Controlling Processing for Persimmon Product Texture

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

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JANTANA SUNTUDPROM

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Abstract

'Fuyu' sweet persimmon is cultivated commercially in the northern part of New Zealand. In 2012, 2,250 tonnes from 50 New Zealander growers was produced with domestic and international market values of $4.0M and $7.1M respectively. Approximately 50 % of persimmon do not meet export standards due to external skin blemishes, and consequently could be processed into alternative products. The global preserved or prepared fruit market has been gradually increasing and is estimated at US $34B.

Many food products such as jams, marmalades, spreads, fillings and toppings are produced by using fruit purée as a key ingredient. Texture and colour are important quality attributes in these products which influence customer acceptability. The properties of fruit purée are dependent on the input fruit quality. Changes after harvest in terms of physicochemical and sensory properties significantly influence the quality of manufactured products and its properties as an ingredient. Processing persimmon into a desired product is a challenge due to the limited knowledge of the relationship between postharvest quality of fruit and their processed characteristics. The overall objective of this research was to create generalised guidelines for the effects of persimmon fruit quality and processing conditions on the resulting textural properties of a persimmon product. This research informs manipulation of processes in order to achieve the required product properties that result in positive sensorial experiences.

During postharvest shelf life at 20 °C whole persimmon firmness reduced on average with large variation. Persimmon skin turned from a pale green-yellow to orange-red. A correlation of hue angle between persimmon skin and tissue was developed. In addition, influences of fruit colour on final product quality were demonstrated and provide a guideline for future work in order to investigate the effects of β-cryptoxanthin changes during fruit ripening on final product colour characteristics.

β-galactosidase was found to be the predominant enzyme in persimmon ripening and its activity increased approximately 3 times at fully-ripe state. As ripening progressed, pectinmethylesterase (PME) activity increased and later declined, while no significant change in polygalacturonase (PG) activity was detected, even though total galacturonic acid (GalA) content declined. Increasing β-galactosidase and PME with decreasing total GalA during ripening resulted in a flowable purée and low purée yield when processing more ripe fruit due to cell wall components of fruit tissue being degraded and solubilised.

After puréeing, persimmon gels spontaneously formed at 20 °C which was symptomatic of calcium gel behaviour. In contrast, the degree of methylesterification of persimmon was observed to be 53 % on average which could be classified as a high methoxyl pectin. Ethylenediaminetetraacetic acid (EDTA) chelation prevented the gel structure forming by sequestering calcium ions. Heating tissue to 73 °C for 30 minutes prior to tissue puréeing resulted in the strongest gels in all samples. Heat treatment and processing activated PME resulting in long chains of GalA which are sensitive to calcium crosslinking. Interestingly, combining 25 % of mature-green tissue with 75 % of overripe tissue resulted in increasing gel strength dramatically by approximately 85 % in comparison to gel made from 100 % of overripe tissue. This strong gel from combining tissue is thought to be due to calcium crosslinks created between the long chains of methyl free GalA from mature-green tissue and the short chains of methyl free GalA presented in overripe tissue.

This research develops general guidelines for how fruit maturity and heat treatment influence persimmon purée product final quality attributes. Textural characteristics of persimmon purée product can be manipulated to achieve the final desired product quality by controlling fruit ripeness and processing conditions (EDTA addition, temperature and combining different types of tissue). Based on a preliminary study, the investigation of the degree of blockiness of persimmon pectin is suggested for future work in order to characterise in the structures of persimmon pectin which impart gelling mechanism and the subsequent result on gel characteristics. This

future work would be beneficial for the improvement of processing guidelines to achieve the desired final product quality attributes.

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Abbreviations

IU	International unit
µg	microgram
mg	milligram
g	gram
GalA	galacturonic acid
HMP	high methoxyl pectin
LMP	low methoxyl pectin
DMe	degree of methylesterification
PME	pectinmethylesterase
PG	polygalacturonase
Hz	hertz
s	second
G′	storage modulus
G″	loss modulus
G*	complex modulus
η*	dynamic viscosity
° δ	loss tangent
Pa	Pascal
mm	millimeter
∅	diameter
H	height
FW	fresh weight
DW	dry weight
w	weight
v	volume
TSS	total soluble solids
DM	dry matter
HG	homogalacturonan
RG I	rhamnogalacturonan I

RG II	rhamnogalacturonan II
E_a	activation energy
R	gas law constant
T	absolute temperature
k	reaction rate constant
kg_f	kilogram force
CIE L*C*h*	colour parameter measured by spectrophotometer (lightness, chroma and hue angle)
NaOH	sodium hydroxide
DNS	dinitrosalicylic acid
H_2SO_4	sulfuric acid
NaCl	sodium chloride
MeOH	methanol
W_{flesh}	weight of flesh persimmon tissue
W_{can}	can weight
W_{dry}	weight of flesh persimmon tissue after drying
WHC	water holding capacity
m_a	filter paper weight
m_p	purée weight
m_f	weight of filter paper after 15 minutes
EDTA	ethylenediaminetetraacetic
ω	angular frequency
Hz	hertz
M.W.	molecular weight
A.W.	atomic weight
M	molarity
J	Joule

Research Background

1.1 Introduction

Persimmon was introduced to many countries in temperate zones at the end of the nineteenth century (Kitagawa & Glucina, 1984; Roy et al., 1995). 'Fuyu' (*Diospyros kaki L.* cv Fuyu) is a non-astringent persimmon (Childers et al., 1995) and is cultivated commercially in the northern part of New Zealand (Lyle, 2006). In 2012, 2,250 tonnes from 50 New Zealand growers was produced, with the domestic market value being $4.0M (Aitken & Hewett, 2012). 'Fuyu' persimmon is the main variety which is exported to many places including Australia, Malaysia, Thailand, Singapore, Hong Kong, Canada and the European Union (Turk, 2012). The export market price is increasing, currently being valued at $7.1M in 2012 (Aitken & Hewett, 2012). However, there are 50 % of persimmon which do not meet the export standard but could be processed into alternative products.

Fruit purée is an important ingredient for many food products such as jams, marmalades, spreads, fillings and toppings. The global trade in preserved or prepared fruit is currently estimated at US $34B (Anon., 2013c). The biggest exporter and importer of preserved or prepared fruit are Brazil (US $2.5B) and Japan (US $2.1B) respectively. Generally, consumer acceptability of food products produced from fruit purées is influenced by texture and colour (El-Zoghbi, 1994; Meullenet, 2009). Particularly, textural attributes are crucial for sensory acceptance and are heavily influenced by for example, the type of fruit. However, the qualities of fruit purée are also dependent on the fruit input quality. Naturally, after harvest, fruit have variability in maturity and quality factors such as colour, flavour and firmness. After harvest, fruit remain physiologically active, further changing their physicochemical and sensory properties. Like any other food processing industry, the quality of the

end product can be affected significantly by variation in the raw material fruit quality and properties.

Processing persimmon into a desired product is a challenge due to the limited knowledge of the relationship between postharvest quality of fruit and the resulting processed product characteristics. Additionally, persimmon is a good model for other fruit as it is consumed over a wide range of textural profiles, from crisp like an apple, to almost gooey soft like a ripe tomato, meaning that there is a large range in the input material to investigate. Development of guidelines of the effects of persimmon fruit quality on the resulting properties of a persimmon purée will enable manipulation of manufacturing processes in order to achieve the required product properties. Outcomes from this research could benefit commercial applications for developing persimmon processing and technology.

1.2 Research aim and objectives

This study aimed to provide the knowledge and mechanisms for the effects of fruit ripeness and processing conditions on the resulting textural characteristics of persimmon purée. The study focused on textural qualities as they are significant for sensory acceptance. The overall objective was to generate guidelines of the effects of factors on persimmon product final quality attributes. Hence, the objectives were:

i) To character the postharvest changes of persimmons as a means to describe the potential physical and physicochemical qualities of the fruit as a raw material.

ii) To determine the effects of the persimmon ripeness and manufacturing on yield, physicochemical and textural characteristics of persimmon product

iii) To define processing effects on the persimmon product textural characteristics and their underlying mechanisms.

CHAPTER 2

Literature Review

2.1 Persimmon

2.1.1 Persimmon production

The Japanese or Oriental persimmon (*Diospyros kaki*) originated in China (Kitagawa & Glucina, 1984). Before 1900, persimmon was introduced to Japan, where it is simply called kaki (Kitagawa & Glucina, 1984). Persimmon was introduced to many countries in temperate zones at the end of the nineteenth century such as Italy, Brazil, USA, and Israel (Kitagawa & Glucina, 1984; Roy et al., 1995). Persimmon is classified into two types: non-astringent (e.g. 'Fuyu', 'Jiro', 'Suraga' and 'Mizushima') and astringent (e.g. 'Hachiya', 'Yotsumizo' and 'Hiratranenashi') varieties (Roy et al., 1995). Non-astringent persimmon can be eaten from crisp to soft. Astringent persimmon composes of tannin that results in a tartness. These tannins reduce during ripening and hence astringent persimmon need to be fully ripened before consumption (Taira et al., 1998). Persimmon is described as a typical or true berry – a simple fruit derived from a single pistil with essentially fleshy pericarp throughout with the exocarp forming merely a thin skin (Cronquist, 1971). Persimmon has a superior ovary therefore the fruit often has the remnant of the calyx at the base (Lyle, 2006). Additionally, persimmon is classified as a climacteric fruit (Crisosto et al., 1999).

'Fuyu' has been cultivated in New Zealand since the 19th century (Kitagawa & Glucina, 1984). In 2012, the production was 2,250 tonnes from a total of 50 growers (Aitken & Hewett, 2012). The domestic market value was $4.0M. New Zealand persimmon was exported to many places, for example, Australia, Malaysia, Thailand, Singapore, Hong Kong and Canada (Turk, 2012). The value of the export market was $7.1M in 2012 (Aitken & Hewett, 2012). However, there are

approximately 50 % of persimmon that do not meet export quality standards and hence are available to be processed into alternative products.

2.1.2 Nutritional and compositional constituents

Table 2.1 shows typical nutritional and compositional elements of persimmon fruit. Persimmon fruit contains high vitamin A (5400 IU) and vitamin C (52 mg.100 g^{-1} FW). Vitamin A and C in persimmon fruit are 6 and ~ 2.3 times higher than vitamin A and C in tomato fruit reported by Thakur et al. (1996). Persimmon contains a comparable dietary fibre (86.7 %) to Japanese pear (87.5 %) (Takekawa & Matsumoto, 2012). Persimmon comprises calcium at 9 mg.100 g^{-1} FW which is less than pear (13 mg.100 g^{-1} FW) (Kadam et al., 1995). The high vitamin A and dietary fibre properties of persimmon could be considered as functional properties which have a potential for developing innovative food products. Persimmon contains a wide range of carotenoids from 2.8-8.3 mg.100 g^{-1} FW. Beta-cryptoxanthin is the most abundant carotenoid being about 20-30 % of the total carotenoids (Zhou et al., 2011). Carotenoids are not only responsible for fruit colour but are also precursors of vitamin A (Sánchez-Moreno et al., 2003).

2.2 Postharvest ripening

As ripening progresses, physicochemical and physical changes occur. Processing of different ripeness would be expected to obtain products with different properties. Developing more understanding of the physicochemical, physical and textural change characteristics associated with ripening would create a knowledge base for the fruit processing sector on the potential processed product outcomes.

Table 2.1 Nutritional constituents and composition of persimmon (*Diospyros kaki*).

Constituents and composition (per 100 g of fresh weight of edible part)	
Vitamin A (IU)	5400 [i]
Vitamin C (ascorbic acid) (mg)	52 [ii]
Organic acid (malic acid) (%)	0.13-0.14 [iii]
Protein (mg)	610 [iii]
Dietary fibre (%)	86.7 [viii]
Condensed tannin (g)	14.93 [viii]
Calcium (mg)	9 [iv]
Phosphorus (mg)	27 [x]
Potassium (mg)	203 [x]
Magnesium (mg)	11 [x]
Pectin (%)	0.52-1.07 [v] (water soluble pectin~64.69%)
Total sugar (mg)	14340 [ii]
Reducing sugar (mg)	13900 [ii]
Sucrose (mg)	420 [ii]
Fructose (mg)	7030 [ii]
Glucose (mg)	6870 [ii]
° Brix	16.2 [ii]
pH	5.3-5.5 [ii]
Carotenoids (mg)	2.8-8.3 [vi]
Lycopene (mg)	0.75-4.1 [vi]
β-cryptoxanthin (mg)	0.194-1.566 [iv]
Phenolic compound (g.100 g^{-1} dry weight)	8.5 [vii]
Water (%)	82.03 [iii]
Food energy (calories)	79 [iv]

Source: [i] Daood et al. (1992), [ii] Itoo (1971) , [iii] Winton & Winton (1935) , [iv] Turk (2004) , [v] Roy et al. (1995) , [vi] Brossard & Mackinney (1963) , [vii] Van Buren (1970), [viii] Takekawa & Matsumoto (2012), [ix] Zhou et al., (2011), [x] Celik & Ercisli (2008)

2.2.1 Colour changes

Postharvest colour changes are caused by loss of chlorophyll and concomitant synthesis of the carotenoids and anthocyanins (Wills et al., 1998; Kays & Paull, 2004b). Colour changes measured as °Hue (from 100.1 to 38.1) and chroma (from 27.2 to 49.2) during tree ripening of 'Hana Fuyu' persimmon has been reported (Payne et al., 1991). Carotenoids are developed from phytoene after multiple reactions involving dehydrogenation, cyclization, hydroxylation, oxidation, and epoxidation (Yano et al., 2005). β-cryptoxanthin is rich in some ripe fruit for example 'Sun rise' papaya (3182 μg β-cryptoxanthin.100 g^{-1} FW; Bhaskarachary et al., 1995), Satsuma mandarin (2986 μg β-cryptoxanthin.100 g^{-1} FW; Yano et al., 2005), red chilli (894 μg β-cryptoxanthin.100 g^{-1} FW) and orange pepper (238 μg β-cryptoxanthin.100 g^{-1} FW; Breithaupt & Bamedi, 2001), but it is not detected in orange carrots (Bhaskarachary et al., 1995; Aizawa & Inakuma, 2007). β-cryptoxanthin is present in particular on the skin of persimmon (Kitagawa & Glucina, 1984). At fully-ripe persimmon skin consist of 55 μg β-cryptoxanthin.100 g^{-1} FW (Breithaupt & Bamedi, 2001). Payne et al. (1991) found increasing β-cryptoxanthin from 8 to 59 μg.100 g^{-1} FW during on tree ripening of 'Hana Fuyu' persimmon.

