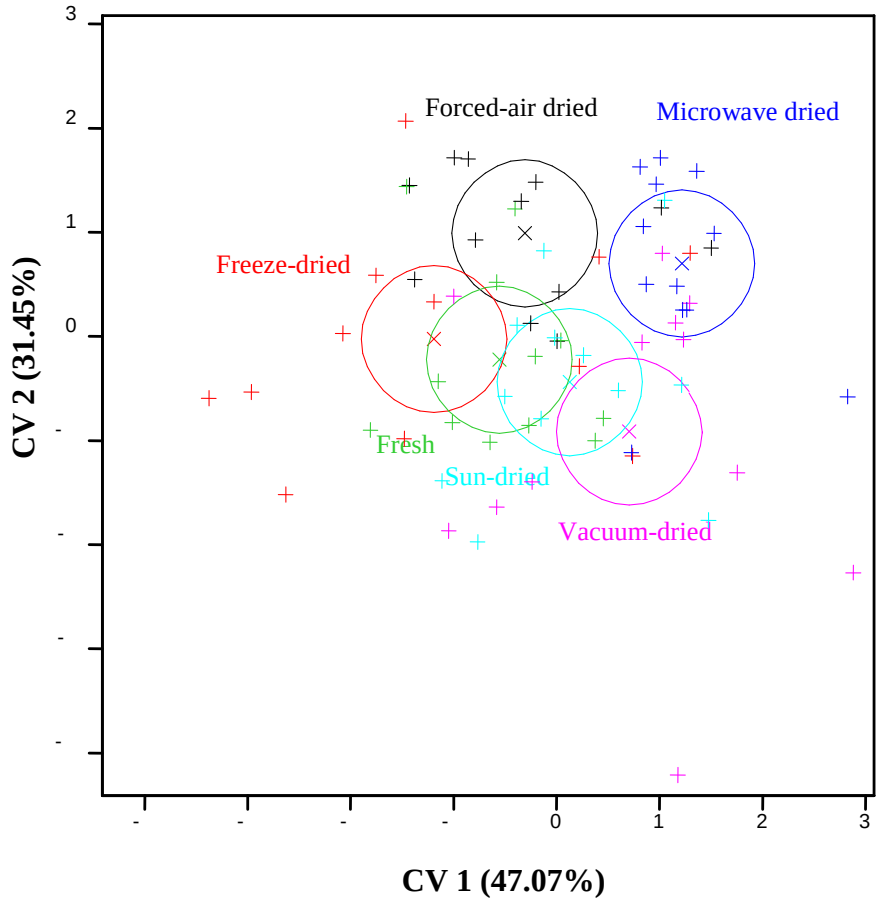
5.3.4.3. Mango kernel

The canonical variate (CV) plot (Fig 5.12) shows the canonical variate analysis in kernel of 6 antioxidant assays for the first two eigenvalues. The first canonical variate dimension

explained 47.07% of the data and the second dimension 31.45%. Both CV1 and CV2 accounted for 78.49 % of the total variance ratio. CV1 was primarily driven by L-ORAC with the highest negative loading coefficient) whilst FRAP, TPC, ABTS, DPPH and H-ORAC were more important in CV2 (Appendix 2.2.3a).

In CV1, which is dominated by L-ORAC (negative), the vacuum and microwave dried kernel showed higher CVA scores than the fresh products. This finding indicated that the fresh kernel had higher L-ORAC values than vacuum and microwave dried kernel products. Of the five dried kernel products, the freeze dried product had significantly higher ($p < 0.05$) L-ORAC than any other dried kernel product.

Figure 5.12. Canonical variates analysis of fresh and dried kernel products produced by five different drying treatments.



In regards to CV2, the circles of the fresh kernels and dried kernels overlapped except for the forced-air and microwave dried kernels which are positive in CV2. The loading coefficients of TPC and FRAP, were negative in CV2 and consequently forced-air and microwave dried kernels had the significantly lower TPC and FRAP values ($p < 0.05$) than the other dried kernel products (Appendix A.2.2.3b). There were no significant differences ($p < 0.05$)

between fresh and vacuum, sun and freeze dried kernel samples. However, of the drying treatments, vacuum drying was the most effective and significant ($p < 0.05$) in retaining FRAP and TPC values compared to the freeze, microwave and forced-air drying treatments.

5.4. Discussion

Previous reports have shown that the temperature used in a drying treatment affects the stability of the chemical components of food and often results in a reduction of antioxidants such as polyphenols, ascorbic acid, tocopherols and carotenes in the dried products. The reduction is due to chemical or enzymatic changes that cause volatilisation or thermal decomposition of specific molecules (Larrauri, 1997; Jang *et al*., 2007). There could be several possible interactions between components in the food during the heat treatment. Such interactions are dependent on (i) the presence or the absence of hydroxyl groups in the B ring of flavonoids (Sichel *et al*., 1991); (ii) whether compounds are liberated or released (Jeong *et al.*, 2004); (iii) caramelisation; (iv) formation of Maillard reaction products (MRPs) and (v) the interaction between components from the breakdown of cellular constituents (Nicoli *et al*., 1997; Liu *et al*., 2008). The kinetics of these interactions depends on the chemical and structural composition of the starting material which will vary between the flesh, peel and kernel. Thus, the content of antioxidants such as ascorbic acid, carotenoids, tocopherols, phenolic compounds in dried mango flesh, peel and kernel will vary according to the drying treatment.

5.4.1. Effects of drying on total phenolic content and antioxidant capacity of mango flesh

The antioxidant activities of phenolic compounds vary depending on the chemical structure, concentration and oxidized state of the phenolics (Nicoli *et al.,* 1999). There was a significant ($p < 0.05$) increase in TPC in freeze, forced-air and microwave dried flesh products. Freeze drying has been previously reported as a suitable treatment to retain nutrients such as phenolics in a dried product (Venskutonis, 1997). During the freeze drying process, the development of ice crystals within the tissue matrix accelerates the rupturing of the cell structure which results in a better solvent access and consequently a higher extraction of phenolic compounds from flesh samples (Shih *et al*., 2009). A higher TPC was found in forced-air and microwave dried flesh samples than in the corresponding fresh product which could be due to the formation of new phenolic compounds by the non- enzymatic conversion of phenolic precursor molecules to phenolic compounds (Soong and Barlow, 2004) or to the breakdown of cellular constituents during the thermal treatment which releases bound

phenolics to free phenolics (Dewanto *et al*., 2002; Randhir *et al.,* 2008; Boateng *et al*., 2008; Vega-Galvez *et al*., 2009).

In contrast, the decrease in ABTS and DPPH scavenging activity, H-ORAC and L-ORAC that occurred in most dried mango flesh products could indicate that the phenolic compounds that are released during the drying treatments are not effective in their ABTS and DPPH scavenging ability or in inhibiting the peroxylradical induced oxidation as measured by ORAC. The exception is that the ABTS scavenging activity of the forced-air dried flesh product was not significantly different from fresh flesh.

A reduction in the levels of ascorbic acid and carotenoids are known to occur in mango flesh during the drying treatments with or without heat. These changes could offer an explanation for the lower antioxidant capacity of the dried flesh products (Ndawula, 2004; Rocha Ribeiro, 2007). Another possible explanation for the reduction in L-ORAC values is that the lipophilic antioxidants such as phospholipids, carotenoid and tocopherols are degraded at the elevated drying temperatures (Azizah *et al*., 1999).

Unlike the data from ABTS, DPPH, H-ORAC and L-ORAC, there were no significant differences in FRAP between the fresh and dried mango flesh products, except for forced-air dried flesh. The difference in the assays results might be due to the fact that the ABTS, DPPH and ORAC assays involve free radicals reacting with phenolic and browning pigment compounds, whilst the FRAP assay measures the total reducing power of any electron donating substance. The latter is not directly related to free radical scavenging. Furthermore, in the FRAP assay, not all reductants that are able to reduce Fe^{3+} are antioxidants and some antioxidants that can effectively scavenge free radicals may not efficiently reduce Fe^{3+}. That is, the FRAP assay is an indirect test of total antioxidant power and not specific for radical scavenging as the ABTS, DPPH and ORAC assays (Ou *et al*., 2002; Wangcharoen and Morasuk, 2007). Interestingly, the FRAP values for the forced-air dried flesh, were significantly higher than fresh flesh which is similar to the TPC results.

In summary all the drying treatments (except for the forced-air drying effects on ABTS values), decreased the antioxidant capacity as measured by ABTS, DPPH, ORAC whilst forced-air, freeze and microwave drying increased TPC and FRAP values. Among the drying treatments, forced-air drying was the most effective treatment for flesh based on the TPC, DPPH, ABTS, FRAP and H-ORAC levels of all the dried flesh products. The exceptions to

this observation are the DPPH and H-ORAC levels in the freeze and microwave dried flesh products, respectively.

PCA showed that the high loadings of ABTS, DPPH, H-ORAC and L-ORAC in PC1 and the higher score of fresh flesh in PC1 indicated that fresh flesh had higher antioxidant values measured by ABTS, DPPH, H-ORAC and L-ORAC than those of the dried flesh. Besides, the high loadings of TPC and FRAP and higher score of forced air dried flesh relative to fresh and other dried flesh sample in PC2 suggested that forced air dried samples had high TPC and FRAP values

The results from PCA and CVA are in agreements with those obtained by ANOVA (5.3.1 and 5.3.2) and confirmed that dried flesh showed lower ABTS, DPPH, H-ORAC and L-ORAC values than fresh flesh samples. Forced-air dried flesh however had higher TPC and FRAP values than fresh flesh or other dried flesh samples.

5.4.2. Effects of drying on total phenolic content and antioxidant capacity of mango peel

Similar to flesh, forced-air, freeze and microwave drying increased the TPC of peel compared to fresh peel. The explanation for the increase in TPC could be due to the release and formation of new phenolic products. There were significant ($p < 0.05$) changes in antioxidant capacity as measured by the different methods between fresh and dried peel. There was a significantly ($p < 0.05$) higher FRAP in microwave and freeze dried peel compared to fresh. In addition, there were significantly lower H-ORAC values in forced-air, freeze and vacuum dried peel and significantly ($p < 0.05$) lower L-ORAC in forced-air compared to fresh peel. Nevertheless, there were no significant changes in ABTS and DPPH values between fresh and dried peel examined. This finding is in agreement with the results reported by Berardini *et al*. (2005) who showed that existing antioxidants were lost and new antioxidants produced during thermal treatment processes and that the changes could contribute to the stability of antioxidants in dried peel products. Mango peels are rich sources of flavonol O- and xanthone C-glycosides (Berardini *et al*., 2005; Schieber *et al*., 2003). Research has shown that there is a significant loss of peel flavonol glycosides on drying which is dependent on the extent of the heat treatment. In contrast, some xanthone glycosides are formed during the drying process (Berardini *et al*., 2005).

Of the drying treatments in this research, microwave drying, followed by freeze drying were the most effective processes as indicated by the high TPC, FRAP and H-ORAC values in the dried peel products.

The results from PCA reinforced that in general, the drying treatments influenced the antioxidant capacity of mango peel and microwave and freeze drying were more effective in increasing levels of phenolic compounds, reducing power activity and free radical scavenging activity whilst fresh peel products exhibited higher H-ORAC values. There were no differences in L-ORAC between fresh and dried peel, except for forced-air dried products which had lower values than fresh peel.

The results from CVA are in agreements with those analysed by ANOVA (5.3.1 and 5.3.2) and by PCA (5.3.3) confirming that the drying treatments influenced the antioxidant capacity of mango peel. Freeze and forced-air drying treatments significantly ($p < 0.05$) increased TPC, DPPH (not found by ANOVA) and FRAP, but significantly ($p < 0.05$) decreased H-ORAC and L-ORAC values compared to fresh peel. Of the drying treatments, microwave, forced-air and freeze air drying in general are ideal drying treatments for mango peel.

5.4.3. Effects of drying on total phenolic content and antioxidant capacity of mango kernel

Whilst forced-air, freeze and microwave drying increased TPC of mango flesh and peel, no significant differences in TPC were observed between any dried mango kernel samples and fresh kernel suggesting that the chemical and structural composition of the starting material is a contributing factor to the final phenolic content of the dried product. The main phenolic compounds of mango seed kernel are phenolic acids (Soong and Barlow, 2004; Maisuthisakul, 2008) which exist as free phenolic acid (42-56%), esterified phenolic acid (10-19%) and insoluble bound phenolic acid (15-20%) (Schieber *et al*., 2000). Free phenolic acid usually increases in dried products as the heating time and temperature increases. A decline indicates that some phenolic acids are probably destroyed by the heat treatment. Thus, the observation that there is no significant differences between the TPC of fresh and dried kernel products could be explained by some phenolic compounds being generated during the thermal treatment whilst others are reduced and destroyed by the elevated temperature during the drying process.

There were some significant changes in antioxidant capacity between fresh and dried kernel. The ABTS, DPPH and FRAP values of vacuum dried kernel and ABTS and DPPH of freeze dried kernel increased in comparison to fresh kernel. In contrast, L-ORAC in vacuum and microwave dried kernel products decreased. These changes suggested that the drying treatments influenced the antioxidant capacity of kernel in different ways.

Some drying treatments can increase or decrease the phenolic and antioxidant compounds of kernel, consequently, no significant changes in TPC or antioxidant capacity of kernel will occur. The observed stability of TPC and antioxidant capacity in kernel could therefore be due to a loss of existing antioxidants and the formation of new antioxidant products as a result of the drying treatments. It has already been demonstrated that high temperatures and long exposure times can result in a reduction of phenolic compounds and free radical scavenging activities. Furthermore, natural lipophilic antioxidants such as phospholipids, tocopherols and carotenoids, which occur in mango kernel, could be degraded and release some phenolic compounds. There were however no significant differences between the various dried mango kernel products and fresh kernel in TPC and free radical scavenging activities.

Of the five drying treatments, vacuum drying at a low temperature (60^{o}C) for a short time (12 hours) or freeze drying (-50^{o}C) for 48 hours would be expected to minimise the loss of antioxidants and consequently contribute to the retention of antioxidant compounds. The antioxidant compounds retained by vacuum drying are probably the hydrophilic antioxidants such as ascorbic acids and phenolic acids but not lipophilic antioxidants as indicated by the low L-ORAC levels (Figure 5.6c). The low L-ORAC levels were also obtained with microwave dried kernel suggesting that the high drying treatment temperature might destroy the lipophilic antioxidants in mango kernels. The TPC and antioxidant capacity of mango kernels (ABTS/DPPH radical scavenging activities, FRAP reducing power, hydrophilic and lipophilic peroxylradical induced oxidation/ORAC inhibiting activities) are both sensitive to thermal treatments. Consequently, vacuum drying and freeze drying are the most effective treatments for delivering higher ABTS, DPPH, FRAP and H-ORAC values in dried kernel compared to fresh kernel. However, freeze drying is ideal, because the process retains lipophilic antioxidants in mango kernels.

The results from PCA are in agreements with those from one-way ANOVA. The freeze dried and vacuum dried kernel products showed the highest antioxidant capacities as measured by all the assays except for L-ORAC, compared to fresh and any other dried kernel product. There were no differences in L-ORAC between fresh and dried kernel, except for microwave and vacuum dried kernel. Freeze dried kernel had higher L-ORAC than any other dried kernel. There were no differences in L-ORAC between fresh and dried kernel products. Freeze dried kernel showed higher L-ORAC than microwave and vacuum dried kernel products.

Similar to one-way ANOVA and PCA, the CVA results showed there were no significant differences in L-ORAC between fresh and dried kernel, except for microwave and vacuum dried kernel. Additionally, freeze dried kernel had higher L-ORAC values than any other dried kernel product. Of the drying treatments, CVA also confirmed that vacuum drying was more effective than forced-air, microwave and freeze drying in retaining antioxidant capacity, except for lipophilic antioxidants.

5.5. Conclusions

- The effects of five different drying treatments on TPC and antioxidant capacity of fresh mango flesh, peel and kernel were determined. Sun, forced-air, freeze, vacuum and microwave drying influenced the TPC and antioxidant capacity of mango flesh, peel and kernel. Overall, the results obtained from the three statistical analyses, one-way ANOVA, PCA and CVA on the effects of drying treatments on TPC and antioxidant capacity of fresh mango flesh, peel and kernel, were similar and consistent.

- Drying treatments generally decreased the antioxidant capacity of flesh as measured by ABTS, DPPH, H-ORAC and L-ORAC which was possibly due to chemical or enzymatic changes from the volatilisation or thermal decomposition of specific antioxidant molecules. Forced-air drying is the preferred method for drying flesh as it increased the TPC and FRAP. Phenolic compounds which act as antioxidants could be formed and released during heat treatment at 65^{o}C for 72 hours from processes such as caramelisation, Maillard reaction and the interaction between components from the breakdown of cellular constituents.

- The drying treatments influenced the antioxidant capacity of peel. Microwave and freeze drying were more effective in retaining TPC and FRAP than fresh and any other dried peel product (except for forced-air dried peel). Fresh peel products exhibited higher H-ORAC and L-ORAC than dried peel. Of the drying treatments, microwave and freeze drying were more effective than any other drying treatment.

- There were no significant ($p \geq 0.05$) differences in TPC between fresh and dried kernel suggesting that the drying treatment did not influence kernel TPC. A possible explanation is that some phenolic compounds are generated during the thermal treatment whilst others are destroyed by the elevated temperature during the drying

process. However, vacuum drying and freeze drying were the most effective treatments for delivering higher ABTS, DPPH, FRAP and H-ORAC values in dried kernel compared to fresh kernel. Freeze drying is also more effective process in retaining lipiphilic antioxidants than any other drying treatment, except for forced-air drying. The effectiveness of vacuum and freeze drying was expected since antioxidant capacity (ABTS/DPPH radical scavenging activities, FRAP reducing power, ORAC hydrophilic and lipophilic peroxylradical induced oxidation) of mango kernels are sensitive to thermal treatments.

CHAPTER 6

RELATIONSHIPS BETWEEN PHYSICOCHEMICAL CHARACTERISTICS AND ANTIOXIDANT CAPACITY

6.1. Introduction

Mango flesh, peel and kernel have been shown to contain high levels of antioxidants (Ribeiro *et al.*, 2008). The antioxidant levels vary depending on a number of factors such as cultivar, growing location, climate, cultural practice, maturity and post-harvest practices such as processing, transportation and storage (Melgarejo *et al.*, 2000; Ozkan, 2002; Zarei *et al*., 2010). The physicochemical characteristics and antioxidant capacities of mangoes from five different cultivars were compared in chapter 4. The differences in physicochemical characteristics and antioxidant capacities could be attributed to either genetic differences between the cultivars or to the different maturity stages of the mangoes at the time of study. In this chapter, data from the Tommy Atkins cultivar (chapter 5) was used to investigate the relationship between physicochemical characteristics and antioxidant capacities of mangoes.

In general, the physicochemical parameters of fruit can be used as indicators of their stage of maturity. As a climacteric fruit, mangoes are generally harvested when they are still unripe (green), and their ripening is then completed during their time to market (Lechaudel and Joas, 2007). As mangoes ripen: (i) the flesh firmness decreases (softens) due to changes in the cell walls, (ii) skin/peel colour changes from green to yellow or red mostly because of destruction of the green pigment, (iii) sugars increase due to conversion of starch to sugars and (iv) acidity decreases due to breakdown of acids in the flesh. Also vitamin C, moisture content and fresh weight of the fruit fractions might either decrease or increase during on-tree maturation, ripening and senescence (Beaulieu and Lancaster, 2007). The physiological changes that occur during storage also lead to variations in physicochemical characteristics, quality and antioxidant properties of mangoes. Consequently, any observed differences in maturity or physicochemical characteristics of the fruit should reflect variations in the physiology and biochemistry such as changes in the amounts of TPC or antioxidant quantities in mangoes. Several studies have already demonstrated that there is a significant correlation between the physicochemical properties, antioxidant activity and phenolic content of fruits. Drogoudi et al. (2008) reported that apple peel with darker, redder and bluer colour and apple flesh with lighter colour and lower soluble solid content exhibited higher antioxidants and nutritional values. Walkowiak-Tomczak *et al.* (2008) also suggested that plums with darker colour displayed higher anthocyanins. Wang and Lin (2000) found that blackberries and

strawberries had the highest antioxidant capacity measured by ORAC assay during the green stages whilst red raspberries had the highest capacity at the ripe stage. Also, total anthocyanin content increased with maturity for all blackberry, raspberry and strawberry. By determining the relationships between physicochemical properties (mostly based on maturity indices) and antioxidant capacities, specific physicochemical parameters may be able to be used as indicators of the antioxidant capacity of the flesh, peel and kernel components of mangoes (Manthey and Perkins-Veazie, 2009). Although the antioxidants of mangoes have been the subject of several studies, there is little information on the relationship between the physicochemical characteristics of the mango fruit and the antioxidant capacities of mango flesh, peel and kernel. There is, therefore, a need to assess whether there is a relationship between mango maturity as measured by physicochemical parameters such as colour, dry matter, firmness/texture, pH, total soluble solids (°Brix), acids and vitamin C with the antioxidant properties of mango flesh, peel and kernel.

It has been suggested that phenolic compounds are major contributors to the antioxidant activities of foods of plant origin (Zielinski and Kozlowska, 2000; Zhao *et al.*, 2008). Therefore, there should be a relationship between the TPC and the antioxidant capacity of fruits such as mangoes. Furthermore the measurements of antioxidant capacity are based on different principles such as scavenging radicals or decomposing peroxides or chelating metal ions. Consequently, the assays often produce different results for the same samples within or between laboratories (Connor *et al.*, 2002; Ou *et al.*, 2002; Thaipong *et al.*, 2006). The correlations between the various antioxidant assays are also inconsistent. However, a few reports have indicated there is a correlation between antioxidant capacity and the total phenolic content of mango flesh, peel and kernel (Ajila, 2007b; Ribeiro, 2008; Maisuthisakul and Gordon, 2009; Manthey, 2009). Up to date, there is also little published information on whether the physicochemical properties of mango fruit can be used as an indicator of the potential of mango flesh or peel or kernel as viable food sources of antioxidants. The aims of this chapter are to

- Evaluate the relationships between different physicochemical characteristics of Tommy Atkins mangoes
- Investigate the relationships between data from the six different methods of assaying the antioxidant capacities of mango flesh, peel and kernel.
- Determine the relationships between the physicochemical parameters of mangoes with the total phenolic content (TPC) and antioxidant capacity of flesh, peel and kernel from the fruit.

6.2. Materials and methods

6.2.1. Determination of physicochemical characteristics of Tommy Atkins mangoes

Seventy two fresh Tommy Atkins mangoes were assessed for their physicochemical characteristics based on their maturity score, colour, firmness, total soluble solid (TSS), titratable acidity (TA), vitamin C, moisture content and fruit, flesh, peel and kernel fresh weights. The determinations of the physicochemical characteristics of mangoes are described in 3.3.

6.2.2. Determination of antioxidant capacity of mango fractions

Six antioxidant assays were applied to mango flesh, peel and kernel (from 72 Tommy Atkins mangoes) as described in 3.6. They were TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC.

6.2.3. Statistics

a. Pearson's correlation analysis and principal component analysis

All assays were conducted in triplicate. The Pearson's correlation analysis and principal component analysis (PCA) were performed as outlined in 3.17.6 and 3.17.4 to determine the correlations:

(i) among physicochemical parameters: the matrix consisted of 72 fresh mangoes and 16 physicochemical variables (maturity score, firmness, TSS, TA, TSS:TA ratio, vitamin C, moisture content, L*, a*, b*, chroma, hue, total weight, % flesh, % peel and % kernel).

(ii) among antioxidant capacity assays: the matrix consisted of 72 fresh fruits (216 flesh, peel and kernel samples) and six antioxidant assays (TPC, ABTS, DPPH, FRAP, H-ORAC, L-ORAC).

(iii) between fruit physicochemical parameters of mango fruits and antioxidant capacities of flesh, peel and kernel. The matrix consisted of 16 physicochemical property variables (maturity score, firmness, TSS, TA, TSS:TA ratio, vitamin C, moisture content, L*, a*, b*, chroma, hue, total weight, % flesh, % peel and % kernel) in the mango fractions that showed no significant interaction effects from the treatments and the variables from the 6 antioxidant assays (TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC). A total of 22 variables were used to examine the correlation.

The data was inputted into Minitab 16 to determine the correlation coefficients, their significance and principal component analysis (PCA) by standardising the variables.

Correlations were presented as Pearson's correlation coefficients R which were considered significant when $p < 0.05$ based on a two tailed test.

PCA is a useful statistical method for visualising and interpreting large datasets by forming fewer composite variables (principal components). PCA was used to obtain a simplified overview of the relationships between data sets and to double check the results from Pearson's correlation analysis. The Varimax rotation method was also used in principal component analysis (PCA) to maximise the sum of the variances of the squared loadings to obtain all the coefficients (squared correlation with factors) which can be either extremely large or small with few intermediate values (Kaiser, 1958).

b. Interaction effects

Data on antioxidant capacities were evaluated first before determining the correlations since Chapter 5 indicated there were significant effects of drying treatments on antioxidant capacity values of mango samples so it is difficult to make a general statement about the correlations of antioxidant assays used for measuring all of 72 mangoes from different treatments. Therefore, the simple effect tests (as described in 3.17.4) were implemented in MINITAB for each mango treatment (fresh or dried) on the correlations between two individual assays (see Appendix A.3.1). Only data from the treatments which showed non-significant ($p \geq 0.05$) interaction effects were used for determining correlations. The interaction effects and data treatment were performed by the same procedure for each mango fraction (flesh, peel and kernel).

c. Repeatability of assays

Seventy two dried mango flesh, peel and kernel fractions (72 mangoes x 3 fractions) were assayed for antioxidant levels as measured by six different methods. Each assay was performed in triplicate. The repeatability of each assay was evaluated as described in 3.17.8. It is noted that there were significant effects of drying treatments on antioxidant capacity of mango flesh, peel and kernel, the number of mangoes used for evaluating the assay repeatability varied after the interaction effects were tested as indicated in 6.2.3b.

6.3. Results

The relationships between physicochemical characteristics and antioxidant capacities were investigated by determining whether there were any correlations between the three sets of data. Specifically whether there were any correlations between (i) the physicochemical parameters; (ii) the antioxidant assays and (iii) the physicochemical parameters of the fruit

and antioxidant capacity of flesh, peel and kernel. The results are presented from the Pearson's correlation analysis and principal component analysis (PCA).

6.3.1. Pearson's correlations between physicochemical characterisitcs of mangoes

The differences in the physicochemical characteristics between the five different mango cultivars were investigated in Chapter 4. In the present chapter, the physicochemical parameters, of 72 mangoes from the cultivar Tommy Atkins were investigated and the correlations between their physicochemical properties were evaluated. Although the mangoes were from the same batch (mean physicochemical properties in Table 6.1), the physicochemical characteristics or maturity of each mango were actually different which enabled the relationships of the physicochemical properties and maturity to be evaluated. Particularly, the hue angle (41.8 ± 2.1) obtained from 72 mangoes in Table 6.1 were slightly different from the hue angle (from 12 freeze-dried mangoes) which was 53.1 ± 6.8 ($233.1^{o} - 180^{o} = 53.1^{o}$) obtained in Table 4.1.

Table 6.1. The physicochemical properties of Tommy Atkins mangoes (n = 72).

Parameters	Value ± SE
Maturity score	2.5 ± 0.1
TSS (˚Brix)	6.0 ± 0.3
TA (%)	0.9 ± 0.0
TSS:TA	7.3 ± 0.6
Firmness (kg cm^{-2})	40.5 ± 3.6
Vitamin C (mg 100 g^{-1})	32.1 ± 0.4
Moisture of flesh (%)	85.7 ± 0.4
Total weight (g)	328.4 ± 3.8
Percentage of flesh (g)	82.2 ± 0.4
Percentage of peel (g)	6.3 ± 0.2
Percentage of kernel (g)	5.3 ± 0.1
Chroma	34.5 ± 0.9
Hue (^{o})	41.8 ± 2.1
L*	40.2 ± 0.8
a*	18.7 ± 1.3
b*	25.3 ± 0.9

Table 6.2. Correlation coefficients of physicochemical parameters of Tommy Atkins mangoes (n = 72).

Parameters	TSS	TA	TSS:TA	Firmness	Vitamin C	Moisture of flesh	Total weight	Flesh %	Peel %	Kernel %	Chroma	Hue	L	a	b
Maturity	0.34**	-0.54***	0.50***	-0.72***	-0.55***	0.58***	0.02	0.12	-0.18	0.26*	0.08	0.27*	0.19	-0.13	0.2
TSS	-	-0.29*	0.86***	-0.21	-0.28*	0.24*	0.27*	-0.03	-0.03	0.03	0.2	0.12	0.17	-0.03	0.22
TA		-	-0.64***	0.41***	0.85***	-0.48***	0.07	0.28*	-0.05	-0.41***	0.11	-0.04	0.03	0.16	0.07
TSS:TA			-	-0.30**	-0.59***	0.34**	0.12	-0.12	-0.05	0.23	0.12	0.09	0.11	-0.08	0.13
Firmness				-	0.32**	-0.69***	0.05	-0.21	0.27*	-0.26*	-0.18	-0.25*	-0.25*	0.06	-0.23
Vitamin C					-	-0.50***	0.18	0.24*	-0.01	-0.35**	0.12	-0.15	-0.08	0.21	-0.02
Moisture of flesh						-	-0.03	0.14	-0.14	0.04	0.18	0.15	0.18	0.04	0.15
Total weight							-	0.05	-0.03	-0.18	0.04	-0.2	-0.23*	0.2	-0.2
% flesh								-	-0.81***	-0.33**	0.41***	0.24*	0.36**	0.07	0.40**
% peel									-	-0.13	-0.34**	-0.12	-0.21	-0.1	-0.25*
% kernel										-	0.2	-0.07	0.23	0.06	0.22
Chroma											-	0.01	0.56***	0.50***	0.62***
Hue												-	0.65***	0.69***	0.66***
L* of peel													-	0.34**	0.97***
a* of peel														-	0.32**
b* of peel															-

*Correlation is significant at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

6.3.1.1. Pearson's correlations between physicochemical properties and maturity score

Table 6.2 shows there was a significant ($p < 0.001$) negative and strong correlation between firmness and maturity score; significant ($p < 0.001$) positive and moderate correlations between maturity score and moisture content, TSS:TA ratio and TSS and significant ($p < 0.001$) negative and moderate correlations between maturity score and vitamin C and TA.

There were also significant ($p < 0.05$) and relatively weak correlations between maturity score with hue angles; L*; b* and percentage of kernel in the mango fruit. There were no correlations ($p \geq 0.05$) between maturity score and chroma; a*; total fruit weight; percentages of mango flesh and peel.

6.3.1.2. Pearson's correlations between the physicochemical characteristics

There were significant correlations between individual physicochemical parameters. Table 6.2 shows significant ($p < 0.001$) positive and strong correlations between TSS and TSS:TA; TA and vitamin C; significant ($p < 0.001$) negative and relatively strong correlations between firmness and moisture content; TSS:TA and TA. There were also significant ($p < 0.05$) positive and moderate correlations between firmness and TA; moisture content and TSS:TA; firmness and vitamin C: significant ($p < 0.05$) negative and moderate correlations between vitamin C and TSS:TA ; vitamin C and moisture content; TA and moisture content; firmness and TSS:TA.

In general, there were no significant correlations between any of the colour parameters or any other measured characteristic. The exception is that there was a significant ($p < 0.05$) but weak correlation between colour and firmness in which firmness was negatively correlated with L*; b* and hue. There were also significant ($p < 0.001$) and strong correlations between L* with b* or hue angle and weak-to-moderate correlations between L* and chroma.

6.3.2. Pearson's correlations between the antioxidant assays

Before determining the correlations between the six different antioxidant assays (data from 72 mangoes from fresh and 5 drying treatments) it was important to determine if there were any interaction effects between the treatments by assessing whether one variable (antioxidant values from an assay) is dependent or independent of another variable (fresh or dried treatments). Thus, the interaction effects between the drying treatments and the antioxidant levels measured by the six different assays were statistically tested using GLM (Appendix A.3.1). The data from fresh mango samples was identified to have an interactive effect

between the treatment conditions of mango samples with the antioxidant assay results. The data on the antioxidant levels in the fresh mangoes (n = 12) responsible for the interaction effects were therefore excluded from the statistical comparison. Only the data from the remaining 60 mangoes were used to examine the interrelationship between the antioxidant capacities measured by the six assays. The mean antioxidant capacity of mango flesh, peel and kernel from the six assays are in Table 6.3. The Pearson's correlation coefficients are in the Table 6.4, 6.5 and 6.6 for flesh, peel and kernel respectively.

Table 6.3. Total phenolic content and antioxidant capacity of mango flesh, peel and kernel (Mean ± SE, n = 72).

Antioxidant assays	Flesh	Peel	Kernel
TPC (mg GAE 100 g^{-1} DW)	662.5 ± 24.1[a]	6144.3 ± 180.0[b]	11496.6 ± 648.9[c]
ABTS (µmol TE g^{-1} DW)	56.0 ± 2.2[a]	752.2 ± 17.5[b]	1930.2 ± 97.5[c]
DPPH (µmol TE g^{-1} DW)	42.0 ± 2.3[a]	728.3 ± 18.1[b]	1863.2 ± 106.2[c]
FRAP (µmol Fe^{2+} g^{-1} DW)	39.4 ± 1.5[a]	356.6 ±13.8[b]	796.3 ± 52.1[c]
H-ORAC (µmol TE g^{-1} DW)	46.4 ± 2.4[a]	434.2 ± 13.7[b]	837.1 ± 59.8[c]
L-ORAC (µmol TE g^{-1} DW)	1.7 ± 0.2[a]	5.3 ± 0.2[b]	9.7 ± 0.6[c]

Means in the same row with the different superscripts (a-c) are significantly different ($p < 0.05$).

The TPC (measured by Folin–Ciocalteu) of flesh samples was significantly ($p < 0.001$) correlated with antioxidant levels measured by ABTS, DPPH, FRAP and H-ORAC. There was no significant correlation ($p \geq 0.05$) between TPC and L-ORAC. There was a relatively strong correlation between TPC and ABTS ($p < 0.001$) or FRAP ($p < 0.001$) and a significant moderate correlations with DPPH ($p < 0.001$) and H-ORAC ($p < 0.001$) with TPC.

The four assays ABTS, DPPH, FRAP and H-ORAC were positively correlated with each other ($p < 0.001$). There was a significant ($p < 0.05$) but weak correlation between DPPH and H-ORAC in flesh. The highest correlation coefficients were between ABTS and FRAP ($p < 0.001$) and the lowest correlation was between DPPH and H-ORAC ($p < 0.05$). There was no significant correlation between L-ORAC and any other assay in flesh.

Table 6.4. Pearson's correlation coefficients between results from the six antioxidant assays for flesh (n = 60).

	TPC	ABTS	DPPH	FRAP	H-ORAC	L-ORAC
TPC	-	0.74**	0.56**	0.74**	0.56**	-0.14
ABTS		-	0.64**	0.88**	0.69**	0.01
DPPH			-	0.57**	0.29*	-0.26
FRAP				-	0.62**	-0.01
H-ORAC					-	0.02
L-ORAC						-

Correlation is significant at *$p < 0.05$; **$p < 0.001$.

In peel, TPC was significantly ($p < 0.001$ or $p < 0.05$) correlated with antioxidant capacities as measured by ABTS, DPPH, FRAP, H-ORAC and L-ORAC. There were significant ($p < 0.001$) and relatively strong correlations between TPC with DPPH, FRAP, ABTS and H-ORAC and significant ($p < 0.05$) and moderate correlations between TPC with L-ORAC.
All the antioxidant assays: ABTS, DPPH, FRAP, H-ORAC and L-ORAC were significantly and positively correlated in peel. In particular, the ABTS, DPPH and FRAP assays were highly significant ($p < 0.001$) and strongly correlated with each other. There was a highly significant ($p < 0.001$) and moderate correlation between H-ORAC with the other assays.

Table 6.5. Pearson's correlation coefficients between results from the six antioxidant assays for peel (n = 60).

	TPC	ABTS	DPPH	FRAP	H-ORAC	L-ORAC
TPC	-	0.72**	0.78**	0.76**	0.64**	0.34*
ABTS		-	0.84**	0.79**	0.57**	0.30*
DPPH			-	0.78**	0.70**	0.34*
FRAP				-	0.57**	0.32*
H-ORAC					-	0.53**
L-ORAC						-

Correlation is significant at *$p < 0.05$; **$p < 0.001$.

The TPC of kernel was significantly ($p < 0.001$) and very strongly correlated with their antioxidant levels as measured by ABTS, DPPH, FRAP and H-ORAC. There was no significant ($p \geq 0.05$) correlation between TPC and L-ORAC. Four of the antioxidant assays were significantly ($p < 0.001$) and strongly positively correlated with each other except for L-ORAC which showed no significant ($p \geq 0.05$) correlations with any of the other antioxidant assays, except for DPPH.

Table 6.6. Pearson's correlation coefficients between results from the six antioxidant assays for kernel (n = 60).