Lycopene also accumulates during fruit ripening. Payne et al. (1991) found increasing lycopene from < 1 to 167 μg.100 g^{-1} FW during on tree ripening of 'Hana Fuyu' persimmon. Lycopene is found in ripe fruit such as guava (4383 μg.100 g^{-1} FW), papaya (2481 μg.100 g^{-1} FW), red fleshed watermelon (6184 μg.100 g^{-1} FW; Yano et al., 2005).

Polyphenol oxidase (PPO) acts on two substrates, a monohydroxyphenol to hydroxylate at the *o*-position of the original hydroxyl group and on *o*-dihydroxyphenols by remove the hydrogen of the hydroxyl groups to form benzoquinones (Ramírez et al., 2002). Polyphenol oxidase is a key enzyme influencing the quality of fruit and vegetable after harvesting and during storage (Artes et al., 1998; Waliszewski et al., 2007). Brown colour development on marked

and bruised fruit skin is caused by benzoquinones formed by *o*-diphenol oxidase reacting with oxygen, sulfhydryl compounds, amines, amino acids and proteins (Ramírez et al., 2002; Muñoz & Barcelo, 2004). Lee et al., (2005) found that persimmon storage at 20° C for 4 weeks developed bruising as a result of an increase in polyphenol oxidase activity, influencing visual deterioration.

Overall, this information indicates that as the red skin of ripe 'Fuyu' persimmon develops not only due to β-cryptoxanthin accumulation but also lycopene accumulation which could potentially be transferred to a processed product.

2.2.2 Total soluble solids (TSS), dry matter (DM), pH and organic acid

Detached produce continue to be biologically active, resulting in the consumption of starch, sugars and organic acid to produce energy used by synthetic reactions in living cells (Wills et al., 1998). Once ripening has begun, there is a series of irreversible degradation processes which cause physicochemical changes in total soluble solid (TSS), organic acid, pH and dry matter (DM) (Nath et al., 2006; Valero & Serrano, 2010b).

TSS generally increases as ripening progresses due to conversion of starch into sugar (Kays & Paull, 2004b). Increasing TSS during ripening has been reported for tomato (Kaur et al., 2006), kiwifruit (Jordan et al., 2000), peaches (Shinya et al., 2013), date plum persimmon (Glew et al., 2005) and 'Rojo Brillante' persimmon (Plaza et al., 2012). Fruit with high DM content at harvest tends to develop high TSS once ripe (Young et al., 1993; Jordan et al., 2000; McGlone et al., 2003; Palmer et al., 2010). The DM of 'Fuerte' avocado decreases during ripening while a slight increase is observed in 'Hass' avocado (Ozdemir & Topuz, 2004).

During ripening, organic acids decline due to consumption by respiration. As a result pH increases and flavour, taste and odour are influenced (Kays & Paull, 2004b). However, changes in organic acid and pH as ripening progresses differ depending on the fruit. Increasing organic acid during ripening is reported for tomato (Kaur et al.,

2006) and sweet cheery (Serrano et al., 2005) while decreasing trends are reported for apricot, nectarine and plum (Valero & Serrano, 2010a). Unlike any other fruit, 'Fuyu' persimmon is reported to have an organic acid peak during ripening (Senter et al., 1991; Clark & MacFall, 2003). Increasing pH during ripening has been reported for tomato (Kaur et al., 2006), kiwifruit (Jordan et al., 2000) and peaches (Shinya et al., 2013). In contrast, the tomato (Bui et al., 2010), guava (Abreu et al., 2012) and 'Rajo Brillante' persimmon (Plaza et al., 2012) show no significant change in pH during ripening.

After harvest, fruit continue to be biological active resulting in increasing TSS and a tendency to decrease DM with reducing organic acid and increasing pH. These chemical changes are expected to carry though to processed product quality. Understanding how raw fruit material qualities influence processed product can create knowledge which may be useful for industrial process design.

2.2.3 Firmness

Overall, firmness is one of the most measured textural qualities which is used as a criterion to define fruit maturity. Firmness reduction during persimmons ripening is reported at ≈ 82 % 'Fuyu' (Luo, 2006), ≈ 78 % 'Qiandaowuhe' (Luo, 2007), ≈ 90 % 'Giombo' (Blum et al., 2008) ≈ 69 % 'Rojo Brillante' (Plaza et al., 2012) and ≈ 83 % 'Yangfeng'(Yin et al., 2012) of initial firmness. Similarly, tomato (Bui et al., 2010), avocado (Obenland et al., 2012), peach (Shinya et al., 2013) and papaya (Ong et al., 2013) undergo significant softening during ripening. While many fruit show similar magnitudes of softening, persimmon is unique of that it is consumed across the firmness range. However, the length of time postharvest plays a key role in firmness determination for this study

Firmness of fruit reduces during ripening as a result of microstructure changes (Salvador et al., 2007). Fruit softening has generally been related to the degradation of the pectin, especially the middle lamella region, by pectinmethylesterase (PME), polygalacturonase (PG), β-galactosidase and pectate lyase (Valero & Serrano, 2010b). Overall, there is no single enzyme reaction that accounts for the textural

change during fruit ripening. This suggests that sequential and cooperative reactions occur between a number of enzymes.

Enzyme reactions occur with substrate specificity and are active within specific ranges of pH, temperature and substrate concentration (Kilara & Desai, 2001). The enzyme reaction is affected by both activators and inhibitors which are present in the complex structure of enzyme molecules. The optimum pH ranges of pectolytic enzymes are 4.0 to 7.0 (Huber et al., 2001). With increasing temperature, the enzymatic reaction rate increases to a maximum and then decreases with further increasing temperature (Bayindirli, 2010). Additionally, the catalytic activity of some enzymes depends on the presence of an organic compound (Bayindirli, 2010) which are called a coenzyme, that loosely attaches to the enzyme. If the enzymes are attached firmly by a covalent bond (e.g. calcium, cupper, ferrous, manganese, magnesium and zinc), it is called a prosthetic group.

At early maturity, parenchyma cells of the mesocarp are dense with small intercellular spaces which are filled with air (Salvador et al., 2007). Cells are bound together closely by the cell wall. Large vacuoles developed inside the cells which are full of soluble material. As maturity advances the parenchyma degrades progressively. Additionally, some intercellular spaces are filled by solutes. The typical cell structure is lost resulting in loss of intercellular adhesion of parenchyma in the structure of fruit at more advance stages.

2.2.3.1 Pectin characteristics

Generally, pectic substance are classified into four types; protopectin, pectic acid, pectinic acid and pectin (Rodrigues, 2012). Pectins are widely found in fruit, (especially citric and apple) and are a mixture of anionic heteropolysaccharides (Willats et al., 2001; Rao & Lopes da Silva, 2006). Generally, plant cell walls are composed of 60 % water and about 40 % solids. This solid portion consists of various polymeric, cellulose, hemicellulose, pectin, protein and some minor small molecule compounds which vary from plant to plant (Kays & Paull, 2004a). Generally, it is reported that the fruit cell wall contains more pectin than

hemicellulose (Waldron, 2004). Pectin is found especially in the middle lamella layer (Van Buren, 1991; Wang et al., 2002).

Pectin is a primary cell wall polymer which consists of various types of polysaccharides (Fry, 1988). Pectin structure has been described as a series polymer containing smooth and hairy blocks (Voragen et al., 1995; Flutto & Caballero, 2003). The smooth region of pectin is known as homogalacturonan (HG) with the hairy regions being rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) (Figure 2.1). In some fruit such as lemon and apple, pectin may also consist of xylogalacturonan which is rich in xylose (Voragen et al., 1995).

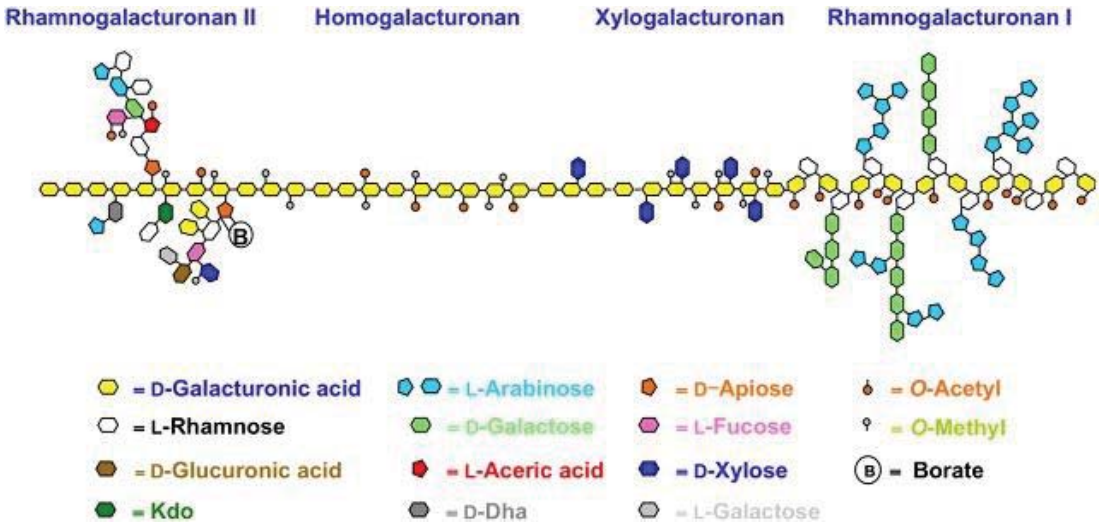


Figure 2.1 Pectin structure is mainly composed of rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II), homogalacturonan (HG) and xylogalacturonan (Scheller, 2011).

Typically, HG in the cell wall is the most abundant polysaccharide at about 65 % of pectin whilst RG I and RG II constituent 25-35 % and less than 10 % respectively (Harholt et al., 2010). Homogalacturonan (HG) is composed of a linear polygalacturonic acid backbone (Mohnen, 2008) of α-(1-4)-D-galacturonic acids. A proportion of these galacturonic acid will have methyl ester groups at the C-6 carboxyl position (Pilnik & Rombouts, 1981; Tucker & Grierson, 1987; Harholt et al., 2010). The HG chain may contain about 100-200 galacturonic acid (GalA)

residues (Willats et al., 2001). Approximately 95 % of GalA residues are present in the smooth region (de Vries, 1988).

The hairy regions of RG I and RG II consist of many sugars, predominantly being rhamnose (Rha), galactose, and arabinose. RG I has a backbone which consists of alternating disaccharides at the O-4 of the Rha residues (Mohnen, 2008). RG II has a complex structure of three pectic polysaccharides which consists of 13 different sugars and over 20 different linkages (Gilbert, 2010). Galactose is a main constituent of the side chains on RG backbone (Willats et al., 2001; Wang et al., 2002). Rha residues in the RG backbone can be substituted with β-1,4-galactan branched arabinan and/or arabinogalactan side chains (Harholt et al., 2010).

Fruit ripening induces modification of the cell wall polysaccharides by pectic enzymes (Nakamura et al., 2003). During ripening, pectin is de-esterified by PME. Specifically methylester groups of methylated galacturonic acids resulting in methyl free GalA residues (Pilnik & Rombouts, 1981). Consequently, PME action results in decreasing the degree of methylesterification (DMe) of pectin (Stoforos et al., 2002). During ripening of fruit such as strawberry, date, mango and guava, the DMe decreases significantly (El-Zoghbi, 1994). In contrast, inconsistent changes of DMe in apple during postharvest ripening have been reported (Fischer & Amadò, 1994).

After PME methylesterification, methyl free GalA residues are depolymerised by polygalacturonase (PG), causing shortening of the pectin chain (Fischer & Bennett, 1991; Wang et al., 2002). The more methylesters removed by PME, the more GalA residues can be liberated by PG (Daas et al., 1999). In addition, pectin becomes more soluble resulting in softening fruit (Tong et al., 2000; Salvador et al., 2007). Pectin changes during ripening of papaya (Shiga et al., 2009), grape (Oanh et al., 2010), Chinese bayberry (Sun et al., 2013) and avocado (Huber & O'Donoghue, 1993) have demonstrated significant increase in water soluble pectin as ripening progressed. Redgwell et al. (1992) demonstrate significant degradation of water insoluble pectin of kiwifruit. In contrast, GalA content did not change for peaches during ripening at 25 °C for 5 days (Yoshioka et al., 2011). During persimmon softening, water-soluble

pectin increased due to the extensive solubilization and depolymerization by PME and PG (Cutillas-Iturralde et al., 1993; Wakabayashi, 2000; Megumi et al., 2002).

2.2.3.2 Pectinmethylesterase (PME)

During fruit ripening, pectolytic enzymes are stimulated leading to pectin degradation (Nemec, 2008). Pectolytic enzyme hydrolysis during ripening results in physical and chemical changes which lead to conversion, modification and degradation of pectin (Fischer & Bennett, 1991; Nath et al., 2006). PME is a key enzyme that causes pectin change in ripening fruit. The role of PME is to linearly demethylesterify methylester groups of polygalacturonic acid on the pectin resulting in methyl free GalA residues (Pilnik & Rombouts, 1981; Fischer & Bennett, 1991; Micheli, 2001). Importantly, linear PME hydrolysis increases blocks of GalA residues on the pectin chain which subsequently interact with calcium (Bacic et al., 1988; Thakur et al., 1997; Micheli, 2001; Flutto & Caballero, 2003; Mark & Olga, 2004; Mohnen, 2008; Cautela et al., 2010).