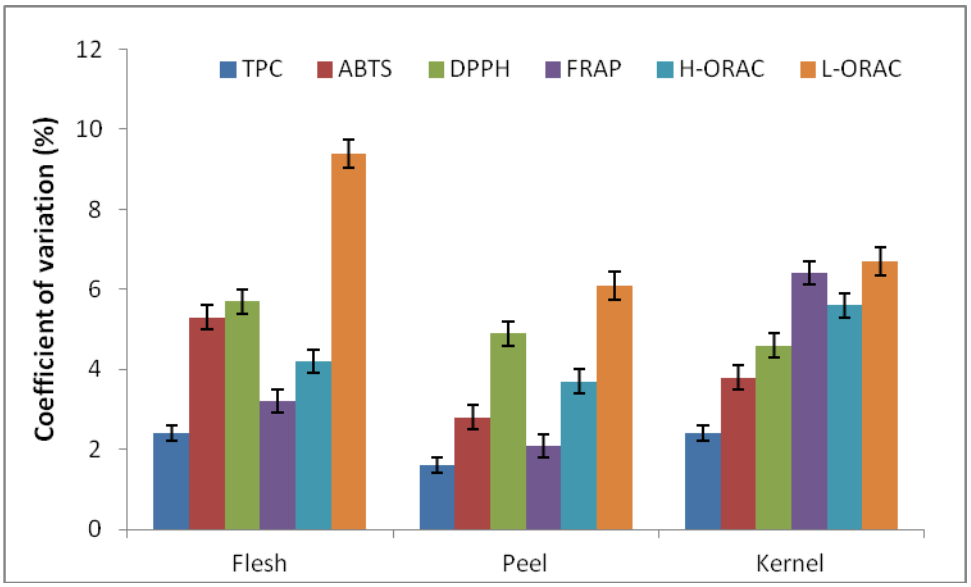
	TPC	ABTS	DPPH	FRAP	H-ORAC	L-ORAC
TPC	-	0.98**	0.96**	0.91**	0.90**	0.26
ABTS		-	0.96**	0.94**	0.94**	0.23
DPPH			-	0.87**	0.89**	0.28*
FRAP				-	0.91**	0.12
H-ORAC					-	0.19
L-ORAC						-

Correlation is significant at *$p < 0.05$; **$p < 0.001$.

6.3.3. Repeatability of antioxidant assays

The six assays TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC were selected to determine the antioxidant capacity of mangoes. The coefficients of variation of all the six antioxidant assays for flesh, peel and kernel are in Figure 6.1. All the methods had a satisfactory coefficient of variation of less than 10%.

Figure 6.1. Coefficients of variation (± SE) in six antioxidant assays for dried mango flesh, peel and kernel (n = 60).



6.3.4. Pearson's correlations between physicochemical characteristics and antioxidant capacities

The correlation coefficients between the physicochemical characteristics and antioxidant capacity in flesh determined by the six assays are shown in Table 6.7.

In flesh, there was a significant ($p < 0.05$) negative and weak correlations between TSS and TSS:TA with FRAP and a significant ($p < 0.05$) positive and weak correlations between vitamin C with FRAP and L-ORAC. There were significant ($p < 0.05$) positive and weak correlations between the percentage of flesh with ABTS and FRAP and a significant ($p < 0.05$) negative and weak correlations between the percentage of peel with all the measurement of antioxidant capacity (except for TPC).

The correlation coefficients between the physicochemical properties and antioxidant capacities in peel are in Table 6.8. There were significant ($p < 0.05$) and negative weak correlations between TA and Vitamin C with the lipophilic antioxidants, highly significant ($p < 0.001$) negative and moderate correlations between the percentage of peel with TPC and antioxidant capacity of peel measured by ABTS, DPPH, FRAP and H-ORAC. Interestingly, there were significant ($p < 0.01$ or $p < 0.05$) positive and weak correlations between the percentage of flesh with the TPC and antioxidant capacity of measured by ABTS, DPPH and FRAP.

The correlation coefficients between the physicochemical properties and antioxidant capacity in kernel are shown in Table 6.9. There were more significant ($p < 0.001$ or $p < 0.01$) correlations between the physicochemical properties of the fruit and antioxidant levels in kernel than were observed with flesh or peel. For example, there were significant ($p < 0.001$) negative and moderate correlations between the maturity score, total soluble solids and TSS:TA with TPC and antioxidant levels of kernel measured by ABTS, DPPH, FRAP and H-ORAC (Table 6.9). Furthermore, there were significant ($p < 0.01$ and $p < 0.05$) positive and moderate correlations between the firmness and total titratable acidity of fruit with TPC and antioxidant levels of kernel measured by ABTS, DPPH, FRAP and H-ORAC (Table 6.9). There was a significant ($p < 0.01$ and $p < 0.05$) positive and weak correlation between vitamin C and TPC and antioxidant capacities measured by ABTS and DPPH with vitamin C (Table 6.9). Significant ($p < 0.01$ and $p < 0.05$) negative weak correlations were found between the percentage of kernel with the TPC and antioxidant capacities of kernel measured by ABTS, DPPH, FRAP, H-ORAC and L-ORAC. In particular, there were significant ($p < 0.05$) positive

weak correlations between the percentage of peel with the TPC and the antioxidant capacity measured by all the assays, except for L-ORAC.

Table 6.7. Correlation coefficients between physicochemical properties and antioxidant capacities in flesh (n = 60).

Parameters	Antioxidant assays					
	TPC	ABTS	DPPH	FRAP	H-ORAC	L-ORAC
Maturity	-0.01	0.03	0.02	-0.19	0.06	-0.16
TSS	-0.13	-0.08	-0.05	-0.30*	-0.08	0.10
TA	0.04	0.01	-0.06	0.15	0.03	0.20
TSS:TA	-0.14	-0.07	-0.08	-0.31*	-0.08	0.09
Firmness	0.03	-0.02	0.02	0.08	-0.07	0.21
Vitamin C	0.16	0.18	0.01	0.29*	0.10	0.30*
Moisture	-0.22	-0.09	-0.14	-0.14	-0.05	-0.16
Total weight	0.14	0.10	-0.10	0.05	0.08	0.06
% Flesh	0.18	0.34**	0.18	0.34**	0.20*	0.24
% Peel	-0.16	-0.42**	-0.26*	-0.30*	-0.27	-0.28
% Kernel	0.03	0.12	0.19	-0.07	0.09	-0.07
Chroma	0.03	0.08	0.07	0.05	0.01	0.09
Hue	-0.12	-0.28*	-0.04	-0.27*	-0.21	-0.16
L* of peel	-0.19	-0.28*	-0.02	-0.29*	-0.25	-0.17
a* of peel	0.08	0.21	0.04	0.20	0.08	0.06
b* of peel	-0.12	-0.20	0.02	-0.23	-0.18	-0.05

Correlation is significant at *$p < 0.05$; **$p < 0.001$.

Table 6.8. Correlation coefficients between physicochemical characteristics and antioxidant capacity in peel (n = 60).

Parameters	Antioxidant assays					
	TPC	ABTS	DPPH	FRAP	H-ORAC	L-ORAC
Maturity	0.10	0.05	0.15	0.02	0.10	0.11
TSS	0.08	0.16	0.07	0.03	-0.02	-0.03
TA	-0.17	-0.20	-0.22	-0.17	-0.18	-0.31*
TSS:TA	0.12	0.20	0.14	0.06	0.07	0.21
Firmness	0.15	0.07	0.06	0.15	0.06	0.11
Vitamin C	-0.22	-0.21	-0.20	-0.21	-0.24	-0.39**
Moisture	-0.04	0.02	0.06	-0.02	0.07	0.00
Total weight	-0.07	-0.12	-0.10	-0.16	-0.14	-0.17
% Flesh	0.28*	0.32*	0.30*	0.31*	0.22	-0.04
% Peel	-0.49***	-0.48***	-0.52***	-0.41**	-0.44***	-0.16
% Kernel	0.22	0.08	0.24	0.10	0.27	0.26
Chroma	0.01	0.20	0.06	0.07	-0.05	-0.04
Hue	0.15	0.07	0.06	0.12	0.10	0.10
L* of peel	0.08	0.14	-0.05	0.07	0.03	0.11
a* of peel	-0.15	-0.04	-0.03	-0.07	-0.10	-0.13
b* of peel	0.12	0.18	0.03	0.11	0.05	0.06

Correlation is significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 6.9. Correlation coefficients between physicochemical characteristics and antioxidant capacity in kernel (n =60).

	Antioxidant assays					
Parameters	TPC	ABTS	DPPH	FRAP	H-ORAC	L-ORAC
Maturity	-0.58***	-0.55***	-0.55***	-0.51***	-0.47***	-0.11
TSS	-0.50***	-0.53***	-0.50***	-0.48***	-0.55***	0.08
TA	0.41**	0.37**	0.42**	0.25	0.28*	0.11
TSS:TA	-0.49***	-0.51***	-0.50***	-0.44***	-0.49***	0.02
Firmness	0.44***	0.43**	0.42**	0.38**	0.35**	-0.13
Vitamin C	0.33*	0.28*	0.34**	0.17	0.22	0.16
Moisture	-0.32*	-0.30*	-0.32	-0.20	-0.21	0.03
Total weight	-0.13	-0.17	-0.10	-0.25	-0.19	0.12
%Peel	0.32*	0.33*	0.26*	0.42*	0.30*	-0.05
%Flesh	-0.14	-0.16	-0.10	-0.26	-0.17	0.19
%Kernel	-0.36**	-0.35**	-0.36**	-0.33*	-0.29*	-0.34**
Chroma	0.06	0.03	-0.01	0.02	0.01	0.09
Hue	-0.05	-0.01	-0.04	-0.02	0.01	0.15
L* of peel	0.07	0.08	0.03	0.12	0.07	0.18
a* of peel	0.05	0.00	-0.01	-0.01	-0.03	-0.08
b* of peel	0.01	0.03	-0.01	0.04	0.03	0.14

Correlation is significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

6.3.5. Correlations using principal component analysis (PCA)

PCA was used to gain an overview of the interrelationship among physicochemical parameters and antioxidant capacity as measured by the different assays. As described in chapter 5, principal components analysis is a statistical technique to simplify data by reducing the number of variables. In PCA, linear combinations of the original variables are derived which can explain the maximum amount of variations in the data set and which are orthogonal to each other. Principal component analysis (PCA) divides the data into distinct sets that best describes the relationship of the measured parameters. In this chapter, PCA was used on the physicochemical properties data set and the antioxidant level data set using Minitab 16.

6.3.5.1. Correlations between physicochemical properties using PCA

The matrix (72 mangoes x 16 variables) was used to perform PCA with the weighting method of standardisation. Six principal components were extracted that accounted for 85.8% of the total variation. These components were selected because their eigenvalue exceeded 1.0 and explained more than 80% of total variance. PC1 described the statistical relationship that accounts for the greatest amount of sample variation, followed by PC2, PC3, PC4, PC5 and

PC6. Each component described, in decreasing order, less variation in the sample set. The data from the first 3 of these PC accounted for 62.4% of the variance in the 16 variables (PC1= 27.7%, PC2 = 21%, PC3 = 13.7). The variable loadings for components PC1, PC2 and PC3 were extracted (Appendix A.3.2.1).

Figure 6.2 shows the projection of the 72 mangoes and 16 variables in the plane defined by the two principal components. The plot of PC1 vs. PC2 provides a visualisation of the relationships between the variables. The loadings in the PCA plot express the level of correlations between principal components with the original variables and the level of correlations among the variables.

Figure 6.2. Loading plot of the relationships between physicochemical property parameters in mangoes (n = 72).

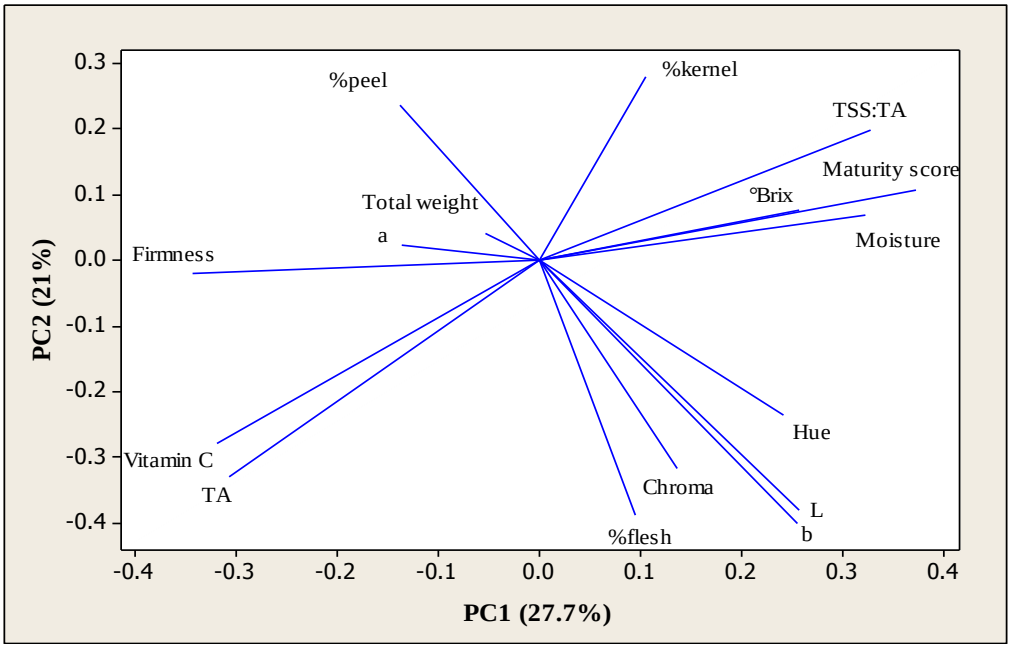


Figure 6.2 shows that the maturity score, TSS, TSS:TS ratio, moisture content, hue angle, L* and b* were positively correlated in PC1. The maturity score, TSS, TSS:TS ratio and moisture had similar vector directions indicating there is a strong relationships between these parameters. The similar vector directions of Hue, L* and b* suggests there is strong correlation between these colour parameters. But, the vector directions of these colour parameters are different from the maturity score vector. Therefore, there is either no relationship or very weak one between colour and maturity. Firmness, TA and Vitamin C were negatively correlated with PC1 in which firmness was highly correlated with maturity score whilst TA and vitamin C appeared to be highly correlated with each other. These

observations indicate that firmness, TA, and Vitamin C are good indicators of the maturity stage of mangoes.

The second component (PC2) explained an additional 21.0% of the variation and describes the relationships between the percentages of mango fractions. The percentage of flesh is highly negatively correlated with the percentage of peel and negatively moderately correlated with the percentage kernel. The directions of the vectors suggest that PC1 explains the variability in the maturity parameters whilst PC2 describes the variability in the percentage of the mango fractions.

The third component PC3 (loading plot of PC3 is not shown but explains an additional 13.6% of the variation) was mostly dominated by the colour variables of the mango fractions in which there was a positive strong correlation between L* with b* and hue angle. This finding indicates that lighter mango peel is more yellow and darker ones are more orange-red.

The short length of the vectors for total weight and a* value of mangoes indicate that these variables were correlated with other principal components rather than PC1, PC2 and PC3. However, as mentioned earlier, the other PCs account for a small amount of the variations, and are not presented. The total weight and a* value are therefore not taken into account in this evaluation.

6.3.5.2. Correlations between antioxidant activity assays using PCA

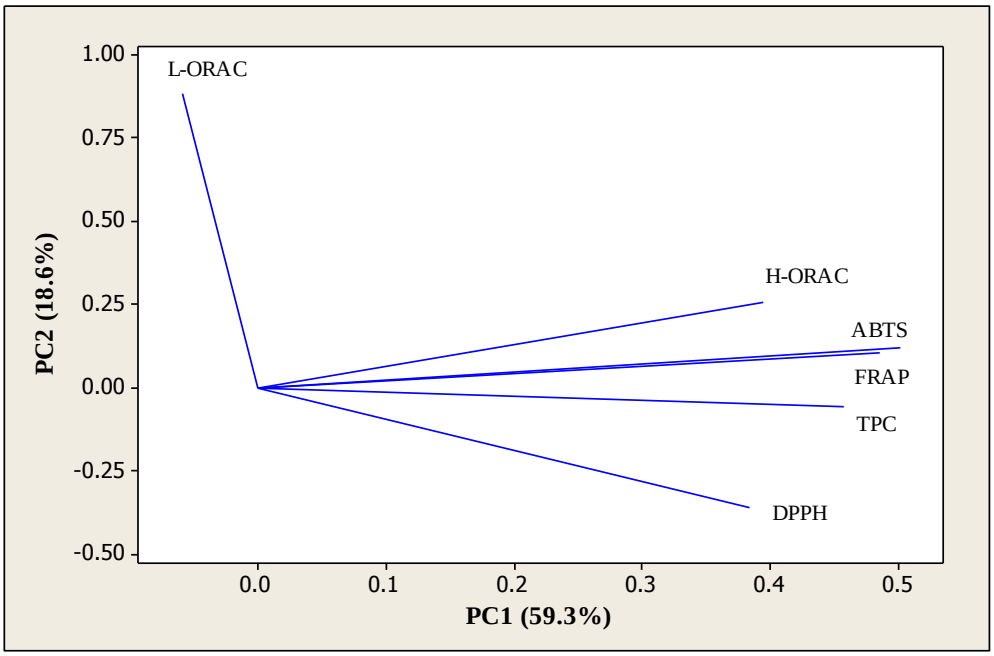
As indicated previously, due to the interaction effects, data from the 12 fresh mango fractions were excluded from this analysis. Data from 60 mango fractions were used to evaluate the correlations regardless of drying treatments being applied. The 60 mango fractions (flesh, peel and kernel) that underwent the five drying treatments were used for PCA. The matrix (60 mangoes x 6 assay variables) was used to perform PCA by standardising the variables (TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC assays).

a. Correlations between antioxidant activity assays for mango flesh

Three principal components were extracted that accounted for 88.2% of the total variation. These three components (PC1= 59.3%, PC2 = 18.6%, PC3 = 10.2%) were selected because their eigenvalues exceeded 1.0 or explained more than 80% of the total variance (Appendix A.3.2.2). The variable loadings for components PC1, PC2 and PC3 were extracted and the high weighting scores indicate that there is a tight association with that principal component.

Figure 6.3 shows the projection of the 60 mango flesh and 6 variables in the plane defined by the two principal components.

Figure 6.3. Loading plot of the relationships between antioxidant assays for mango flesh (n = 60).



In the loading plot (Figure 6.3), all the antioxidant assays, except for L-ORAC, were strongly positively correlated with PC1 and had similar vector directions, indicating that a strong relationship exists between the variables. Moreover, TPC, ABTS, DPPH and FRAP were found to have a similar loading on PC1 indicating that these assays were closely related. The high loading of TPC on PC1 suggests that phenolics in mango flesh are good antioxidants. The two vectors for ABTS and FRAP visually overlap suggesting there is a very strong positive correlation between the ABTS and FRAP assays in flesh. In contrast, the vector direction of L-ORAC was orthogonal to PC1 and had a low loading. Furthermore, L-ORAC was strongly positively correlated with PC2 with the highest weighting on PC2 (Appendix A.3.2.2) confirming that there is no correlation between L-ORAC and any other antioxidant assay in mango flesh.

b. Correlations between antioxidant activity assays for mango peel

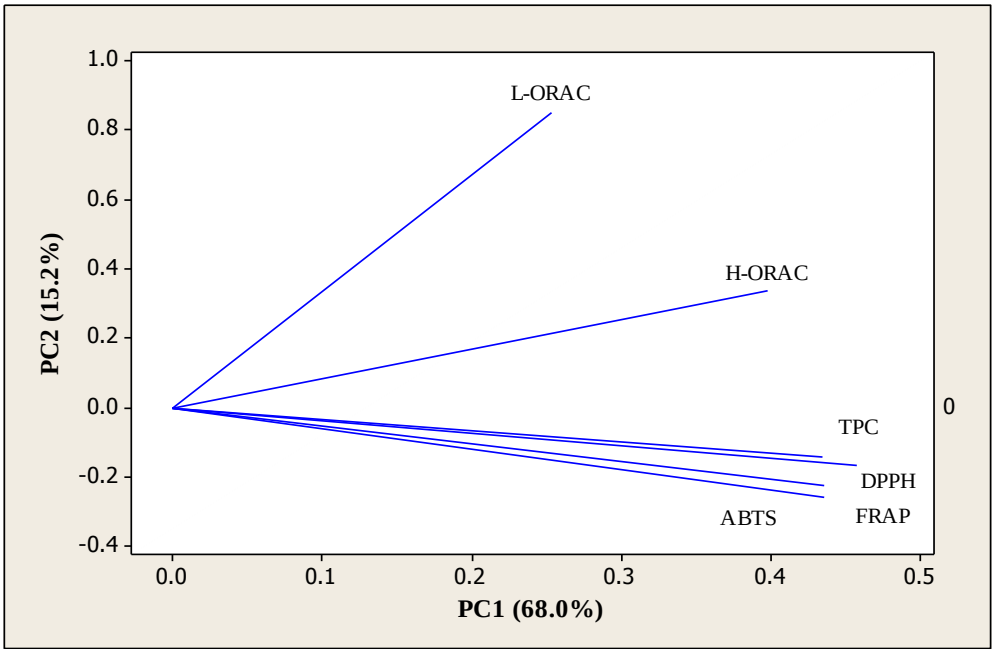
Two principal components were extracted that accounted for 83.2% of the total variation. PC1 described 68.0% and PC2 an additional 15.2% of the variation in the sample set. These two components were selected because their eigenvalues exceeded 1.0 and explained more than

80% of total variance. The variable loadings for components PC1 and PC2 were applied and the high weighting scores indicate a tight association with that principal component.

Figure 6.4 shows the projection of the 60 mango peel and 6 variables in the plane defined by the two principal components.

In the loading plot (Figure 6.4), all the antioxidant assays, except for L-ORAC, were positively correlated with PC1 in which TPC, ABTS, DPPH and FRAP assays were strongly correlated. Moreover, TPC, ABTS, DPPH and FRAP were found to have a similarly loaded on PC1 confirming that these assays were closely related. The high loading of TPC on PC1 suggests that phenolic compounds in mango peel are good antioxidants. Though H-ORAC and L-ORAC were correlated with PC1, they had lower loadings on PC1 compared to the other assays. H-ORAC was moderately correlated whilst L-ORAC was weakly correlated with the other assays. Along PC2, L-ORAC showed a high loading whilst the other assays exhibited a low loading indicating that there is no correlation between the lipophilic antioxidants with the hydrophilic antioxidants and total antioxidant capacities of mango peel.

Figure 6.4. Loading plot of the relationships between antioxidant assays for mango peel (n = 60).



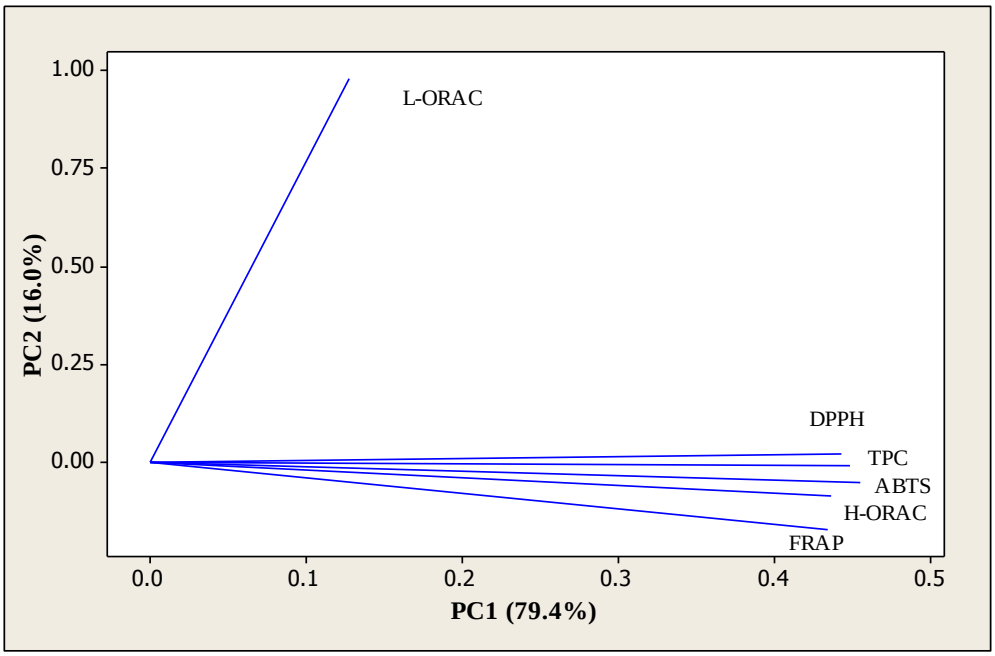
c. Correlations between antioxidant activity assays for mango kernel

Two principal components were extracted that accounted for 95.3% of the total variation. PC1 described 79.4% whilst PC2 described an additional 16% of the variation in the sample set.

These two components were selected because their eigenvalues exceeded 1.0 and explained more than 80% of total variance. The variable loadings for components PC1 and PC2 were applied and the high weighting scores indicate a tight association with that principal component.

Figure 6.5 shows the projection of the 60 mango kernel and 6 variables in the plane defined by the two principal components. The loading plot in Figure 6.5 is similar to that obtained with peel. All the antioxidant assays for kernel, except for L-ORAC, were positively correlated with PC1. However, unlike peel, all of the assays which included TCP, ABTS, DPPH, FRAP and H-ORAC were strongly correlated. Moreover, TPC, ABTS, DPPH, FRAP and H-ORAC were found to be similarly loaded on PC1 indicating that the assays were closely related and the high loading of TPC on PC1 suggests that phenolic compounds in mango kernel are also good antioxidants. The direction of the L-ORAC vector was orthogonal to PC1 and had the low loading on PC1. Along PC2, L-ORAC showed a high loading whilst the other assays exhibited low loadings indicating that there are no correlations between the lipophilic with hydrophilic or total antioxidant capacities in mango kernel.

Figure 6.5. Loading plot of the relationships between antioxidant assays for mango kernel (n = 60).



6.3.5.3. Correlations between the individual physicochemical parameters and antioxidant activities using PCA

PCA was performed on the physicochemical properties and the antioxidant assay data sets to obtain a simplified overview of the relationships between the variables of physicochemical properties and the antioxidant assay variables in a reduced dimensional plot.

As indicated previously, only 60 mango fractions (flesh, peel and kernel fractions) regardless of drying treatments applied from the seventy two mangoes were used for PCA. The matrix (60 mangoes x 22 variables) was used to perform PCA by standardising the variables (antioxidant assays). The matrix consisted of 16 physicochemical property variables (maturity score, firmness, TSS, TA, TSS:TA ratio, Vitamin C, moisture content, L*, a*, b*, Chroma, Hue, total weight, % flesh, % peel and % kernel) and 6 antioxidant assay variables (TPC, ABTS, DPPH, FRAP, H-ORAC and L-ORAC). The correlations between the various physicochemical parameters and antioxidant assays were investigated in section 6.3.2 and 6.3.3 so in this section, the relationships between the physicochemical parameters and the antioxidant assay variables in mango flesh, peel and kernel were evaluated. But, due to the large number of variables in one plane, and the amount of generated data for one figure, the visual interpretation of the PCs was improved by using the Varimax rotation in which the extraction of two components only was examined and one variable was related , at the most, to one other variable (Appendix A.3.2.3).

a. Correlations between physicochemical parameters and antioxidant activity in flesh

Six principal components were extracted that accounted for 78% of the total variation and were selected because their eigenvalues exceeded 1.0. The PC1-PC2 plane contained the largest variation of all the possible planes and was used to plot the variables of physicochemical parameters and antioxidant capacity levels in flesh. Varimax was used in principal component analysis (PCA) to maximise the sum of the variances of the squared loadings.

Figure 6.6. Loading plot of physicochemical parameters and antioxidant assays for mango flesh (n = 60).

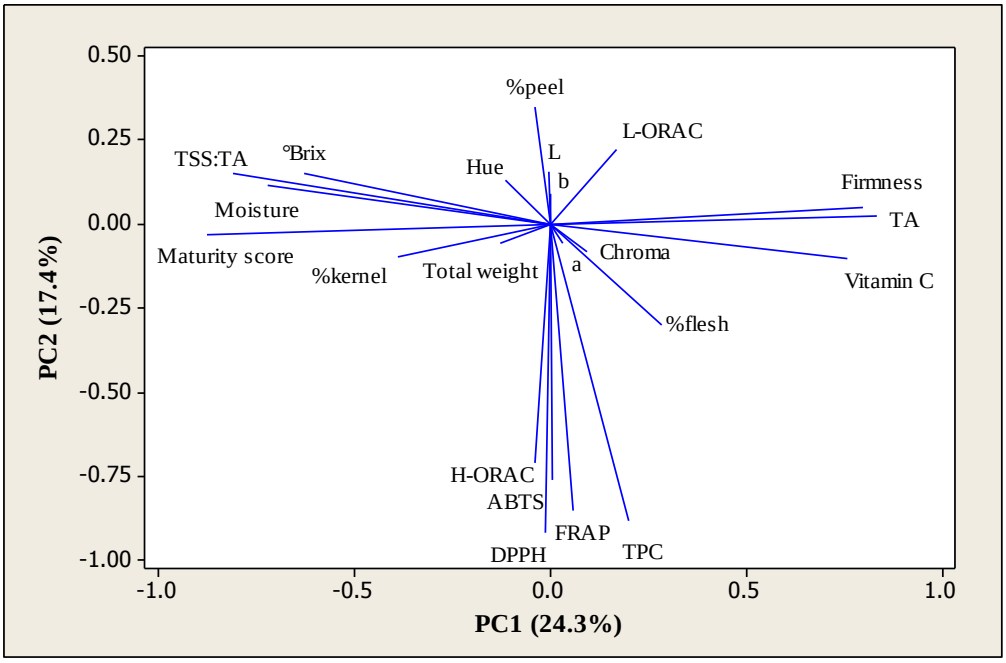


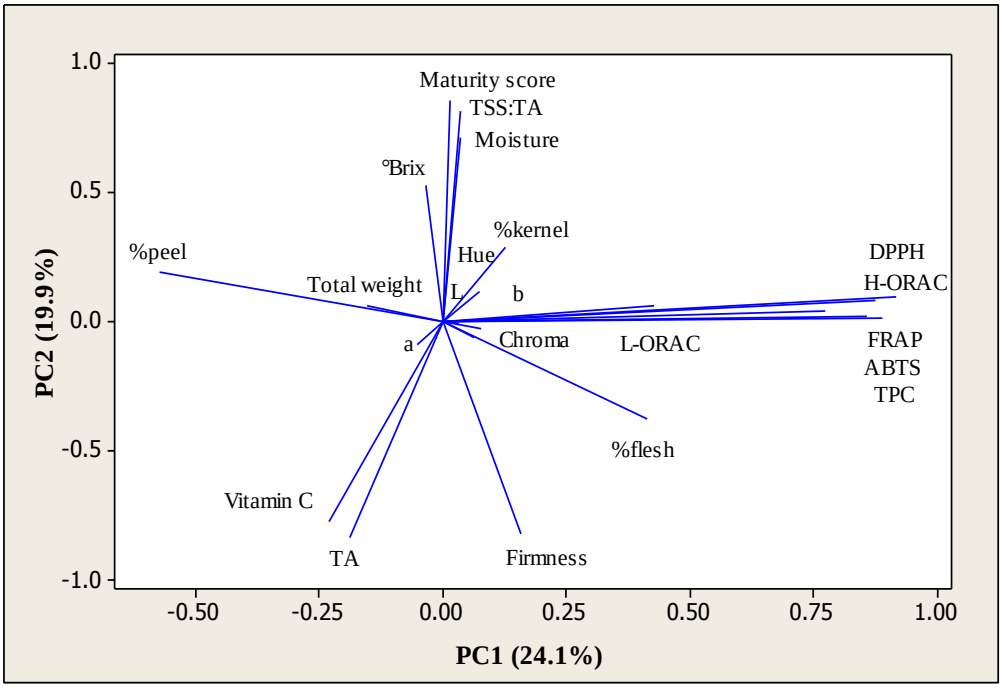
Figure 6.6 shows there was no correlation between any antioxidant assays in PC1 with the physicochemical variables. The orthogonality between the antioxidant assays, except for L-ORAC, with the measured maturity parameters maturity score, firmness, TSS, TA, TSS:TA ratio, Vitamin C, moisture content, total weight and % kernel implied there was no correlation between the two groups. It should be noted that PC1 represents the maturity parameters whilst PC2 describes the antioxidant assays. Although some colour space values were correlated with PC2, the short length of the vectors indicated there was no correlation between colour and antioxidant capacity. The percentage of peel was positively correlated with PC2 and had a medium loading on PC2 suggesting a weak-to-moderate negative correlation between the percentage of peel and the antioxidant capacity of mango flesh. A weak positive correlation between the percentage of flesh and the antioxidant assay was also observed.

b. Correlation between physicochemical characteristics and antioxidants in mango peel

Six principal components were extracted that accounted for 80.2% of the total variation. These components were selected because their eigenvalues exceeded 1.0 and explained more than 80% of the total variance. Similar to flesh, the PC1-PC2 planes contained the largest variation of all the possible planes and were used to plot the variables in peel. Varimax was used to maximise the sum of the variances of the squared loadings.

Figure 6.7 shows the projection of the 60 mango peel and 22 variables in the plane defined by the two principal components (factors) that were rotated by Varimax. The antioxidant assay groups were strongly positively correlated according to their PC1 characteristics. However, the L-ORAC had a low loading on PC1 indicating there was a negligible or weak correlation with the other assays. Moreover, the orthogonality between the antioxidant assays, except for L-ORAC, and the physicochemical variables suggest that there was no correlations between the antioxidant assays and the physicochemical parameters. An exception to this conclusion is that the antioxidant assay group was correlated with the percentage of peel. However, the correlation is weak-to-moderate due to a low loading of the percentage of peel on PC1. It should also be noted that PC1 defined the antioxidant assays variables whilst PC2 described the physicochemical parameters of mango peel.

Figure 6.7. Loading plot of physicochemical parameters and antioxidant assays for mango peel (n = 60).

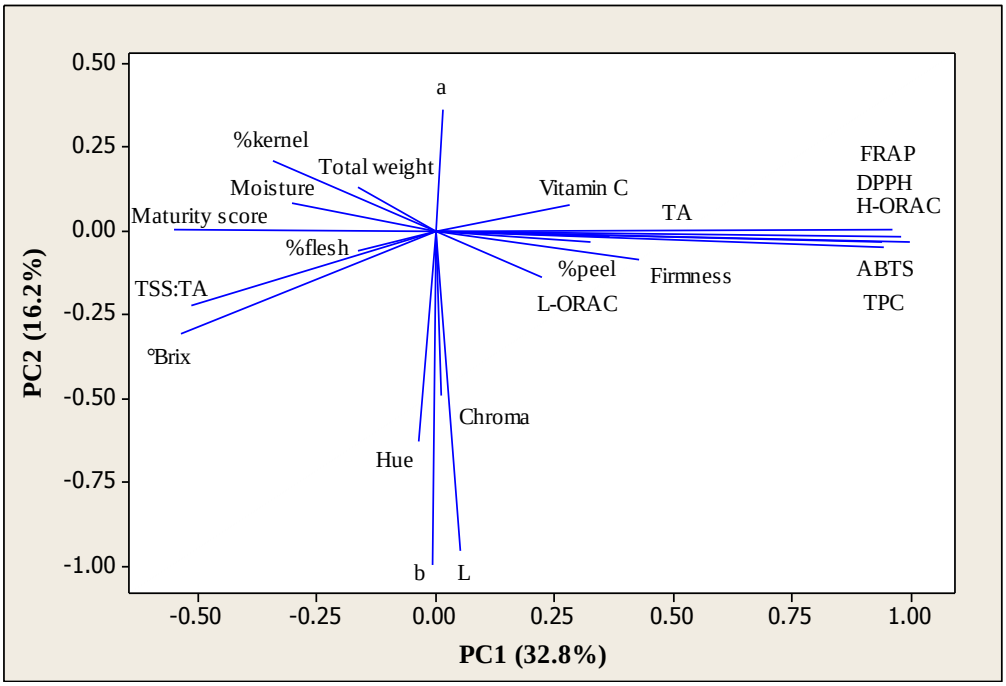


c. Correlation between physicochemical properties and antioxidants in mango kernel

Six principal components were extracted that accounted for 83.1% of the total variation. These components were selected because their eigenvalues exceeded 1.0 and explained more than 80% of the total variance (Appendix A.3.2.3). Similar to flesh and peel, Varimax was used in principal component analysis (PCA) to maximise the sum of the variances of the squared loadings (Appendix A.3.2.3).

Figure 6.8 shows the projection of the 60 mango kernels and 22 variables in the plane defined by the two principal components (factors) that were rotated by Varimax. The figure shows that all the antioxidant assay variables, except for L-ORAC and several physicochemical variables were correlated with PC1. However, the antioxidant assay variables were strongly correlated with PC1 with the high loadings on PC1 ranging from 0.941 to 0.999 whilst the other physicochemical parameter variables had low loadings (< 0.5 or > -0.5). Therefore, there is only a weak-to-moderate correlations between these antioxidant assays with maturity score, TSS:TA ratio, TSS, moisture content, firmness, TA and vitamin C. The maturity score, TSS and TSS:TA ratio also had high loadings on PC1 suggesting there is a higher correlation with the antioxidant capacity than with the other physicochemical parameters. There is a weak negative correlation between the antioxidant assays and the percentages of kernel. Meanwhile, PC2 was mostly dominated by the colour space values suggesting there is no correlation between the colour of mangoes and antioxidant capacity of mango kernel.

Figure 6.8. Loading plot of physicochemical parameters and antioxidant assays for mango kernel (n = 60).



6.4. Discussion

Overall, the results from the Pearson's correlation analysis (6.3.1) and principal component analysis (6.3.2) are in agreement. There were some slight differences. Namely the Pearson's correlation analysis results revealed that mango flesh with either low TSS or TSS:TA ratio or

high vitamin C exhibited relatively high FRAP values. Mango peel with low TA or vitamin C also exhibited relatively high lipophilic antioxidants.

6.4.1. Correlations between physicochemical characteristics of mangoes

The results illustrated that there was a relationship between specific physicochemical properties such as firmness, TSS, TA, TSS:TA ratio, moisture content and vitamin C with the maturity of mangoes. These findings are in agreement with previous studies (Kalra and Tandon, 1983; Slaughter., 2009; Jha *et al.*, 2010**;** Padda *et al.*, 2011). Specific details are discussed below.

6.4.1.1. Correlations between physicochemical properties and maturity score

Firmness has been considered a reliable indicator of mango maturity and has been used as a key indicator of when to harvest mangoes (Padda *et al.*, 2011). Firmness decreases as fruits mature and more rapidly as they ripen. This statement was confirmed in this study by the significant ($p < 0.001$) negative and strong correlations between firmness and maturity score. As indicated in chapter 4, firmness reflects the stage of maturity of mangoes. The differences in firmness are probably due to changes in the structure of pectin polymers in the cell wall during the ripening process (Kalra *et al.*, 1995).

Total soluble solid and titratable acidity in fruits are also reliable indicators of maturity. A significant moderate and positive correlation between TSS and maturity score was found in this study. The changes in soluble solids are probably due to the conversion of starch to sucrose, fructose and glucose which also increases the sweetness of fruits (Lechaudel and Joas, 2006). Jha *et al.* (2006) suggested that TSS increases slightly as the fruit matures and increases more after the fruit reaches the mature green stage.

A moderate and negative correlation between TA and maturity score was also found. This finding is similar to that reported by Lechaudel and Joas (2006), indicating that the acidity of the fruit decreases due to the breakdown of acids in the fruit during maturation. Lechaudel and Joas (2006) suggested that the decrease in mango acidity with increasing maturity was associated with a decrease in citric acid. Thus, the moderate and negative correlation between TA and maturity score probably depends on the citric acid concentrations in mangoes.

Another suggested index for maturity is the TSS:TA. A moderate and positive correlation between the TSS:TA ratio and maturity score was observed. Ripe mangoes exhibited a high TSS:TA ratio compared to green ones. This is in agreement with the research of Wanitchang

et al. (2011) who reported a higher TSS and a lower TA content in ripe mangoes. Thus, TSS:TA increases as the mangoes ripen. The TSS:TA is reported to be the best parameter to predict the maturity of mangoes and also to be an excellent indicator of the taste and palatability of fruits (Mahayothee *et al*., 2004).

There have been few reports in the literature on the relationship between moisture content and maturity score of mangoes. However, Padda *et al.* (2011) suggested that the moisture content of mangoes could be a reliable indicator of their maturity and quality during the ripening phase due to the relationship of moisture content with other maturity parameters such as TSS, TA and firmness (Saranwong *et al.*, 2004). This study confirmed this hypothesis by the observed significant ($p < 0.001$) positive and moderate correlation between moisture content and maturity score ($R = 0.577$).

There was also a significant negative and moderate correlation between vitamin C and maturity score. These results are in full agreement with the reports that vitamin C decreases during fruit maturation (Kalra and Tandon, 1983; Toor and Savage, 2005; Abourayya *et al*., 2011).

Normally, colour is an important contributor to the visual appearance of fruit and is often used in many grading standards for fruit quality. Tommy Atkins, which has a red skin colour (reddish blush) due to the anthocyanin, peonidin-3-galactoside (Proctor and Creasy, 1969), develops more colourful peels than other mango cultivars which are green or yellow skinned. Mitcham and McDonald (1992) stated that Tommy Atkins develops a more red (a*) and yellow (b*) peel pigmentation than any other cultivar (as described in chapter 4), and that these pigments contribute to Tommy Atkins being visually more appealing to consumers. However, the peel colour of Tommy Atkins remains unchanged during the ripening and post harvest stages. The results in this research confirm that there was no significant correlation between a* ($p \geq 0.05$) and b* ($p = 0.043$) with maturity score. There were also weak correlations between maturity score and hue angle or L* and a non-significant ($p \geq 0.05$) correlation between maturity score and chroma. These findings are in agreement with Malevski *et al.* (1977) who found that peel colour, as measured with a colorimeter, was an unreliable index of maturity in mangoes (Slaughter, 2009). Padda *et al*. (2011) stated that changes in peel colour are not always correlated with maturity, ripeness or internal eating quality. The latter facts are supported by this research.

There were no significant correlations between the maturity of mangoes with the total fruit weight and the percentages of mango fractions except for kernel. It should be noted however that the percentage of kernel in the whole fruit was higher in ripe than green mangoes.

6.4.1.2. Correlations between physicochemical properties

There were significant correlations between the measured physicochemical properties confirming that some of the measured parameters are reliable indicators of maturity.

In this study, significant ($p < 0.01$) positive and strong correlations were observed between the TSS: TA ratio and TSS or TA which was due to the simultaneous increase in TSS being associated with a decrease in TA.

In addition, there was a significant ($p < 0.01$) negative correlation between firmness and moisture content. This is probably due to the mangoes with a low moisture content having more cell wall material and higher osmotic potential and consequently exhibits higher flesh firmness (Palmer, 2010). An observation in this research was that as TA decreased firmness decreased which agrees with previous reported work on fruits (Kader *et al.,* 1982; Wills *et al.,* 1983; Meredith *et al.,* 1989). This significant correlation was supported by a significant ($p < 0.05$) negative correlation between firmness and the TSS:TA ratio, reflecting the fact that as TSS increases firmness decreases. The results are similar to those on peach reported by Delwiche and Baumgardner (1983).

The moderate relationship between moisture content and TSS:TA is probably due to the high proportion of sugars, starch and organic acids contributing to the dry matter of the fruit.

There are few literature reports on the relationship between titratable acidity and vitamin C in mangoes. There are several acids that can contribute to total acidity such as citric, malic, tartaric, oxalic, a-ketoglutaric and ascorbic acids. However, ascorbic acid is usually present in a very low concentration and does not normally affect the total titratable acidity. Interestingly, in this study, the titratable acidity levels were significantly ($p < 0.001$) correlated to ascorbic acid levels. The vitamin C levels not only showed a very high and significant ($p < 0.001$) correlation with the titratable acidity ($R = 0.85$) but also a significant ($p < 0.05$) and moderate correlation with TSS:TA and moisture content. The results suggest that ascorbic acid has an important role in determining the maturity of mangoes.

Prior to this study, there have been no detailed publications on the relationships between the physicochemical characteristics in mangoes. These studies on the correlations between the

physicochemical properties of mangoes need to be verified across a range of mango cultivars other than Tommy Atkins.

Although peel colour is an unreliable index of maturity in mangoes, there were correlations between some peel colour measurements such as L* with b* and hue angle. These findings indicate that mangoes with a light appearance have either more yellow or more orange-red colour.

6.4.2. Correlations between the antioxidant capacity assays

Most phenolic compounds are hydrophilic antioxidants and secondary metabolites in fruits (Macheix *et al.*, 1990; Thaipong *et al.*, 2006).The observed high correlations between TPC and antioxidant assays indicate that the phenolic compounds in mango flesh, peel and kernel are major contributors to the ABTS radical cation scavenging activity, DPPH radical scavenging activity, FRAP reducing power and the inhibiting oxygen radicals in the hydrophilic ORAC. A positive correlation between the total phenol content and antioxidant capacity in mangoes has been previously reported (Soong and Barlow, 2004; Khammuang and Sarnthima, 2008; Khammuang and Sarnthima, 2011**).** Cam *et al.* (2009) also reported a high correlation between ABTS and DPPH with TPC in pomegranates. A high correlation between TPC and antioxidant capacity, as measured by ABTS and DPPH, has also been found in wine (Paixao *et al.*, 2007). Previous researchers have found that the correlations between antioxidant capacity as measured by ABTS, DPPH, FRAP and ORAC assays were high with the correlation coefficients ranging from 0.68 to 0.97 (Thaipong *et al*., 2006). Connor *et al.* (2002) found a high correlation between ORAC and FRAP in blueberries and Awika *et al.* (2003) high correlations between ORAC, ABTS and DPPH in sorghum.

The results from the present study suggest that phenolic compounds in mango flesh, like other fruits, are the major contributors of ABTS radical cation scavenging activity, DPPH radical scavenging activity, FRAP reducing activity power and hydrophilic ORAC.

The antioxidant activity of polyphenolic compounds depends on the B-ring hydroxyl configuration which is the most significant determinant of the scavenging activity of ROS (Everette and Islam, 2012). The high correlations between Folin–Ciocalteu with the other antioxidant assays indicate that the total phenolic content can be used as indicator for hydrophilic antioxidant capacities of mango flesh, peel and kernel.

ORAC measure antioxidant capacity with H-ORAC and L-ORAC measuring hydrophilic and lipophilic antioxidants, respectively (Huang *et al.*, 2002; Pellegrini *et al.*, 2003; Prior *et al.*,

2003, Huang *et al.,* 2005; Islam *et al.,* 2009; Walker and Everette, 2009). The major constituents responsible for hydrophilic antioxidant capacity are phenolics and anthocyanins and for lipophylic capacity, carotenoids and tocopherols (Teow *et al.,* 2007). Although these assays measured the antioxidant capacity of the same samples, their mechanism of fighting free radicals and oxidants (scavenging radicals or decomposing perosides or chelating metals) are different. Consequently, the results were slightly different (Table 6.3) depending on the sample and the assay conditions. Furthermore, the measured antioxidant activity is often the sum of several antioxidant scavenging free radicals. Thus the reported differences in antioxidant levels in samples and the interactions between antioxidants is often a reflection of assay differences (Everette and Islam, 2012) and consequently they may not actually be correlated to each other.

In this study, the results from assaying flesh, peel and kernel with ABTS, DPPH, FRAP and H-ORAC showed that the assays were positively correlated with each other (p <0.001), except for a significant (p < 0.05) weak correlation between DPPH and H-ORAC with the flesh samples. This suggests that the antioxidant compounds in flesh, peel and kernel that scavenged ABTS, scavenged DPPH, reduced FRAP and inhibited the peroxyl-radical-induced. The strong correlations between the hydrophilic antioxidant levels as measured by ABTS, DPPH, FRAP and H-ORAC indicates that all these assays have similar capacity to predict the antioxidant capacities of mango flesh, peel and kernel.

These findings are similar to those of Everette and Islam (2012) who found a significant correlation between ABTS, DPPH and hydrophilic ORAC but contrast to the studies by Ou *et al.* (2002) and Huang *et al.* (2002), who found there was no agreement between the antioxidant assays.

In general, in this research, TPC, ABTS, DPPH, FRAP and H-ORAC assays in flesh, peel and kernel were significantly (p < 0.001) positively correlated to each other. The correlations between the antioxidant assays were higher in the kernel than peel than flesh. It should be noted that the strong correlations between the five assays TPC, ABTS, DPPH, FRAP and H-ORAC assays were strong in kernel and between the four assays TPC, ABTS, DPPH and FRAP were strong in flesh and peel. These differences could be due to the differences in extraction procedures as TPC, ABTS, DPPH, FRAP use a very simple 80% acetone extraction procedure and ORAC use a procedure based on acetone/water/acetic acid (70:29.5:0.5).

In this study, the antioxidant capacity was determined for both hydrophilic and lipophilic compounds. Normally, only hydrophilic antioxidants are analysed due to their high contribution to the total antioxidant capacity of mangoes (Kabuki *et al.*, 2000; Wu *et al*., 2004; Ajila *et al.*, 2007; Gonzalez-Aguilar *et al.*, 2007; Kim *et al.*, 2007; Rocha Ribeiro *et al.*, 2007; Barreto *et al.*, 2008; Masibo and He, 2008; Manthey and Perkins-Veazie, 2009; Pitchaon, 2009; Robles-Sanchez *et al.*, 2009; Matsusaka and Kawabata, 2010; Khammuang and Sarnthima, 2011; Ma *et al*., 2011). Robles-Sanchez *et al.* (2009) measured antioxidant capacity of "Ataulfo" mangoes for the first time using both H-ORAC and L-ORAC and the total antioxidant activity was measured by adding H-ORAC and L-ORAC. The study suggested that L-ORAC contributed only 1% to the total antioxidant capacity which is comparable to those results obtained by Wu *et al.* (2004) who reported 988 μmol TE per 100 g FW and 14 μmol TE per 100 g FW for H-ORAC and L-ORAC, respectively.

This is the first study in which both hydrophilic and lipophilic antioxidant activites of different mango fractions were measured and the correlations between the hydrophilic and lipophilic antioxidants in mango flesh, peel and kernel were determined. The lipophilic antioxidant capacity in all the mango flesh, peel and kernel fractions were much lower than the hydrophilic capacity. However, it should be noted that the lipophilic antioxidants generally have a greater bioactivity than hydrophilic antioxidants because of their ability to pass more effectively through lipoprotein cell membranes (Huang *et al*., 2002; Davis and Auten, 2010). There are several lipophilic antioxidants (such as carotenoid, tocopherol and phospholipids) found in flesh, peel and kernel. Another explanation for the significant ($p < 0.05$) correlation between the lipophilic and hydrophilic antioxidant capacity in peel is the differences in the composition and concentration of the lipophilic antioxidants in peel compared to flesh and kernel (Ajila *et al.*, 2007b). To understand these differences more detailed analyses of the specific lipophilic antioxidant compounds in mango fractions is required in the future.

6.4.3. Repeatability of antioxidant assays

It is clear that no single antioxidant assay can truly reflect the total antioxidant activity of a particular food (Prior *et al*., 2005) due to the chemical diversity of antioxidants in foods. Therefore, it will be misleading to make a comparison using one assay only for assessing the antioxidant activity of mangoes. Nevertheless, the most appropriate assay method would be that with good correlation with the other assays (as described in 6.3.2) and which is repeatable. The assay with the minimum coefficients of variation in the flesh, peel and kernel

fractions was the TPC assay and that with the maximum coefficients of variation was the L-ORAC assay. The coefficients of variation for TPC assays in flesh, peel and kernel were 2.4, 1.6 and 2.4%, respectively. There were low coefficients of variation for the FRAP assay of flesh (3.2%) and peel (2.1%) and the ABTS assay of kernel (3.8%).

In general, all the assays showed good repeatability with the coefficients of variation ranging from 1.6 to 9.4%. The TPC assay for flesh, peel and kernel, the FRAP assay for flesh and peel and ABTS for kernel all yielded high repeatability and could be used to determine the antioxidant capacity in mango fractions with confidence. The strong correlations between the TPC, ABTS and FRAP assays applied to the flesh, peel and kernel fractions (investigated in 6.3.2.) confirmed the efficiency and effectiveness of these antioxidant assay methods for determining the antioxidant capacity of the mango fractions. Furthermore, these three assay methods are suitable for aqueous antioxidants and are reliable, fast, and relatively simple to use.

Although all the antioxidant assays showed good results in terms of their significant to moderate to strong correlations the three antioxidant assays TPC, ABTS and FRAP are the most suitable methods to determine the antioxidant capacity of mango flesh, peel and kernel.

6.4.4. Correlations between physicochemical properties and antioxidant activities

In section 6.3.1, significant correlations between physicochemical properties such as firmness, TSS, TS, TSS:TA, vitamin C, moisture content were found. These parameters are considered good indicators of mango maturity. There were only a few significant ($p < 0.05$) weak correlations in flesh and peel and some significant ($p < 0.05$) weak-to-moderate correlations in kernel that were linked to maturity as expressed through physicochemical parameters and antioxidant capacities. Nevertheless, the results do clearly demonstrate that some of the phenolic compounds and antioxidant compounds are affected by maturation and ripening.

The results also revealed that mango flesh with either a low TSS or TSS:TA ratio or high vitamin C exhibited relatively high FRAP values. In peel, the mangoes that showed low TA or vitamin C contained relatively high lipophilic antioxidants. However, these specific relationships were not observed by principal component analysis. The kernels from less ripe mangoes showed low maturity scores, TSS, TSS:TA ratio and high TA, firmness and exhibited relatively high TPC and antioxidant capacity as measured by all the assays, except for L-ORAC. The mangoes kernels with high vitamin C also showed relatively high TPC and antioxidant capacities as measured by ABTS and DPPH.

However, the correlations between maturity and antioxidant capacities were different between the flesh, peel and kernel fractions with some parameters showing correlations in one fraction and not in another. There have been very few reports on the correlations between maturity and the antioxidant activities in mango fractions indicating that the total phenolic concentrations of mango fruits or other fruits decrease during ripening. For example, Celik *et al.* (2008) stated that the green stage of cranberry fruits had the highest antioxidant capacity. Furthermore, the DPPH values of *Mangifera pajang* Kosterm fruit pulp and fruit juice powder extracts were strongly correlated with ascorbic acid levels ($R = 0.97$, $p < 0.01$) as reported by Ibrahim *et al.*(2010). In this research the total antioxidant capacity as measured by TPC, ABTS and DPPH in kernels were high compared with the contribution of Vitamin C to antioxidant capacity.

Normally, colour is one of the most important indicators of maturity (Drake *et al.,* 1982) and is mainly influenced by the concentration and distribution of various compounds such as anthocyanins, carotenoids, flavonoidss, betalains, and chlorophylls in peel (Gao and Mazza, 1995). It is often noted that during fruit ripening the increase of antioxidants is often accompanied by sweetness improvements, fruit softening and a decrease in tartness and astringency particularly in fruit seeds. Antioxidant pigments are therefore a visual indicator of the quality of fruit fractions (Kalt *et al*., 2004). In contrast, these same pigments can act as scavengers of radical species (Wang *et al.,* 1997) and contribute to antioxidant activity (Rapisarda *et al.,* 1999; Kasım *et al*., 2011).

From the results in 6.3.2, the research showed that colour is not a good indicator of maturity as indicated by the significant correlations between maturity score with a* ($p \geq 0.05$) and b* ($p = 0.043$), respectively. The Tommy Atkins mangoes which had red skin colours (reddish blush) due to the anthocyanin, peonidin-3-galactoside (Proctor and Creasy, 1969) maintained their colour during the storage period of the experiments. In this study there was no significant correlation between colour and the antioxidant capacities of flesh, peel or kernel. More studies are required across a variety of mangoes to determine the correlations between the maturity of mangoes and antioxidant activities of flesh, peel and kernel.

Interestingly a significant ($p < 0.05$) correlation was identified between the percentages of mango flesh, peel and kernel with antioxidant capacity. Mangoes with a high percentage of flesh or low percentage of peel exhibited high antioxidant capacity in flesh and peel. Those mangoes with a low percentage of kernel or high percentage of peel exhibited high antioxidant capacity in kernel. These results suggest that mangoes with a high percentage of

flesh and low percentages of peel and kernel yield the highest levels of antioxidant capacity. However, these correlations were quite low and further works need to be done to support these findings.

6.5. Conclusions

- The results from the Pearson's correlation analysis (6.3.1) are in agreement with those obtained by principal component analysis (6.3.2) in all the mango fractions from Tommy Atkins cultivar.

- In regards to the relationship between the various physicochemical properties, the similarity between the results from Pearson's correlation analysis and PCA strongly support that the physicochemical characteristics such as firmness, total soluble solid (TSS), titratable acidity (TA), TSS:TA ratio, vitamin C and moisture content are strongly correlated with the maturity stage of the Tommy Atkins mangoes. Surprisingly, despite the important role of the visual appearance of mangoes attracting consumers, colour is not a good indicator of maturity for Tommy Atkins. The significant and strong correlations between the various physicochemical properties suggest that one particular physicochemical parameter can reflect the characteristic of one other parameter. Thus, with the measurement of any parameter, it is possible to accurately determine the maturity of the fruit of Tommy Atkins. The data also demonstrate the relationship between the physicochemical properties of mangoes with the ripening stages.

- The correlations between the antioxidant capacity assays and between TPC and the antioxidant capacity assays in flesh, peel and kernel using PCA were similar to the conclusions from the Pearson's correlation analysis in section 6.3.2 and 6.4.2. The strong correlations between TPC with antioxidant levels as measured by ABTS, DPPH, FRAP and H-ORAC indicates that phenolic compounds in mango flesh, peel and kernel are good sources of antioxidants. Moreover, the strong correlations between the antioxidant assays including TPC suggest that one assay can be selected to monitor the antioxidant capacity of mango flesh or peel or kernel. In particular, the strongest correlation was between ABTS and FRAP in flesh, between ABTS, DPPH and FRAP in peel and between all the antioxidant assays, except for L-ORAC, in kernel. In addition, weak-to-moderate correlations were found between the lipophilic antioxidants (L-ORAC) and hydrophilic antioxidants in mango peel. To understand

these differences in more detail, further investigations and analyses of specific lipophilic antioxidant compounds in mango fractions should be carried out across a wide variety of mangoes.

- The correlations between the maturity properties and antioxidant capacities varied in flesh, peel and kernel. From Pearson's correlation analysis the results revealed that mango flesh with either low TSS or TSS:TA ratio or high vitamin C exhibited relatively high FRAP values. Mango peel had low TA or vitamin C and exhibited relatively high lipophilic antioxidants. However, these specific relationships were not observed by the principal component analysis. In contrast, in mango kernel, the correlations between the physicochemical properties and antioxidant capacities were similar using both Pearson's correlation analysis and principal component analysis. Kernels from the less ripe mangoes which had low maturity scores, TSS, TSS:TA ratio and high TA and firmness were relatively high in TPC and antioxidant capacity as measured by all the assays, except L-ORAC. The kernel of mangoes with high vitamin C also showed relatively high TPC and antioxidant capacities as measured by ABTS and DPPH.

- When considering the correlations between maturity as assessed by physicochemical parameters and antioxidant capacities there were only a few significant ($p < 0.05$) weak correlations in flesh and peel and one significant ($p < 0.05$) weak-to-moderate correlations in kernel. The results demonstrate that some of the phenolic compounds and antioxidant compounds in kernel are affected by the maturity stages.

- Furthermore, both Pearson's correlation and PCA suggest that the mangoes with a high percentage of flesh and a low percentage of peel and kernel tend to have the highest antioxidant capacity in the flesh and peel but not the kernel. The correlations are weak-to-moderate but it would be useful tool to estimate the antioxidant capacity of kernel by using the outside physicochemical characteristics of the mango fruit.

CHAPTER 7
ANTIOXIDATIVE EFFECTS OF MANGO KERNEL AND PEEL ON SHELF-LIFE OF PORK PRODUCTS

7.1. Introduction

Supermarkets and consumers require good quality meat throughout the shelf life of the product. Any technique to extend the shelf life of fresh meat or meat products is very important to the food industry. Techniques to extend shelf life often involve limiting the lipid oxidation or microbiological contamination of the product. Lipid oxidation is a major contributor to the deterioration in meat quality during refrigerated storage and is often associated with colour and odour changes that directly affect the consumer acceptance of meat products.

Meat colour is a strong visual indicator that influences the selection of food by consumers at the point of purchase and is influenced by pre-slaughter factors such as the species, age of animals, sex, diet and exercise and post-slaughter by the oxidative state of the pigment myoglobin. Oxymyoglobin (MbO_2) is myoglobin bound to oxygen and is bright pink (red) in normal fresh pork. Deoxymyoglobin (Mb) is myoglobin without oxygen and has a purplish colour in vacuum packaged pork. Metmyoglobin (MMb) is formed from the oxidation of the iron in myoglobin. It has a brownish colour which consumers find undesirable. Ferric haem pigments in meat can promote the oxidation of tissue lipids (Greene, 1969) which produces free radicals that subsequently decompose haem and cause discoloration (Haurowitz *et al.*, 1941; Greene, 1969).

The development of oxidative off-flavour (rancidity) is also a serious problem during the storage of meat products (Gray *et al.*, 1996). Lipid oxidation is responsible for the breakdown of fatty acids to produce volatile odour compounds that contribute to rancidity and undesirable flavours (Mottram, 1987). The autoxidation of lipids can also occur in raw meat at room temperature or under refrigeration and produce off-flavours (Farmer, 1994). Warmed over flavour (WOF) often refers to the characteristic off-flavour that develops in cooked or raw meat (Sato and Hegarty, 1971; Pearson *et al.*, 1977; Pearson and Dutson, 1994; O'Sullivan *et al.*, 2003). Numerous volatile compounds contribute to the smell and taste of food and can now be analysed by a sophisticated instrument system such as HS-SPME-GC-MS. However, the correlation between the analytical profile of volatile

compounds and odour perception by consumers is not strong due to the interactions that can occur between the odour components. Thus the aroma assessment of pork products by human sensory analysis is advisable.

Pork products, due to their relatively high content of unsaturated fatty acids, oxidise more rapidly than beef or lamb. Pork products manufactured from minced meat, such as pork patties and sausages are particularly prone to lipid oxidative changes that ultimately result in the products turning brown, becoming rancid and producing off-odours.

Recently, the undesirable changes in lipid oxidation have been reduced by adding synthetic or natural antioxidants that have the ability to neutralise free radicals in processed foods. Currently, there is a market trend to utilise natural antioxidants as food additives because of observed safety and toxicity problems associated with synthetic antioxidants such as BHA, BHT and TBHQ (Buxiang and Fukuhara, 1997; Jo *et al.*, 2006). Mango seed kernel and peel have been suggested as potential sources of natural antioxidants since they contain polyphenols, anthocyanin, carotenoid, vitamin C and vitamin E (Soong, *et al.*, 2004; Berardini *et al.*, 2005; Ajila *et al.*, 2007). There is, however, no data on the effects of mango peel and kernel on lipid oxidation associated with colour and odour changes in pork products stored refrigerated.

The objective of the present study was to assess the antioxidant effects of mango peel and kernel on the colour, odour and lipid oxidative stability of pork sausages and patties during refrigerated storage. BHT supplemented sausages and patties were used as commercial synthetic supplemented controls and pork sausages and patties without any antioxidants were also used as controls. The pork products were tested for microbiological safety as microbial spoilage is a primary factor associated with meat quality.

7.2. Materials and methods

7.2.1. Chemicals and reagents

Chemicals and reagents were used as listed in 3.16.

7.2.2. Preparation of mango peel and kernel additives

Mango peel and kernel from Catchu cultivar were used as antioxidant additives in thi study since they showed a high antioxidant capacity among the Vietnamese mangoes. Peel and

kernel were freeze-dried and ground into fine powder and then stored in the freezer at -40^{o}C before use.

7.2.3. Preparation of pork sausages and patties

Pork patties and sausages were prepared and manufactured as described in 3.8. Antioxidant additives (mango kernel, peel and BHT) were added (at day 0) into the pork mince for making sausages and patties.

7.2.4. Proximate analysis

Fat, protein and moisture content of pork mince were determined by the methods described in 3.9.

7.2.5. TBARS

Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS) in pork and pork products by Maraschiello *et al.* (1999) as described in 3.10. TBARS values of pork products with 3 different fat contents (high, medium and low) were determined.

7.2.6. Colour

Colour measurements were made on the surface of raw pork patties with a Hunter colorimeter (Hunter Associated Labs., Inc. Reston, VA). The method of colour measurement was described in 3.12. Colour of pork products with 3 different fat contents (high, medium and low) was measured.

7.2.7. Myoglobin

Myoglobin (deoxymyoglobin [Mb]), oxymyoglobin (MbO_2) and metmyoglobin (MMb) in stored raw pork patties and sausages were determined using the procedure of Krzywicki (1982). Only pork products with high fat content were used for determining myoglobin.

7.2.8. Volatiles

The volatile compounds in minced pork products were determined using a HS-SPME-GC-MS technique as reported by Ramirez *et al.* (2004) with some modifications as described in 3.1.3. Only pork products with high fat content were used for measuring volatiles. The pork sausages with and without antioxidant additives (mango kernel and BHT) were measured at day 0 and day 4.

7.2.9. Odour

Odour acceptability was assessed by the researcher using a 5-point hedonic scale as described in 3.12 (Das *et al.*, 2011). Pork products with high fat content were used for evaluating odour.

7.2.10. Microbiology

Microbiological characteristics of mango peel, kernel and pork products (sausages and patties) with high fat content were determined by Hills Laboratories, New Zealand as outlined in 3.15.

7.2.11. Statistical analysis

One-way, two-way, three-way ANOVA, interaction effects and PCA were performed as described in 3.17.

7.3. Results

7.3.1. Proximate composition

Table 7.1 shows the proximate composition of fresh pork mince at three fat levels: low, medium and high. The fat content of the low, medium and high fat pork mince were 9.1, 22.2 and 47.5% DW, respectively. The crude protein contents were was 89.0, 76.4 and 51.9 %, DW respectively. The moisture contents of the low fat, medium and high fat pork mince were 75.2, 73.9 and 65.1%, respectively.

Table 7.1. Composition analysis of pork minces.

Composition	% Moisture (FW)	% Crude Protein (DW)	% Crude Protein (FW)	% Fat (DW)	% Fat (FW)
Low fat mince	75.2	89.0	22.1	9.1	2.3
Medium fat mince	73.9	76.4	19.9	22.2	5.8
High fat mince	65.1	51.9	18.1	47.4	16.5

DW: dry weight; FW: fresh weight

7.3.2. TBARS

7.3.2.1. Pork sausages

In order to determine the effects of the three factors, antioxidant treatment, storage time and fat content, on lipid oxidation, measured by TBARS, the interactions between the three factors were analysed by a three-way ANOVA. Analysis of variance of TBARS values for pork sausages stored over 10 days showed significant ($p < 0.05$) effects for the three-factor

interactions as well as significant ($p < 0.001$) effects for the two-factor interactions and the three main factors (Table 7.2). In this case, only the three-factor interactions were examined and interpreted while ignoring the two-factor interactions and main effects. Two methods were used to interpret the three-factor interactions between antioxidant treatment, storage time and fat content. Firstly, the three-way analysis was divided into a two-way analysis of antioxidant treatment and storage time and was tested at each level for the third factor fat content (high, medium and low). Secondly, the results of three-way analysis were confirmed by an interaction plot between three factors; antioxidant treatment, storage time and fat content. From this plot, all the statements regarding the effects on TBARS were given in regards to the interactions between antioxidant treatment, storage time and fat content.

Table 7.2. Three-way ANOVA statistical analysis using General Linear Model for TBARS values of pork sausages influenced by treatment, storage time and fat content.

Source of variance	Degree of freedom	*p*-value
Storage	5	< 0.001
Treatments	3	< 0.001
Fat content	2	< 0.001
Storage x Treatments	15	< 0.001
Storage x Fat content	10	< 0.001
Treatments x Fat content	6	< 0.001
Storage x Treatments x Fat content	30	< 0.05

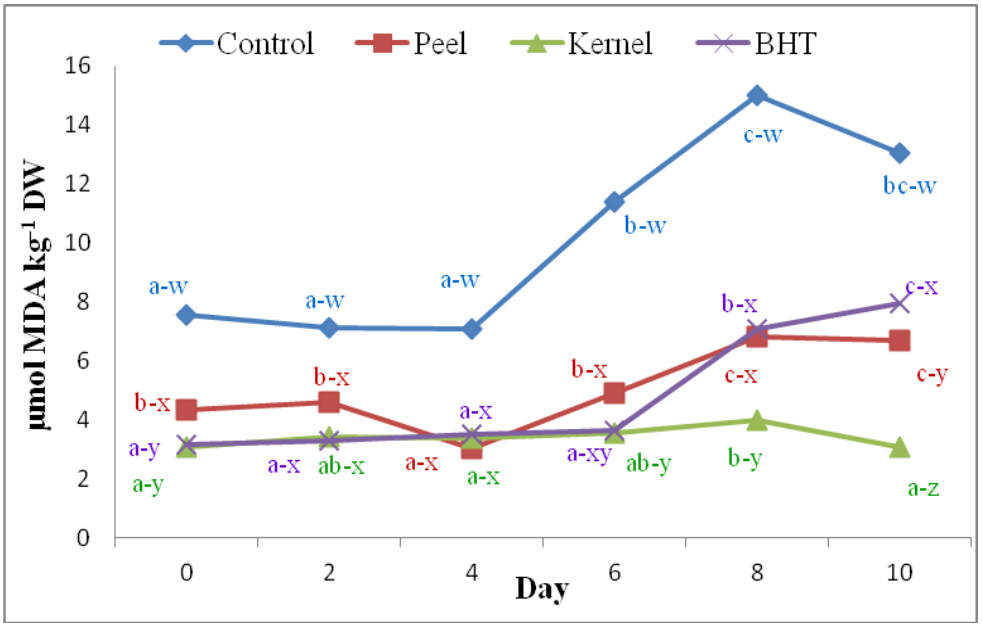
Treatments: control, kernel added, peel added and BHT added.
Storage: day 0, 2, 4, 6, 8 and 10. Fat content: low, medium and high.

7.3.2.1.1. Three-factor interaction effects evaluated by two-factor analyses

a. Pork sausages with low fat content

The addition of mango kernel or peel or BHT to the low fat sausages was an effective antioxidant treatment as illustrated by the significantly lower TBARS values in the treated sausages compared to the controls over the 10 day storage period (Figure 7.1). At day zero TBARS values of the low fat sausages with added kernel or peel or BHT were lower than the control sausages. TBARS increased significantly at day 6 and continued to slightly increase to day 10 compared to day zero (7.6 µmol MDA kg^{-1} DW in low fat) in the control sausages without any antioxidants whilst increases in the sausages treated with peel or BHT were only observed at days 8 and 10.

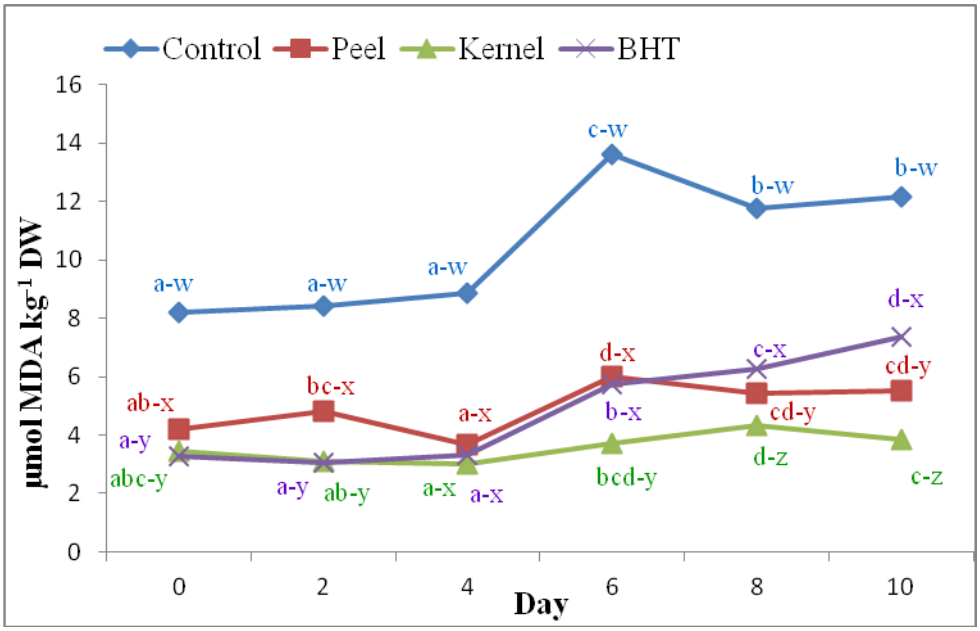
Figure 7.1. TBARS values of low fat (9.1%) pork sausages (n = 3) over 10 day storage.



Different letters (w-z) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units:µmol MDA/kg DW.

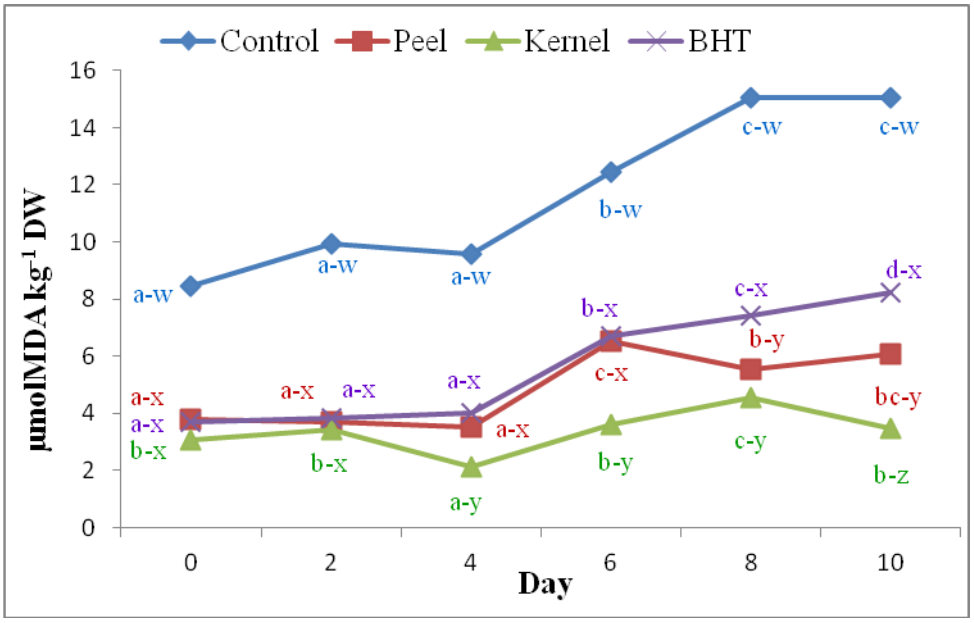
The sausages treated with peel slightly increased at day 8 then stabilised at day 10 whilst those treated with BHT tended to gradually increase up to day 10. In contrast, those sausages treated with kernel showed no significant changes over the 10 day storage period, except for day 8, relative to day zero. At day 10, TBARS were the highest in the control sausages (13.0 µmol MDA kg^{-1} DW in low fat), followed by those treated with BHT (6.3 µmol MDA kg^{-1} DW in low fat), peel (6.7 µmol MDA kg^{-1} DW in low fat) and kernel (3.1 µmol MDA kg^{-1} DW in low fat).