Studies reported increasing PME activity during ripening of avocado (Wakabayashi et al., 2000). Marangoni et al. (1995) demonstrated increasing PME activity in tomato during ripening. Additionally, high PME distribution in tomato tissue during colour break stage is reported (Blumer et al., 2000). 'Qiandaowuhe' persimmon also showed a peak of PME activity during ripening at 20 °C for 4 days with a dramatic decrease in firmness (Luo, 2007). Increasing PME activity as fruit ripens have also been reported for 'Taubate' (Awad, 1985) and 'Rojo Brillante' persimmons. Similarly, Alonso et al. (1997) demonstrated high PME activity in ripe Spanish persimmon. In contrast, PME activity in apricot is reported to decrease during postharvest ripening (Cardarelli et al., 2002).

2.2.3.3 Polygalacturonase (PG)

The function of PG during fruit ripening is to hydrolyse α-1,4 linked galacturonan linkages of the pectin structure without disturbing the α-1,2 linkage (Fischer & Bennett, 1991). Endo-PG hydrolyses pectin polymer randomly whilst exo-PG

cleaves off GalA monomers or digalacturonides from the non-reducing end (Benen & Visser, 2003). PG is usually known as an endo-acting enzyme that cause pectin solubilization resulting in fruit softening and shortening pectin chains (Tong et al., 2000; Wang et al., 2002; Salvador et al., 2007; Valero & Serrano, 2010b).

PG activity increases during the climacteric stage in papaya (Paull & Chen, 1983). A fully-ripe avocado has PG activity three times greater than when fruit reach the edible soft stage (Awad & Young, 1979). PG activity increases during kiwifruit ripening (Tavarini et al., 2009). 'Qiandaowuhe' persimmon also showed a peak of PG activity during ripening at 20 °C for 5 days, coinciding with a dramatic decrease in firmness (Luo, 2007). However, PG activity was either not detected or did not show any significant correlation during persimmon softening in another two reports (Kang et al., 1998; Luo, 2005). Similarly, strawberry and apple can ripen without any detectable depolymerisation of pectin (Goulao & Oliveira, 2008).

2.2.3.4 β-galactosidase characteristics

Cell wall enzymes degrade polysaccharides resulting in fruit softening (Fischer & Bennett, 1991). Ali et al. (1995) found that β-galactosidase may contribute to pectin debranching due to its action on galactose. de Veau et al. (1993) and Van Buggenhout et al. (2009) found that β-galactosidase hydrolysis led to a significant decline in cell wall integrity resulting in tissue softening. β-galactosidase activity increases rapidly during 'Bianhua' persimmon ripening co-incident with decreases in flesh firmness (Luo, 2005). Additionally, Kang et al. (1998) found high activity of β-galactosidase during persimmon ripening. Similarly, Cutillas-Iturralde et al. (1993) and Nakamura et al. (2003), both suggest that β-galactosidase is the crucial enzyme during persimmon ripening. A number of studies have been reported that β-galactosidase causes tissue softening as ripening progresses in banana (Cheng et al., 2011), papaya (Othman et al., 2011), avocado (de Veau et al., 1993), muskmelon (Ranwala et al., 1992), tomato (Wallner & Walker, 1975; Carrington & Pressey, 1996), mango (Ali et al., 1995), and kiwifruit (Ogawa et al., 1990; Wegrzyn & Macrae, 1992; Ross et al., 1993; Tavarini et al., 2009).

2.2.3.5 Other pectolytic enzymes

Pectin lyase and pectate lyase catalyze the nonhydrolytic split by β-elimination of the glycosidic linkage next to the methyl free GalA (Alkorta et al., 1998; Muñoz & Barcelo, 2004). Generally, pectin lyase catalyzes pectins that have high DMe while pectate lyase catalyzes pectin with low DMe. During fruit ripening, pectin lyase causes deterioration resulting in fruit quality reduction. Whilst, pectate lyase cleaving may result in the maceration of plant tissue (Marín-Rodríguez et al., 2002). Pectin lyase is a potential enzyme useful for the fruit juice industry because pectin lyase catalyzes pectin without interrupting the methyl ester groups, hence preserving aroma and flavor (Alkorta et al., 1998; Yadav et al., 2009). Optimum pH and temperature for pectin lyase are 5.5 – 10.5 and 35 – 65 °C respectively (Alkorta et al., 1998; Yadav et al., 2009) .

Cellulase and hemicellulase are pectolytic enzymes which hydrolyze cell wall polysaccharides (Bayindirli, 2010; van den Berg et al., 2010; Rodrigues, 2012). Cellulase is a multienzyme system which compose of endoglucanase, exoglucanase and glucosidase (Brummell, 2006; Prasanna et al., 2007). They hydrolyze cell wall polysaccharide at different positions. Endoglucanase hydrolyzes the β-1,4-link between adjacent glucose residues with random position, exoglucanase splits non-reducing ends of the chain and produces glucose or cellobiose, whilst β-glucosidase breaks cellobiose into glucose molecules (Prasanna et al., 2007). Generally, at unripe stage, cellulose activity is low and then increases during fruit ripening (Brummell & Harpster, 2001). Hemicellulase hydrolyze both glycosides with low molecular weight to monosaccharides and oligosaccharides that cannot be further split (Wakabayashi, 2000; Spiridon & Popa, 2004). These enzymes are used in fruit juice processing to facilitate maceration, clarification and liquefaction in order to improve yields and to reduce processing costs in fruit juice industries.

2.3 Processed fruit products

2.3.1 Fruit jams

Fruit jam is defined as mixtures of fresh or frozen fruit or fruit pulp or purée or juice and sweetening agents (O'Beirne, 2003). A jam should have a soluble solid content of at least 65 % with the essential ingredients being a fruit component, pectin, acid and sugar (Smith, 2003). The global trade in jams, fruit jellies and marmalades is US $15B (Anon., 2012). Pectin is the key component to produce jam with 0.5-1.1 % added (O'Beirne, 2003). Pectin is utilised as the gelling agent in fruit jam and stabilizing polymer in other food products (Itoo, 1971; Mohnen, 2008).

Gelling mechanism in food industry involve two types: acid-sugar and calcium gels. High methoxyl pectin (HMP) usually forms hydrogen-bridge bonding networks in acid and high sugar condition, referred to as an acid-sugar gel (Bacic et al., 1988; Hui et al., 2004). Typically, HMP are produced in the presence of 55-75 % methylated pectin with low pH at 2.5-3.5 (Lopes da Silva & Rao, 2006). Low methoxyl pectin (LMP) pectin forms an ionic crosslink with calcium resulting in a calcium gel (Bacic et al., 1988; Hui et al., 2004). LMP forms gels in a wide range of pH (2.0-7.0) whether sugar is present or not (Yapo & Koffi, 2006). LMP gels form under the presence of calcium which crosslink between pairs of carboxyl groups of pectin molecules and are often described as an egg box model (Bacic et al., 1988; Thakur et al., 1997; Flutto & Caballero, 2003).

Pectin properties such as molecular weight and degree of methylesterification (DMe) are considered when producing a gel, as different pectin properties result in alternative product textural properties (Oakenfull, 1991). Higher molecular weight leads to firmer acid-sugar gels whilst DMe influences strength of calcium gel (deMan, 1999). The DMe also influences flavour perception. Guichard et al. (1991) reported that HMP may induce an undesirable typical flavour and intensity of flavour and taste, while LMP has less influence.

Jam pH is generally at 2.8-3.4 depending on soluble solid content (O'Beirne, 2003). The acid acts as a stabilizer of the interaction between pectin and sugar and balances the sweet and sour taste of the product (Zhao, 2012). Additionally, pH is used to determine the temperature at which jellies set (i.e. slow set and rapid set). Commercial pectins gain the maximum firmness at a pH of 3.0-3.15 and 3.30-3.50 (Smith, 2003).

The best quality jams are produced from fresh or frozen fruit (O'Beirne, 2003). Fruit jams may contain 35-45 % fruit content. Carbonell et al. (1991a, 1991b) found a strong relationship of fruit content and soluble solids on gel strength of strawberry jam. In contrast, they found no relationship of fruit content and soluble solids on gel strength of peach jam. Dietary fibre is applied to strawberry jam in order to increase the market value and act as a self-stabiliser (Grigelmo-Miguel & Martín-Belloso, 1999).

2.3.2 Fruit juice

Fruit juice is an important processed product of the fruit industry. The global fruit juice market was at US $79B in 2009, and it is predicted to increase to a total of US $93B by 2014 (Barkla, 2011). Fruit juice for the New Zealand export market has increased continuously at 33 % since 2011, with the recent market values at $59.2M in 2012 (Aitken & Hewett, 2012). Presently, organic fruit juice is more popular in the fruit juice market (Barkla, 2011). Additionally, 100 % fruit juices are considered to be an integral part of the 5+ a day program which is cosponsored by the World Health Organisation (WHO) (Anon., 2013a).

Fruit juices can be made from temperate (e.g. orange, apple, and berry etc.) and tropical (e.g. pineapple and papaya etc.) fruit (Rutledge, 2001). Juice manufacturing begins with sorting fruit, then washing, grinding, heating or enzyme treatment, pressing, filtering, pasteurising, bottling, cooling and storing (Padilla-Zakour et al., 2012). Cloudy juices do not undergo clarification after juice extraction, while a clear juice product needs to remove the pectin by enzyme treatment (Mark & Olga, 2004). Typically, depectinization reduces the viscosity and slipperiness of pulp before

decanting and pressing steps. Pectinmethylesterase (PME) is used to reduce the molecular weight of the cloud particles in fruit juice via methylesterification (Cautela et al., 2010). The quality of fruit juice depends on the quality of the raw material and the maturity stage (Mark & Olga, 2004).

2.3.3 Fruit purée

Fruit purée is a key ingredient in many food products for example bakery products, dairy products, jams and marmalades, topping, spreads and fruit juices. Global trade in preserved or prepared fruit purée is currently estimated at US $34B (Anon., 2013c). The biggest exporter and importer of preserved or prepared fruit is United States of America (US $3.0B and US $5.4B respectively).

Fruit purée is produced by blending fruit flesh (Rutledge, 2001). The purée processing line consists of peeling, size reducing, heating, finishing and adding starch or sugar to get the desired consistency (Balestra et al., 2011). Texture of purée is an important property as it can influence the textural properties of the final product and is a key factor that influences customer acceptability and preference (Guinard & Mazzucchelli, 1996).

Different fruit result in different textural characteristics of fruit purées (Table 2.2). For instance apple purée has a stronger structure (higher storage modulus, G′) in comparison to banana and apricot purées. In addition, fruit maturity demonstrated a strong effect on textural property of the resulting purée such as white guava (Sánchez et al., 2009). Unripe white guava purée has approximately 75 % stronger texture in comparison to purée produced from ripe white guava. However, there are no current publications on persimmon purée textural properties.

Table 2.2 Textural properties (G′, G″) of fruit purées.

Products	Measurement conditions							sources	notes
	Probe geometry	%Strain	Freq. (Hz)	Shear rate (s-1)	Temperature (°C)	G′ (Pa)	G″ (Pa)		
Apple purée	A six blade vane		0.01-6.37	2.14-214 214-2.14	20	600	180	(Espinosa et al., 2011)	Reported at 1 Hz
Apple purée	Parallel plate (60 mm, 1 mm gap)		0.1-10	0.1-100, 100-0	20	~300	~60	(Ahmed & Ramaswamy, 2007)	Reported at 1 Hz
Banana purée	Parallel plate (60 mm, 1 mm gap)		0.1-10	0.1-100, 100-0	20	~150	~70	(Ahmed & Ramaswamy, 2007)	Reported at 1 Hz
Apricot purée	Parallel plate (60 mm, 1 mm gap)		0.1-10	0.1-100, 100-0	20	~110	~18	(Ahmed & Ramaswamy, 2007)	Reported at 1 Hz
White guava purée (removed seed)	Aluminium cylinder ∅ 0.6 cm.	0.01	4		20	5,202±17 (unripen, pH 4.1) 1,319±21 (ripened, pH 4.4)	2,370±78 (ripened) 1,172±23 (ripened)	(Sánchez et al., 2009)	
Jaboticaba pulp (particle size 200 nm)	Plate-plate, ∅ 4 and 6 cm, rough surface, 2 mm gap		0.01-10	0-300, 300-0		~190	~28	(Sato & Cunha, 2009)	Reported at 1 Hz
Jaboticaba pulp (particle size 855 nm)	Plate-plate, ∅ 4 and 6 cm, rough surface, 2 mm gap		0.01-10	0-300, 300-0		~700	~80	(Sato & Cunha, 2009)	Reported at 1 Hz

2.3.4 Canned fruit

Canning is the thermally processing of food packed in hermetically sealed container, to create an environment free of microorganisms (Anon., 2013b). Canning is an effective technique to sterilise food in order to prevent enzymatic and microbial spoilage (Ramaswamy, 2004).

The target microorganism for canned food are *Clostridium botulinum* and *Clostridium perfringens* as they are heat resistant, spore-forming and anaerobic pathogens (Cramer, 2013). They can produce deadly botulism toxin when stored under anaerobic ambient conditions if they are not destroyed by heat treatment. There are other heat-resistant anaerobic microorganisms that need attention, for instance *Bacillus stearothermphilus, Bacillus thermoacidurans* and *Clostridium thermosaccolyticum* (Awuah et al., 2007; Meng & Ramaswamy, 2007).

There are a wide range of canned fruit for example apricot, berries, grapefruit, pineapple, grapes, peaches, pears, plumes and prunes. Manufacturing processes for canned fruit begin at washing and peeling fruit raw materials, then blanching, grading or sorting, filling, exhausting, sealing, retorting, cooling, labelling and storage (Ramaswamy & Marcotte, 2005). Canned food legislation stipulates that low-acid canned foods have the final equilibrium pH > 4.6 and water activity > 0.85 while acidified canned-foods have the final equilibrium pH ≤ 4.6 and water activity > 0.85 (Anon., 2013b).