Figure 7.2. TBARS values of medium (22.2%) fat pork sausages (n = 3) over 10 day storage.



Different letters (w-z) within the same storage day are significantly different ($p < 0.05$). Different letters (a-d) within the same treatment are significantly different ($p < 0.05$). Units:µmol MDA/kg DW.

Figure 7.3. TBARS values of high fat (47.4%) pork sausages (n= 3) over 10 day storage.



Different letters (w-z) within the same storage day are significantly different ($p < 0.05$). Different letters (a-d) within the same treatment are significantly different ($p < 0.05$). Units:µmol MDA/kg DW.

b. Pork sausages with medium and high fat contents

The results were similar to those obtained with the low fat pork sausages. Namely, the TBARS were significantly lower in the medium or high fat sausages supplemented with

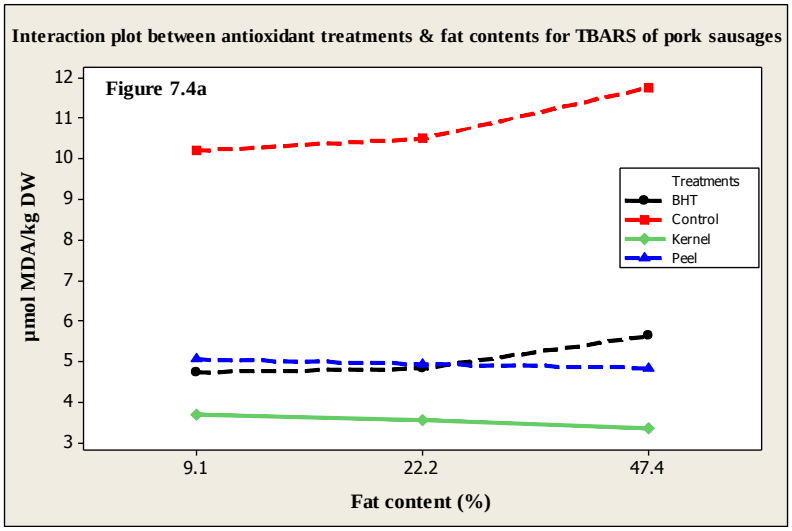
mango kernel or peel or BHT than the control sausages over the 10 day storage period (Figure 7.2 and 7.3). At day zero the TBARS values of both the medium and high fat sausages treated with kernel or peel or BHT were lower than the control sausages (8.2 and 8.5 µmol MDA kg^{-1} DW in medium and high fat, respectively). However, in the medium and high fat sausages at day 6, TBARS increased significantly compared to day zero in the control sausages and in the sausages treated with mango peel or BHT.

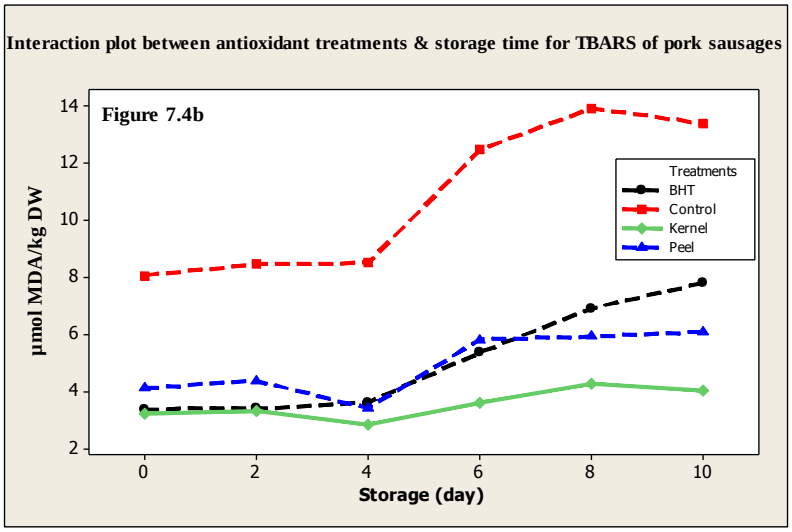
Similar to the low fat sausage results, the medium or high fat sausages supplemented with kernel showed no significant changes in TBARS over the 10 day storage period, except for day 8, relative to day zero. However, unlike the low fat sausages, those treated with peel stabilised their TBARS from day 6 to 10 whilst in those sausages treated with BHT, TBARS gradually increased from day 6 to 10. At day 10, TBARS values were highest in the control sausages (12.1 and 15.0 µmol MDA kg^{-1} DW in medium and high fat, respectively), followed by those treated with BHT (7.4 and 8.2 µmol MDA kg^{-1} DW in medium and high fat, respectively) then peel (5.5 and 6.1 µmol MDA kg^{-1} DW in medium and high fat, respectively) then kernel (3.8 and 3.5 µmol MDA kg^{-1} DW in medium and high fat, respectively).

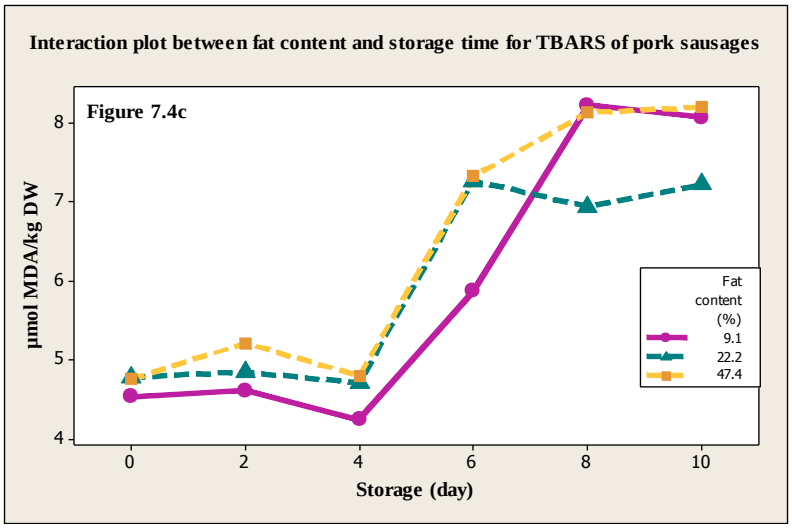
7.3.2.1.2. Three-factor interaction effects evaluated by the interaction plot

The results are illustrated by the interaction plots in Figure 7.4a, 7.4b and 7.4c. Figure 7.4a shows that the addition of the antioxidants to pork sausages, in each instance maintained TBARS, regardless of fat content over the 10 day storage period. The supplementation with kernel showed the highest antioxidative effect, followed by peel or BHT compared to the control sausages (Figure 7.4b), regardless of fat content (Figure 7.4a). However, the fat content of the sausages slightly influenced the TBARS values at day 6, onwards when the effects of kernel on maintaining TBARS values were more pronounced than peel or BHT (Figure 7.4b) in the medium and high fat sausages (Figure 7.4c).

Figure 7.4. Interaction effects of treatment, fat content and storage time on TBARS values of pork sausages.







7.3.2.2. Patties

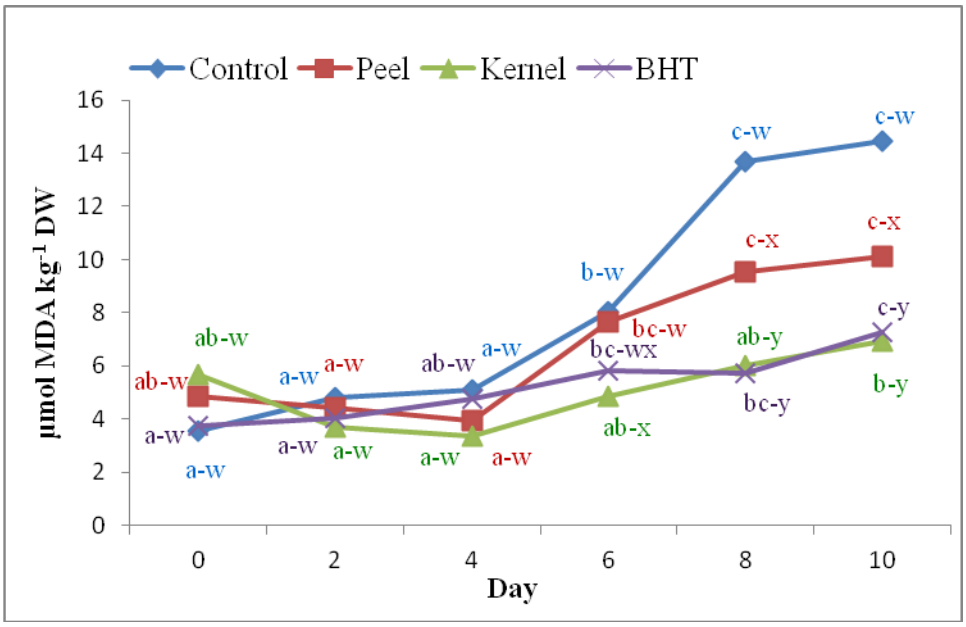
The three-way ANOVA of TBARS data for pork patties with different fat content, with and without antioxidants and stored for up to 10 days showed there were significant ($p < 0.05$) effects for the three-factor interactions as well as significant ($p < 0.001$) effects for the two-factor interactions and the three main factors (Table 7.3). The three-factor interactions on the TBARS of pork patties was evaluated and interpreted whilst ignoring the two-factor interactions and main effects. The effects of antioxidant treatment and storage time on the TBARS of pork patties were determined using a two-way ANOVA at each level for the third factor fat content (low, medium and high). Then the results were confirmed by the interaction plots between the three factors.

Table 7.3. Three-way ANOVA statistical analysis using General Linear Model for TBARS values of pork patties influenced by treatment, fat content and storage time.

Source of variance	Degree of freedom	*p*-value
Storage	5	< 0.001
Treatments	4	< 0.001
Fat content	2	< 0.001
Storage x Treatments	20	< 0.001
Storage x Fat content	10	< 0.001
Treatments x Fat content	8	< 0.001
Storage x Treatments x Fat content	40	< 0.01

Treatments: control, kernel added, peel added and BHT added.
Storage: day 0, 2, 4, 6, 8 and 10. Fat content: low, medium and high.

Figure 7.5. TBARS values of low fat (9.1%) pork patties (n = 3) over 10 day storage.



Different letters (w-y) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units: µmol MDA/kg DW.

Figure 7.6. TBARS values of medium fat (22.2%) pork patties (n = 3) over 10 day storage.

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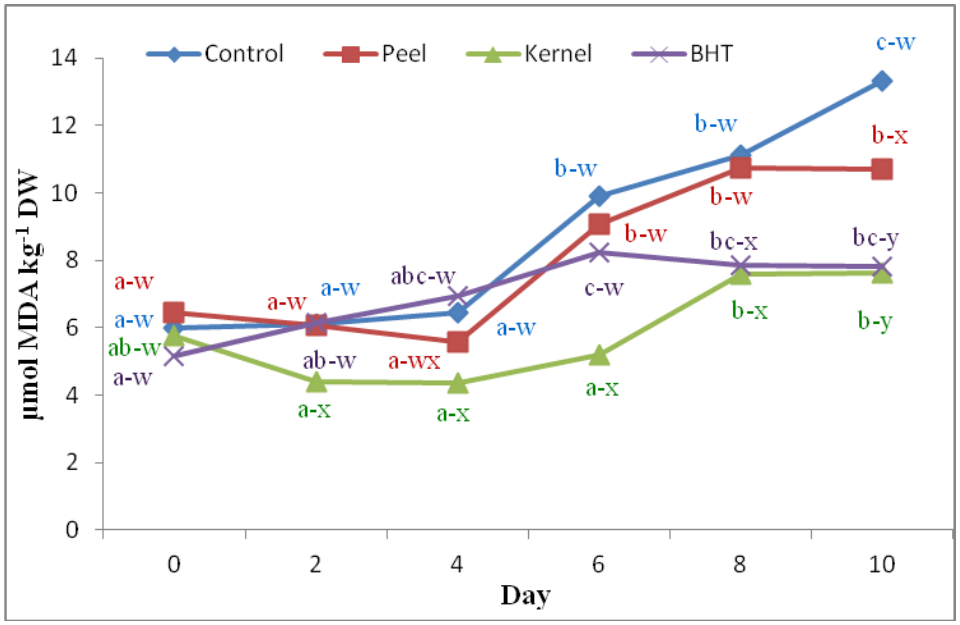

Different letters (w-y) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units: µmol MDA/kg DW.

Figure 7.7. TBARS values of high fat (47.4%) pork patties (n = 3) over 10 day storage.

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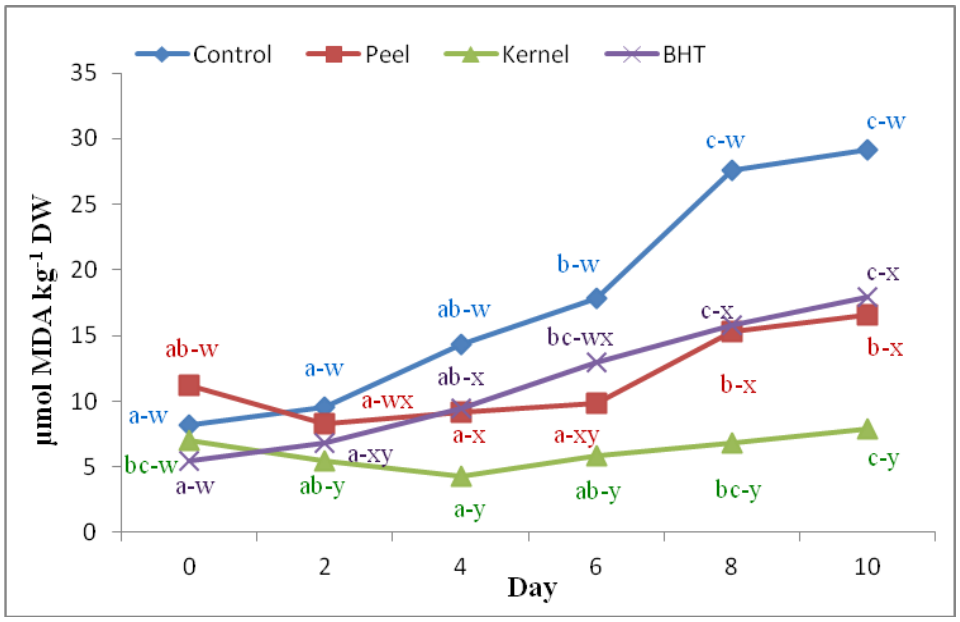


Different letters (w-y) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units: µmol MDA/kg DW.

7.3.2.2.1. Three-factor interaction effects evaluated by two-factor analyses

a. Pork patties with low (Figure 7.5) and medium (Figure 7.6) fat content

At day zero the TBARS values of the control patties and treated patties were similar to each other, regardless of fat content. Over the 10 days of storage, the low and medium fat patties showed similar TBARS results which were influenced by the antioxidant treatments and storage times. The TBARS values of both the low and medium fat patties at day 6 increased significantly when compared to day zero in the control patties (3.6 and 6.0 µmol MDA kg^{-1} DW in low and medium fat, respectively) and those containing either peel or BHT. In contrast, those patties with low and medium fat content treated with kernel showed no significant changes over the 10 day storage period relative to day zero. At day 8, the control patties significantly (p <0.05) increased their TBARS whilst those treated with antioxidants stabilised their TBARS values for the remainder of storage period. At day 10, TBARS values were the highest in the control patties (14.5 and 12.3 µmol MDA kg^{-1} DW in low and medium fat, respectively), followed by those treated with peel (10.1 and 10.7 µmol MDA kg^{-1} DW in low and medium fat, respectively) then BHT (7.3 and 7.8 µmol MDA kg^{-1} DW in low and medium fat, respectively) or kernel (6.9 and 7.6 µmol MDA kg^{-1} DW in low and medium fat, respectively).

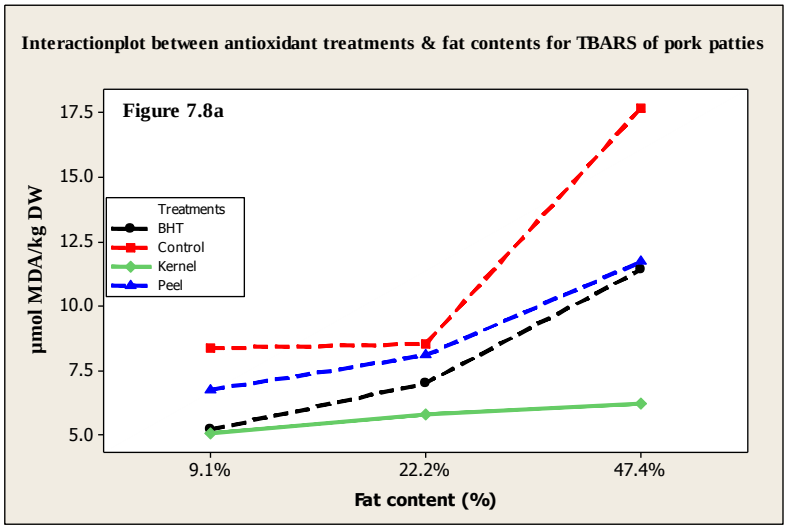
b. Pork patties with high (Figure 7.7) fat content

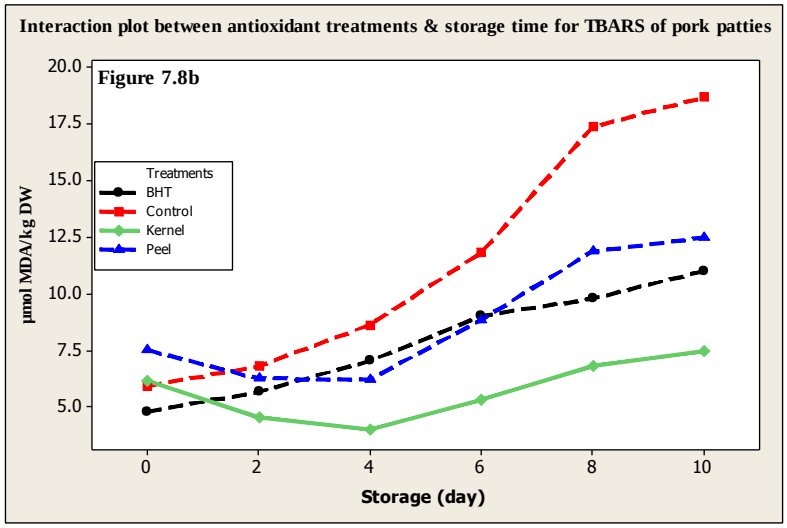
Unlike the low and medium fat patties, TBARS increased significantly in the control high fat patties at day 4 of the storage compared to day zero (8.2 µmol MDA kg^{-1} DW in high fat). Increases in TBARS also occurred in the patties with added BHT at day 6. In contrast, those patties with added kernel or peel showed no significant changes over the 10 day storage period relative to day zero. The patties with added peel had significantly higher TBARS than those with added kernel across the storage period, except for days 0, 2 and 6. From day 6, the TBARS values dramatically increased in the control patties and remained stabilised in those patties with added antioxidants for the remainder of the storage period. At day 10, TBARS values were highest in the control patties (29.2 µmol MDA kg^{-1} DW in high fat), followed by those treated with peel (16.6 µmol MDA kg^{-1} DW in high fat) or BHT (17.9 µmol MDA kg^{-1} DW in high fat) and then kernel (7.9 µmol MDA kg^{-1} DW in high fat).

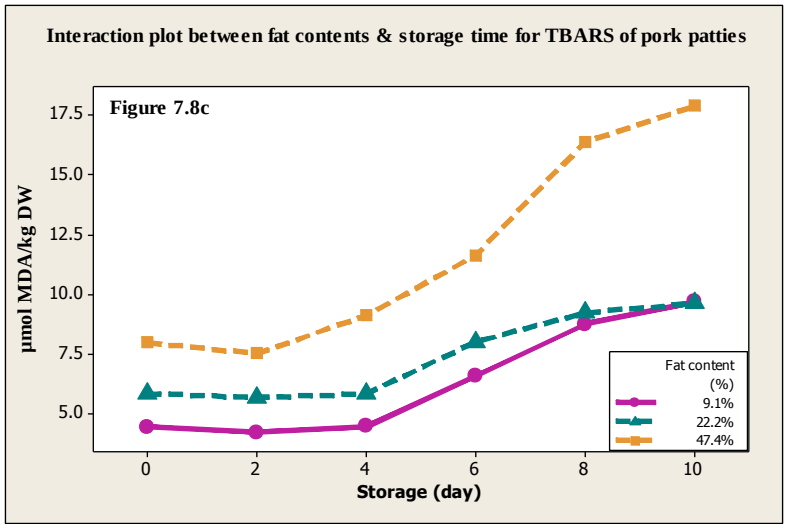
7.3.2.2.2. Three-factor interaction effects evaluated by the interaction plot

The interaction results are illustrated and confirmed by the interaction plots in Figure 7.8a, 7.8b and 7.8c which show the effects of the three antioxidant treatments on reducing the TBARS of pork patties after 4 days of storage, particularly those patties with a high fat content (Figure 7.8a and 7.8b). At some stages of storage e.g. at day 4 (Figure 7.8c), the more superior effects of kernel on reducing TBARS values compared to peel or BHT were more pronounced as illustrated in the high fat patties (Figure 7.8a).

Figure 7.8a, 7.8b and 7.8c. Interaction effects of treatments, fat content and storage time on TBARS values of pork patties.







From day 6 to day 10 of storage, kernel provided the highest antioxidative effects, followed by BHT then peel compared to controls (Figure 7.8b) but the differences between the antioxidant treatments were dependent on the patty fat content (Figure 7.8a).

7.3.3. Colour

A three-way ANOVA was used to determine possible interactions between the three factors antioxidant treatment, storage time and fat content as well as the effects of these factors. The interaction plots were used to examine and interpret the results. The main effects of antioxidant treatment, storage time and fat content and their interactions for the colour characteristics (a* values and hue angle value) of pork sausages and patties are shown in Table 7.4, 7.5, 7.6 and 7.7.

7.3.3.1. Sausages

7.3.3.1.1. a* values

Analysis of variance of the a* values (redness) (Table 7.4.) for pork sausages with different fat contents and antioxidant treatments during storage for 10 days showed there were no statistical significant ($p \geq 0.05$) interactions between the three-factors or any two-factor interactions, except for a significant ($p < 0.01$) interaction between treatment and storage time. Thus, for the a* values of pork sausages, only the interaction between treatment and storage time and the main effect (fat content) were analysed. The interaction was analysed using two-way ANOVA and the results showed that the differences in a* value (redness) between the control sausages and those receiving antioxidant treatments were dependent on storage time (Figure 7.9). The fat content at three different levels (low, medium and high) were analysed using one-way ANOVA.

Table 7.4. Three-way ANOVA statistical analysis using General Linear Model for a* values of pork sausages influenced by treatment, fat content and storage time.

Source of variance	Degree of freedom	*p*-value
Storage	5	< 0.0001
Treatments	3	< 0.0001
Fat content	2	< 0.0001
Storage x Treatments	15	< 0.01
Storage x Fat content	10	> 0.05
Treatments x Fat content	6	> 0.05
Storage x Treatments x Fat content	30	> 0.05

Treatments: control, kernel added, peel added and BHT added.
Storage: day 0, 2, 4, 6, 8 and 10. Fat content: low, medium and high.

a. **Treatment and storage time interaction (Figure 7.9a)**

All the sausages decreased their a* values from day 0 to day 6. There was a rapid decline in the a* values of the control sausages, particularly at day 2. Indeed, the control sausages tended to continually decrease a* values to day 10 whilst the sausages with additives, particularly those with kernel, tended to stabilise a* up to day 10, except for day 8.

b. **Significant main effect (Fat content) (Figure 7.9b)**

The medium fat sausages had higher a* values than the low or high fat sausages regardless of treatment and storage time.

Figure 7.9a. Interaction effects of antioxidant treatment and storage time on a* values of pork sausages.

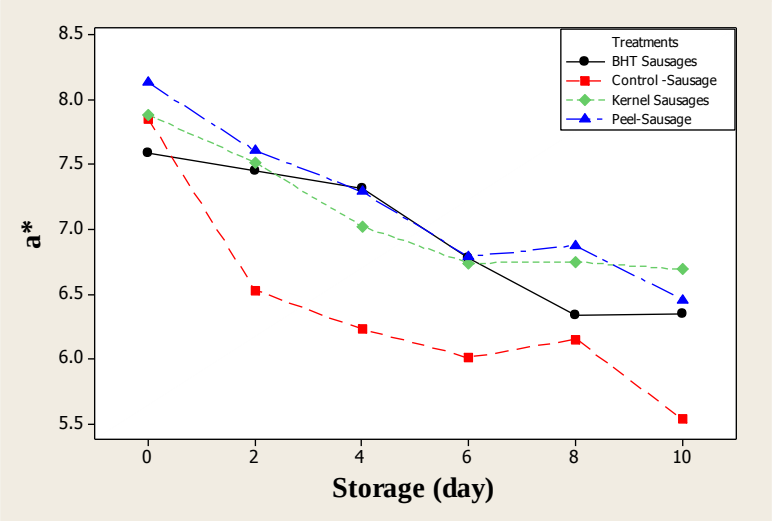
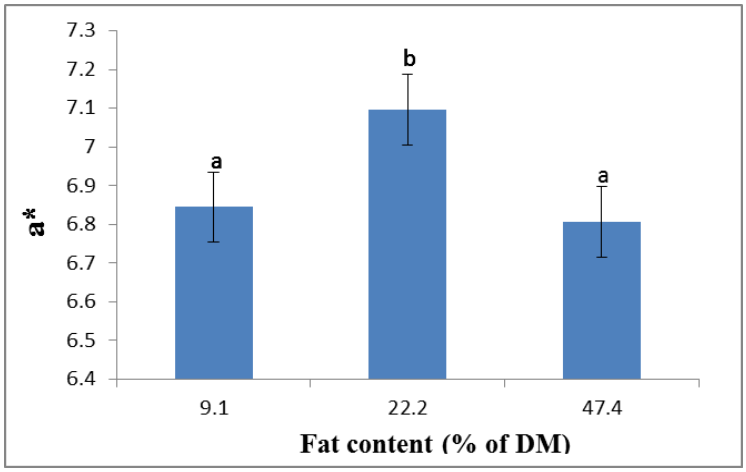


Figure 7.9b. Effects of fat content on a* values (mean ± SE) of pork sausages.



Different letter a,b show significant differences at p < 0.05.

7.3.3.1.2. Hue angles

The analysis of variance of Hue values for sausages with different fat contents treated with antioxidants or stored for 10 days showed there was no statistical significant ($p \geq 0.05$) interaction between the three-factors (Table 7.5.).

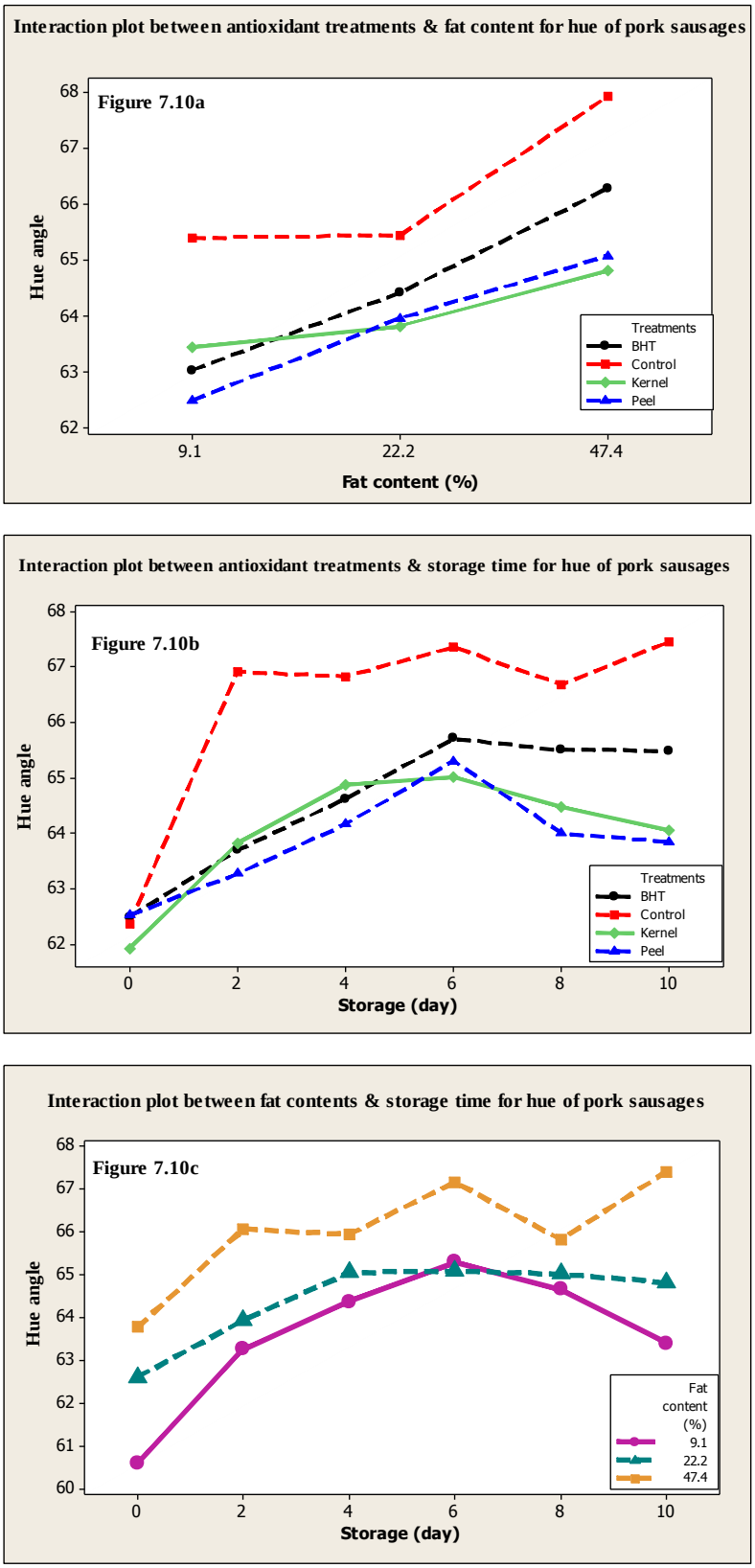
Table 7.5. Three-way ANOVA statistical analysis using a General Linear Model for hue values of pork sausages influenced by treatment, fat content and storage time.

Source of variance	Degree of freedom	*p*-value
Storage	5	< 0.0001
Treatments	3	< 0.0001
Fat content	2	< 0.0001
Storage x Treatments	15	< 0.05
Storage x Fat content	10	< 0.05
Treatments x Fat content	6	< 0.05
Storage x Treatments x Fat content	30	> 0.05

Treatments: control, kernel added, peel added and BHT added.
Storage: day 0, 2, 4, 6, 8 and 10. Fat content: low, medium and high.

However, all the two-factor interactions were significant ($p < 0.05$) indicating that the effect of one factor is dependent on another factor.

Figure 7.10a, 7.10b and 7.10c. Interaction effects on hue angles of pork sausages.



a. Treatment by fat content interaction for hue

There was a significant (p = 0.043) interaction between treatment and fat content. In general, as fat content increased, the hue angle values increased in all the sausages, except that the hue

angles of the medium and those of the low fat control sausages or kernel supplemented sausages were similar (Figure 7.10a).

b. Treatment by storage time for hue

No significant differences in hue angles were found between the control sausages and those with additives at day 0 (Figure 7.10b). The hue angles increased dramatically in the control sausages at day 2 and remained similar over the remainder of the storage time. The hue angles increased gradually from day 0 to day 6 for the sausages with antioxidants and after day 6 decreased slowly to day 10. However, the hue angles decreased more rapidly in the sausages with kernel or peel than those with BHT. Despite hue remaining constant from day 2 to 10 in the control sausages, these sausages showed larger hue angles relative to the sausages with additives over the 10 day storage period.

c. Fat content by storage time for hue

The higher fat sausages showed higher hue angles, except at day 6 and 8 when the hue angles of low and medium fat sausages were similar. Although the hue angles of the sausages with different fat content changed over the storage period, the effects of fat content on hue angles over 10 days of storage were inconsistent (Figure 7.10c).

7.3.3.2. Patties

7.3.3.2.1. a* values

The analysis of variance of a* values for pork patties with different fat content, antioxidant treatment and storage time showed there were significant ($p < 0.01$) effects associated with the three main factors as well as significant ($p < 0.001$) interactions between them (Table 7.6).

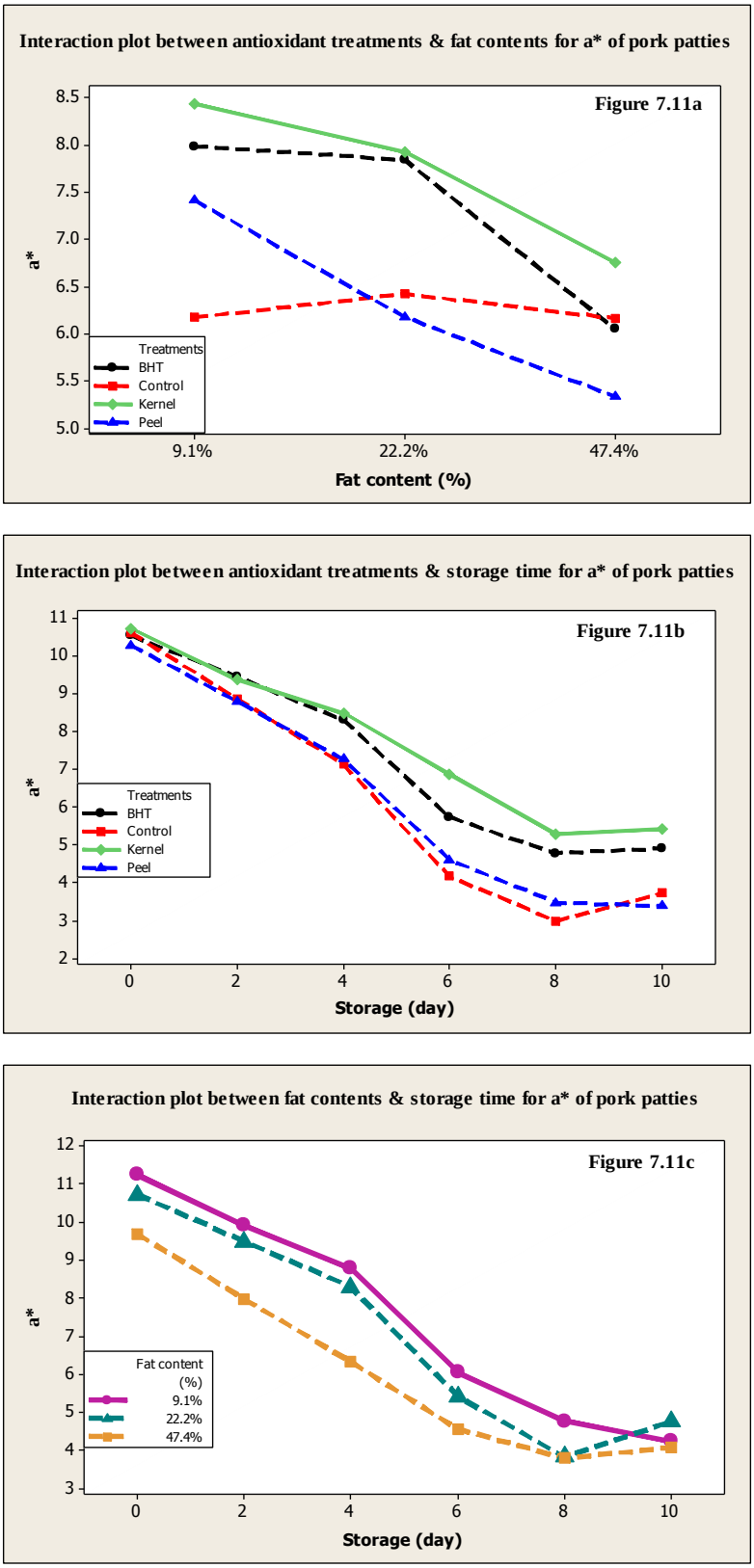
By using the three-way ANOVA analysis, a three-factor interaction was found statistically significant ($p < 0.05$). Thus the two-factor interactions and main effects were ignored. The interaction plot was used to interpret the results as determined with the sausages in 7.3.2.1. From the plot, all the statements regarding the effects on TBARS were given in regards to the interactions between antioxidant treatment, storage time and fat content.

Table 7.6. Three-way ANOVA statistical analysis using a General Linear Model for a* values of pork patties influenced by treatment, fat content and storage time.

Source of variance	Degree of freedom	*p*-value
Storage	5	< 0.0001
Treatments	3	< 0.0001
Fat content	2	< 0.0001
Storage x Treatments	15	< 0.0001
Storage x Fat content	10	< 0.0001
Treatments x Fat content	6	< 0.0001
Storage x Treatments x Fat content	30	< 0.0001

Treatments: control, kernel added, peel added and BHT added.
Storage: day 0, 2, 4, 6, 8 and 10. Fat content: low, medium and high.