2.4 Fruit maturity influences fruit product quality

Raw material quality effects the quality of processed products, meanwhile processing may or may not improve processed product quality (Mishra & Gamage, 2007). Fruit maturity in particular is a key factor influencing final product quality. Fruit purée quality can be affected by fruit maturity in terms of colour stability, browning, acidity, soluble solids-acid ratio and viscosity.

The colour stability of a food product is a crucial property which is influenced largely by changes as ripening progresses (Wills et al., 1998). Colour stability of strawberry jam was reduced when produced from immature fruit in comparison to fully-ripe fruit (Spayd & Morris, 1981; Mazur et al., 2014). Producing strawberry nectar made from ripe and fully-ripe strawberry ensures the product has more colour stability and fully-ripe strawberry is suggested to be the best maturity to produce a more colour stable product (Gossinger et al., 2010). In contrast, combining ripe fruit with immature fruit at 25 and 50 % results in a reduction of colour stability in strawberry jam (Spayd & Morris, 1981). Guava purée was less susceptible to browning when produced from fruit of a specific maturity (Yusof et al., 1988). Additionally, colour of peach purée could be improved by removing immature fruit (Gonzalez et al., 1992a). Canning more mature peach results in higher yellow or orange-yellow colour with a good flavour quality (Kader et al., 1982).

By removing immature fruit, the acidity decreased and soluble solids-acid ratio increased in peach purée (Gonzalez et al., 1992b). High quality dried apricots can be produced from full maturity apricot that has the highest sugar-acid ratio (McBean et al., 1971). However, high soluble solid in ripe red pepper causes yeast and lactic flora growth resulting in a fermented dehydrated product (Gallardo-Guerrero et al., 2010). A thick canned tomato paste is a result of early harvesting of tomato in comparison to harvest during ripening (Akbudak, 2010).

The viscosity of guava purée increased when processing more mature fruit (Yusof et al., 1988). A thicker canned tomato paste with greater serum viscosity is reported being caused by early harvest tomato in comparison to harvest during ripening (Akbudak, 2010). High linear correlation between consistency and optical density is reported in tomato purée made from ripe tomatoes (Haley & Smith, 2003). Texture of minimally processed peaches is reported to be firmer when produced from peach harvested corresponding to the green-yellow colour in comparison to more mature stage (fully yellow colour) (Martins et al., 2013).

In summary, it is well documented that fruit maturity is the important factor which highly influences quality attributes of processed product. Persimmon can be

consumed over a wide range of fruit textures. It is possible that textural effects could be observed in persimmon purées. To date, there is lack of information of the influence of maturity on final product quality for persimmon processing. Hence, one of the objectives of this study is to determine the effect of fruit maturity on resulting purée properties.

2.5 Thermal processing and effects on qualities of fruit products

There are a number of thermal processing application such as blanching, pasteurization, sterilization and cooking (Catherine & Jean-François, 2012). In food processing, thermal process involves elevating the temperature which subsequently results in changes such as colour and texture (Ahmed & Shivhare, 2005). Figure 2.2 displays a typical fruit canning process which illustrates steps of blanching and thermal processing to achieve different outcomes.

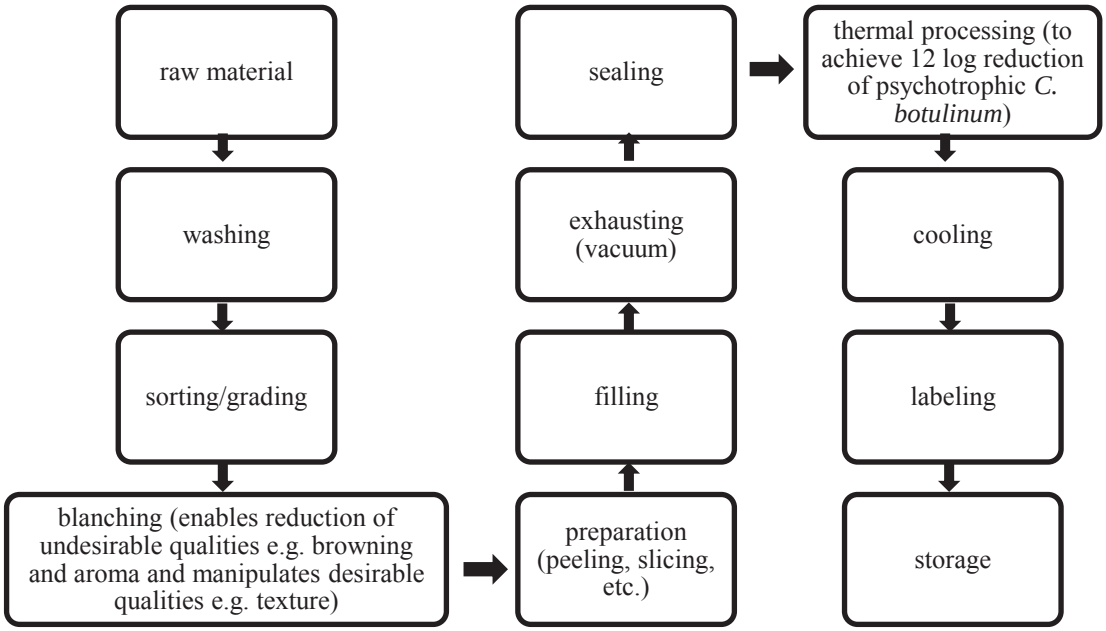


Figure 2.2 Typical fruit canning operation (adapted from Armstrong (2003) and Ramaswamy (2004).

Blanching is a relatively mild thermal treatment which is associated with solid foods, with the purpose being to improve the product stability during storage but not sufficient to establish storage stability at room temperature (Heldman & Hartel, 1997). Alternatively, other thermal processes aim to destroy microbial pathogens (Berk, 2009a; Lewis & Jun, 2012). Thermal processing applies either to food in hermetic containers or food in bulk prior to packaging (Berk, 2009b).

2.5.1 Temperature effect on reaction rate

Thermal treatment affects reaction rates of chemical, physical and biological changes (Karel & Lund, 2003). In food processing, the Arrhenius equation (Eq 2.1) is a thermodynamically based description of the temperature dependency of reaction rate which is most widely used (Giannakourou & Taoukis, 2006; Villota & Hawkes, 2006).

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \qquad \textbf{Eq 2.1}$$

Where k_0 represents the Arrhenius equation constant, E_a is the activation energy ($J.mol^{-1}$), R is the gas law constant (8.3144 $J.mol^{-1}K$), T is absolute temperature and k is the reaction rate.

2.5.2 Blanching applications and the effects on physicochemical, biological and textural characteristics

The purpose of blanching is to modify the macromolecule structure and microbial status of the product (Gökmen, 2010; Chamorro & Vidaurreta, 2012). Blanching consists of heating food to a predetermined temperature holding at the temperature for a defined period of time, and later rapidly cooling or passing to the next processing unit (Grandison, 2012). This operation is often used prior to the processing operation in processing plants. A number of studies have applied blanching in order to investigate the effect on preserved product qualities. Blanching temperature and holding time vary depending on sample size and type of fruit.

Many effects of blanching on enzyme and microbial activity have been reported. Peroxidase (POD) and polyphenoloxidase (PPO) activities are completely inactivated when blanched at 94 °C for 7 minutes in mango slices resulting in reduction of browning (Ndiaye et al., 2009). POD and PME activities are completely inactivated when blanching sliced carrot at 95 °C for 5 minutes (Gamboa-Santos et al., 2012) while PME of canned carrot shows the highest activity when blanching at 77 °C for 10 minutes (Lee et al., 1979). Heating potato tissue to between 65 to 80 °C results in sharply increasing cell permeability which can contribute to increasing PME hydrolysis as PME can access the substrate faster (Angersbach et al., 1999). Blanching at 65 °C for 15 minutes increases PME activity in green and red bell pepper slices (Ni et al., 2005). The concentration of *L. innocua* on red bell pepper reduces significantly after blanching at 60 °C for 3 minutes while blanching for 1 minute at 50 °C reduced the total mesophiles considerably in strawberry fruit (Alexandre et al., 2011).

Likewise, many quality effects as a result of blanching have been documented. Blanching at 94 °C for 7 minutes causes approximately 23 % reduction in lightness of mango purée (Ndiaye et al., 2009). Hue angle of blueberry juice increases when blanched at 100 °C for 3 minutes (Rossi et al., 2003). Blanching 'Rosa' and 'Espada' mango pulp at 75 or 100 °C for 3 minutes reduced ascorbic acid by 53 and 72 % respectively (Soares & Jose, 2013). Blanching (100 °C for 3 minutes) increased total anthocyanins in blueberry juice with no effect on physicochemical composition (Rossi et al., 2003). Blanching sliced carrot at 60 °C for 40 minutes resulted in less total soluble solids losses (26.5 %) in comparison to blanching at 95 °C for 5 minutes (31.4 %) (Gamboa-Santos et al., 2012).

Effects of blanching on pectin content and DMe have been described. Prestamo et al. (1998) reported that blanching caused little increase in total pectin substance of frozen and frozen blanched carrot. While, Lo et al. (2002) demonstrated that blanching carrot cube at 70 °C for 71 minutes resulted in reducing water soluble pectin and methanol content. More recently, Latorre et al. (2013) demonstrated that blanching carrot at 90 °C for 7 minutes did not affect change of GalA content. Blanching broccoli florets at 60 °C for 40 minutes resulted in an approximate 10 %

increase in GalA content with an approximate 35 % decrease in DMe (Christiaens et al., 2012a).

Textural change of fruit as influenced by blanching has also observed. Blanching apple at 100 °C for 90 s reduced the storage modulus (G′) by 10 times due to alterations in the micro and ultrastructure of the tissue (Loredo et al., 2013). G′ reduced by 15 % in fresh melon when blanched by saturated water vapour for 1.5 minutes (Martinez et al., 2005). Blanching at 70 °C for 15 min carrot and green bell pepper increased firmness by approximately 30 and 20 % respectively (Ni et al., 2005). Blanching carrot at 74 °C for 20-30 minutes resulted in a firmer texture than blanching at 100 °C for 4-5 minutes (Lee et al., 1979).

Despite well documented blanching effects on macromolecule structure and microbial status of food, there is lack of information on persimmon textural and biological properties. Studies that develop more understanding of blanching effects on persimmon textural and biological characteristics are therefore needed.

2.5.3 Thermal processing and the effects on physicochemical, biological and textural characteristics

Thermal processing can extend the shelf life of food products by means of eliminating microbial pathogen by exposing the product to elevated temperatures for a sustained period to time (Stoforos, 1995; Ahmed & Shivhare, 2005). Subsequently, thermal processing may affect changes in terms of toxin inactivation, colour, texture, aroma and nutritional properties (Manganaris et al., 2005; Ramaswamy & Marcotte, 2005).

Vegetative cells including yeast and mould spores can be inactivated by temperatures at 75 °C (Smelt et al., 2002). Thermal processing of 0.21 minutes at 121 °C reduces the population of *Clostridium botulinum* by 12 decimal reductions (Chen & Ramaswamy, 2003; Weng, 2005). Vegetative cells of bacteria are more likely to be heat sensitive at low pH conditions (Ritz et al., 1998). Silva and Gibbs (2004) report heat resistance of spoilage microorganism for orange juice, peach, and

tomato paste are *Bacillus megaterium* (spores), *Clostridium butyricum* (spores), and *Bacillus coagulans* (spores) respectively. Design of thermal processing conditions depends on the type of products and the target microorganism. Thermal treatment for tomato paste are 100 °C, *D-value* = 0.8 minutes, for orange juice 90 °C, *D-value* = 1.1 minutes, *z-value* = 11.5 °C and for peach product 90 °C, *D-value* = 3.5 minutes, *z-value* = 9.5 °C.

Vásquez-Caicedo et al., (2007) reported no changes of β-carotene and vitamin A concentrations in mango purée and nectar when heating at 93 °C for16 minutes, but hue angle reduced by 20 % and POD activity reduced by 96 % (Vásquez-Caicedo et al., 2007). Heating cashew apple juice at 90 °C for 1, 2, and 4 hours caused isomerisation and oxidation resulting in carotenoid degradation influencing the colour of product (Zepka & Mercadante, 2009). Additionally, studies reported that low temperature blanching (60 °C, 40 minutes) prior to carrot cooking (100 °C, 20 minutes) resulted in a firmer carrot texture (Lemmens et al., 2009; de Roeck et al., 2010)

2.6 Summary and research opportunity

'Fuyu' persimmon is the main variety which is exported to many countries around the world with currently increasing export market value. However, there is approximately 50 % of persimmon which do not meet export standards which could be processed into alternative products.

Fruit purée is an important ingredient for many food products such as toppings, jams, spreads, marmalades, juices, dairy products and fillings. Global trade in preserved or prepared fruit is currently estimated at US $34B. Not unlike other food processing industries, the quality of processed fruit products can be significantly affected by variation in fruit quality as an ingredient. This is due to fruit having variability in maturity and quality factors such as colour and firmness at the time of harvest. Additionally after harvest, fruit remain physiologically active causing further changes to their physical, physicochemical and textural properties. In particular pectolytic enzymes (PME, PG, β-galactosidase) remain active, resulting in alteration

of pectin structure and subsequent fruit softening. Manufacturing from mature or ripe fruit may result in products that differ in terms of textural properties. To date, there is a lack of information in how ripeness influences processed product property. More studies should be conducted to inform fruit processing industries to design processes to produce desired product attributes.

Thermal processing is commonly used to kill microbial pathogens and to inactivate undesirable enzymes which contribute to subsequent product quality changes. Blanching changes physicochemical, biological and textural characteristics. Textural attributes are in particular a crucial factor that influences customer acceptability. From this review, there is lack of understanding of the underlying mechanism of how textural properties of a manufactured product change as influenced by blanching.

There is a good opportunity to produce a persimmon product using persimmon from a wide range of input fruit and investigate the influences on the textural characteristics of manufactured product. Developing understanding and subsequent processing guidelines for effects of persimmon fruit quality on the resulting properties of a persimmon product will enable manipulation in order to achieve required product properties. Outcomes from this research could benefit commercial development of persimmon processing.