Figure 7.11a, 7.11b and 7.11c. Interaction effects on a* values of pork patties.



No significant difference in a* values were found between the control and treated patties at day 0 (Figure 7.11b). However, lower a* values were found in the high fat patties with added antioxidants (Figure 7.11a). a* values of all the patties tended to decrease from day 0 to day 8,

regardless of their fat content (Figure 7.11c), followed by a stable phase to day 10, except for the control patties which increased at day 10 (Figure 7.11b & 7.11c), particularly those with medium fat content (Figure 7.11c). The patties treated with kernel or BHT decreased their a* values less rapidly than the controls or those with peel in the first 4 days but the effects of the antioxidant treatments were minor in the late stages of storage.

7.3.3.2.2. Hue values

The three-way ANOVA analysis of hue angles of pork patties with different fat contents or treatments or storage up to 10 days showed there were significant ($p < 0.01$) effects associated with the three main factors and significant ($p < 0.001$) interactions between them (Table 7.7). A three-factor interaction was found statistically significant ($p < 0.05$) thus the two-factor interactions and main effects were ignored (Table 7.7). The interaction plot was used to interpret the results. From this plot, all the statements regarding the effects on TBARS were given in regards to the interactions between antioxidant treatment, storage time and fat content.

Table 7.7. Three-way ANOVA statistical analysis using General Linear Model for hue angles of pork patties influenced by treatment, fat content and storage time.

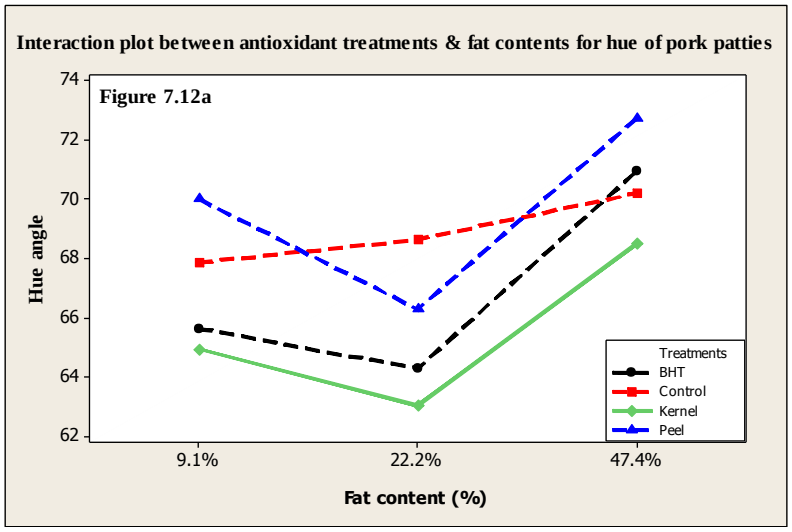
Source of variance	Degree of freedom	*p*-value
Storage	5	< 0.0001
Treatments	3	< 0.0001
Fat content	2	< 0.0001
Storage x Treatments	15	< 0.0001
Storage x Fat content	10	< 0.0001
Treatments x Fat content	6	< 0.0001
Storage x Treatments x Fat content	30	< 0.0001

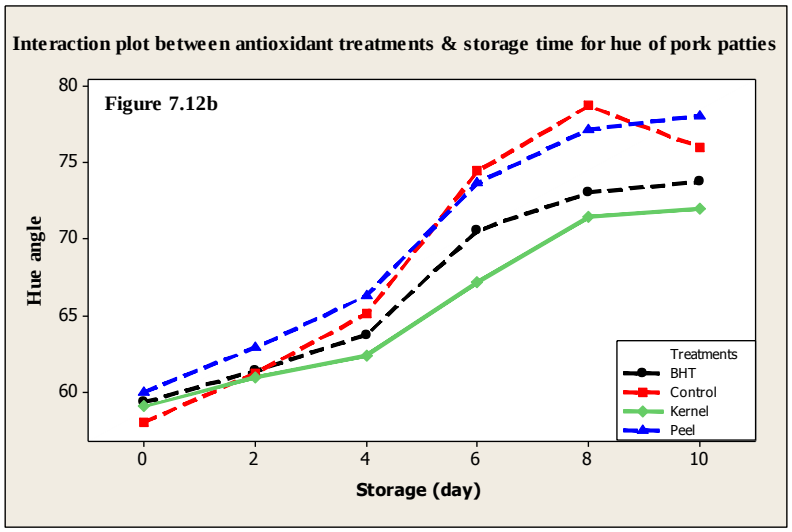
Treatments: control, kernel added, peel added and BHT added.
Storage: day 0, 2, 4, 6, 8 and 10. Fat content: low, medium and high.

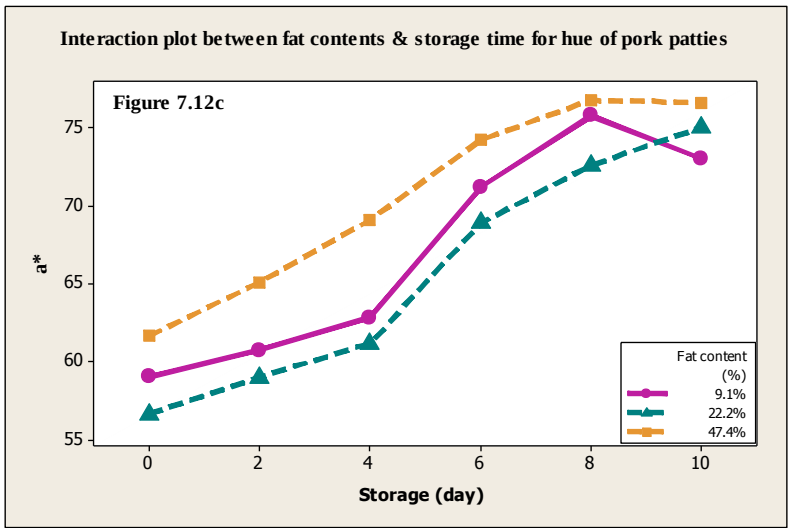
No significant difference in hue angle was found between the control and treated patties at day 0 (Figure 7.12b). However, as fat content increased, hue angle increased in all the patties, except that the hue angles of medium and low fat control patties were similar. The hue angles of all the patties gradually increased from day 0 to day 8 at all fat content levels and remained constant at the end of the storage period, except for the decrease in hue angles of the control patties, particularly the medium fat patties at day 10 (Figure 7.12c). The hue angles in the control patties and those supplemented with peel, followed by the BHT treated increased

more rapidly than those in the patties with kernel during the first 4 days, particularly in the pork patties with a high fat content (Figure 7.12a & 7.12c). The effects of treatment and fat content on the hue angles of pork patties were minor in the last 2 days of storage.

Figure 7.12a, 7.12b and 7.12c. Interaction effects on hue angles of pork patties.







7.3.4. Myoglobin

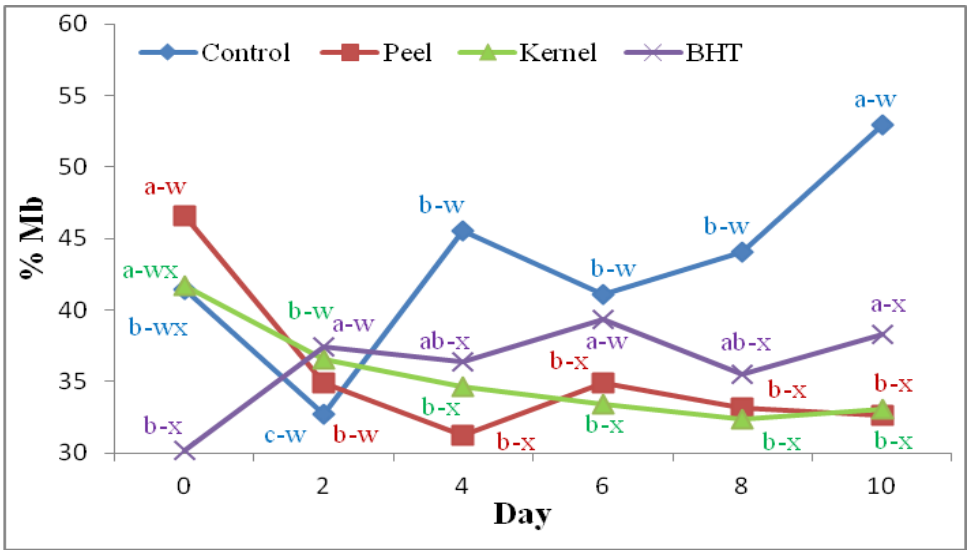
7.3.4.1. Sausages

The total myoglobin concentration (Mb, MbO_2 and MMb) of pork sausages (only high fat pork was investigated) at day 0 was 1.7g/kg meat. The colour of the pork sausages varied depending on the type of myoglobin (Mb, MbO_2 and MMb).

Changes in the type of myglobin (Mb, MbO_2 and MMb) in pork sausages over a 10 day storage period showed significant differences (p <0.05) between the treatments (Figure 7.13a, 7.13b and 7.13c). The addition of mango kernel or peel or BHT to the sausages immediately influenced the proportions of Mb, MbO_2 and MMb at day 0. At day 0, the proportion of Mb was significantly lower in the sausages with added BHT than those with added peel. However, at day 2, the proportions of Mb were similar in all the treatments. In the control sausages after 2 days storage, the Mb increased progressively from 32.7 (day 2) to 52.9% (day 10) whilst there were no changes in the sausages with supplements across the 10 days of storage (Figure 7.13a).

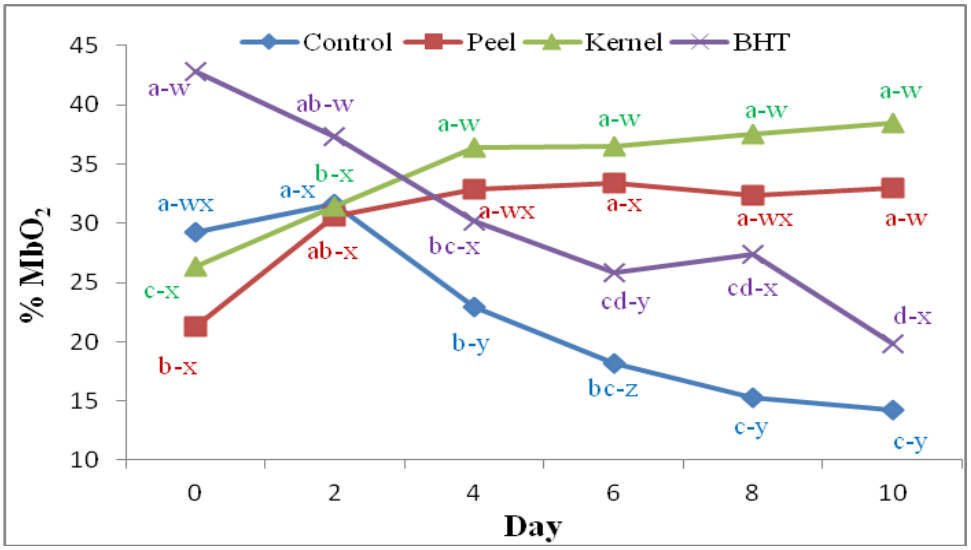
The proportion of MbO_2 was significantly higher in the sausages with added BHT than those with added peel at day 0 and day 2. After 2 days MbO_2 decreased gradually from 31.6 (day 2) to 14.3% in the control sausages and remained stable in the sausages with kernel or peel or BHT. In contrast, there was a significant and progressive decrease in the sausages with added BHT from day 2 to 10. In addition, after day 2, the sausages with added BHT had a lower proportion of MbO_2 than those with added kernel or peel (Figure 7.13b).

Figure 7.13a. Deoxymyoglobin (%) of pork sausages (n = 3) with different antioxidant treatments over 10 day storage.



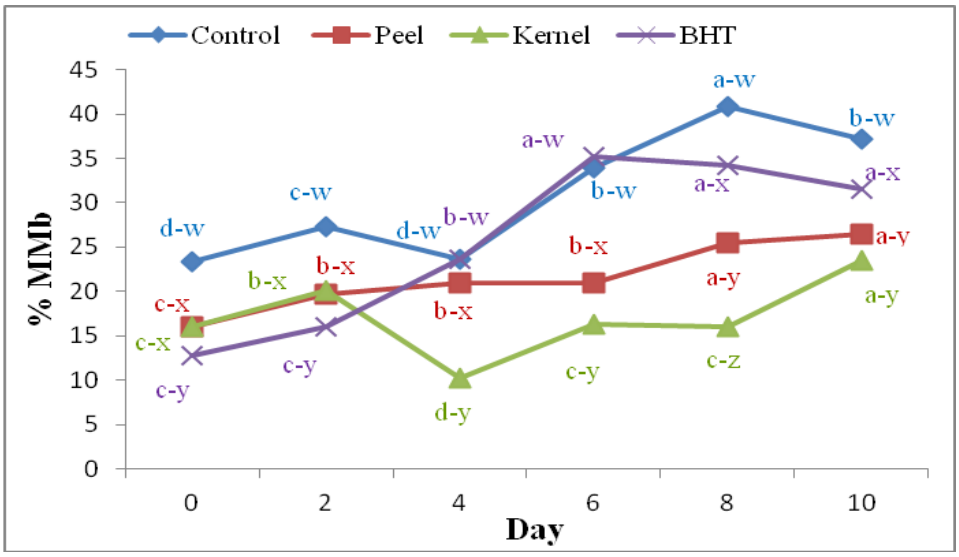
Different letters (w-x) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units: %.

Figure 7.13b. Oxymyoglobin (%) of pork sausages (n = 3) with different antioxidant treatments over 10 day storage.



Different letters (w-z) within the same storage day are significantly different ($p < 0.05$). Different letters (a-d) within the same treatment are significantly different ($p < 0.05$). Units: %.

Figure 7.13c. Metmyoglobin (%) of pork sausages (n = 3) with different antioxidant treatments over 10 day storage.



Different letters (w-z) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units: %.

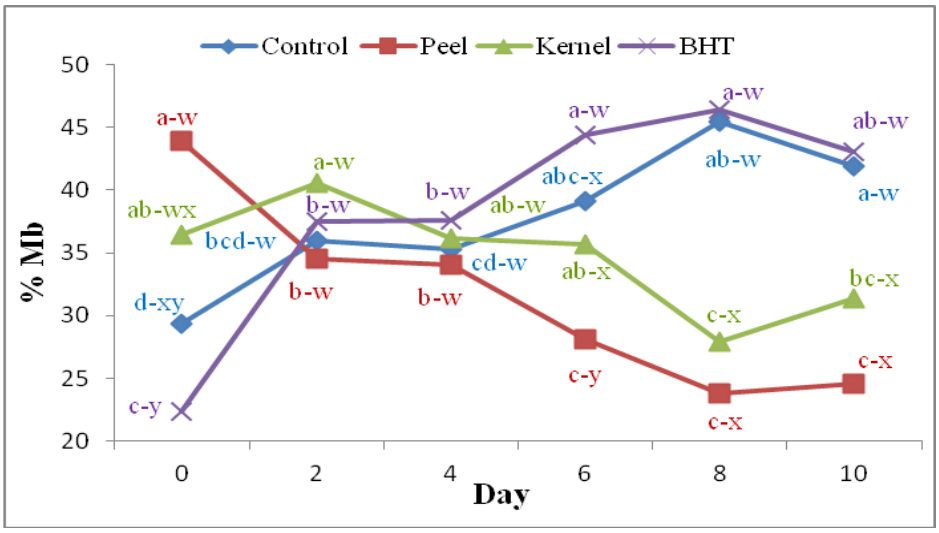
In the first 2 days of storage, there were significant differences in MMb in the sausages. The control sausages had the highest MMb, followed by those with added kernel or peel then added BHT. After 4 days of storage, the proportion of MMb in the control sausages gradually increased from 23.7 (day 4) to 40.9% at day 8 followed by a slight decrease at day 10 (37.1%). The sausages with added BHT increased up to 6 days of storage, and then stabilised thereafter. In contrast, the MMb remained relatively unchanged over the 10 day storage period in the sausages with either added kernel or peel. The proportions of MMb were lower in the sausages supplemented with mango kernel or peel than in the control sausages (Figure 7.13c).

7.3.4.2. Patties

The total myoglobin contentration (Mb, MbO_2 and MMb) of pork patties (only high fat pork was investigated) at day zero was 1.4 g/kg meat which was significantly lower than that of the pork sausages. The colour of meat depended on the form (Mb, MbO_2 and MMb) of myoglobin in the meat. The changes in myglobin (Mb, MbO_2 and MMb) in pork patties showed significant differences ($p < 0.05$) over the storage time and with the treatment (Figure 7.14a, 7.14b and 7.14c). The addition of mango kernel or peel or BHT to the patties influenced the proportions of Mb, MbO_2 and MMb at day 0. The proportion of Mb was significantly lower in the patties with BHT than those with added kernel or peel at day 0. However, at day 2 and 4, the proportions of Mb were similar across all the patties tested. However, after 4 days storage, the Mb increased gradually in the patties with added BHT and

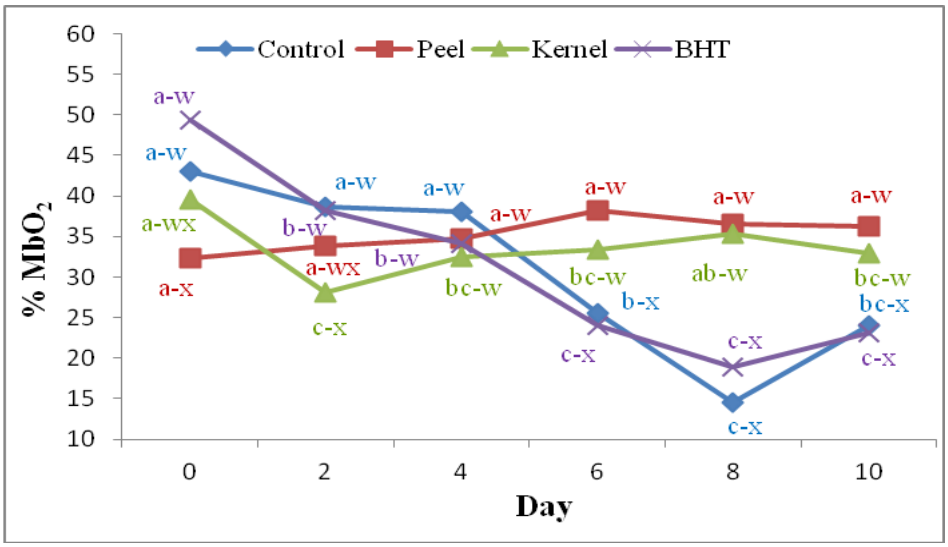
the control patties whilst in the patties with added kernel or peel, there was a slow decrease up to day 8. The proportion of Mb in the control patties at day 0 and day 8 were 29.4% and 45.4%, respectively. The proportion of Mb in all the patties remained constant from day 8 to day 10 (Figure 7.14a).

Figure 7.14a. Deoxymyoglobin (%) of pork patties (n = 3) with different antioxidant treatments over 10 day storage.



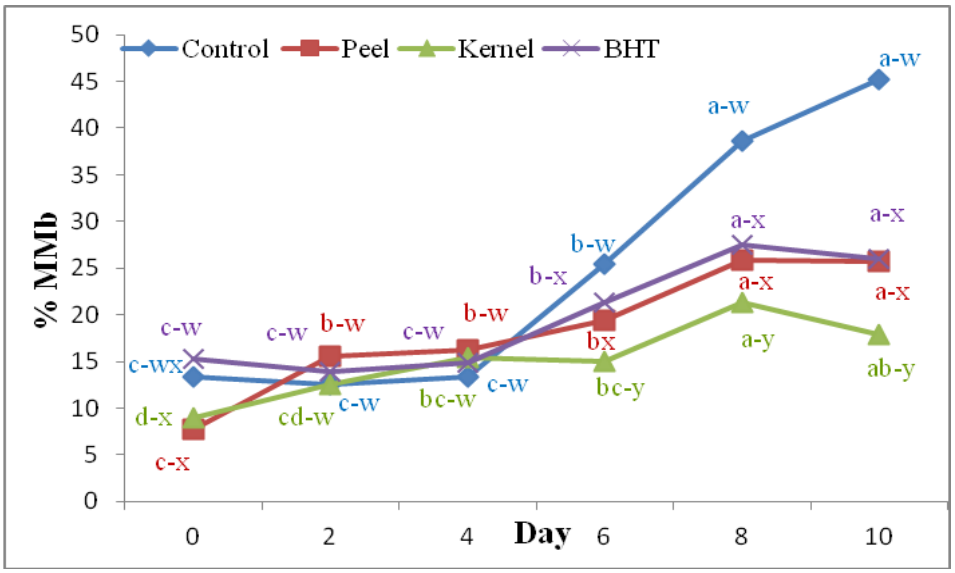
Different letters (w-y) within the same storage day are significantly different ($p < 0.05$).
Different letters (a-c) within the same treatment are significantly different ($p < 0.05$).
Units: %.

Figure 7.14b. Oxymyoglobin (%) of pork patties (n = 3) with different antioxidant treatments over 10 day storage.



Different letters (w-x) within the same storage day are significantly different ($p < 0.05$).
Different letters (a-c) within the same treatment are significantly different ($p < 0.05$).
Units: %.

Figure 7.14c. Metmyoglobin (%) of pork patties (n = 3) with different antioxidant treatments over 10 day storage.



Different letters (w-y) within the same storage day are significantly different ($p < 0.05$). Different letters (a-c) within the same treatment are significantly different ($p < 0.05$). Units: %.

The proportion of MbO_2 was significantly higher in the patties with added BHT than those with added peel at day 0. However, at day 2 and 4, the proportions of MbO_2 were similar in all the patties tested, except for those with added kernel which started to decrease at day 2 but remained constant over the 4 to 10 day storage period. The patties with added peel exhibited stable MbO_2 over the 10 day storage period. The control and patties with added BHT, however, decreased gradually from day 0 to day 8 and increased slightly at day 10. The proportion of MbO_2 in the control patties at day 0 and day 8 were 43% and 14.6%, respectively (Figure 7.14b).

There were no significant changes in the proportions of MMb in all the patties between day 2 and 4. Differences in MMb proportion were clearly observed after 4 days when the MMb in control patties increased progressively and rapidly from 13.3% (day 4) to 45.3% (day 10). In contrast, the MMb of those with added peel or BHT slowly increased to day 8 and then remained unchanged to day 10. The patties with added kernel stabilised the MMb proportion from day 4 to day 10, except for a slight increase at day 8. The proportion of MMb in the control sausages was the highest, followed by the sausages with added BHT or peel and then kernel from day 6 to day 10 (Figure 7.14c).

7.3.5. Volatiles

A number of volatile compounds in the pork samples were identified by SPME-GCMS (see the Appendix A.4.1 for the Chromatogram). However, only the important volatiles that have been linked to the lipid oxidation of meat products are listed in Tables 7.8 and 7.9.

7.3.5.1. Pork sausages

Table 7.8. Major volatile compounds in the control pork (47.4% fat) sausages and those supplemented with mango kernel or BHT (n = 2) after refrigerated storage (4ºC) for 4 days.

	Treatments	Control		Kernel		BHT	
		Area (TIC x 10^5)	Area %	Area (TIC x 10^5)	Area %	Area (TIC x 10^5)	Area %
Retention time (min)	Volatiles						
3.6	Pentane	53.1 ± 37.4	0.4	14.1 ± 9.7	0.1	6.9 ± 3.5	0.1
3.8	Hexane	134.2 ± 3.4	1.2	67.1 ± 58.4	0.5	224.1 ± 36.9	1.7
4.7	Heptane	13.8 ± 7.4	0.1	7.8 ± 1.3	0.1	14.3 ± 2.1	0.1
4.8	2-Propanone	302.4 ± 10.2^{a}	2.6	234.1 ± 6.5^{b}	2.1	250.5 ± 3.1^{b}	1.9
6.2	Octane	240.2 ± 73.4^{a}	2.1	78.1 ± 22.9^{ab}	0.7	37.3 ± 6.3^{b}	0.3
11.3	1-Butanol	65.8 ± 12.6	0.6	35.1 ± 8.0	0.3	116.5 ± 73.8	0.9
13.2	1-Pentanol	5126.8 ± 25.6	44.0	4710.1 ± 838.1	41.5	6291.3 ± 504.8	47.1
15.0	3-hydroxy-2-butanone	5352.5 ± 536.2	45.8	5898 ± 995.1	52.0	6120.7 ± 691.9	45.7
15.7	1-Hexanol	209.6 ± 100.3	1.8	79.1 ± 24.2	0.7	200.6 ± 56.0	1.5
19.2	2-ethyl-1-hexanol	123.6 ± 10.5^{ab}	1.1	144.5 ± 24.2^{a}	1.3	52.9 ± 6.8^{b}	0.4
24.4	Hexanoic acid	47.8 ± 5.5	0.4	60.3 ± 17.5	0.5	51.4 ± 19.6	0.4
	Total volatiles	11669.9 ± 572.3		11328.3 ± 1873.6		13366.7 ± 1107.6	

Values are the mean ± SE.
a,b: Means with different superscripts within a row are significantly different ($p < 0.05$).
nd: not detected.

In Table 7.8, eleven volatile compounds were identified and quantified in the pork sausages with certainty. The major volatiles were alcohols such as 1-pentanol, 1-hexanol and 2-ethyl-1-hexanol and ketones such as 3-hydroxy 2- butanone and 2-propanone. Of all the compounds in the control sausages, 3-hydroxy-2-butanone and 1-pentanol together accounted for 90% of the volatiles

The levels of individual volatiles and total volatiles in the control sausages and those with added kernel or BHT were not significantly ($p \geq 0.05$) different except for 2-propanone,

octane and 2-ethyl-1-hexanol. The 2-propanone was lower in both the kernel and BHT supplemented sausages and octane and 2-ethyl-1-hexanol were lower in the BHT treated sausages than in the control sausages.

7.3.5.2. Pork patties

Table 7.9. Major volatile compounds in the control pork (47.4% fat) patties and those supplemented with mango kernel or BHT (n = 2) stored refrigerated (4ºC) for 4 days.

	Treatments	Control		Kernel		BHT	
		Area (TIC x 10^5)	Area %	Area (TIC x 10^5)	Area %	Area (TIC x 10^5)	Area %
Retention time (min)	Volatiles						
3.319	Pentane	802.5 ± 16.4^a	8.9	272.8 ± 130.7^b	3.3	299.6 ± 98.7^b	4.6
3.807	Hexane	353.0 ± 23.2^b	3.9	733.5 ± 32.0^a	8.5	827.4 ± 150.1^a	14.6
4.611	Propanal	63.0 ± 10.3	0.7	nd	0.0	nd	0.0
4.779	Heptane	32.9 ± 10.8	0.4	33.2 ± 3.1	0.4	47.2 ± 15.4	0.9
4.919	2-Propanone	805.5 ± 308.5^b	8.8	2701.1 ± 490.0^a	31.5	465.0 ± 458.7^b	5.2
6.398	Octane	48.8 ± 3.8	0.5	85.0 ± 55.73	0.9	25.2 ± 2.3	0.4
8.226	Pentanal	975.9 ± 42.5	10.8	nd	0.0	nd	0.0
10.963	Hexanal	3300.6 ± 169.3	36.6	nd	0.0	nd	0.0
11.884	1-Butanol	33.0 ± 2.7^a	0.4	11.7 ± 6.2^b	0.1	17.7 ± 6.2^{ab}	0.3
13.25	Heptanal	344.7 ± 47.1	3.8	nd	0.0	nd	0.0
14.268	1-Pentanol	1313.4 ± 78.6	14.6	1922.1 ± 249.4	22.3	3695.2 ± 1265.0	55.6
15.102	3-hydroxy-2-butanone	269.2 ± 43.2^b	3.0	2829.1 ± 1158.2^a	30.4	693.5 ± 509.2^{ab}	13.9
16.511	1-Hexanol	127.8 ± 14.0^a	1.4	46.0 ± 7.9^b	0.5	31.4 ± 7.1^b	0.6
20.61	2-Ethyl-1-Hexanol	143.2 ± 10.6^a	1.6	56.8 ± 13.9^b	0.7	93.8 ± 17.9^b	1.5
25.249	Hexanoic acid	411.1 ± 57.1^a	4.5	109.5 ± 18.7^b	1.3	140.2 ± 19.6^b	2.3
	Total volatiles	9024.4 ± 368.4		8800.7 ± 648.2		6336.2 ± 1296	

Values are the mean ± SE.
a,b Means with different superscripts within a row are significantly different ($p < 0.05$).
nd: not detected.

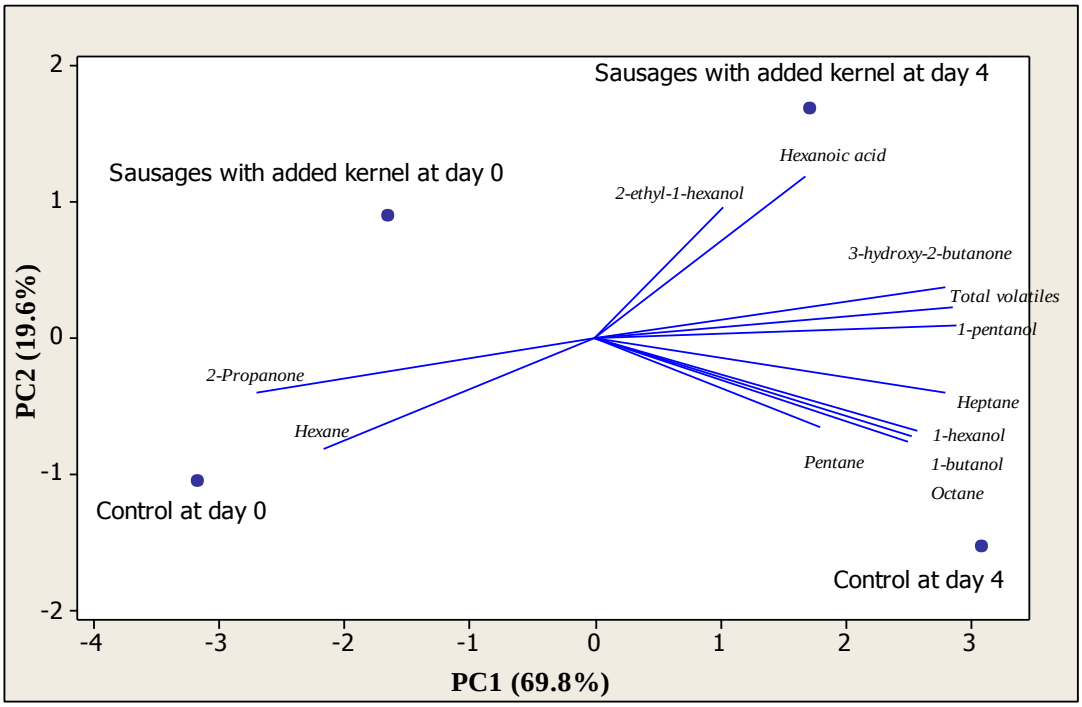
Fifteen major volatile compounds were identified with certainty in the pork patties which included hydrocarbons, aldehydes, alcohols, ketones, and carboxylic acids. The three compounds in control patties with the highest percentage were hexanal, pentanal and 1-pentanol, which together accounted for 62% (Table 7.9).

There were significant ($p < 0.05$) differences in the levels of the volatiles depending on the treatment. The aldehydes; hexanal, pentanal, propanal, and heptanal and the alcohols

1-hexanol, 1-hexanol-2-ethyl and 1-butanol together with pentane and hexanoic acid were all significantly lower in the patties supplemented with kernel or BHT. In contrast, hexane and ketones such as 2-propanone and 3-hydroxy-2-butanone were significantly higher in the patties supplemented with kernel.

7.3.5.3. Effects of mango kernel on reducing volatiles during 4 days of storage

Figure 7.15. Biplot of first two principal components obtained from PCA showing the relation between selected volatile compounds and PC1 and PC2 and comparison of pork sausages (n = 2) with and without kernel between day 0 and day 4.



The results of volatile obtained at day 0 and day 4 were used to investigate the antioxidative effects on reducing volatiles. Figure 7.15 shows the first 2 principal components which accounted for 89.4% of the total variation across the samples (PC1 and PC2 accounted for 69.8% and 19.6% of the variance, respectively). When the volatile distributions were taken into account, the major compounds that positively contributed to the PC1 dimension were 3-hydroxy-2-butanone, 1-pentanol, heptane, 1-hexanol, 1-butanol, pentane and octane whilst on the negative side of the PC1 axis was 2-propanone and hexane. The important compounds along the PC2 axis were hexanoic acid and 2-ethyl-1-hexanol.

The control sausages at day 0 were located on the negative side of PC1 (PCA score < -3) indicating a negative correlation with the volatile compounds. The total volatiles were located on the positive side of PC1 with a positive correlation with 2-propanone and hexane. The

sausages with added kernel at day 0 were located in the left upper quadrant of the plane and were not positively associated with any volatile indicating the low level of volatile compounds identified in these sausages in the present study.

In contrast, the control sausages at day 4 were on the positive side of PC1, in the same direction as pentane, octane, 1-butanol, 1-hexanol, heptanes; had a high score (PCA score > 3) on PC1 and was highly associated with 1-pentanol, 3-hydroxy-2-butanone and total volatiles indicating there were higher levels of these volatiles in the control than any other sausages. The sausages with added kernel at day 4 were located in the right upper quadrant of the plane, which showed higher levels of hexanoic acid and 2-ethyl-1-hexanol in these sausages than all the other sausages.

7.3.6. Odour

7.3.6.1. Sausages

The odour acceptability was assessed by the same person using a 5 point hedonic as outlined in 3.14. The sausages, irrespective of their composition, showed a decrease in their odour scores over the 10 days of chilled storage. The sausages without additives decreased odour from 5 to 0 after 10 day storage. Nevertheless, the odour score of sausages with added kernel had acceptable score (≥ 3) for up to 6 days of storage whilst those with added peel or BHT only had an acceptable score (≥ 3) for up to 4 days of storage. The control sausages had an undesirable score (< 3) and unpleasant odour after 4 days of storage. Over the 10 day storage period the sausages supplemented with kernel had a higher odour scores (= 2) than the control sausages (= 0) or sausages supplemented with BHT (= 0) or peel (= 1). The odour score of sausages with added peel was higher than the score of sausages with added peel or BHTwas observed between the sausages with added peel and those with added BHT at day 10.

7.3.6.2. Patties

The odour score of all the patties decreased over the 10 day storage period. The odour score of patties with added kernel had acceptable odour scores (score ≥ 3) for up to 6 days of storage compared to those with added peel or BHT which had only acceptable score (≥ 3) for up to 4 days of storage. In contrast, the control patties had unacceptable odour scores (< 3) and an unpleasant smell after 4 days of storage.

Over 10 days, the kernel supplemented patties had higher odour scores (= 2) than the control patties (= 2) or patties supplemented with BHT (= 2) or peel (= 1). The patties with added peel had a slightly higher odour score than those with added BHT after 4 days of storage.

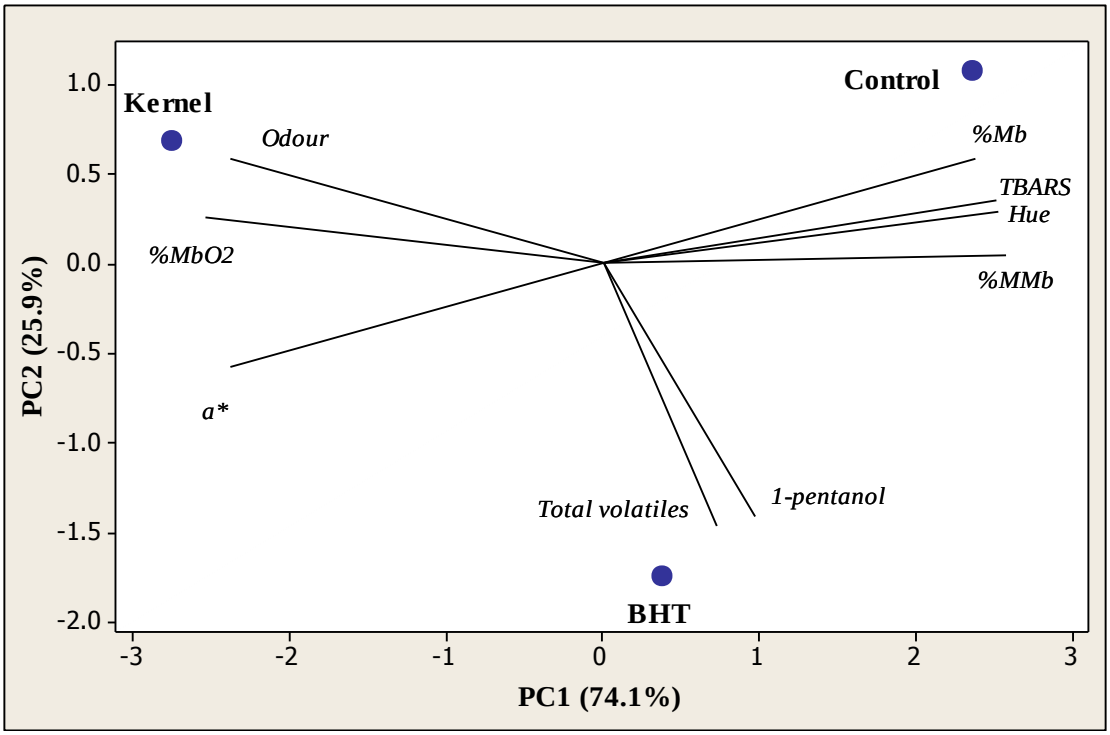
7.3.7. The relationships between the attributes of pork products with lipid oxidation

The attributes TBARS, colour, odour, myoglobin and volatiles of the pork products (only pork products from 47.4% fat mince were investigated) were correlated with lipid oxidation and to each other. The results of all the attributes were obtained at day 10, except for those of volatiles which were obtained at day 4. The volatile compounds 1-pentanol and hexanal were found to be the most common volatiles contributing to lipid oxidation in pork sausages and patties, respectively in this study. Therefore, these tow compounds and the total volatiles were used for the investigation. PCA was used to reduce the original variables (all attributes) to a fewer variables (principal components) that incorporate the maximum amount of variation. The PCA biplot is a point-vector plot where the attributes (variables) are represented by vectors (lines) and the samples are points. The relationships between attributes are determined based on the correlations between these variables (attributes) with the principal components (PC1, PC2, PC3, etc.) and the relative length of their vectors. PCA was applied giving a better visualisation for discriminating between the samples with different treatments which are represented as points. The samples (points) that have the highest absolute loading score values on a PC indicate the attributes which are strongly correlated with the sample on a PC.