CHAPTER 3

Postharvest Characteristics of 'Fuyu' Persimmon (*Diospyros kaki* L.)

3.1 Introduction

'Fuyu' persimmons are harvested when they are firm and yellow-orange but not fully ripe (Turk, 2004) with a flattened shape (Childers et al., 1995). As persimmons are a climacteric fruit, they undergo the ripening process after harvest and remain biologically active causing a series of physiological, biochemical and sensory quality changes, including softening and changes in colour, total soluble solids (TSS), titratable acid (malic acid), pH and dry matter (DM) (Valero & Serrano, 2010b). Cell wall material, especially the middle lamella, becomes loose and softer (Tucker & Grierson, 1987) due to pectolytic enzymatic hydrolysis (Fischer & Bennett, 1991) resulting in a significant loss in firmness. Colour changes are caused by the loss of chlorophyll and concomitant synthesis of carotenoids and anthocyanins (Wills et al., 1998; Kays & Paull, 2004b). TSS generally increase as a result of conversion of starch into sugar while the content of organic acids is reduced due to consumption as a substrate for respiratory metabolism (Kays & Paull, 2004b).

The state of maturity at the time of fruit harvest is an important factor that affects postharvest and processed product characteristics. The harvesting season for persimmon in New Zealand is usually between April and June (Mason et al., 1989). Due to a short season, persimmons are harvested in bulk. Harvesting fruit at either immature or overripe can lead to extensive losses (e.g. rot) due to increased susceptibility to physiological disorders (Mishra & Gamage, 2007). Poor and

variable raw material qualities have the potential to influence processed product quality. These issues may or may not be able to be rectified by processing. The objectives of the study in this chapter were to investigate the changes in 'Fuyu' persimmon properties during postharvest shelf life and to define a range of persimmon characteristics that can be used or manipulated in order to obtain desirable processed persimmon products.

3.2 Materials and methods

3.2.1 Materials

Persimmon (*Diospyros kaki,* cv. Fuyu) was supplied from a commercial grower located in Whangarei, New Zealand in June 2009. Fruit were transported at ambient temperature to the laboratory at Palmerston North. The fruit were stored at 0 ± 1 °C and 85 % relative humidity (RH) until used. Figure 3.1 illustrates preparation of persimmons for determination of postharvest qualities. Three sets of persimmon were prepared for non-destructive (Set A) and destructive measurements (Set B). Both sets were stored at 20 °C, RH ≈ 70 % for 17 days. Postharvest characteristics were measured at days 0, 3, 6, 10, 13 and 17. Set A was assessed for colour changes only. A subset of Set B was withdrawn at intervals and was subjected to the measurements of firmness, TSS, pH and titratable acidity. Set C was stored at 20 °C, with air atmosphere, RH ≈ 70 % and 15 persimmons were measured for changes in their skin-tissue colour daily for 15 days. The remaining persimmons in Set C were analysed for the dry matter (DM) content and mineral concentration.

3.2.2 Fruit firmness

The tissue firmness of persimmons was evaluated using an electronic penetrometer (Willowbank Electronics, New Zealand) equipped with a 7.9 mm round (Magness-Taylor) probe. Fruit skin (0.6 mm thick) was removed at two opposite positions on the fruit equator prior to measurement (Celik & Ercisli, 2008). Flesh firmness was

expressed as the average peak force (kg_f) required to puncture the tissue at a speed of 20 $mm.s^{-1}$ to a 8 mm depth (Salvador et al., 2007).

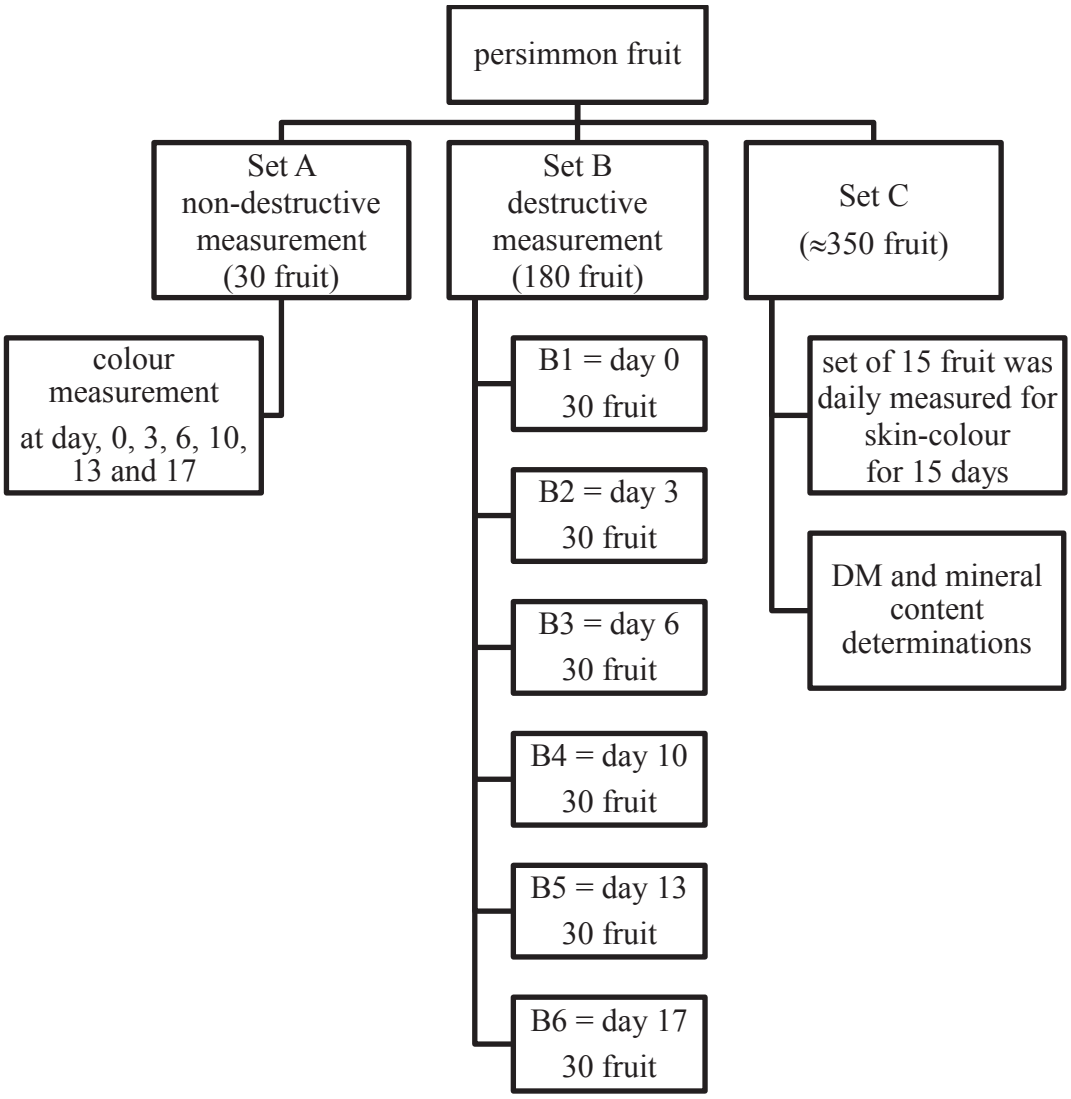


Figure 3.1 Fruit sample preparations for postharvest quality measurement.

3.2.3 Skin colour and skin-tissue colour

The skin colour of persimmon was determined using a spectrophotometer (CM-2000d, Konica Minolta Sensing, Osaka, Japan) set up under the conditions of 10 ° observer and standard illuminant D65 with a measurement head diameter of 8 mm. The calibration was done with a standard white tile (CM-A145, Konica Minolta,

Osaka, Japan) prior to sample measurement. CIE L*C*h* colour parameters (lightness, chroma and hue angle) were measured from three different areas of fruit skin marked around the equator of the persimmon. Then, 0.6 mm thick of the marked skin was removed followed by the colour measurement at three different spots on the flesh tissue around the fruit equator.

3.2.4 Total soluble solids (TSS)

Persimmon juice was extracted from representative persimmon tissues (approximately 20 g per persimmon) using a garlic press with a layer of cheese cloth at the bottom. The TSS of the extracted persimmon juice was measured using a hand-held refractometer (Pocket PAL-1, Atago, Japan). Calibration was conducted using distilled water prior to sample measurement.

3.2.5 pH

The pH of extracted persimmon juice was measured using a pH meter (Sentron 1001 pH system, Sentron Europe BV, Roden, The Netherland) that had been calibrated using pH 4 and 7 buffer solutions prior to analysis.

3.2.6 Titratable acidity

Malic acid is the predominant acid in 'Fuyu' persimmon (Senter et al., 1991). Titratable acidity of persimmon juice was determined by titration with 0.1 M NaOH to the end point of pH 7.1. Ten representative juice samples were assessed on each measurement occasion. Each juice sample was extracted from 3 persimmons. Juice samples (1 mL) were added into 50 mL of distilled water and titrated using a titrator (Mettler DL21, Mettler-Toledo AG, Greifensee, Switzerland). The titrator was calibrated with pH 4.0 and 7.0 buffer solutions prior to sample measurement. A blank (51 mL of distilled water) was also measured at the end point of pH 7.1. The content of titratable acidity was calculated as the percentage of malic acid using Eq 3.1 (Sadler & Murphy, 2003).

$$\text{Titratable acid (Malic acid)(w/ v)} = \left(\frac{(M)(V_1 - V_{blank})(\text{Eq wt})}{(V_2)(1000)}\right)(100) \quad \textbf{Eq 3.1}$$

Where

M = molarity of titrant (NaOH)
V_1 = volume of titrant (NaOH) used for titration of sample (mL)
V_{blank} = volume of titrant used for titration of blank (mL)
Eq wt = Equivalent weight of predominant acid (mg/mEq) (67.05 $mg.mol^{-1}$ Eq for malic acid)
V_2 = volume of sample (mL)
1000 = conversion factor

3.2.7 Dry matter (DM)

Ten persimmons from Set C (Section 3.2.1) were removed from storage randomly and their firmness was measured (Section 3.2.2). The DM content was determined by drying persimmon flesh tissue. Flesh persimmon tissue (approximately 5 g) (W_{flesh}) was dried in a dehydrator (Model 4926 T, Excalibur Products, Florida, USA) at 70 °C for 48 hours by placing sample in an aluminium dish of a known weight (W_{can}). After 48 hours, the dry sample was cooled in a desiccator for 30 min before measuring the dry weight of sample ($W_{dry\ +\ can}$). The DM content was calculated using Eq 3.2.

$$\text{DM} = \frac{W_{dry+can} - W_{can}}{W_{flesh}} \text{ x } 100 \qquad \textbf{Eq 3.2}$$

3.2.8 Mineral concentrations

The concentration of minerals in persimmon was determined by The Nutrition Laboratory, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North. Three sets of persimmons in duplicate from Set C (Section 3.2.1) were analysed at three different stages of maturity (average firmness of 1.1, 4.9, and 9.1 kg_f). Samples were peeled, cut into small chunks (approximately size of 10 x 50 x 10 mm) and snap frozen with liquid nitrogen. Later, frozen samples were freeze dried and ground using a pestle and mortar. The freeze dried persimmon powder

samples were sent to the Nutrition Laboratory for mineral measurements. Mineral concentrations (calcium, magnesium, potassium and sodium) were analysed using inductively coupled plasma-optical emission spectroscopy (ICP-OES). All mineral content were expressed as g.100 g^{-1} FW.

3.2.9 Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (version 9.2 (TS2M3), SAS Institute Inc., Cary, NC, USA). Data were analysed by analysis of variance (ANOVA). Comparison of mean was performed by using standard error values and least significant difference (LSD) to evaluate significant differences at $P = 0.05$. Correlation was evaluated using linear regression performed at $P \leq 0.001$ using a SigmaPlot Systat (version 11, SigmaPlot Systat software Inc., San Jose, CA, USA).

3.3 Results and Discussion

3.3.1 Changes in firmness of persimmon during postharvest shelf life

The firmness of persimmon during postharvest shelf life reduced continuously on average with large variation (Figure 3.2). Initially, the firmness of approximately 50 % of persimmon were > 8 kg_f while the majority were > 5.5 kg_f. After 6 days at 20 °C, the average firmness dropped to approximately 5 kg_f but the variation between samples was still large, spanning across the entire range of firmness. The average firmness continued to decrease and the firmness level spanned over the entire range at days 10 and 13. At day 13, the majority of persimmons were fully-ripe and overripe with the firmness values of 0.1-4 kg_f while some persimmons were completely soft and their firmness reached the minimum detectable threshold value of 0.1 kg_f. After 17 days at 20 °C, the distribution of firmness was reduced with 50 % of persimmon having a firmness < 1.5 kg_f.

Storing persimmon at 20 °C for 6 days resulted in firmness reduction. Softening could be caused by progressive parenchyma degradation resulting in loss of intercellular adhesion of parenchyma in the structure of fruit (Salvador et al., 2007). The sharp reduction in firmness of persimmon after 6 days at 20 °C was similar to that observed by (Salvador et al., 2006) for 'Rojo Brillante' persimmon. This could possible imply that 'Fuyu' persimmon firmness reduces rapidly due to similar progressive degradation of cellular structure.

A high variability in the rate of persimmon ripening during postharvest shelf life can potentially influence the properties of processed persimmon purée products. As shown in Figure 3.2, storing persimmon at 20 °C for ripening caused a continuous firmness reduction. The results suggested that storing mature persimmons (firmness at 7-9 kg_f) for 6 days at 20 °C caused about 50 % of persimmon to ripen (4-7 kg_f). When stored for 13 days at 20 °C, 50 % of persimmon was overripe (0.1-2 kg_f).

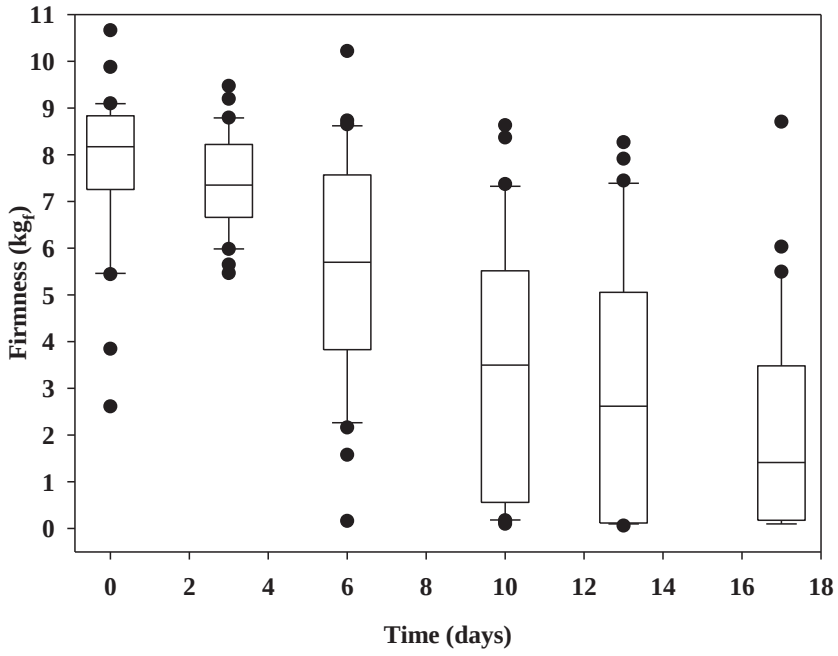


Figure 3.2 Box plots displaying the distribution of persimmon firmness during postharvest shelf life at 20 °C for 17 days. Each box represents a population of 30 fruits and dots are individual fruit. Top bars = maximum values, top bar of the box = the third quartile, middle bars represent the mean values, bottom bar of the box = the first quartile and bottom bars = minimum values.

These results can be used in future work to develop the relationship between persimmon ripeness, firmness and the quality of purée products made from persimmons at different stages of ripening.

A large variability in the firmness of individual persimmons within a population was observed during the entire postharvest ripening period tested in this study. The firmness of individual fruit was consequently chosen to be an independent parameter for later studies.

3.3.2 Changes in skin colour of persimmons during postharvest shelf life

During postharvest storage, persimmon skins turned from a pale green-yellow to orange-red (Figure 3.3) when stored at 20 °C for 17 days. Their colour was measured, in terms of lightness, chroma and hue angle (Figure 3.4).

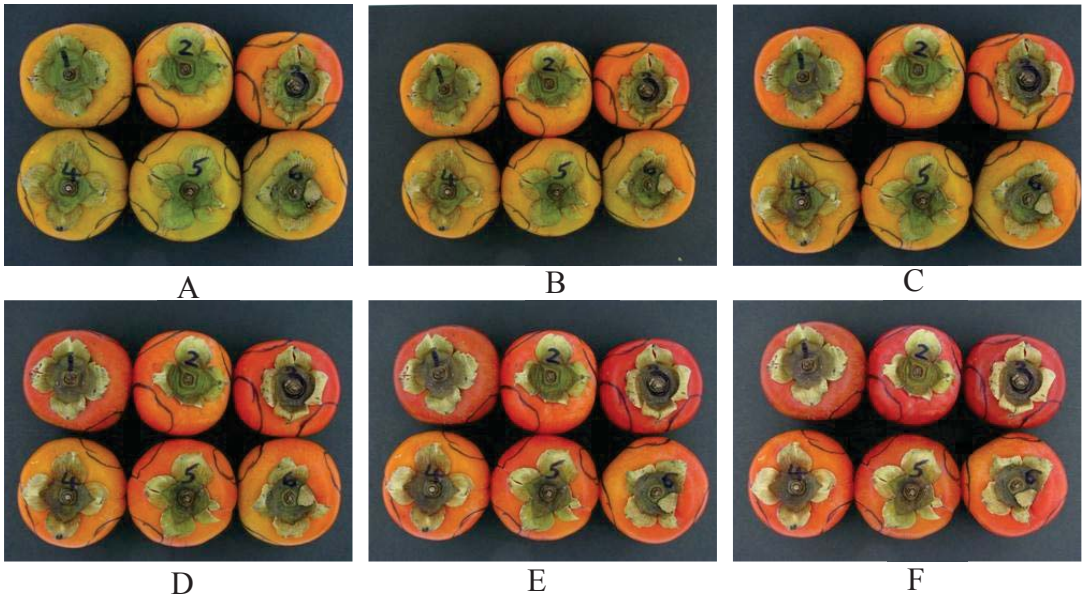


Figure 3.3 Visual observations of colour change in persimmons during postharvest storage at 20 °C for 17 days. Skin colour was measured after (A) 0, (B) 3, (C) 6, (D) 10, (E) 13 and (F) 17 days at 20 °C.

The lightness value of persimmon decreased gradually over time during storage, from 60 to 39 over 17 days (Figure 3.4A). The variability in the lightness values of

persimmon skin colour within a group of samples measured at different storage times tended to be larger with longer storage time. The chroma value of persimmon skin colour, representing colour saturation and intensity, also reduced gradually from 67 to 40 during storage but a high variability occurred later in storage (Figure 3.4B).

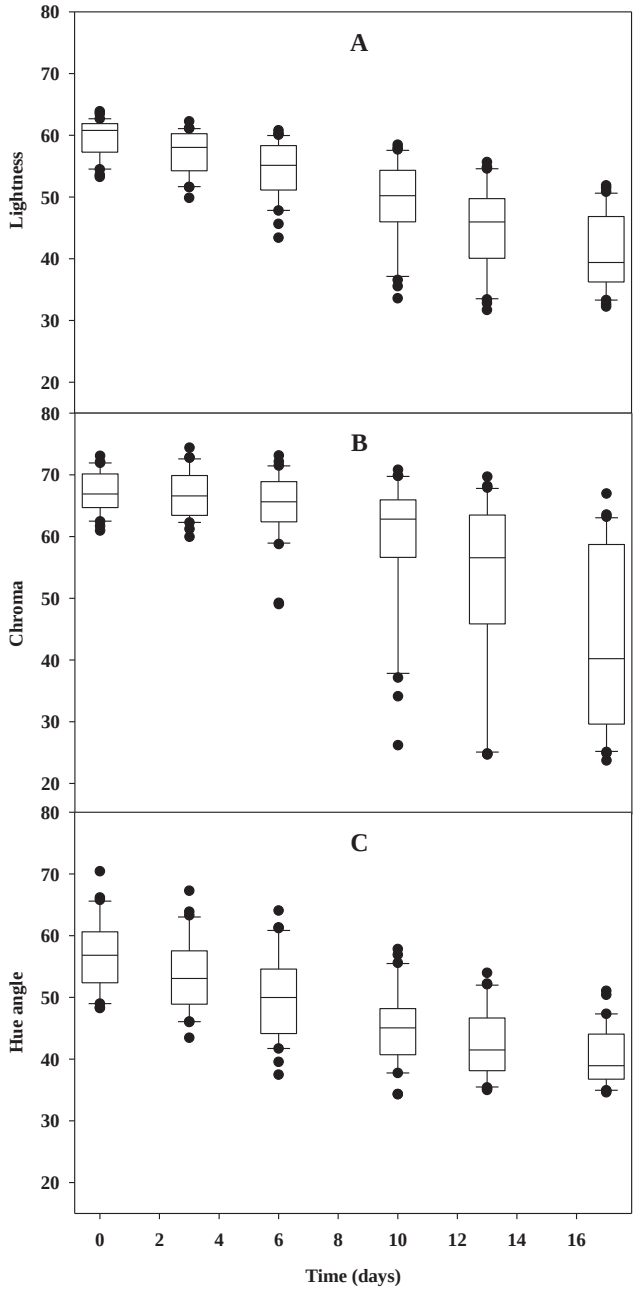


Figure 3.4 Changes in persimmon skin colour during postharvest shelf life at 20 °C for 17 days. Colour measurements were done on interval days on marked skin of 30 persimmons. A, B and C represent lightness, chroma and hue angle respectively.

The hue angle of persimmon skin declined from about 57 ° initially to about 38 ° after 17 days with a relatively small variation (Figure 3.4C), indicating that the persimmon skin colour changed from yellowish orange to red. This result suggests that all fruits developed colour in a similar manner with the distinguishable differences between fruits being attributable to differences in their ripeness during storage after postharvest. Similar results were reported for skin colour changes in 'Shiraz' persimmon during storage at 1 °C for 56 days (Shahkoomahally & Ramezanian, 2013) and during storage at 18 °C for 14 days (Lyon et al., 1992). Niikawa et al., (2007) found that during 'Fuyu' persimmon ripening carotenoids, β-cryptoxanthin, zeaxanthin and lycopene accumulate.

Overall the results indicate that the skin colour of persimmon turned from pale green-yellow to dark orange-red. This implies that these changes in colour observed between persimmons at different stages of ripening could affect the colour of persimmon purée product. Therefore, it is important to understand how changes in postharvest skin colour as well as other textural and sensory properties (e.g. firmness, flavour, acid, sweetness) could be affected by postharvest processing, handling and storage conditions in order to achieve the desired eating quality of processed persimmon products.

3.3.3 Correlations between skin colour and tissue colour

The colour of persimmon flesh was also measured and compared with the colour of persimmon skin. Figure 3.5 shows that the correlation coefficient (R) for lightness, chroma (colour intensity) and hue angle (colour) between the skin and flesh samples was 0.783, 0.610 and 0.836, respectively. A reasonably good relationship for hue angle between samples suggests that changes in colour measured based on a hue angle of skin can provide a good prediction of colour changes of the flesh tissue.

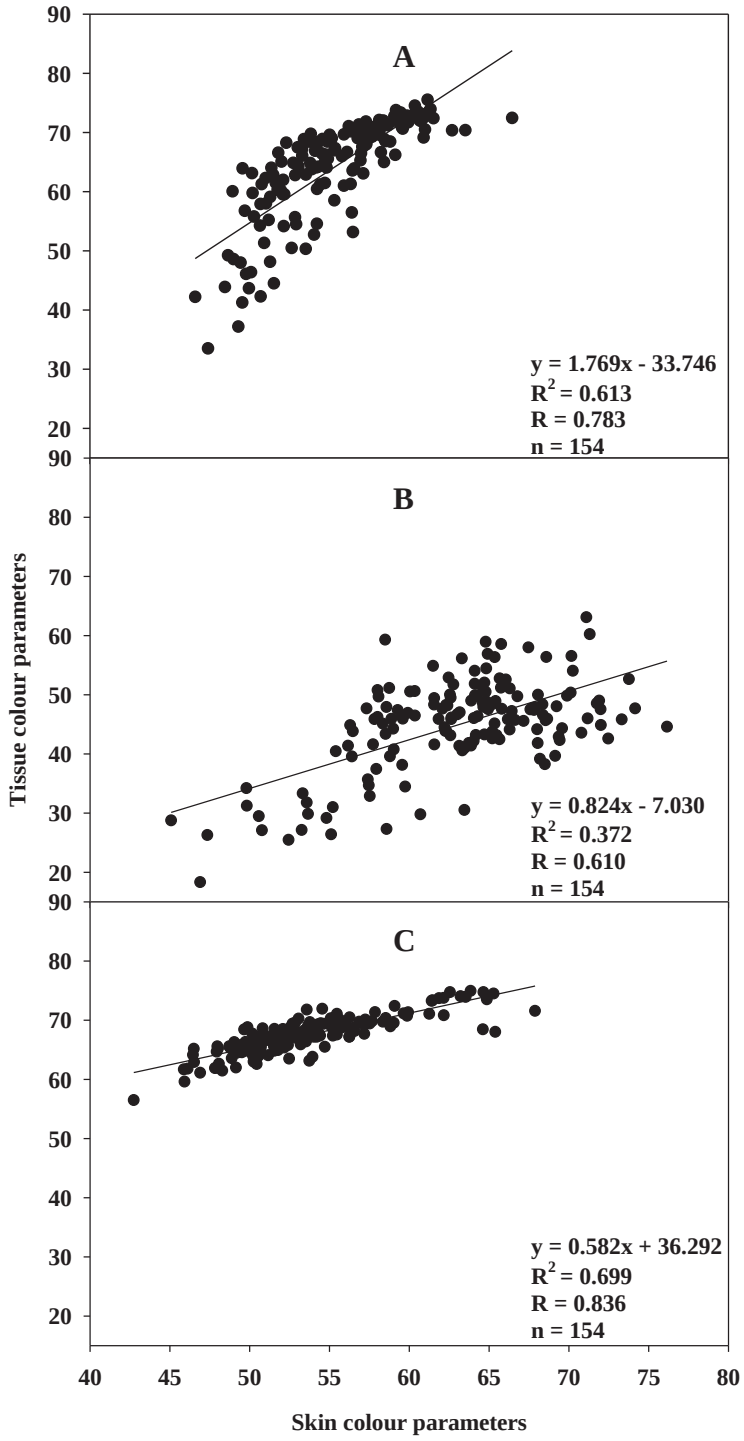


Figure 3.5 Correlation between skin colour (x-axis) and tissue colour (y-axis) of persimmon stored at 20 °C for 15 days. Colour parameters were measured from three different points of fruit skin marked around the equator of persimmon. Then, a 0.6 mm thick of the marked skin was removed prior to measure flesh colour. A, B and C represent correlations of lightness, chroma and hue angle, respectively.

Typically, changes in colour hue and colour intensity during the postharvest shelf life of persimmon are more variable in persimmon skin than flesh tissue. This is also evident in this work (see Figure 3.5C). Valero & Serrano (2010b) reported that during ripening, the concentration of pigments varies widely being higher in skin than in flesh tissue. Some differences in colour between the skin and flesh parts of persimmons is due to difference in their carotenoid concentration being more concentrated in the skin than in the flesh tissue (Payne et al., 1991).