7.3.7.1. Sausages

Figure 7.16 shows the first 2 principal components accounting for 100% of the total variation across the samples (PC1 and PC2 accounted for 74.1% and 25.9% of the variance, respectively).

Figure 7.16. Biplot of first two principal components obtained from PCA showing the relation between attributes and PC1 or PC2 and comparison between pork sausages (47.4% fat content) with and without antixodant additives.



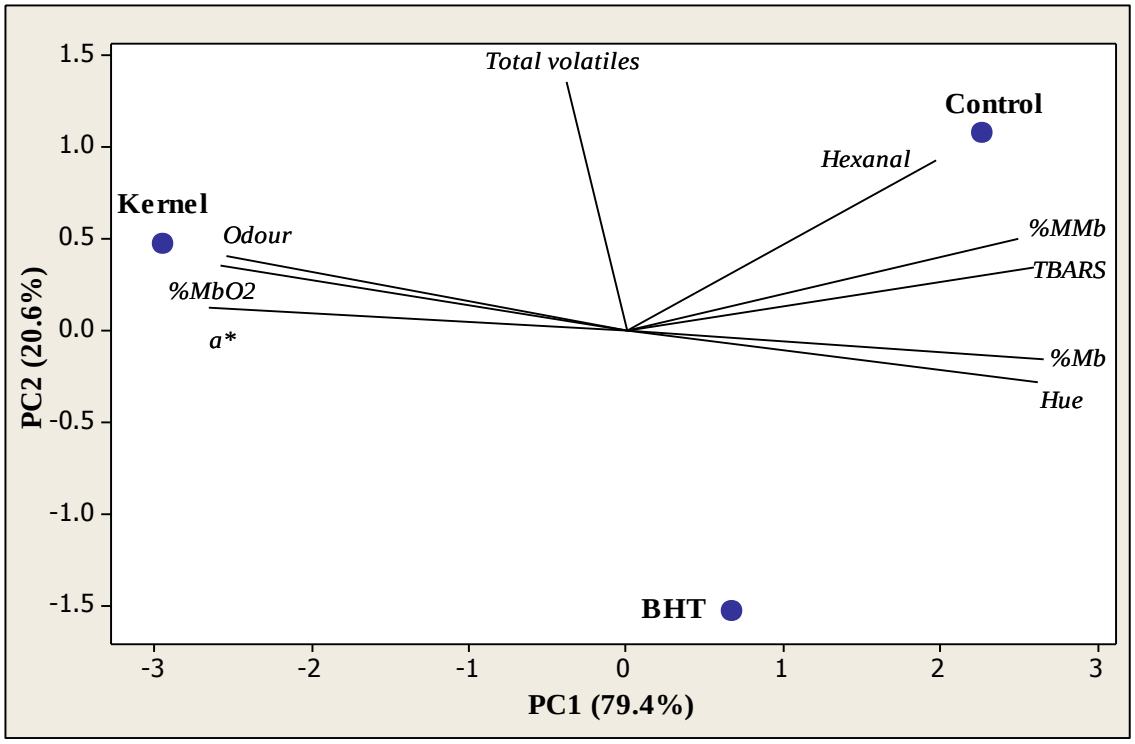
When PC1 was taken into account, the values that positively contributed to PC1 were Mb, MMb, TBARS and Hue whilst on the negative side were odour, MbO_2 and a* values. The important attributes along the PC2 axis were the total volatiles and 1-pentanol.

The control and kernel supplemented sausages were located on PC1 and BHT supplemented sausages on PC2. The control sausages on the right upper quadrant (PCA score > 2) were positively correlated with Mb, MMb, TBARS and Hue. In contrast, the sausages with added kernel was located on the negative side of PC1 (PCA score < -2) with positive correlations with MbO_2, odour score and a*. Sausages with added BHT were located in the lower part of the plane and in the same direction as total volatiles and 1-pentanol.

7.3.7.2. Patties

Figure 7.17 shows the first 2 principal components accounted for 100% of the total variation across the samples. (PC1 and PC2 accounted for 79.4% and 20.6% of the variance, respectively).

Figure 7.17. Biplot of first two principal components obtained from PCA showing the relation between attributes and PC1 or PC2 and comparison between pork patties (47.4% fat content) with and without antixodant additives.



The PC1 was dominated by most of the attributes except for total volatiles which were correlated with PC2. Hexanal was correlated with Mb, MMb, TBARS and hue and was located on the right side of PC1. Odour, MbO_2 and a* values were located on the left side of PC1 and were highly correlated to each other.

The control patties were in the right upper quadrant (PC1score > 2) and positively correlated with Mb, MMb, TBARS whilst the patties with added kernel were located on the negative side of PC1 (PC1 score < -2). BHT supplemented patties were located on the lower part of PC2 and were negatively correlated with the total volatiles.

7.3.8. Microbiology analysis

The microbiological characteristics of mango peel and kernel were analysed for TVC, E.coli, Bacillus cereus, yeasts and moulds (Table 7.10).

Table 7.10. Microbiological characteristics of mango peel and kernel.

Mango fractions	TVC cfu g^{-1}	E.coli cfu g^{-1}	Bacillus cfu g^{-1}	Yeasts cfu g^{-1}	Moulds cfu g^{-1}	Yeasts & Moulds cfu g^{-1}
Peel	190,000	< 5	< 10	50	50	100
Kernel	600	< 5	< 10	< 50	< 50	< 50

Fresh pork mince (used to make sausages and patties) at day zero and pork sausages and patties with 1% of mango kernel at day zero and after cold storage at 4°C for 4 and 10 days were analysed for TVC, E.coli, staphylococcus aureus, clostridiums, yeasts, moulds and salmonellae (Table 7.11).

Table 7.11. Microbiological characteristics of pork products containing kernel.

Pork samples	TVC cfu g^{-1}	E.coli cfu g^{-1}	Staphylococci cfu g^{-1}	Clostridiums cfu g^{-1}	Yeasts & Moulds cfu g^{-1}	Salmonellae per 25g
Mince	1,700,000	> 1500	< 10	< 10	3600	nd
Sausages + kernel day 0	370	< 5	< 10	< 10	3500	nd
Sausages + kernel day 4	2,200,000	< 5	< 10	< 10	nd	nd
Sausages + kernel day 10	< 100,000	< 5	< 10	< 10	nd	nd
Patties + kernel day 0	2,200,000	> 1500	< 10	< 10	3700	nd
Patties + kernel day 4	9,900,000	> 1500	< 10	< 10	nd	nd
Patties + kernel day 10	73,000,000	< 5	< 10	< 10	nd	nd

nd: Non- detected
cfu g^{-1}: colony-forming units per gram

The TVC in fresh mince, pork sausages and patties with added kernel at day 0 ranged from 3.7×10^5 to 2.2×10^6 cfu g^{-1}. The TVC of pork patties increased dramatically to concentrations of 9.9×10^6 and 73×10^6 cfu g^{-1} after 4 and 10 days of storage, respectively.

The concentration of TVC in pork sausages were 2.2 x 10^6 and less than 10^5 cfu g^{-1} at day 4 and 10, respectively.

E.coli was detected in fresh pork mince and pork patties at day 0 and 4 at concentrations higher than 1500 cfu g^{-1}. However, E.coli in sausages at day 0, 4 and 10 and patties at day 10 were less than 5 cfu g^{-1}.

Pathogenic bacteria such as staphylococci and coliforms were recovered from pork mince, pork sausages and patties but were less than 10 cfu g^{-1}. The concentrations of yeasts and moulds in pork mince, pork sausages and patties at day zero ranged from 3500 to 3700 cfu g^{-1}. However, yeasts and moulds were not detected in the pork sausages and patties at day 4 and 10. Salmonellae were not detected in any of the pork samples tested.

7.4. Discussion

7.4.1. Proximate composition of pork mince

The fat content of mince is an important factor affecting the extent of lipid oxidation which is a major factor influencing the shelf life of meat products (Asghar *et al.,* 1988; Ladikos and Lougovois, 1990; Park *et al.,* 2008; Ismail *et al.,* 2009). The fat, protein and moisture content of pork mince with different fat levels were similar to the results reported by Tan *et al.* (2012). There was a negative correlation between the fat and crude protein and moisture content of the minced pork. Pork mince with a high fat content had a low protein and low moisture content. The results are in agreement with those reported by other authors, who found similar correlations between the intramuscular fat content and concentrations of the other chemical components in meat (Park *et al.,* 2001; Wichacz *et al.,* 1998; Daszkiewicz *et al.,* 2005).

7.4.2. TBARS

There were lower TBARS values and rates of TBARS production in sausages supplemented with mango kernel, peel and BHT compared to the control sausages suggesting that the supplements were inhibiting the lipid oxidation in pork sausages over the 10 day storage period regardless of their fat content. However, the TBARS values of the sausages treated with peel or BHT (except for low fat sausages) started to increase at day 6 whilst those supplemented with kernel remained unchanged indicating that kernel was more effective than peel or BHT in reducing lipid oxidation regardless of the sausage fat content. In particular, the

higher inhibitory effects of kernel relative to peel or BHT were more pronounced in the high and medium fat sausages at day 6.

In pork sausages, peel conferred antioxidative effects to the same extent as kernel in maintaining the TBARS values of the pork patties from day 8 to day 10. The increase in TBARS in sausages treated with BHT after day 6 and for the remainder of the storage period indicated that the concentration of BHT (0.01%) is insufficient to prevent lipid oxidation after this time. Sausages treated with kernel had the lowest TBARS values at day 10 of all the sausages demonstrating that kernel is a good active source of antioxidants that can extend the shelf life of pork sausages. Peel also has antioxidative effects on sausages but showed less effective than kernel.

Similar to the sausage results, patties supplemented with kernel showed higher antioxidative effects on reducing TBARS than peel or BHT after day 4. However, the patties treated with BHT had stable TBARS from day 6 to day 10 and had similar TBARS values as those with kernel after 6 days, except for the high fat patties which suggests that high fat patties might produce more lipid oxidation products and that BHT was less effective than kernel to scavenge the free radicals from lipid oxidation. Thus, kernel was the most effective source of antioxidant evaluated. Furthermore, the high inhibitory effects of kernel were independent of product fat levels and compared to BHT, the effects were more pronounced in the high fat patties.

Finally, the fact that there was no significant ($p \geq 0.05$) difference in the TBARS of pork patties or sausages with kernel at day 0 and day 10 indicates that the dried kernel product was extremely effective in restricting lipid oxidation in pork products. Indeed, mango kernel may contain some antioxidant compounds such as tocopherol and carotenoids that quench lipid oxidation. Soong *et al.* (2004) also reported that mango seed kernel has potent antioxidant activity with not only phenolic compounds but also lipophilic antioxidants such as phytosterols and tocopherols). They suggested that mango seed kernel could be used as a functional food ingredients and therapeutic functional food products.

It should be noted that lipid oxidation is often initiated by the unsaturated fatty acids of membrane phospholipids which leads to the production of hydroperoxides. The latter are susceptible to further oxidation to secondary reaction products such as MDA. Although unsaturated fatty acids are the main factor contributing to lipid oxidation, the fat content is

considered an important factor particularly in meat products exposed to air during storage (Ahn, *et al.,* 1998). The fat content in the present study influenced the TBARS values of the untreated and treated sausages and patties throughout the storage period. The present study showed that the TBARS values of patties were higher in the high fat content products. Furthermore, at some stages of storage e.g. day 6 for sausages (Figure 7.4c) and day 4 for patties (Figure 7.8c) the benificial effects of kernel on reducing TBARS were more pronounced in the high fat content products. These findings are in agreement with the study of Ahn *et al.* (1998) who reported that high fat is a factor accelerating the oxidation of lipid in raw meat during storage.

Overall, the addition of 1% mango kernel or 1% peel or 0.01% BHT to sausages and patties were effective as antioxidants over a 10 day storage period, regardless of product fat content. Both mango kernel and peel, as mentioned in chapter 2, contain large amounts of antioxidants such as vitamins C, E, flavonoids, tannins, coumarins, curcuminoids, xanthons, phenolics and terpenoids (Soong, *et al.,* 2004; Berardini *et al.,* 2005; Ajila *et al.,* 2007). The list of molecules possesses strong scavenging abilities that capture free radicals and chelate metals. Mango kernel was found to possess a high antioxidant capacity and to contain high concentrations of both hydrophilic and lipophilic antioxidants than peel (chapter 4) and consequently, was more superior at inhibiting lipid oxidation. Furthermore, although BHT has often been used to prevent the negative effects of lipid peroxidation by scavenging chain carrying peroxyl radicals or diminishing the formation of initiating lipid radicals, mango kernel was more effective than BHT in the present study. This is probably due to the various kinds of antioxidants in mango kernel being dissolved in both the water and lipid phases thereby enhancing the antioxidative effects on muscle systems. Abdalla *et al.* (2007) also suggested that mango seed kernel extracts could be used as a source of natural antioxidants.

7.4.3. Colours

The a* value, or degree of redness, is the most easily measurable non-destructive indicator of fresh meat discoloration. In general, *a** values will decrease with display time. Mathematical formulae using the *a** and *b** values can predict hue (true colour) angle calculated as tan−1(*b**/*a**) which, if plotted as a function of storage can be correlated with the visual appearance of products (Clydesdale, 1978). There were no differences in a* and hue angles between the control and treated sausages at day zero suggesting that the addition of mango kernel or peel or BHT did not physically impart the redness (a* value) or true colour (hue angle) of the pork sausages regardless of fat content. The colour of the sausages started to

change dramatically at day 2, particularly, the redness of the control sausages which faded very rapidly.

Changes in hue angles during the storage of pork sausages, which are shown in Figure 7.10b confirm that storage and treatments influence colour and that the changes have an opposite trend to those for redness or a* values, particularly at day 2. In addition, although all sausages tended to be less red in the subsequent storage days, those with additives kernel, peel and BHT had a redder colour and maintained the redness in a stable form from day 6 to the end of the storage period; thus demonstrating a positive effect on pork products. In general, as fat content increased, the redness of all of sausages decreased regardless of the treatments, except for the similar hue angles of medium and low fat control sausages or kernel added sausages (Figure 7.10a), over the storage time, except for day 6 (Figure 7.10c).

As with the pork sausages, there were no differences in the a* values and hue angles between the control patties and those with additives at day zero suggesting that the addition of mango kernel or peel or BHT had no physical affect on the redness and true colour of the pork patties irrespective of the fat content. The fat content influenced the colour of patties at day zero. Namely the redness decreased more rapidly in the high fat patties. The decreases in a* values and increase in hue angles indicate that the pork patties tended to be less red regardless of fat content, as storage time increased. The addition of kernel or BHT stabilised the colour and maintained the redness of the pork patties.

It should be noted that the pork products tended to be less red (red and true red colour) in the subsequent storage days. The redness of the pork sausages decreased dramatically at day 2 and decreased slightly thereafter whilst the redness of the pork patties decreased gradually from day 0 to day 8 and remained constant at day 10. This finding was similar to those from the study of Zhu and Brewer (1998) who found that a* values decreased in fresh pork on storage. This could possibly be attributed to the myoglobin and oxymyoglobin in pork products being lost by oxidation which turns the pigment to a brown colour attributed to metmyoglobin. In addition, as fat content increased, the redness of pork sausages and patties, in general, decreased over the storage period. This finding is in agreement with Georgantelis *et al.* (2007) who stated that more lean patties had more myoglobin which supports the moderately higher a* values. The additions of antioxidants inhibited the decrease in redness and discoloration of pork sausages and patties. Kernel had the highest effect on maintaining the redness and colour of pork sausages and patties, followed by peel or BHT. Peel was more

effective in maintaining the redness and colour in sausages but BHT was more effective in patties.

7.4.4. Myoglobin

As mentioned earlier in 7.4.3., loss in redness and discoloration could possibly be attributed to the myoglobin (Mb) and oxymyoglobin (MbO_2) in pork products being lost by oxidation which turns the pigment to a brown colour attributed to metmyoglobin. Thus different forms of myoglobin were determined. The total myoglobin contentration (Mb, MbO_2 and MMb) of pork sausages and pork patties at day zero were 1.7 and 1.4g kg^{-1} meat, respectively. The differences could be due to the longer processing of sausages that exposed the mince longer to the air thereby increasing MbO_2 concentration in the pork sausages at day 0. However, the total myoglobin contentrations (Mb, MbO2 and MMb) of pork sausages and pork patties at day zero were similar to the findings of Agullo *et al* (1990) for pork ribs (1.8 g kg^{-1}) and those of Ginger, *et al.* (1954) for pork (1.44g kg^{-1}). Nevertheless, the results of the present study are slightly lower than the pork values (2 - 7.5 g kg^{-1}) reported by Fox and Condon (1982). These differences could be attributed to the type of muscle analysed (e.g. leg, back, ribs and shoulder) and the processing of the meat (e.g. fresh table cut or mince). In the present study, the pork sausages and patties were produced from minced shoulder meat only. The mincing process reduces the myoglobin and destroys the cellular integrity which liberates a variety of prooxidants which can accelerate the discoloration process (Faustman and Cassens, 1990).

It should be noted that myoglobin can exist in three forms: purple reduced myoglobin (Mb), red oxymyoglobin (MbO_2) and brown metmyoglobin (MMb). The actual colour of pork products depends on the proportion of three forms of myoglobin. The changes of these three forms showed a similar trend over the 10 days storage period in this research. Namely, the addition of the antioxidants (mango kernel, peel and BHT) into the pork sausages and patties changed the three forms of myoglobin immediately which resulted in significant differences between the control samples and those with added peel or BHT at day zero. This could be due to either the immediate effect of BHT increasing MbO_2 and reducing Mb and MMb or possibly the interference of mango peel pigment on the colour of myoglobin. There were no significant differences in the proportion of myoglobin between the control pork samples and those with added kernel suggesting that the colour of mango kernel did not physically impact on the myoglobin colour of the pork products and that the antioxidant aspect of kernel had no immediate action on the pork products.

However, after day zero, there were inconsistent changes in Mb, MbO_2 and MMb of pork sausages and patties at day 2 and 4, respectively due to the complex changes in the formation and loss of the three myoglobin forms. The three forms of Mb, MbO_2 and MMb are constantly being interconverted, particularly in the presence of oxygen, during storage which results in changes in meat colour (Giddings, 1974; van Laack *et al*, 1990; Zhu and Brewer, 1998). Furthermore, the products were packed under retail meat display conditions which may cause low oxygen tension, a condition suitable for the oxidation and reduction of pigments which occurs simultaneously and continuously for a significant time post mortem. There was noticeable blooming (or the formation of MbO_2) of the pork sausages and patties in the first 2 and 4 days storage and oxidation of Mb and MbO_2 to MMb, respectively. Meanwhile, the MMb reducing activity in the pork sausages and patties reduced MMb to Mb which can be subsequently oxygenated to bright red MbO_2. The formation and loss of myoglobin from these processes resulted in the inconsistent changes in Mb and MbO_2 to MMb during the first 4 days of storage.

For the remainder of the storage period after 4 days, the proportion of MbO_2 showed an opposite trend to Mb and MMb in the control sausages and control patties. The proportions of Mb and MMb tended to increase and MbO_2 decrease in the case of control and BHT added pork products. The observed results can be attributed to the oxidation of MbO_2 to MMb after the first 4 days of storage. A factor responsible for meat discolouration and MbO_2 oxidation is lipid oxidation (Brown and Mebine, 1969; Gray *et al.*, 1996) which produces free radicals and aldehydes that react with MbO_2 and consequently accelerates the accumulation of MMb.

Mancini and Hunt (2005) suggested that pork sausages and patties have a natural reducing capacity due to endogeneous reductants such as NADH, muscle oxygen scavenging enzymes and reducing enzyme systems (Mancini *et al.* , 2005) which all contribute to a decrease in MMb formation. However, over an extended storage period, due to the consumption of these reductants and the inactivation of reducing systems induced by the formulation of metabolic by-products, the reducing capacity is decreased which results in a rapid accumulation of MMb on the surface of the products. This is responsible for the loss in pink colour.

During the extended storage period, oxygen consumption which is controlled by the activity of oxygen-utilising enzymes and the activity of mitochondria (Faustman and Cassens, 1990; Zhu and Brewer, 1998) is decreased which leads to the low-oxygen partial pressure that is the likely cause of the oxidation of MbO_2 to MMb (Mancini and Hunt, 2005).

In addition, the observed changes in Mb, MbO_2 and MMb after 8 to 10 days of storage could be attributed to the oxidation of the ferrous-species of myoglobin to the brown ferric-species MMb due to the prolonged exposure to air and the low oxygen pressure (Lindahl *et al.*, 2006). Despite being packed in the oxygen-permeable plastic bags, the pork products particularly the pork patties placed in the petri dishes covered tightly with lids were stored in conditions with low oxygen tension. The low oxygen tension, which is created by the increase in oxygen consumption rate during storage (Bendall and Taylor, 1972), can result in rapid formation of MMb at meat surfaces from MbO_2 through the ferric redox state at low-oxygen partial pressures. Subsequently, there is a conversion of MbO_2 to Mb during the storage conditions with low oxygen tension. This explains why the Mb proportion had the similar trend as MMb and increased after 8 to 10 days of storage.

Lipid oxidation is responsible for pigment and myoglobin oxidation. Consequently, the presence of mango kernel or peel or BHT which act as reductants and exhibit antioxidative effects, inhibited the discolouration of the pork products. The colour stabilising effects of kernel, peel and BHT may also be the result of their ability to chelate transition metals that are involved in free radical generation and free radical scavenging which retards the oxidation of MbO_2 to MMb, and as a consequence, reduces or at least maintains the increase in Mb. These effects of the additives were inconsistent in the first 2 days in the sausages and 4 days in the patties but were more pronounced at later storage times when the control pork samples started to discolour.

Metmyoglobin, the oxidised meat pigment, is responsible for the undesirable brown colour of fresh meat. Greene *et al.* (1971) reported that 40 % MMb caused meat rejection by consumers. Although the limit of 40% MMb was established for beef as reported by Greene *et al.* (1971), this limit was also applied for pork in the study of Calhoun *et al.* (1999). No information regarding the percentage of MMb of pork to be rejected by consumers, therefore, it is assumed that less than 40% of MMb was acceptable for pork in the present study. The findings showed that all the sausages and patties treated with antioxidants (kernel, peel and BHT) over the 10 day storage period were acceptable whilst the control sausages at day 8 and the control patties at day 10 were unacceptable.

The pork sausages and patties with added kernel showed the highest effects of retarding the formation of MMb and Mb as well as maintaining the proportion of MbO_2, followed by those with added peel then BHT. Although BHT, a synthetic antioxidant, reduced the oxidation of

MbO_2 to MMb and formation of Mb in both pork sausages and patties during storage, its effect was lower than kernel or peel. Furthermore, due to the potential toxicity, the application of BHT is limited by law to certain meat products (e.g. sausages or ground pork) at 0.01% (Lee *et al.,* 2005). To apply BHT to pork sausages or patties, a combination of BHT with another antioxidant such as Vitamin E or kernel or peel is recommended. Both kernel and peel are good sources of antioxidants but kernel has a higher antioxidant capacity than peel and therefore a more substantial effect on stabilising the colour than peel.

7.4. 5. Volatiles

7.4.5.1. Pork sausages

The majority of the volatile compounds listed in Table 7.8 have been previously found in pork sausages (Estevez *et al.,* 2003; Olse, *et al.*, 2005) with 1-pentanol (44% of the total area of volatiles) and 3-hydroxy-2-butanone (45.8% of the total area of volatiles) being the major volatiles. However, aldehydes, such as hexanal and pentanal, are the two major volatiles unique to the autoxidation of lipids in meat (Selke *et al.,* 1980), and were not identified in the sausages in this study. This could be due to their reduction to other volatiles such as 1-hexanol (Jaar *et al.,* 1999) and/or hexanoic acid (Jo and Ahn, 2000) and 1-pentanol (Yamashita *et al.,* 1977). Although, no aldehydes were identified, various alcohols were observed in significant amounts. Mottram (1985) found that the headspace volatile compounds of pork (pork was cooked until the centre temperature in the meat reached 70°C) were dominated by aldehydes and alcohols originating from the thermal oxidation of lipids and concluded that alcohols could be used as indicators of lipid oxidation in pork products. However, due to their higher odour threshold, which is defined as the minimum concentration at which the compound can be detected by the sense of smell, compared to aldehydes, the influence of alcohols on aroma development is lower than aldehydes. Mottram *et al.* (1984) also found that the headspace volatiles of the untreated or the salt pork (no heating applied) contained essentially the same components as those of cooked pork from which aldehydes and alcohols dominated the headspace volatiles of the samples although there were quantitative differences.

The ketones such as 3-hydroxy-2 butanone and 2-propanone which are also lipid oxidation products were found to have a major contribution (e.g. 45.8% by 3-hydroxy-2-butanone) to the total volatiles of pork sausages in the present study. In addition, some hydrocarbons such as pentane, hexane, heptane and octane that have been identified as major constituents of the headspace volatiles (Yasuhara and Shibamoto, 1990) were present in pork sausages in significant amounts. A significant level of hexanoic acid in pork sausages is due to the

reduction of hexanal. The presence of the volatiles produced from lipid oxidation in pork sausages with and without treatment with antioxidants, after 4 days, indicates that lipid oxidation has occurred over the storage period.

The effect of BHT or mango kernel in sausages on reducing 2-propanone, octane and 1-pentanol (effective in kernel only) indicates that mango kernel and BHT have influenced the production of volatile compounds that are linked to undesirable off-flavours in pork sausages. However, there were non-significant ($p \geq 0.05$) differences in the levels of the majority of the volatile compounds between the control sausages and those supplemented with kernel or BHT. These findings suggest that kernel and BHT have an antioxidative effect in terms of reducing volatiles but the effect is minor at day 4 of storage.

7.4.5.2. Pork patties

The predominant volatile compounds in the control pork patties were hexanal, 1-pentanol and pentanal (Figure 7.9). Unlike pork sausages, some aldehydes, such as hexanal, pentanal, propanal and heptanal, were positively identified in the control pork patties. Aldehydes play an important role in contributing to the aroma of pork products due to their low perception threshold and their distinctive odours (Pugliese *et al.,* 2010). These volatiles appear at high levels under aerobic conditions (Jo and Ahn, 2000). The hexanal levels were very high (contribution of 36.6 % in the total volatiles) among the volatiles identified in the pork patties which is understandable as hexanal is a major product of the autoxidation of n-6 fatty acids. Supplementation of patties with mango kernel or BHT reduced these aldehydes to undetectable levels suggesting that the additives have inhibited lipid oxidation.

Furthermore, similar to the sausage results, several alcohols were identified indicating that the aldehydes have been reduced to alcohols, particularly to 1-pentanol which was observed in significant amounts and contributed to 14.6% of the total area of the volatiles in the control pork sausages.

In addition, the ketones (also lipid oxidation products) such as 2-propanone and particularly 3-hydroxy-2 butanone were found in patties. They also made a substantial (11.8%) contribution to the total volatile content of control pork patties in the present study. A significant level of hexanoic acid was observed in the pork patties and is probably due to the oxidation of hexanal. Jo and Ahn (2000) stated that the hexanal level is an indicator of lipid oxidation and that the levels decrease dramatically during storage due to its oxidation to

hexanoic acid. Indeed, the oxidation and transformations between volatiles compounds often makes it difficult to explain some of the profile changes in the volatiles and the impact of the changes on product quality. Nevertheless, despite these changes, the absence of the aldehydes as well as the low levels of alcohols in the pork patties with the additives strongly suggests that the additives have inhibited lipid oxidation.

7.4.5.3. Antioxidative effects of mango kernel on reducing volatiles over 4 days of storage

In order to compare the volatile compounds of pork sausages with and without kernel added over the storage period, the data from volatile analyses of pork sausages at day zero and day 4 were compared. The levels of most of volatiles in the sausages with or without kernel added at day 4 were higher than those at day 0, except for 2-propanone and hexane which indicates that the volatiles of pork sausages increase as the storage time increases. The higher levels of 2-propanone and hexane in the control sausages at day zero are consistent with the results found in the pork sausages at day 4 and confirm that kernel is effective in reducing these two volatile compounds.

In addition, the high levels of volatile compounds such as 1-hexanol, 1-butanol, 1-pentanol, heptanes, octane, pentane, 3-hydroxy-2-butanone and total volatiles in the control sausages at day 4 compared to kernel supplemented sausages at day 4 indicate that the inclusion of kernel is effective in reducing the production of volatiles from lipid oxidation after a 4 day storage period. However, the sausages with kernel at day 4 had higher levels of hexanolic acid and 2-ethyl-1-hexanol.

7.4.6. Odour

Lipid oxidation is a major cause of the deterioration in the quality of meat or meat products (Vasavada and Cornforth, 2006) and contributes to the rancid odour. Aroma is often characterised by the presence of volatiles, whose concentrations need to be over a specific threshold to generate an odour recognisable by consumers (Garcia-Gonzalez *et al.*, 2008). In this research, the odour was assessed by the researcher who used a 5-point hedonic scale to score the pork products.

The changes in the odour profiles of the patties with different treatments over the storage period were similar to the profiles observed with the sausages. This is expected since any lipid oxidation will contribute to the formation of volatiles such as aldehydes, alcohols and ketones, which have low sensory thresholds and consequently contribute to the odour and flavour

typically associated with rancidity (Frankel, 1998; Olsen *et al.*, 2005). Other products of lipid oxidation and degradation could also contribute to the unpleasant odour, particularly if pork is stored under aerobic conditions.

The pork sausages and patties supplemented with kernel or peel or BHT had higher odour acceptance than the control samples over the 10 day storage period. It is probable that the antioxidants in mango kernel, peel and BHT acted as scavengers of the free radicals produced from lipid oxidation which resulted in the more acceptable odour than from the control samples. Furthermore, the antioxidative effects on maintaining the pleasant odour were highest in mango kernel followed by peel and then BHT.

Odour is the most important parameter that affects the consumer perception of the quality of stored chilled meat. The reported subjective odour scores, however, were only evaluated by one person. To evaluate whether the changes in the quality of pork products are due to lipid oxidation, the results of odour scores should be evaluated alongside changes in TBARS, colour, myoglobin, volatiles and microbial changes in the same samples.

7.4.7. Relationships between the attributes of pork products with lipid oxidation

The findings in sections 7.3.2 to 7.3.6 suggest that lipid oxidation influences the attributes TBARS, colour, myoglobin and odour of pork sausages and patties over the 10 day storage period. Lipid oxidation also influences the levels of volatiles in pork sausage and patties at day 4. To determine whether there were any correlations between the measured attribute changes and lipid oxidation as measured by the various assays, all the measured attributes and assays of pork sausages and patties at day 10 of chilled storage were combined and evaluated by PCA. Unfortunately, the volatiles of the pork products were only determined at day 4 but the data was included in the analysis for a preliminary evaluation of the relationships between the volatiles and lipid oxidation.

The loading plot (Figure 7.18) showed that TBARS, Hue, Mb and MMb were highly correlated to each other and negatively correlated with MbO_2, odour score and a* values in the pork sausages. These findings suggest that TBARS, hue angles, Mb and MMb increased whilst MbO_2, odour score and a* value decreased when lipid oxidation occurred after 10 days of storage. In pork sausages after 4 days of storage, total volatiles and 1-pentanol, were virtually orthogonal to PC1, indicating there was no association with any other attributes located in PC1.

Similarly, in pork patties (Figure 7.19) there was no correlation between the total volatiles after 4 days storage with any of the other attributes. This could be due to the fact that the lipid oxidation after 4 days storage was low and thus the volatiles produced from this process changed less dramatically than the other attributes affected by the lipid oxidation after 10 days storage. Hexanal released from pork patties was highly correlated to MMb, Mb, TBARS and hue. This is not surprising since hexanal is a major product of the autoxidation of n-6 fatty acids in pork patties and like other aldehydes, hexanal produces an unpleasant odour due to their low perception threshold and distinctive odour (Pugliese *et al.,* 2010). The absence of hexanal in the pork sausages could be due to its reduction to 1-hexanol, 2-ethyl-1-hexanol and hexanoic acid as discussed in 7.3.5. Some reports have shown there is a positive relationship between sensory assessment, TBARS and hexanal in pork, beef and turkey (Shahidi *et al.*, 1987; Angelo *et al.,* 1990; Craig, Bowers and Seib, 1991). Dupuy *et al.* (1987) has also reported correlations between volatiles and lipid oxidation in cooked pork.

Pork sausages and patties provide high quality protein, vitamins and minerals for consumers However, during storage, particularly under aerobic conditions, the autoxidation of lipids and the production of free radicals are a consequence of the metabolic changes in a meat biological system (Lee, Hendricks and Cornforth, 1998; Olsen *et al.,* 2005). Lipid oxidation products and free radicals, in particular, contribute to the oxidation of oxymyoglobin to metmyoglobin which results in an undesirable dark brown meat colour (Renerre and Labas, 1987; Lee, Hendricks and Cornforth, 1998). Lipid oxidation breakdown products also cause rancidity due to the production of a complex mixture of aldehydes, ketones, alcohols, hydrocarbons and carboxylic acids. The correlations between TBARS, myoglobin, colour, odour and volatiles are probably a consequence of lipid oxidation in the studied pork products.

Pork products with added kernel showed elevated MbO_2, odour score and a* (redness) and lower Mb, MMb, TBARS and Hue than products with added BHT or the control samples. Kernel inhibited the production of hexanal in pork patties. The total volatiles were highest in the kernel supplemented pork sausages and lowest in kernel supplemented pork patties demonstrating that the total volatiles are not a good indicator of lipid oxidation after 4 days of storage.

Mango kernel, peel and BHT are scavengers of free radicals and the results illustrate that they are effective in retarding lipid and pigment oxidation in the pork sausages and patties up to 10 days of chilled storage. Clearly, the antioxidants in the mango additives and BHT are donating hydrogen to the free radicals and preventing or at least reducing the rate of lipid oxidation. These antioxidant additives may also control the production of any additional free radicals by reducing the activity of ion chelation (Ladikos and Lougovois, 1990). In the previous sections (7.3.2 to 7.3.6), mango fractions, particularly kernel, were identified to be more effective than peel or BHT on reducing TBARS, oxidation of MbO_2 to MMb, hue angles and volatile compounds. In contrast, they increased the redness (a*) and odour scores of pork sausages and patties. PCA analysis confirmed that mango kernel had higher antioxidative properties than BHT in retarding lipid oxidation in pork sausages and patties during chilled storage.

7.4.8. Microbiology

The levels of TVC, E.coli, Bacillus cereus, yeasts and moulds in mango peel and kernel were well below the recommended limits for dried fruits indicating that the freeze drying process used to produce the mango peel and kernel powder was satisfactory. Consequently the products are suitable for inclusion in meat products and do not create any microbiological concerns to the food industry.

Bacteria normally found on the surface of meat are distributed throughout the minced product during the mincing processes (Youssef *et al.*, 1984; Siriken, 2004). This fact together with minced meat being a good medium for the rapid growth of microorganisms means pork sausages and patties are highly susceptible to bacterial contamination and growth will commence if the products are not treated correctly.

The concentrations of TVC in fresh mince was 1.7×10^6 cfu g^{-1} which is within the acceptable levels recommended by the Food Regulators (Food Management Manual, 1995) indicating that the fresh pork mince was safe from TVC.

Similarly, the concentrations of TVC in pork sausages and patties with added kernel at day zero ranged from 3.7×10^5 to 2.2×10^6 cfu g^{-1}, are also within the acceptable levels recommended by the Food Regulators (Food Management Manual, 1995). However, over the storage period, the levels of TVC in the pork patties increased dramatically and attained unacceptable concentrations ($> 5 \times 10^6$ cfu g^{-1}) whilst the concentration in pork sausages

remained below the maximum allowable limit (Food Management Manual, 1995) at day 4 and day 10. These findings indicate that the conditions for making sausages and patties controlled TVC but the aerobic bacteria were produced over the 10 day storage period. The results imply that mango kernel has a potential antimicrobial activity against TVC in pork sausages at day 4 and 10 but such activity was not present in the pork patties.

The count of E.coli in pork mince was above 1500 cfu g^{-1} but in pork sausages with added kernel at day 0, 4 and 10 it was below 5 cfu g^{-1} indicating that the mango kernel has antimicrobial activity against E.coli. In addition, the counts of E.coli in pork patties were above 1500 cfu g^{-1} at day 0 and 4, and less than 5 cfu g^{-1} at day 10 suggesting that kernel may also inhibit E.coli activity in pork patties. The counts of E.coli in pork mince and patties at day 0 and 4 were above 1500 cfu g^{-1} which exceeds the acceptable limits according to Food Management Manual (1995) and the EU meat standards (Anon, 2001.) The results suggest that fresh pork mince and pork patties should be pre-tested for E.Coli and the preparation of pork patties should be monitored and controlled to minimise any E.coli contamination.