Overall the correlation results suggested that the lightness or chroma between skin and tissue samples were less correlated while the correlation of hue angle was relatively high so the hue angle value of skin colour is better to use to predict the colour change or difference in flesh tissue for comparison between samples rather than the lightness or chroma values.

3.3.4 Physicochemical characteristics at postharvest

Changes in some chemical properties (TSS, pH, titratable acid and total solid content) of persimmons were measured during storage for 17 days at 20 °C in air (Table 3.1). The TSS content present in extracted persimmon juice increased during storage and this can be attributed to the breakdown of starch into sugars (Wills et al., 1998). The results were in agreement with Kitagawa & Glucina (1984). On the other hand, several other studies have shown a decrease in TSS during storage of 'Fuyu' at 0-1 °C (Turk, 1993; Cia et al., 2006). Small variations in TSS during 'Fuyu' persimmon ripening was reported by Clark & MacFall (2003).

The initial pH of persimmon before storage was 6.09 which was similar to that reported by Baltacioğlu & Artik (2013). This pH lowered slightly to 5.96 after storage for 17 days at 20 °C in air (RH ≈ 70 %). The titratable acidity (expressed as the percentage of malic acid) was measured by titration of persimmon juice. The value was inconsistent as it increased from 0.011 % to 0.218 % during the first 10 days of storage and then decreased to 0.028 % after 17 days (Table 3.1).

Table 3.1 Changes in physicochemical characteristics of persimmon during postharvest storage at 20 °C for 17 days.

Day	TSS (%)	pH	Titratable acidity (g.100 ml^{-1} juice)	Dry matter (%)
	persimmon juice			persimmon tissue
0	11.6 ^{c}	6.09 ^{a}	0.011 ^{d}	13.2 ^{b}
3	13.9 ^{b}	6.08 ^{a}	0.016 ^{cd}	14.4 ^{ab}
6	13.3 ^{b}	6.07 ^{a}	0.170 ^{b}	14.4 ^{ab}
10	14.1 ^{a}	5.99 ^{b}	0.218 ^{a}	14.2 ^{ab}
13	13.9 ^{ab}	5.97 ^{b}	0.047 ^{c}	15.1 ^{a}
17	14.1 ^{a}	5.96 ^{b}	0.028 ^{cd}	N/A
n	30	30	10	6
LSD	0.7	0.06	0.034	1.74

Means with the different letters within columns are significantly different ($P < 0.05$).

Storage at 20 °C for 10 days resulted in the peak titratable acidity being 20 times greater than the initial fruit before storage. This trend in titratable acid changes was in agreement with other studies for persimmon (Senter et al., 1991; Cia et al., 2006) and Satsuma mandarin (Matsumoto & Ikoma, 2012). A similar pattern of increasing malic acid content was shown in red cherries stored in modified atmosphere packaging at 2 °C during the first week and then it decreased afterwards (Remon et al., 2000).

Matsumoto & Ikoma (2012) reported that the malic acid content increased when fully-ripe Satsuma mandarin fruit was stored at 30 °C but did not occur at lower temperatures (i.e. 5, 10 and 20 °C). This was similar to some other studies carried out with asparagus (Hurst et al., 1994). The accumulation of malic acid was explained by Burdon et al. (2007) as being the result of a metabolic response of fruits to adapt to temperature stress when stored at 18 °C for 3-5 days with 65 % RH plus 2 days at 10 °C. The accumulation of titratable acid in persimmon during postharvest

storage and subsequent decrease could probably be due to an acid metabolism responding to the storage temperature. This process may be a characteristic of the 'Fuyu' cultivar because rapid increasing and decreasing titratable aid were also reported in 'Fuyu' by Senter et al. (1991). There are no reports of such changes for 'Hachiya' (Ozer et al., 2013), 'Ichi Kijiro, 'Jiro', 'Giambo' and 'Aizumi Shiraza' (Senter et al., 1991).

The DM content of persimmon during storage increased (Table 3.1). However, several studies showed no change in the dry matter content. There was no changes in kiwifruit dry matter content during air storage at temperatures between 1.5 and 25 °C (Schotsmans et al., 2007) and in apples when stored in air at 0.5 °C for 6-12 weeks (Palmer et al., 2010; Crisosto et al., 2011). Increasing DM of persimmon during storage observed in this study could possibly be caused by the low relative humidity (average of 70 % at 20 °C) used in this study which caused the loss of water.

Table 3.2 Mineral content of persimmon tissue.

Maturities (firmness)	Mineral concentration (mg.100 g^{-1} FW)			
	Calcium	Magnesium	Potassium	Sodium
Mature-green (9.1 kg_f)	6 ±0.1	5 ±0.3	147 ±27	4 ±0.3
Ripe (4.9 kg_f)	7 ±1	6 ±0.5	141 ±67	6 ±0.7
Overripe (1.1 kg_f)	7 ±0.1	6 ±0.1	164 ± 27	3 ±1

Data are mean ± SD, n = 2

Calcium in 'Fuyu' persimmon was found to be approximately 7 mg.100 g^{-1} FW (Table 3.2) which was comparable with the calcium content observed by Turk (2004). The calcium concentration of persimmon may vary, depending on the variety and tissue type of persimmon as 'Hachiya' persimmon was reported to have 16 mg.100 g^{-1} FW (Celik & Ercisli, 2008) and whole, pulp and peel of 'Triumph' persimmon were shown to contain 9.4, 6.3 and 28.8 mg of calcium per.100 g^{-1} FW, respectively (Gorinstein et al., 2001). A higher concentration of calcium in persimmon skin was also reported by Clark & Smith (1990).

Calcium levels in persimmon can influence ionic interactions in persimmon products. This is because calcium ions induce crosslink formation carboxylic groups of galacturonic acids on pectin chains (Thakur et al., 1997; Flutto & Caballero, 2003).

3.4 Conclusions

Fundamental information of some physical and chemical changes occurring during the postharvest storage of 'Fuyu' persimmon was investigated. Firmness of persimmon reduced during storage. Persimmon skin turned from a pale green-yellow to an orange-red colour. The values of both chroma representing the colour intensity (saturation) and hue angle representing the colour hue decreased during storage. The colour also darkened (resulting in lower lightness) during storage. A strong colour correlation was found for the values of hue angle measured for skin and tissue. This can be a reasonable predictor of relationship between skin and tissue colour changes. This is useful as skin colour can be measured non-destructively.

A trend of increasing TSS with a slight reduction of pH was observed during storage of persimmon. Interestingly, after 10 days of storage at 20 °C, juice pH reduced, associated with a peak in titratable acidity, TSS, rapid firmness reduction and full development of the orange-red skin colour. This peak in titratable acid concentration is expected to carry through to purée products made from those fruits. The DM content showed a slight increase during postharvest storage due to water loss. Mineral concentrations showed a similar level between different stages of fruit ripening. Among the four types of minerals analysed, potassium was the most abundant element, being much higher than the other three minerals (calcium, magnesium, sodium). The calcium level in 'Fuyu' persimmon was comparable with other varieties of persimmon. The calcium content is considered as a useful tool which can contribute to persimmon processing conditions. A further study on the underlying mechanism related to these associated changes is needed in order to explain ripening process of persimmon and could be benefit for postharvest and processing aspects.

The results obtained are useful for manipulation of persimmon maturities in order to achieve the desired raw material fruit properties before processing as changes in the physicochemical properties of fruit as raw materials are likely to affect the qualities of final processed products. Hence, processing of raw material with the same quality attributes can potentially assure the qualities of the final product. Investigations of the factors that influence persimmon purée characteristics are explored in Chapter 4.

Factors that Influence Persimmon Purée Characteristics*

4.1 Introduction

After harvest, fruit remain physiologically active, and hence can ripen, changing their physicochemical, textural and sensory properties from harvest. Despite the use of maturity indices, commercial bulk harvesting of fruit results in variability in maturity, expressed in terms of the quality factors of colour, flavour and firmness. Generally, textural and colour properties of fruit purée are important qualities as they can influence food acceptance (Meullenet, 2009). Fruit naturally have variation in physical and chemical properties. Consequently, processing raw fruit has the potential to result in variation in textural and colour characteristics of the resulting fruit purée. Producing inconsistent purée quality can significantly affect the value of either a consumer or ingredient product.

Raw fruit colour could clearly influence the colour of the manufactured product. At harvest 'Fuyu' persimmon have a pale green-yellow skin. When fully ripe, the orange colour deepens and becomes redder. During ripening, β-cryptoxanthin and zeaxanthin, which are responsible for orange colour increase on persimmon skin (Niikawa et al., 2007). These two compounds were reported at 38-85 % of the total carotenoid content in ripe flesh of persimmon, depending on cultivar (Zhou et al., 2011). Lycopene, responsible for red colour; is mainly accumulated in the persimmon flesh when fully ripe (Niikawa et al., 2007). The lycopene content of persimmon is reported at 0-30 % of the total carotenoid, with 17-23 % found on the skin (Brossard & Mackinney, 1963). These compounds will influence colour of any processed product. As the level can be different between flesh and skin, the resulting colour of a processed product will depend on whether the skin is peeled or not.

* Material from this chapter is included in the paper: Suntudprom, J., East, A.R., Bronlund, J.E., & Lee, S.J. (2011). *Influence of fruit maturity on texture and colour of purée product.* Paper presented at the iCEF11 International Congress on Engineering and Food, Athens, Greece.

These carotenoid compounds can be lost during heating at high temperature (Chutintrasri & Noomhorm, 2007; Zepka et al., 2009; Zepka & Mercadante, 2009; Mertz et al., 2010) and this will be important in the design of a process.

Generally, during ripening and maturation of any fruit, protopectin – the insoluble parent form of pectin substances, is slowly broken down to lower molecular weight fractions (Wills et al., 1998). Subsequently, these pectins become shorter pectin chains and consequently more soluble in water, resulting in a weak matrix structure. This weakened matrix reduces the ability of the structure to hold the soluble fraction, resulting in a decline in the water holding capacity (WHC) of the subsequently manufactured purée. A loss of WHC can also be a result of syneresis of purée due to greater interaction of the ionic components of the purée structure. Syneresis is the spontaneous separation of serum and pulp. For carrot purée degree of syneresis was high when the intercellular adhesion was strengthened (Christiaens et al., 2012b). Overall, changes during fruit ripening could potentially transfer and influence purée physicochemical and viscoelastic characteristics.

Fruit peel tissue is composed of dermal tissue which consists of hard and rough properties (Kays & Paull, 2004a). Including fruit peel in persimmon purée can increase purée yield. Leonard et al., (1976) showed that in pear purée processing, yield is 50 % higher when pears aren't peeled. Persimmon purée yield is likely to be influenced by a similar proportion.

Persimmons lose their firmness significantly (Figure 3.1) and increase total soluble solids and titratable acid (Table 3.1) during ripening. These physicochemical changes in persimmon fruit carry through to influence purée properties. In order to produce a consistent product, the fruit processing industry must understand the influence of fruit ripeness on the subsequent quality of the processed product. This requirement provides an opportunity to identify the key factors influenced by the variability of harvest maturity on manufactured purées. The objectives of this chapter were to evaluate the effects of persimmon maturity on persimmon purée

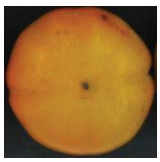
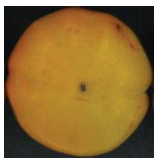
physicochemical and viscoelastic characteristics and to additionally identify the impact of fruit peel on purée yield, physicochemical and viscoelastic characteristics.

4.2 Materials and methods

4.2.1 Purée preparation

Persimmon (*Diospyros kaki,* cv. Fuyu) was supplied by a commercial grower located in Whangarei, New Zealand and manipulated into different maturity stages by storing at room temperature (20 °C, RH 70 %) for different lengths of time. Consequently, five ripening stages were created (Table 4.1). The average fruit firmness was used as an indicator of maturity. Tissue firmness was evaluated using an electronic penetrometer (Willowbank Electronics, New Zealand) equipped with a 7.9 mm round probe (Magness-Taylor).

Table 4.1 Maturity stages of 'Fuyu' persimmon used in this study.

Firmness (kg_f) / Ripeness stages				
0.1-2	2-4	4-7	7-9	>9
Overripe	Fully-ripe	Ripe	Mature	Mature-green
				
Batch 1&2	Batch 3		Batch 4	Batch 5

Fruit of each maturity were processed into purée. Two alternative processes were used that created purée from either whole or peeled persimmon. Whole persimmon purée was created from all five fruit maturities while purées from peeled persimmon were created from 3.0 and 9.1 kg_f maturities only. Persimmons from each of the maturity stages were weighed before and after processing to enable yield estimation. Purée yield was referred to the total weight (kg) of manufacturing for each batch.

To make purée, firstly the calyxes were removed. For the purées made from peeled persimmon, the fruit were then peeled (≈ 1 mm) using fruit hand peeler. Any spoilt parts (i.e. rotten, dark spots, and scars) were also removed prior to cutting into small chunks (approximate size of 10 x 50 x 10 mm) and washed with tap water. Soon after, the persimmon chunks were minced for 5 s using a meat mincer (model PM-98L with a 100 mm mincing head, MAINCA, Barcelona, Spain), followed by blending using a bowl chopper (model C35STP, TALSA, Valencia, Spain) at high bowl speed with low knife speed for 15 minutes. The purée was then filled into 5 kg aluminium foil bags, and were vacuum sealed (model A300/42, Cryovac, Wolfertschwenden, Germany) at 250 mbar with the heat seal at level of 4 and stored at –18 °C until used. Purée samples were also collected from each batch for further analysis into 125 mL clear wide mouth short glass jars capped with a wax seal lid and kept at 5 °C for 17 hours. Since manufacture of each batch was time consuming, the subsequent analysis of the physicochemical and viscoelastic properties of the purées was done on the following day at 20 °C.

4.2.2 Total soluble solids (TSS), pH and titratable acidity

Persimmon purée qualities of TSS, pH and titratable acidity were evaluated as detailed in section 3.2 for whole fruit.