Potential pathogenic bacteria such as staphylococci and coliforms were detected in the pork products but the concentrations were well below the safety limits according to Food Management Manual (1995) indicating that the conditions for preparing and storing the sausages and patties were sufficient to control the pathogenic bacteria. There was no evidence of any inhibitory effect of kernel on these bacteria. However, there were also no changes in the levels of staphylococci or coliforms at day 0, 4 and 10 which could be due to the inhibitory effects of kernel on these bacteria. Alternatively, the pork sausages and patties stored in oxygen-permeable plastic bags at $4^{o}C$ was an unfavourable atmosphere for the growth of bacteria.

Yeasts and moulds were recovered from pork mince, pork sausages and patties at day zero at concentrations below the permissible limits in meat and meat products (Food Management Manual, 1995). The yeasts and moulds were not detected in the pork sausages and patties at day 4 and 10 of storage suggesting that mango kernel had an inhibitory effect on yeasts and moulds in the products. Salmonellae were not detected in any pork samples implying that the procedures for preparing and storing the pork sausages and patties controlled salmonella satisfactory.

7.5. Conclusions

- Lipid oxidation and microbiological contamination are major contributors to the deterioration in meat quality during refrigerated storage. Lipid oxidation was associated with TBARS, colour, odour, myoglobin and volatiles of pork sausages and patties at three different fat levels over a 10 day storage period.

- Proximate analysis of pork mince at three different fat contents (low, medium and high) found that pork mince with a high fat content had a low protein and low moisture content. Lipid oxidation influences the attributes TBARS, colour, odour, myoglobin and volatiles of pork sausages and patties over a10 day storage period.

- TBARS values of pork sausages and patties remained stable in the first 4 days then rapidly increased thereafter during storage. The additions of antioxidants mango kernel orpeel or BHT reduced lipid oxidation in the pork products. However, the antioxidative effects varied according to the pork product type, treatment, storage time and fat level. The addition of antioxidants immediately decreased the TBARS of sausages at day zero and maintained the lower TBARS values compared to control sausages over a 10 day storage period regardless of the fat content. The higher antioxidative effects of kernel compared to peel or BHT were pronounced after 4 days of storage when the sausages with added peel or BHT started to increase their TBARS whilst those with kernel remained constant across the 10 day storage period, regardless of the fat content. The addition of antioxidants did not affect TBARS of pork patties immediately until day 4 thereafter kernel showed the highest effect on inhibiting lipid oxidation, followed by BHT or peel. Peel and BHT showed relatively similar antioxidative effects on reducing TBARS of pork products. However, the peel showed higher antioxidative effects than BHT for sausages (except for low fat pork) whilst lower than BHT for patties (except for high fat pork) in the last 2 days of storage. In general, kernel was more effective than the other additives after 4 days, regardless of the fat content. TBARS values of patties were higher in the higher fat content products. Furthermore, at some stages of storage (day 6 for sausages, day 4 for patties) the higher inhibitory effects on lipid oxidation of kernel compared to BHT were more pronounced in the high and medium fat content products. It is suggested that fat content increased the antoxidative effects of supplements on TBARS values of the pork products.

- There were decreases in redness and increases in discoloration of pork sausages and pork patties during storage. The redness of sausages reduced dramatically at day 2 then remained unchanged across the storage whilst that of patties decreased gradually from day zero to day 10. Mango kernel, peel and BHT are good supplements to stabilise the colour and redness of the pork sausages and patties. Mango kernel showed the highest effect among the additives on maintaining the colour and redness of pork sausages and patties. Peel was more effective in maintaining the redness and colour in sausages but BHT was more effective in patties.

- The fat content of pork products is important since it influences the redness and colour of pork sausages and patties across the storage period. As fat content increased, in general, the redness of pork sausages and patties decreased and the hue angles increased over the storage period. The pork sausages and patties with high fat levels were more susceptible to be oxidised and discoloured. High fat products are, therefore, useful to monitor changes in myoglobin, volatiles and odour characteristics.

- There were inconsistent changes in Mb, MbO_2 and MMb of pork sausages and patties in 2 and 4 days, respectively due to the complex changes in the formation and loss of the three myoglobin forms. The biochemical processes such as oxidation of MbO_2 to MMb, decrease in MMb reducing activity, increase of oxygen consumption rate leading to low oxygen tension thereby resulted in Mb formation from MbO_2 pork products. The pork sausages and patties with added kernel showed the highest effects of retarding the formation of MMb and Mb as well as maintaining the proportion of MbO_2, followed by those with added peel then BHT.

- Odour is the most important parameter that affects the consumer perception of the quality of stored chilled meat. Numerous volatile compounds contribute to the smell and taste of food were analysed by a HS-SPME-GC-MS. 1-pentanol and 3-hydroxyl-2-butanone were the major volatiles in pork sausages whilst hexanal, pentanal and 1-pentanol were the most abundant volatiles found in the pork patties. The aldehydes were not identified in the pork sausages but the reduced forms of aldehydes, particularly alcohols such as 1-pentanol, 1-hexanol and 2-ethyl-1-hexanol, and carboxylic acids (oxidised forms) such as hexanoic acid were found in both pork sausage and patties after 4 days of storage. Whilst the mango kernel and BHT had little antioxidant effects on reducing the volatiles of pork sausages, they decreased

significantly most of the volatiles in pork patties. Furthermore, differences in the levels of volatiles compounds and total volatiles between day 0 and day 4 show that volatiles in pork sausages increased during 4 days of storage. The addition of kernel was more effective than BHT in reducing the levels of volatiles in the pork sausages.

- However, the interactions that can occur between the odour components and the concentrations of key volatiles need to be over a specific threshold to generate an odour recognisable by consumers. Aroma assessment of pork products was assessed by the researcher. Pork sausages and patties were rancid at day 4 of storage. The addition of antioxidant increased the odour score. Namely, peel or BHT maintained the pleasant odour of both pork sausages and patties up to day 4 and kernel up to day 6. Although all the pork products had unacceptable odour after 6 days, kernel showed higher effects on retarding the off- odour than peel than BHT.

- Determining the correlations between the attributes with lipid oxidation by PCA analysis demonstrated that TBARS, hue angles, Mb and MMb increased whilst MbO_2, odour score and a* value decreased when lipid oxidation occurred after 10 days of storage. Hexanal observed at day 4 was also correlated with lipid oxidation in patties after 4 days of storage. The total volatiles of pork products and 1-pentanol found at day 4 in sausages were not correlated with lipid oxidation and other attributes at day 10 of storage. PCA analysis also confirmed that mango kernel had higher antioxidative properties than BHT in retarding lipid oxidation in pork sausages and patties during chilled storage.

- The microbiological profile of pork sausages with added kernel were within the recommended safe limits indicating that the pork sausages with added kernel are safe to consume over a 10 day storage period. However, since the pork patties were contaminated with TVC at day 4 and 10 and with E.coli at day 0 and 4, the Food Management Manual (1995) and EU microbiological meat standards indicate that the pork patties stored for 4 days in the present study should not be consumed. The level of E.coli in fresh pork mince was also above the limit suggesting that fresh pork mince used in any study needs to be pre-tested to determine its acceptability for consumption and further processing. The concentrations of all the microorganisms in mango peel and kernel were below the recommended permissible limits. The levels in pork products with added kernel were either the same or lower than the control levels in

pork mince at day zero implying that kernel and possibly peel are safe to be used as a food additives. The addition of mango seed kernel powder to pork sausages and patties contributed to antimicrobial effects against E.coli, yeasts and moulds and TVC. It is suggested that mango kernel or peel showed antimicrobial activities against staphylococci or coliforms.

- In overall, previous studies have reported effects of antioxidants from seed kernels on the oxidative stability of some kinds of food such as oil and butter-fat. This research investigated for the first time the effects of mango peel and kernel on extending the shelf-life of pork sausages and patties. The conclusion of these findings is that mango kernel and peel can be utilised as a natural antioxidant and antimicrobial product for inclusion in pork products due to the high content of phenolic compounds, hydrophilic and lipophilic antioxidants.

CHAPTER 8
GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

8.1. Physicochemical characteristics, antioxidant capacity and anti-nutrients of mango cultivars

This study found that the Vietnamese mango cultivars Cat Chu, Cat Hoa Loc, Ghep and Nam Dok Mai were soft and suitable for the short term fresh market whilst the Tommy Atkins cultivar imported into New Zealand was fleshier and firmer, and ideally suited for export markets or processing.

Mango flesh, peel and kernel from all the cultivars were good sources of antioxidants with flesh, peel and kernel from Tommy Atkins, peel from Nam Dok Mai and flesh from Cat Chu containing the highest levels of antioxidants. Kernels from all the cultivars contained the highest concentration of total phenolics and antioxidants, followed by peel then flesh, except for Nam Dok Mai, in which peel contained similar antioxidant capacities to the kernels. These findings confirm that mango kernel and peel, which are often discarded as processing waste, can be considered as valuable sources of antioxidants to reduce oxidative damage in food products.

Mangoes, like other fruits, contain tannins and oxalates which have anti-nutitive properties. However, mango flesh from all the cultivars contained low levels of tannins and no oxalates. Kernels followed by peel contained the highest tannin content. Oxalate levels were highest in peel and only found in kernels of Nam Dok Mai and Tommy Atkins.

8.2. Effects of drying on the antioxidant capacity of mango flesh, peel and kernel

Sun, forced air, freeze, vacuum and microwave drying were investigated as methods to dry the components of Tommy Atkins mangoes. Four of the drying treatments reduced the antioxidant capacity, particularly the hydrophilic antioxidants of dried flesh which was attributed to the chemical or enzymatic changes that cause the volatilisation or thermal decomposition of specific molecules linked to the antioxidants. In contrast, forced air drying increased the phenolics and ferric reducing antioxidant activity of dried flesh due to caramelisation, the Maillard reaction or interactions between the cellular components that occur during the drying process.

Fresh peel exhibited higher hydrophilic and lipophilic antioxidant capacities, as measured by ORAC, than dried peel. In contrast, microwave and freeze dried peel, contained higher phenolics and ferric reducing antioxidant power (FRAP) than fresh peel. Microwave drying provides a rapid and effective distribution of heat throughout the peel and this may account for the release of a number of phenolic and antioxidant compounds in the dried products. In freeze drying, the development of ice crystals within the tissue matrix may accelerate the rupture of cell structures and promote solvent access, and consequently, the higher extraction of phenolics from the peel.

Fresh kernel contained higher lipophilic antioxidant capacity than vacuum dried kernel. In contrast, vacuum and freeze dried kernel had higher hydrophilic antioxidant values and antioxidant capacity than fresh kernel because of the lower temperature used. This finding is not surprising since the antioxidant capacity of mango kernels are sensitive to thermal treatments. Because of the variation in the effects of the drying treatments on the antioxidant capacity of dried flesh, peel and kernel, it was concluded that the effect of drying on antioxidants was dependent on the characteristics and type of antioxidants in the specific mango fractions.

8.3. Relationships between physicochemical characteristics and antioxidant capacity

8.3.1. Relationship between the physicochemical characteristics

It was found that the physicochemical characteristics total soluble solid (TSS), TSS:TA, and moisture content of the Tommy Atkins fruit, were positively correlated with the firmness, titratable acidity (TA) and vitamin C and negatively correlated with the maturity stage of the mangoes. It was also observed that any one of these physicochemical parameters could act as an indicator of the characteristics of the other parameters. These results indicate that it would be possible to accurately assess the maturity of the Tommy Atkins by any one of the measured parameters and consequently one parameter could be used to accurately determine the time to harvest mangoes for a specific market.

8.3.2. Relationship between the antioxidant assays

All the antioxidant assays; TPC, ABTS, DPPH, FRAP and H-ORAC were strongly correlated with each other indicating that any one assay could be used to monitor the antioxidant capacity of mango flesh, peel or kernels. However, only a weak-to-moderate correlation was found between the lipophilic (L-ORAC) and hydrophilic (H-ORAC) antioxidants or antioxidant capacity measured by ABTS, DPPH and FRAP in mango peel. This could possibly be due to the differences in the composition and concentration of lipophilic antioxidants in the three fractions. To understand these differences in more detail, further investigations and analyses of specific lipophilic antioxidant compounds in mango fractions should be carried out across a wide variety of mangoes. All the antioxidant assays provided acceptable and repeatable results. Nevertheless, the preferred assay that produced higher repeatability for flesh, peel and kernel was TPC; for flesh and peel was FRAP and for kernels was ABTS

8.3.3. Relationship between the physicochemical characteristics and antioxidant assays

In ripe mangoes, kernel tends to have a low antioxidant capacity and peel a high lipophilic antioxidant capacity compared to unripe mangoes. Thus, mango flesh with a low TSS or TSS:TA ratio or high vitamin C exhibited relatively high ferric reducing antioxidant power. Peel from mangoes with a low TA or vitamin C exhibited relatively high lipophilic antioxidants. Kernel from less ripe mangoes with a low maturity score, TSS, TSS:TA ratio and high TA, firmness and vitamin C were relatively high in TPC and antioxidant capacity. Peel colour (Tommy Atkins) was an unreliable index of mango maturity and antioxidant capacity of the mango fractions.

8.4. Effects of mango peel and kernel on inhibiting lipid oxidation in pork products

The pork products without any additives, covered with high oxygen permeable polyvinyl chloride film, showed small changes in TBARS and myoglobin over the first four days of storage. The colour of the pork sausages decreased in redness and discoloured dramatically by day 2. Off-odour and volatiles increased in the first four days and were unacceptable at day 4. The volatiles 1-pentanol and 3-hydroxyl-2-butanone were the major volatiles obtained from control pork sausages and hexanal, pentanal and 1-pentanol were the most abundant volatiles from the control pork patties at day 4 of storage. Some volatiles, such as aldehydes (e.g. hexanal), were only released in the patties.

The addition of kernel or kernel or BHT to the sausages and patties immediately stabilised the colour and odour of the pork products and the antioxidants in mango kernel or peel (1% w/w) or BHT (0.01% w/w) did not significantly impart any colour or aroma changes on the pork sausages and patties. Thus, kernel and peel are potential sources of natural antioxidants for food products.

The control sauasages rapidly oxidised after 4 days whilst sausages or patties with peel or BHT maintained their pleasant odour up to day 4 and with kernel up to day 6. Of the additives, mango kernel was more effective than peel or BHT in inhibiting lipid oxidation over the 10 days of storage. In general, peel and BHT showed relatively similar antioxidative effects on inhibiting lipid oxidation in the pork products. However, peel was more effective than BHT in reducing undesirable odours, maintaining oxymyoglobin (MbO_2), and retarding the formation of MMb (metmyoglobin) and Mb (deoxymyoglobin) in the pork products. Furthermore, peel was more effective in reducing TBARS, discolouration and redness than BHT in sausages. In contrast, BHT showed higher antioxidative effects in reducing TBARS, discolouration and redness than peel in patties. Pork products with high fat levels were more prone to oxidation and discoloration. The TBARS values of patties and the discoloration and loss of redness in patties and sausages were higher in the high fat content products. It was also found that as the fat content increased from 9.1% to 47.4%, lipid oxidation in the pork products increased and the inhibitory effect of kernel was higher than BHT. A possible explanation was that the high fat patties produced more lipid oxidation products and that BHT was less effective than kernels in scavenging the free radicals generated from the increased lipid oxidation.

The microbiological profile of the pork sausages with added kernels were judged to be within official recommended safety limits indicating that pork sausages with added kernels were safe for consumption over the 10 day storage period. Pork patties were contaminated with TVC and E.Coli as judged by Food Management Manual (1995) and the EU microbiological meat standards and were, therefore, judged as unsuitable for human consumption. One of the causes leading to the high levels of E.coli in pork patties could be the high levels of E.coli in the pork mince supplied. This suggested that fresh pork mince used in any study should be pre-tested to determine its acceptability for consumption and further processing. The concentration of all the measured microorganisms in mango peel and kernel supplemented products were below the recommended permissible limits set by regulatory authorities. Kernels are safe to be used as food additives. Furthermore, the addition of dried mango kernel to pork sausages and patties contributed antimicrobial effects against E.coli, staphylococci, coliforms, yeasts, moulds and TVC suggesting that dried kernel itself possesses antimicrobial properties.

8.5. Recommendations for future research

8.5.1. Optimisation of antioxidant incorporation into meat products

It was found that freeze dried kernel and peel powder added at 1% w/w to pork sausages and patties provided sufficient antioxidants to extend the shelf life of the products from 2 to 6 days according to the quality attributes measured. In future, the freeze dried powder could be solvent extracted to obtain a higher concentration of antioxidants and so reduce any unnecessary toxicants or anti-nutrients. Moreover, due to the lipid insolubility of the dried mango products, there was a reduction in the contact of the dried product with the lipid phase of the food products where the majority of oxidation occurs. The extraction processes should be modified to enhance not only the extraction efficiency of the antioxidants but also to generate an extract that can be dissolved in oil to carry the antioxidants to the lipid phase of meat products. There are many methods available for extraction such as by soxhlet, heat reflux, boiling, traditional and microwave distillation methods as well as supercritical fluid extraction (SFE) using carbon dioxide (CO_2) (Kumar, Krishnaiah and Bono, 2008; Saha *et al.*, 2011). Therefore, two possible future investigations are those that aim:

(i) To optimise the extraction procedures (solvent types and concentrations, time and temperature) to obtain antioxidants from mango peel and kernels.
(ii) To identify suitable carriers that will effectively deliver the antioxidant extract to pork products.

8.5.2. Investigation of the effect of mango peel and kernels on retarding deterioration of pork products

The present study found that lipid oxidation enhanced discoloration of pork products due to oxidation of myoglobin. It was suggested that free radicals produced during lipid oxidation, in addition to transition metals, promote the accumulation of oxidized proteins in muscle due to the strong interactions between proteins and lipids (Wolff *et al.*, 1986; Viljanen, Kivikari and Heinonen, 2004; Descalzo and Sancho, 2008). Protein oxidation can cause protein denaturation and the consequential loss in protein function which is known to be detrimental to meat tenderization (Descalzo and Sancho, 2008). Some studies have indicated that antioxidants were effective in inhibiting both lipid and protein oxidation, Je *et al.*, 2001; Batifoulier *et al.,* 2002; Vijanen *et al.,* 2004; Descalzo and Sancho, 2008). Mango kernels and peel contained high hydrophilic and lipophilic antioxidant capacity and may have the potential to prevent protein oxidation. Therefore, another possible future investigation would be to determine the effects of mango kernel or peel on protein oxidation.

8.5.3. Investigation of antimicrobial activities of mango peel and kernel

In the present study, the main purpose of the microbiological analysis was to test the safety of the pork products prior to consumption. Mango kernel powder showed potential for antimicrobial activity on reducing TVC and inhibiting E.coli growth, staphylococci, coliforms yeasts and moulds in pork products over 10 days of storage. Further studies should investigate the mechanism whereby mango peel and kernels can express antimicrobial activity against microorganisms in meat products. Therefore, an investigation should determine the antimicrobial activities of mango kernel and peel extracts in pork products over long storage periods and the amounts required for the antimicrobial expression in foods other than meat.

8.5.4. Study on the sensory characteristics of pork products with added kernel or peel

Addition of mango kernel or peel powder or extracts can impart the colour and flavour of the pork sausages and patties. Therefore, after the microbiological profiles of pork sausages and patties are tested to ensure their hygiene safety for consumption, a sensory evaluation of these products needs to be conducted.

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APPENDICES

A.1 Appendices: Materials and methods-Chapter 3

A.1.1. Mango fractions: flesh, peel and kernel

a. Whole mango fruit (Tommy Atkins)

b. Mango fruits (Cat Hoa Loc & Cat Chu)

c.Mango fractions

d.Freeze dried peel & kernel from Cat Chu

A.1.2. Water loss (%) and weight (g) of mango fractions flesh, peel and kernel before and after drying

a. Flesh

Drying methods	Weight before drying (g)	Weight after drying (g)	Water loss (%)
Sun dry	213.6 ± 9.0	41.8 ± 3.8	80.6 ± 1.1
Forced air dry	228.5 ± 7.5	39.7 ± 2.0	82.6 ± 0.6
Freeze dry	230.2 ± 6.4	40.5 ± 2.4	82.5 ± 0.8
Vacuum dry	145.6 ± 14.3	22.4 ± 1.9	84.2 ± 0.8
Microwave dry	102.2 ± 11.5	18.1 ± 2.5	82.4 ± 0.5

b. Peel

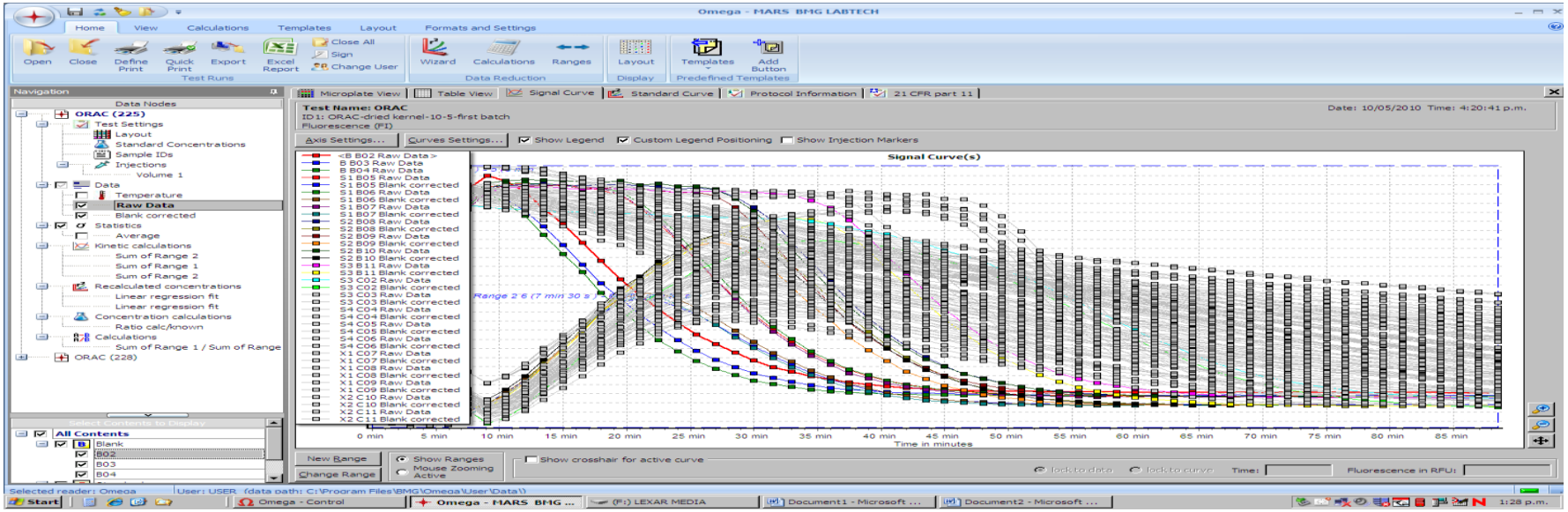
Drying methods	Weight before drying (g)	Weight after drying (g)	Water loss (%)
Sun dry	16.8 ± 1.1	5.2 ± 0.3	68.7 ± 1.0
Forced air dry	16.2 ± 0.4	4.6 ± 0.2	71.4 ± 1.1
Freeze dry	20.7 ± 1.3	5.4 ± 0.4	73.9 ± 1.0
Vacuum dry	22.5 ± 1.8	6.5 ± 0.4	70.6 ± 1.0
Microwave dry	17.6 ± 1.4	5.8 ± 0.4	66.8 ± 0.9

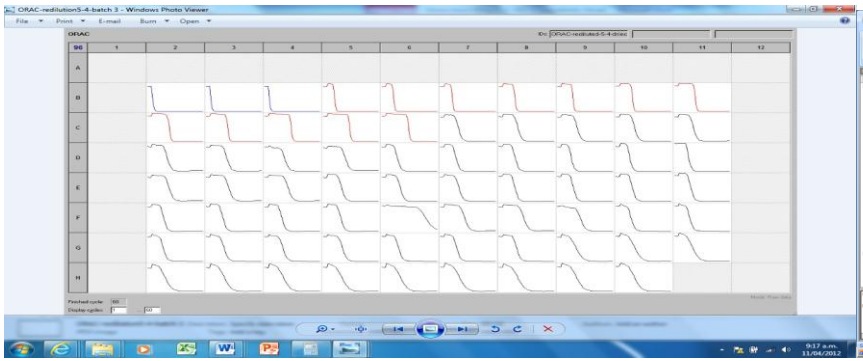
c. Kernel

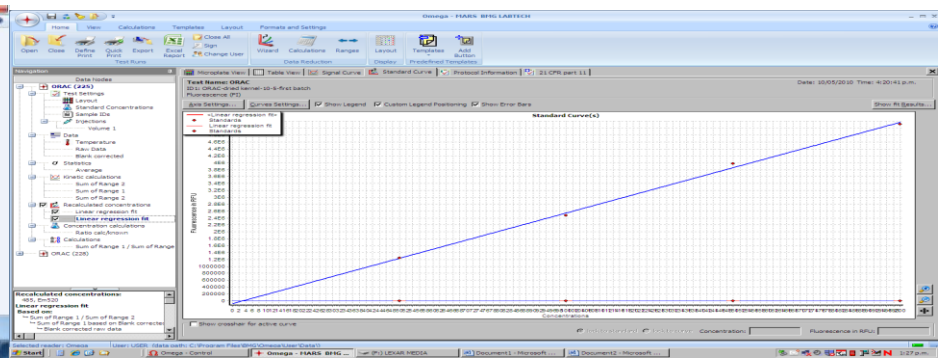
Drying methods	Weight before drying (g)	Weight after drying (g)	Water loss (%)
Sun dry	16.4 ± 1.1	8.2 ± 1.1	50.4 ± 4.8
Forced air dry	18.6 ± 1.2	9.1 ± 1.0	51.2 ± 3.9
Freeze dry	16.2 ± 1.0	7.3 ± 0.9	56.2 ± 3.5
Vacuum dry	16.8 ± 0.9	7.9 ± 1.0	54.1 ± 4.1
Microwave dry	17.7 ± 1.3	11.0 ± 1.3	39.1 ± 5.9

A.1.3. An example of an ORAC output from the plate reader

The curves were plotted by FLUOstar OPTIMA software for a series of standards, phosphate buffer (blank) and diluted fruit extractions, which were prepared as described in the chapter 3. Each line presents the output from a fruit extraction.

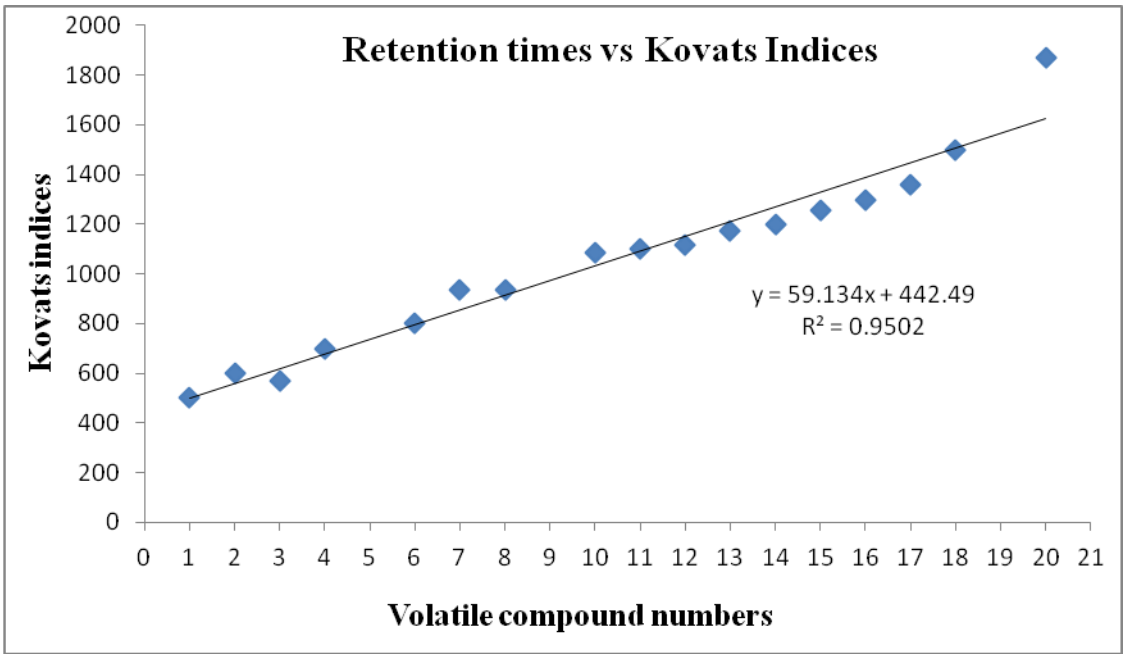






A.1.4 Specifications of the volatiles identified in the pork sausages and patties.

No	Compound name	CAS No	MW	Molecular Formulae	Flavornet - C20
1	Pentane	109-66-0	72.15	C_5H_{12}	500
2	Hexane	110-54-3	86.18	C_6H_4	600
3	Propanal	123-38-6	58.08	C_3H_6O	571
4	Heptane	142-82-5	100.2	C_7H_{16}	700
5	2-propanone	67-64-1	58.08	C_3H_6O	814
6	Octane	111-65-9	114.23	C_8H_{18}	800
7	Pentanal	110-62-3	86.13	$C_5H_{10}O$	935
8	Hexanal	66-25-1	100.1	$C_6H_{12}O$	1084
9	1-butanol	71-36-3	74.12	$C_4H_{10}O$	1145
10	Heptanal	111-71-7	114.19	$C_7H_{14}O$	1174
11	1-pentanol	71-41-0	88.15	$C_5H_{12}O$	1255
12	3-hydroxy-2-butanone	513-86-0	88.11	$C_4H_8O_2$	1287
13	1-hexanol	111-27-3	102.18	$C_6H_{14}O$	1360
14	2-ethyl -1-hexanol	104-76-7	130.23	$C_{18}H_{18}O$	1487
15	Hexanoic acid	142-62-1	116.16	$C_6H_{12}O_2$	1829



A.2. Appendices: Chapter 5

A.2.1. Principal Component Analysis (PCA) of antioxidant assays values for mango fractions using Minitab 16.

A.2.1.1. Flesh

a. Eigenanalysis of the Correlation Matrix

Eigenvalue	3.546	2.185	0.217	0.030	0.021	0.000
Proportion	0.591	0.364	0.036	0.005	0.004	0.000
Cumulative	0.591	0.955	0.991	0.996	1.000	1.000

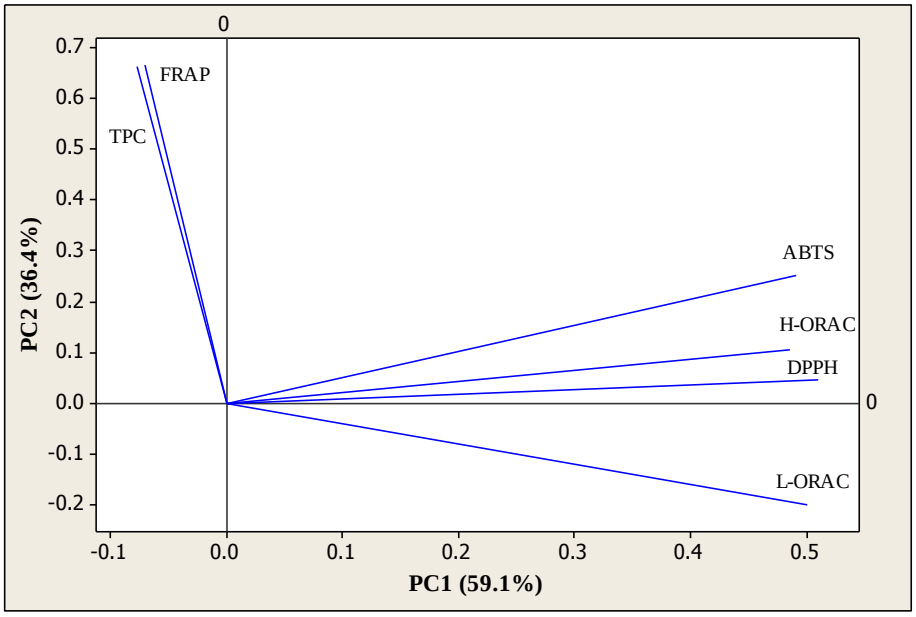
b. Loading coefficients

Variable	PC1	PC2	PC3	PC4	PC5	PC6
TPC	-0.077	0.662	0.159	-0.706	0.044	-0.173
ABTS	0.491	0.252	0.035	0.358	-0.186	-0.729
DPPH	0.510	0.045	0.560	-0.032	-0.436	0.482
FRAP	-0.070	0.667	-0.029	0.555	0.321	0.372
H-ORAC	0.486	0.106	-0.791	-0.204	-0.133	0.260
L-ORAC	0.501	-0.201	0.183	-0.151	0.808	-0.004

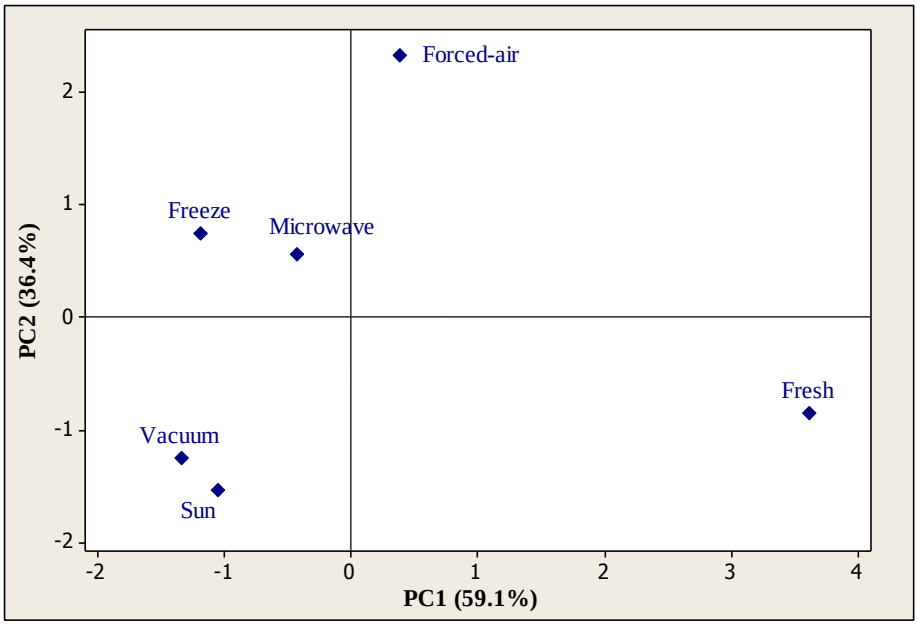
c. PCA score of fresh and dried flesh samples on each PC

	PC1	PC2	PC3	PC4	PC5	PC6
Fresh	3.617	-0.850	0.131	-0.038	-0.021	0.000
Freeze-dried	-1.186	0.745	0.724	-0.183	-0.002	0.000
Forced-air dried	0.389	2.325	-0.048	0.222	0.022	0.000
Vacuum-dried	-1.341	-1.251	-0.048	0.119	-0.229	0.000
Sun-dried	-1.054	-1.535	-0.026	0.092	0.230	0.000
Microwave dried	-0.424	0.565	-0.733	-0.213	0.000	0.000

d. Loading plot reflecting the range of values for the coefficients of PC1 and PC2 applied for flesh



e. Score plot reflecting the range of scores for the fresh and dried flesh



A.2.1.2. Peel

a. Eigenanalysis of the Correlation Matrix

Eigenvalue	3.571	1.682	0.492	0.166	0.089	0.000
Proportion	0.595	0.280	0.082	0.028	0.015	0.000
Cumulative	0.595	0.876	0.958	0.985	1.000	1.000

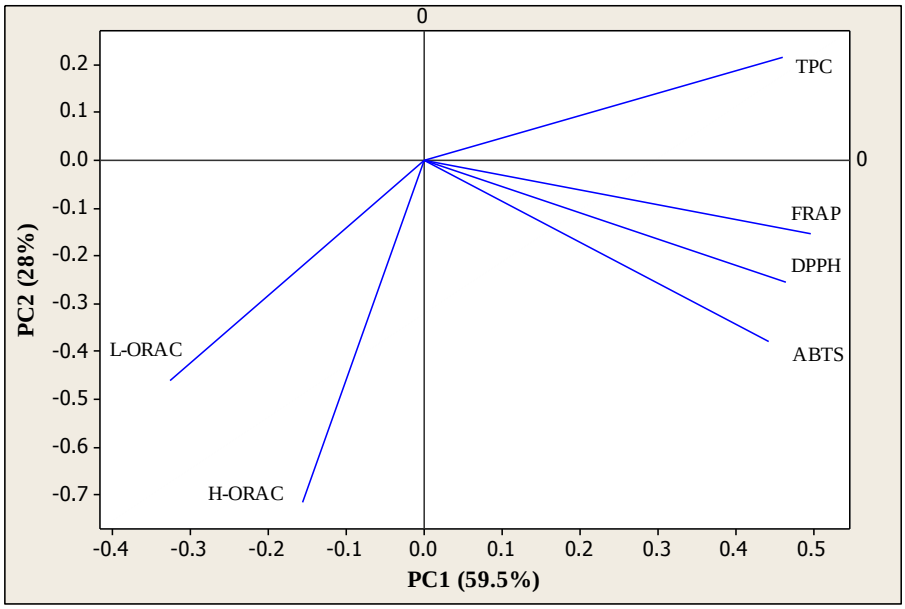
b. Loading coefficients

Variable	PC1	PC2	PC3	PC4	PC5	PC6
TPC	0.460	0.215	0.521	0.197	-0.539	-0.376
ABTS	0.441	-0.378	-0.238	-0.430	0.254	-0.597
DPPH	0.464	-0.255	-0.188	0.761	0.285	0.152
FRAP	0.497	-0.153	0.316	-0.428	0.048	0.667
H-ORAC	-0.157	-0.715	-0.175	0.035	-0.649	0.105
L-ORAC	-0.327	-0.460	0.711	0.112	0.374	-0.154

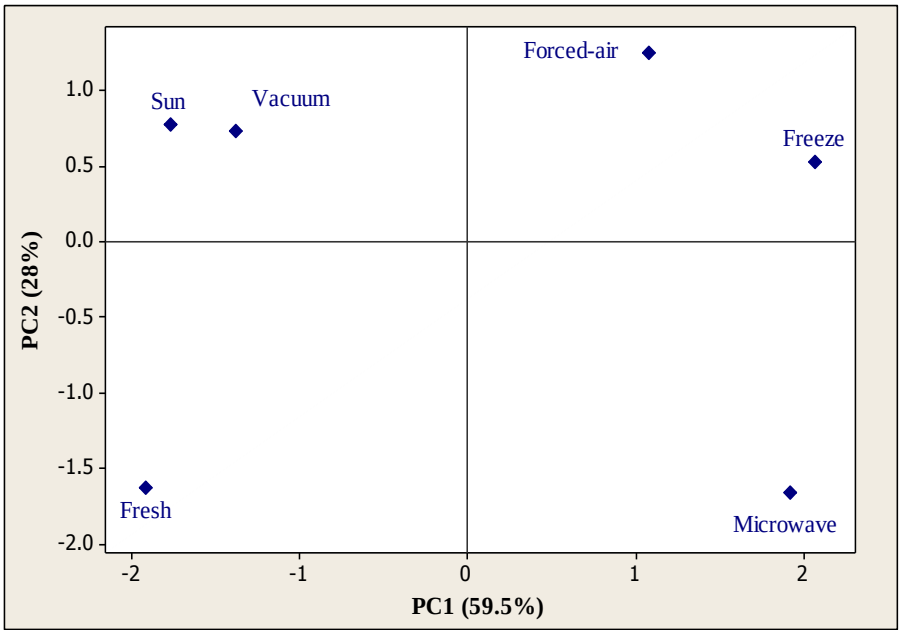
c. PCA score of fresh and dried peel samples on each PC

	PC1	PC2	PC3	PC4	PC5	PC6
Fresh	-1.920	-1.631	-0.406	-0.442	-0.058	0.000
Freeze-dried	2.069	0.532	0.752	-0.507	-0.093	0.000
Forced-air dried	1.080	1.248	-1.196	0.016	-0.020	0.000
Vacuum-dried	-1.380	0.737	0.352	0.086	0.518	0.000
Sun-dried	-1.769	0.775	0.410	0.359	-0.402	0.000
Microwave dried	1.920	-1.662	0.088	0.489	0.055	0.000

d. Loading plot reflecting the range of values for the coefficients of PC1 and PC2 applied for peel



e. Score plot reflecting the range of scores for the fresh and dried peel



A.2.1.3. Kernel

a. Eigenanalysis of the Correlation Matrix

Eigenvalue	4.410	1.067	0.511	0.007	0.005	0.000
Proportion	0.735	0.178	0.085	0.001	0.001	0.000
Cumulative	0.735	0.913	0.998	0.999	1.000	1.000

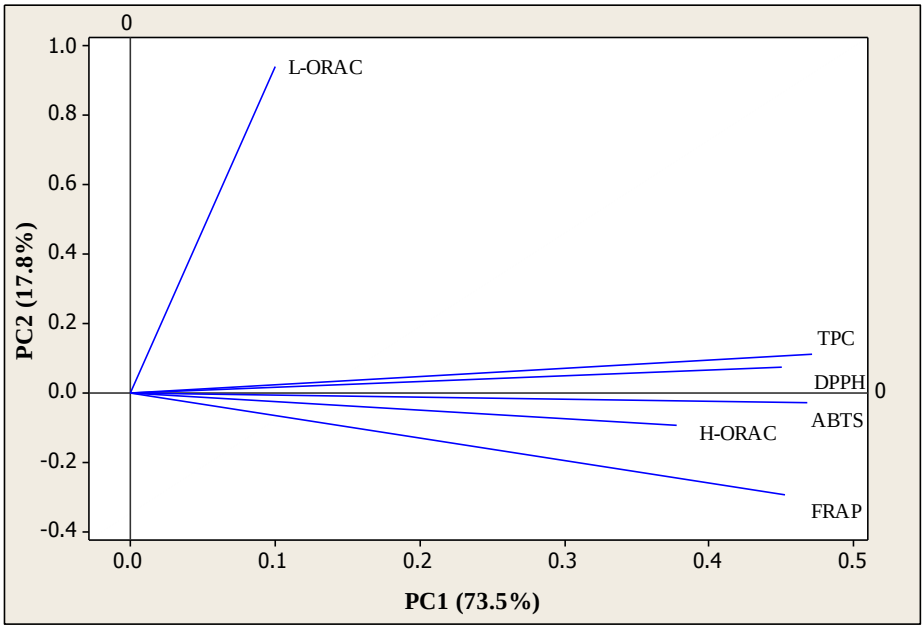
b. Loading coefficients

Variable	PC1	PC2	PC3	PC4	PC5	PC6
TPC	0.471	0.112	-0.095	0.097	-0.845	0.184
ABTS	0.467	-0.028	-0.257	-0.510	0.081	-0.669
DPPH	0.450	0.075	-0.441	-0.124	0.433	0.628
FRAP	0.452	-0.294	0.071	0.764	0.238	-0.252
H-ORAC	0.378	-0.092	0.840	-0.308	0.110	0.192
L-ORAC	0.100	0.941	0.143	0.192	0.154	-0.152

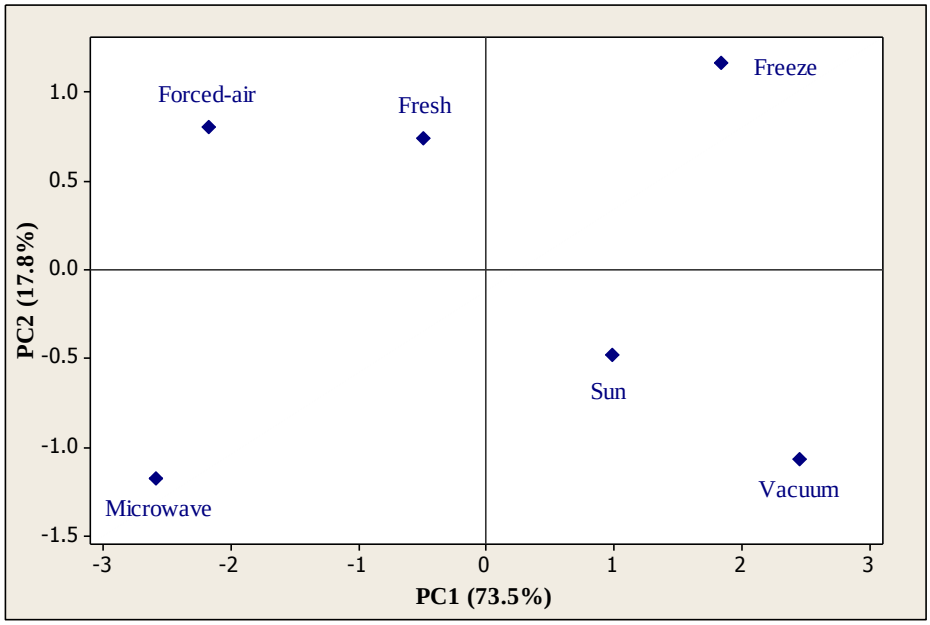
c. PCA score of fresh and dried kernel samples on each PC

	PC1	PC2	PC3	PC4	PC5	PC6
Fresh	-0.498	0.745	1.326	0.020	-0.022	0.000
Freeze-dried	1.837	1.171	-0.461	-0.111	-0.011	0.000
Forced-air dried	-2.176	0.807	-0.601	0.079	0.067	0.000
Vacuum-dried	2.452	-1.066	0.205	0.020	0.089	0.000
Sun-dried	0.979	-0.477	-0.425	0.088	-0.107	0.000
Microwave dried	-2.593	-1.180	-0.044	-0.097	-0.016	0.000

e. Loading plot reflecting the range of values for the coefficients of PC1 and PC2 applied for kernel



e. Score plot reflecting the range of scores for the fresh and dried kernel



A.2.2. Canonical Variate Analysis (CVA) for mango fractions by Genstat 13

A.2.2.1. Flesh

a. Loading coefficients for variables in CV1 & CV2 for flesh

Assays	CV1	CV2
TPC	-0.00379	0.00825
ABTS	0.02883	0.03150
DPPH	0.03138	-0.01388
FRAP	-0.04301	-0.02341
H-ORAC	0.06342	0.00144
L-ORAC	0.50867	0.06860

b. Significant difference among fresh and dried flesh in CV1 and CV2

Drying methods	CV1 score	CV2 score
Fresh	6.735d	0.025b
Freeze dried	-2.9a	0.5196b
Forced-air dried	-0.824bc	1.3998c
Sun dried	-0.782bc	-1.217a
Vacuum dried	-1.585b	1.3998c
Microwave dried	-0.645c	0.4112b

A.2.2.2. Peel

a. Loading coefficients for variables in CV1 & CV2 for peel

Assays	CV1	CV2
TPC	0.00086	-0.00065
ABTS	-0.00487	0.00317
DPPH	0.00260	0.00655
FRAP	0.00152	0.00128
H-ORAC	-0.00761	0.00127
L-ORAC	-0.19227	-0.43542

b. Significant difference among fresh and dried peel in CV1 and CV2

Drying methods	CV1 score	CV2 score
Fresh	-2.0879a	0.1468bcd
Freeze dried	1.4175d	-0.1840abc
Forced-air dried	0.7696cd	0.6122cd
Sun dried	-0.0692b	-0.7657a
Vacuum dried	-0.0564b	-0.4603ab
Microwave dried	0.0264bc	0.6511d

A.2.2.3. Kernel

a. Loading coefficients for variables in CV1 & CV2 for kernel

Assays	CV1	CV2
TPC	-0.00056	-0.00046
ABTS	0.00063	0.00219
DPPH	0.00083	0.00174
FRAP	0.00169	-0.00238
H-ORAC	0.00061	0.00044
L-ORAC	-0.18222	0.00324

b. Significant difference among fresh and dried kernel in CV1 and CV2

Drying methods	CV1 score	CV2 score
Fresh	-0.5553ab	-0.2387ab
Freeze dried	-1.1893a	-0.0404bc
Forced-air dried	-0.3077b	0.9755d
Sun dried	0.1288bc	-0.4534ab
Vacuum dried	0.7069cd	-0.9291a
Microwave dried	1.2165d	0.6862cd

A.3 Appendix: Chapter 6

A.3.1. Interactions of fresh and drying treatments on the correlations between assays

Correlations between each 2 assays	Interactions of fresh and drying treatments on the correlations between assays					
For flesh	Fresh	Freeze dry	Forced-air dry	Sun dry	Vacuum dry	Microwave dry
TPC x ABTS	***	*	N/S	N/S	N/S	**
TPC x H-ORAC	*	N/S	N/S	N/S	N/S	N/S
DPPH x L-ORAC	*	N/S	N/S	N/S	N/S	N/S
FRAP x H-ORAC	**	*	N/S	N/S	N/S	N/S
H-ORAC x L-ORAC	***	N/S	N/S	N/S	N/S	N/S
For peel						
Correlations between each 2 assays for peel						
TPC x H-ORAC	***	N/S	N/S	N/S	N/S	N/S
ABTS x FRAP	**	N/S	*	N/S	N/S	N/S
DPPH x H-ORAC	*	N/S	N/S	N/S	N/S	N/S
FRAP x H-ORAC	**	N/S	N/S	N/S	N/S	N/S
For kernel						
Correlations between each 2 assays for kernel						
ABTS x DPPH	***	N/S	N/S	N/S	N/S	N/S
ABTS x FRAP	***	*	***	N/S	N/S	N/S

*Correlation is significant at *p < 0.05; **p < 0.01; ***p < 0.001; N/S: non-significant*

A.3.2. PCA for determining correlations

A.3.2.1. PCA for determining correlations between physicochemical characteristics

a. Eigen values, proportion of total variability and percent of cumulative variance for the first three principal components

PC	Eigenvalue	% Proportion	% Cumulative
1	4.438	0.277	0.277
2	3.365	0.210	0.488
3	2.176	0.136	0.624

Eigenvalues and variance accounted for (%) by PCA based on correlation matrix

b. Loadings coefficients of PC1, PC2 and PC3 for 16 physicochemical paramaters

Variables	PC1	PC2	PC3
Maturity score	0.372917314	0.108241345	-0.07939252
TSS	0.257357791	0.077975383	-0.16370501
TA	-0.306907937	-0.329165473	-0.02016576
TSS:TA	0.328405997	0.200028461	-0.11965925
Firmness	-0.343521102	-0.020796239	0.128987474
Vitamin C	-0.318964684	-0.279467114	-0.08804258
Moisture content	0.323101085	0.070060671	-0.16004470
Total weight	-0.052822306	0.042508185	-0.31776058
%flesh	0.095064296	-0.388835562	-0.23813002
%peel	-0.137982692	0.237381651	0.278868268
%kernel	0.105101382	0.280210540	0.038580476
Chroma	0.136208273	-0.316990441	-0.35099837
Hue	0.241014302	-0.235062141	0.406793627
L*	0.256966748	-0.381551832	0.183031161
a*	-0.136701032	0.024177732	-0.56607549
b*	0.255430080	-0.400784181	0.15037451

A.3.2.2 PCA for determining correlations between antioxidant assays (an example on flesh)

a. Eigenvalues, proportion of total variability and percent of cumulative variance for the first three principal components

PC	Eigenvalue	% Proportion	% Cumulative
1	3.559	0.593	0.593
2	1.118	0.186	0.780
3	0.612	0.102	0.882

b. Loadings coefficients of PC1, PC2 and PC3 for six antioxidant assay variables in flesh

Variables	PC1	PC2	PC3
TPC	0.434666908	-0.143858065	0.105078562
ABTS	0.435642063	-0.259160884	-0.294743953
DPPH	0.457308881	-0.163774955	0.098719575
FRAP	0.435445820	-0.226200690	-0.346996873
H-ORAC	0.397568325	0.334641920	0.758158796
L-ORAC	0.253920508	0.849803678	-0.443990262

A.3.2.3. PCA for determining correlations between physicochemical characteristics of mango fruits and antioxidant capacity of the fractions (an example of PCA for kernel).

a. Eigenvalues, proportion of total variability and percent of cumulative variance for the first three principal components

PC	Eigenvalues	% Proportion	% Cumulative
1	7.217	0.328	0.328
2	3.574	0.162	0.491
3	3.028	0.138	0.628
4	1.950	0.089	0.717
5	1.458	0.066	0.783
6	1.054	0.048	0.831

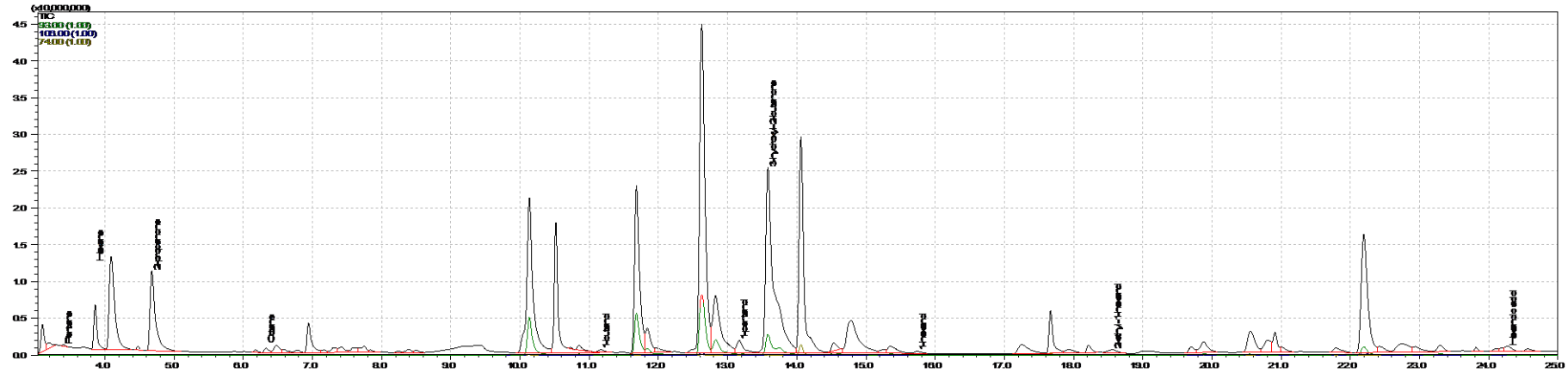
b. Rotated factor loadings of PC1 and PC2 for 16 physicochemical parameter variables and 6 antioxidant assay variables in kernel

Variables	Factor1	Factor2
TPC	0.929	-0.255
ABTS	0.953	-0.230
DPPH	0.914	-0.256
FRAP	0.924	-0.154
H-ORAC	0.937	-0.153
L-ORAC	0.310	0.154
Maturity score	-0.449	0.728
TSS	-0.510	0.427
Firmness	0.281	-0.753
TA	0.149	-0.868
TSS:TA	-0.399	0.692
VitaminC	0.085	-0.807
Moisture content	-0.130	0.744
a*	-0.011	-0.100
b*	-0.005	-0.028
L*	0.071	0.016
Hue	-0.030	0.090
Chroma	0.011	-0.044
Total weight	-0.260	-0.031
%flesh	-0.183	-0.349
%peel	0.276	0.107
%kernel	-0.260	0.314
Variance	**5.433**	**4.223**
%Var	**0.247**	**0.192**

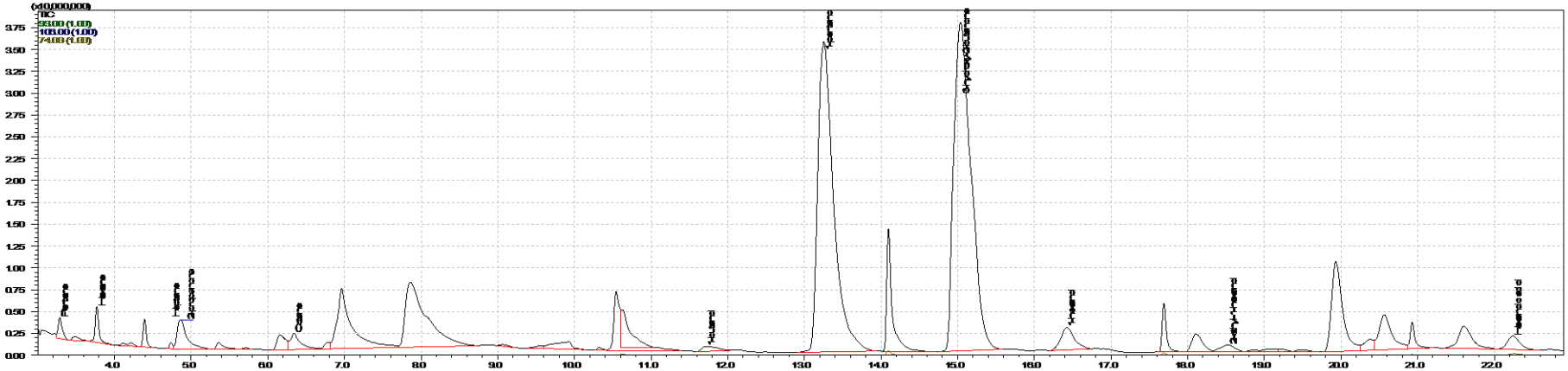
A.4. Appendices: Chapter 7.

A.4.1. Chromatogram obtained by SPME-GC-MS of volatiles produced from pork sausages and patties

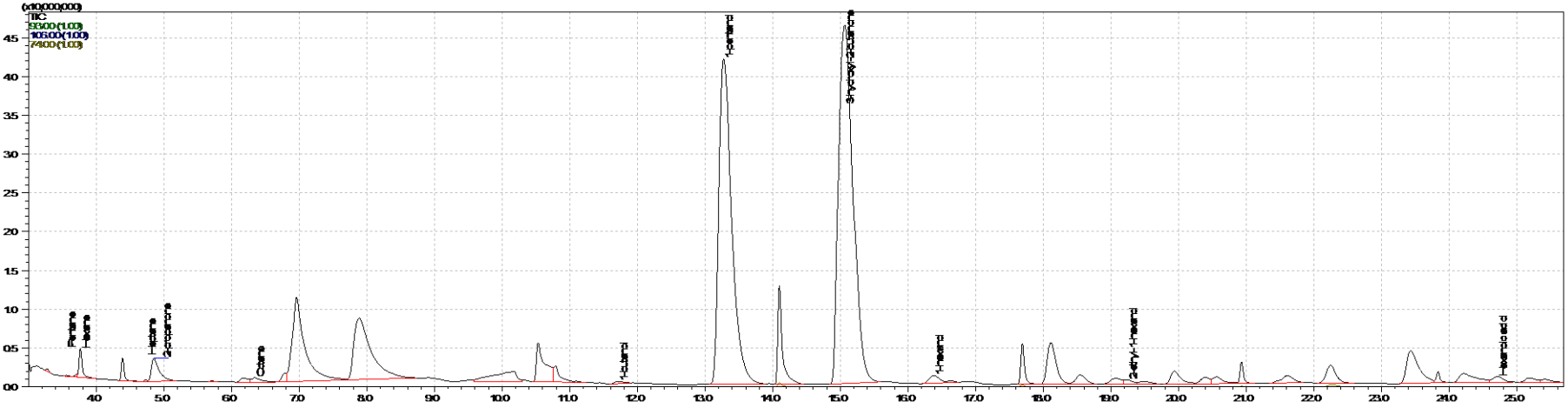
A.4.1.1. Control sausage at day 0



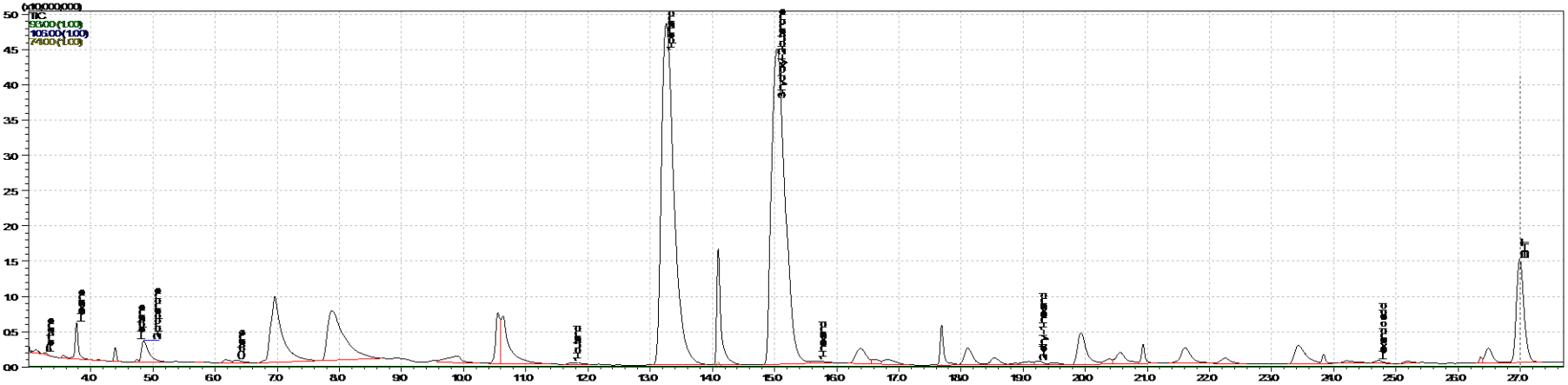
A.4.1.2. Control sausage day 4



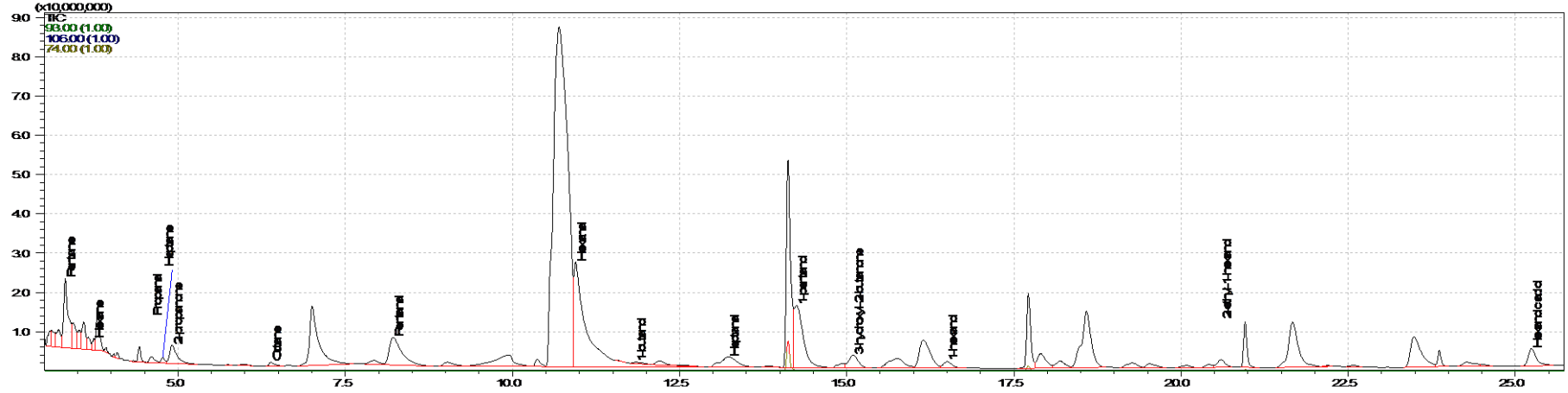
A.4.1.3. Sausages with added kernel at day 4



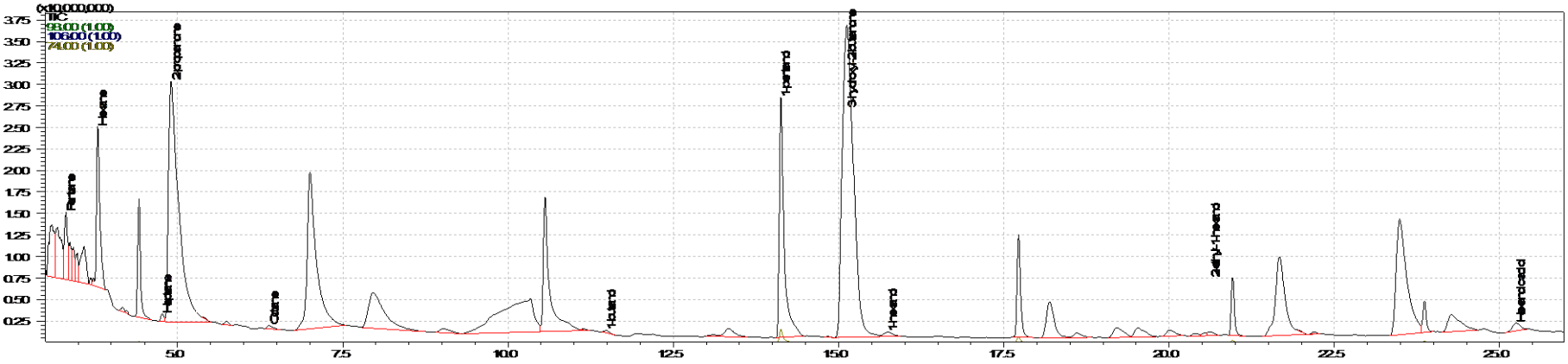
A.4.1.4. Sausages with added BHT at day 4



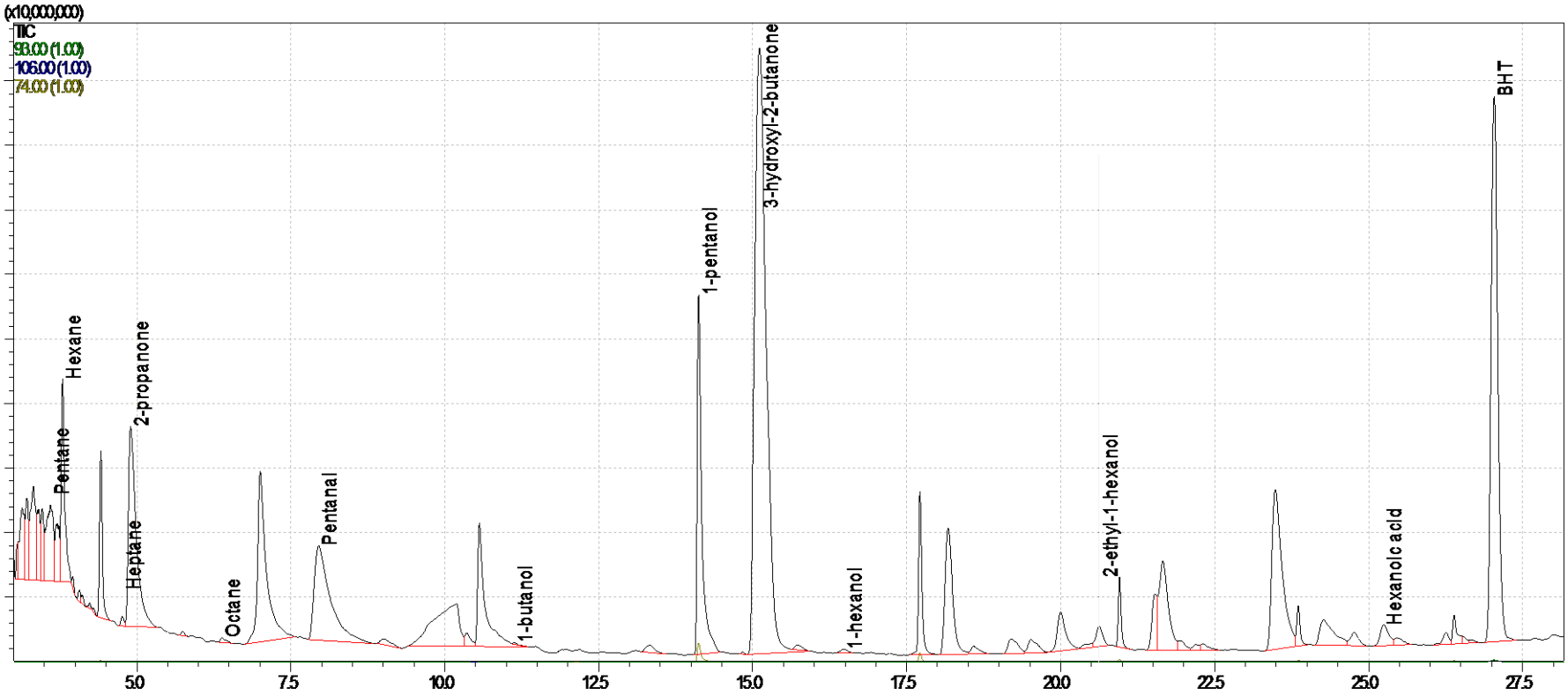
A.4.1.5. Control patties at day 4



A.4.1.6. Patties with added kernel at day 4



A.4.1.7. Patties with added BHT at day 4



A.4.2. Microbiological test results from Hills Lab

Hill Laboratories
BETTER TESTING BETTER RESULTS

R J Hill Laboratories Limited
1 Clyde Street
Private Bag 3205
Hamilton 3240, New Zealand

Tel +64 7 858 2000
Fax +64 7 858 2001
Email mail@hill-labs.co.nz
Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	Lincoln University	**Lab No:**	966628 SPv1
Contact:	Sue Mason	**Date Registered:**	06-Jan-2012
	C/- Lincoln University	**Date Reported:**	22-Jan-2012
	Agriculture & Life Sciences Division	**Quote No:**	47496
	PO Box 84	**Order No:**	4331
	CANTERBURY 7647	**Client Reference:**	Meat Product & Ingredients t
		Submitted By:	Sue Mason

Sample Type: Meat, Poultry and Derived Products

Sample Name:		Fresh Mince Tested Day 0 06/01/12	Kernel Added Sausage Tested Day 0 06/01/12	Kernel Added Sausage Tested Day 4 10/01/12	Kernel Added Sausage Tested Day 10 16/01/12	
Lab Number:		966628.3	966628.4	966628.5	966628.6	
Aerobic Plate Count 35°C	cfu / g	1,700,000	370,000	2,200,000 #1	< 100,000	-
Escherichia coli	cfu / g	> 1,500 #1	< 5 #1	< 5 #1	< 5 #1	-
Staphylococcus aureus	cfu / g	< 10	< 10	< 10	< 10	-
Clostridium perfringens	cfu / g	< 10 #1	< 10 #1	< 10 #1	< 10 #1	-
Yeasts	cfu / g	3,600	3,500	> 15,000 #1	> 15,000 #1	-
Moulds	cfu / g	< 50 #1	< 50 #1	< 50 #1	< 50 #1	-
Yeasts & Moulds	cfu / g	3,600	3,500	-	-	-
Salmonella	per 25g	Not Detected	Not Detected	Not Detected	Not Detected	-

Sample Type: Unspecified Dry Foods

Sample Name:		Mango Peel Powder	Mango Kernel Powder			
Lab Number:		966628.1	966628.2			
Aerobic Plate Count 35°C	cfu / g	190,000	600 #1	-	-	-
Escherichia coli	cfu / g	< 5 #1	< 5 #1	-	-	-
Bacillus cereus	cfu / g	< 10 #1	< 10 #1	-	-	-
Yeasts	cfu / g	50 #1	< 50 #1	-	-	-
Moulds	cfu / g	50 #1	< 50 #1	-	-	-
Yeasts & Moulds	cfu / g	100	< 50	-	-	-

Sample Type: Unspecified Fresh Foods

Sample Name:		Kernel Added Patties Tested Day 0 06/01/12	Kernel Added Patties Tested Day 4 10/01/12	Kernel Added Patties Tested Day 10 16/01/12		
Lab Number:		966628.7	966628.8	966628.9		
Aerobic Plate Count 35°C	cfu / g	2,200,000	9,900,000	73,000,000	-	-
Escherichia coli	cfu / g	> 1,500 #1	> 1,500 #1	< 5 #1	-	-
Staphylococcus aureus	cfu / g	< 10	< 10	< 10	-	-
Clostridium perfringens	cfu / g	< 10 #1	< 10 #1	< 10 #1	-	-
Yeasts	cfu / g	3,700	> 15,000 #1	> 15,000	-	-
Moulds	cfu / g	< 50 #1	< 50 #1	< 50 #1	-	-
Yeasts & Moulds	cfu / g	3,700	-	-	-	-
Salmonella	per 25g	Not Detected	Not Detected	Not Detected	-	-

Analyst's Comments

#1 Statistically estimated count based on the theoretical countable range for the stated method.

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Meat, Poultry and Derived Products			
Test	**Method Description**	**Default Detection Limit**	**Samples**
Staphylococcus aureus	Spread plate, Count on Baird Parker agar, Incubated at 35-37°C for 45-48 hours, Confirmation. Analysed at Hill Laboratories - Microbiology; 101C Waterloo Road, Christchurch. APHA 39.5 4th Ed, FDA BAM 12.	10 cfu / g	3-9
Clostridium perfringens	Spread plate, Count on TSC agar, Incubated anaerobically at 35-37°C for 18-24 hours, Confirmation. Analysed at Hill Laboratories - Microbiology; 101C Waterloo Road, Christchurch. APHA 34.7 4th Ed.	10 cfu / g	3-9
Salmonella	Presence / Absence. Salmonella Enrichment Broths, TECRA, Confirmation. Analysed at Hill Laboratories - Microbiology; 101c Waterloo Road, Hornby, Christchurch. TECRA VIA.	-	3-9

Sample Type: Unspecified Dry Foods			
Test	**Method Description**	**Default Detection Limit**	**Samples**
Aerobic Plate Count 35°C	Count on APC Petrifilm, Incubated at 35°C for 48 hours. Analysed at Hill Laboratories - Microbiology; 101C Waterloo Road, Christchurch. APHA 7.72 4th Ed.	10 cfu / g	1-9
Escherichia coli	Count on E.coli Petrifilm, Incubated at 35°C for 48 hours. Analysed at Hill Laboratories - Microbiology; 101C Waterloo Road, Christchurch. APHA 8.935 4th Ed.	5 cfu / g	1-9
Bacillus cereus	Spread plate, Count on MYP agar, Incubated at 30-32°C for 20-24 hours, Confirmation. Analysed at Hill Laboratories - Microbiology; 101C Waterloo Road, Christchurch. APHA 32.2 4th Ed.	10 cfu / g	1-2
Yeasts & Moulds	Spread plate, Count on DRBC agar, Incubated at 22-25°C for 5 days. Analysed at Hill Laboratories - Microbiology; 101C Waterloo Road, Christchurch. APHA 20.51 4th Ed.	50 cfu / g	1-9

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

S. McKay

Sally McKay BSc
Team Leader - Microbiology