4.2.3 Colour

The colour of persimmon purée was determined using a spectrophotometer (CM-2000d, Konica Minolta Sensing, Osaka, Japan) set up at 10 ° observation with illuminant D65 with measurement head diameter of 8 mm. Purée colour was consistently prepared by filling a plastic petri dish to a height of 15 mm. Colour measurements were conducted from underneath the sample. The calibration was done with a white tile (CM-A145, Konica Minolta, Osaka, Japan) plus a plastic petri dish. During measurement, the petri dish containing purée was covered by a can with a black interior to eliminate transmitted light. The measurement values collected were the CIE L*C*H* colour parameters; lightness, chroma and hue angle.

4.2.4 Water holding capacity (WHC)

Water holding capacity (WHC) is the capacity of purée to hold water over a period of time. WHC was expressed as percentage of weight held in the sample per fresh weight of sample. To evaluate the WHC, persimmon purée was put into a plastic tube (∅ = 30 mm, H = 40 mm) equipped with a layer of cheese cloth at bottom. The tube was placed on top of filter paper of a known weight (m_a) and then 10 g of purée (m_p) was weighed and filled into the tube. After 15 minutes the filter paper was reweighed (m_f) and it was assumed that the gain in weight was a result of water loss from the purée. Water holding capacity was expressed as the percentage of the total weight of the purée sample (Eq 4.1).

$$\mathrm{WHC} = \left[\frac{m_p - (m_f - m_a)}{m_p}\right](100) \qquad \textbf{Eq. 4. 1}$$

4.2.5 Viscoelastic qualities

During the puréeing it was found that the persimmon purée created a gel like structure after subsequent storage at 5 °C. Properties of this gel structure were measured, followed by breaking up of this gel and measuring the flow properties (viscosity). Both textural qualities were assessed using a rheometer (Rheology Advantage, V5.7.0, TA Instruments-Waters LCC, Newcastle, USA). Textural properties (i.e. storage modulus (G′), loss modulus (G″) and viscosity) were determined using a 4-blade vane rotor (∅ = 28 mm, H_{blade} = 42 mm) for oscillatory and flow measurements. The frequencies of oscillatory measurement ranged from 0.1-10 Hz, at 1 % strain. Flow measurement was evaluated at shear rates between 0.01-500 s^{-1}. The chosen strain and shear rate are in the range used for rheological properties of food systems (Pruska-Kędzior & Kędzior, 2006). A strain sweep test was done prior to frequency sweep and flow tests to ensure the strain property of persimmon purée was in the linear viscoelastic region (LVR). Strain sweep tests were run at % strain of 1.45 x 10^{-5} to 1000.0 with at angular frequency of 1.0 $rad.s^{-1}$. All tests were carried out at 20 °C.

4.2.6 Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (version 9.2 (TS2M3), SAS Institute Inc., Cary, NC, USA.). Data were analysed by analysis of variance (ANOVA). Comparison of means was performed by using standard error values (SE) and least significant difference (LSD) to evaluate significant differences at $P = 0.05$.

4.3 Results and discussions

Batches of differently ripened persimmon were created by manipulating firmness at 20 °C. Two further batches of persimmon purée were produced from peeled persimmon at firmness of 3.0 and 9.1 kg_f (batch 3.1 and 5.1 respectively). Each batch continued to express the natural variability in maturity at harvest and hence was composed of a range of firmness of fruit (Figure 4.1A). Variation of firmness was more wide spread in some batches than others.

For batch 1, 50 % of persimmons were very soft and overripe (firmness < 0.1 kg_f). While batch 2, firmness clustered in the range of 0.2 to 0.5 kg_f with the most firm quartile of fruit ranging from 2 to 4 kg_f. The firmness distribution of batch 3 was the most wide spread ranging from 1 to 6 kg_f. In batch 4, there were about 75 % of mature and mature-green persimmons (firmness between 7 to 9 kg_f) and about 25 % of ripe persimmons (firmness between 5 to 6 kg_f). Fruit of batch 5 were normally distributed with an average firmness of about 9.1 kg_f.

During ripening, persimmon skin turns from pale green-yellow to an orange-red colour (Figure 4.1B-D). The lowest and the highest lightness of persimmon skin were found in batches 1 and 5 respectively. Lightness and hue angle increased inconsistently while chroma increased and became less variable with a large variation in batch 1. Overall, fruit ripening influenced lightness, chroma and hue angle and was expected to result in purée differences.

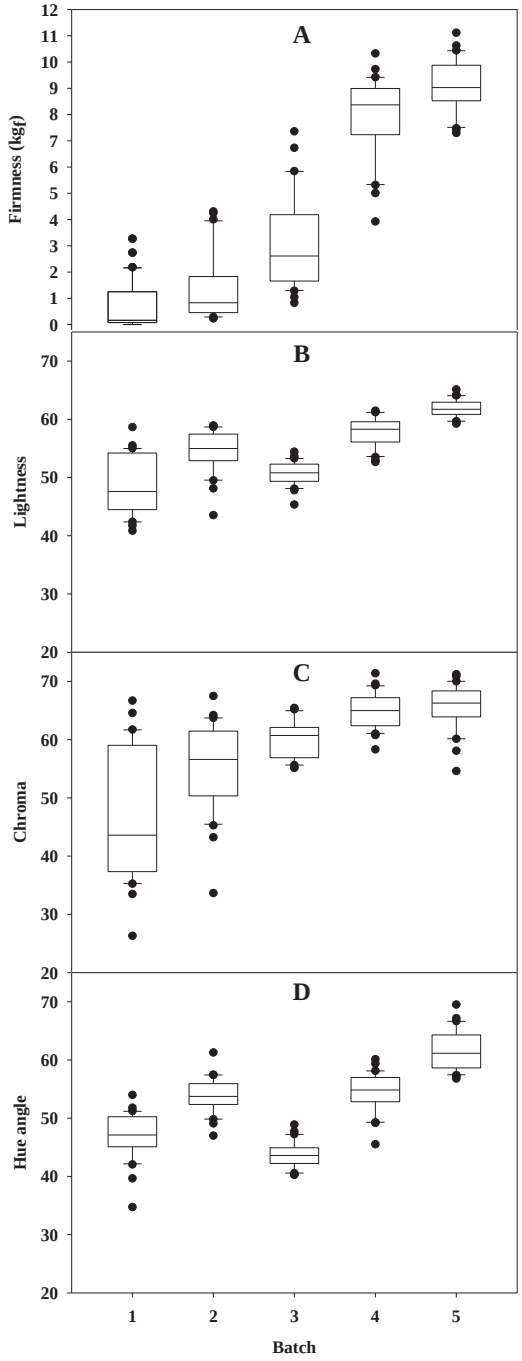


Figure 4.1 Box plot of firmness (A), and persimmon skin lightness (B), chroma (C) and hue angle (D) before processing (n = 30) for batch 1 to 5.

4.3.1 Fruit maturity impacts on purée yields

Fruit at different ripeness can affect purée yield due to altering the ability to handle and manage the fruit during processing. The firmer (9.1 kg_f) fruit had higher yields

in comparison to softer (0.7, 1.3 and 3.0 kg_f) fruit (Figure 4.2) in a fairly consistent pattern across all batches. Low purée yield in batch 1 was affected by the quality of persimmons before processing in terms of ripeness, spoilage, and damage. Batch 1 was manipulated by ripening at 20 °C for 11 days. After this time some fruit had developed rots and damage which required removal before processing (Figure 4.3). Furthermore, persimmons in this batch were very soft making them difficult to handle and manage. Overall, the riper the persimmon, the more the rot, the less the purée yield.

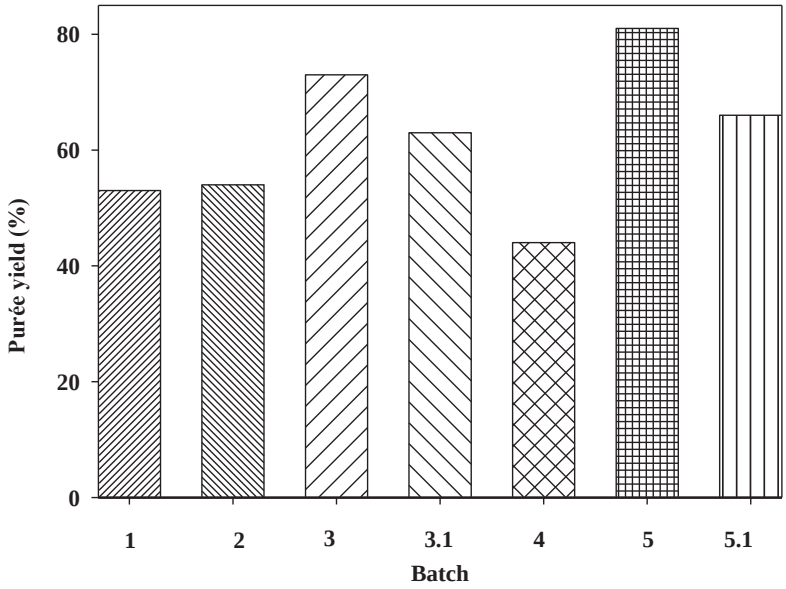


Figure 4.2 Purée yields produced from (unpeeled) persimmons of five maturities (batch 1, 2, 3, 4 and 5) with two alternative batches made by using peeled (batch 3.1 and 5.1) persimmons.

Figure 4.3 Spoilage and damage of persimmon in batch 1: numbers 1-3 display spoilage of persimmon while number 4 displays skin damage which results in later spoilage.

For purée made using peeled persimmons (batch 3.1 and batch 5.1) a lower yield was obtained. Removing persimmon skin reduced purée yield by about 15 % for both batches in comparison to using whole persimmons. This reduction in peeling loss compares favorably to pears where purée yield was reduced by 46 % due to peeling (Leonard et al., 1976).

4.3.2 Physicochemical characteristics of purée as influenced by maturity and peeling

4.3.2.1 Purée colour impacted by maturity

Figure 4.4 shows the visual appearance of persimmon purées made from the 5 different maturity batches. Each batch continued to express the differences in colour as caused by ripening. Colour properties were measured for five batches of persimmon purée made from different maturities of fruit, some containing peel. Purée lightness decreased as fruit ripened whilst there were less consistent trends in changes for both chroma and hue angle (Table 4.2). In particular, batch 3 seemed irregular in comparison to the other batches, which may be symptomatic of the large variability in firmness of the fruit used to manufacture this batch (Figure 4.1A). Significant differences in hue angle were observed, with only batch 3 differing from

the trend of soft fruit resulting in a lower hue angle. Overall, fully-ripe persimmon (3 kg_f) produced a slightly redder (lower hue angle) purée than overripe persimmon (0.7 and 1.3 kg_f) and mature persimmon (7.9 and 9.1 kg_f). Photos of the purées taken in a controlled light environment demonstrate the colour differences of the purées (Figure 4.4). Purées made by ripe and overripe persimmons were darker (less light) than purées produced from mature and mature-green persimmons.

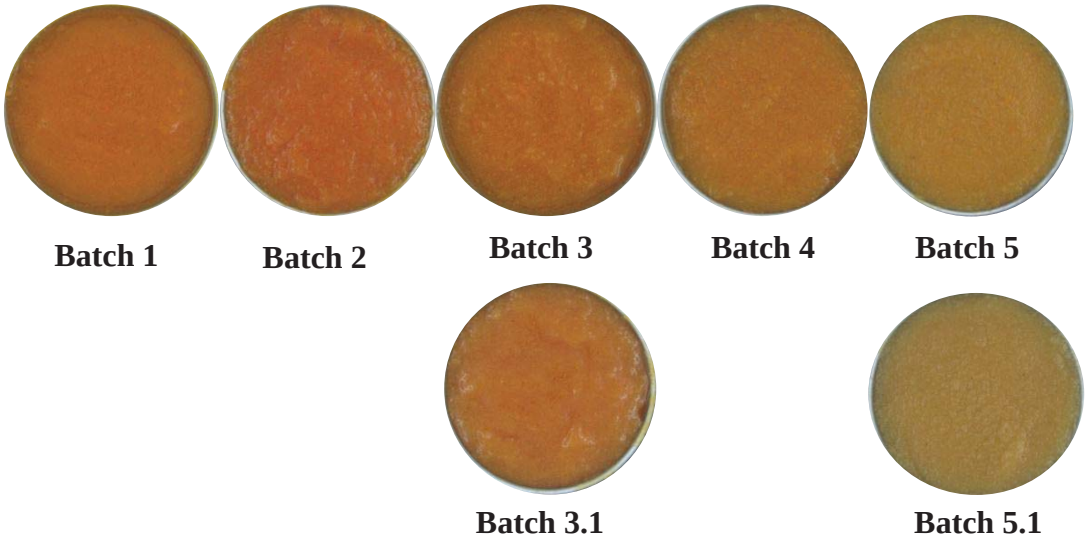


Figure 4.4 Visual appearances of persimmon purées made from different batches with five different maturities. Figures on top row indicate whole persimmon purées and the bottom row indicate peeled persimmon purées.

Table 4.2 Physicochemical and viscoelastic characteristics of purée as influenced by maturity and peeling.

Batches		Average firmness (kg_f)	Purée colour parameters			%TSS	pH	Titratable acidity (g.100 ml^{-1} of juice)	%WHC[(a)]	Storage[(b)] Modulus (G′, Pa)	Loss[(b)] Modulus (G″, Pa)	Viscosity[(c)] (Pa.s)
			Lightness	Chroma	Hue angle							
1	Whole	0.7 (overripe)	43.7 e	22.9 b	61.4 d	12.4 cd	5.5 e	0.032 c	97.6 b	86 e	17.5 e	24.9 c
2	Whole	1.3 (overripe)	44.0 e	22.4 c	63.4 c	11.9 f	5.4 e	0.037 c	96.9 c	85 e	17.8 e	28.4 c
3	Whole	3.0 (fully-ripe)	45.5 c	24.5 a	57.8 e	13.1 a	5.6 c	0.137 a	99.1 a	200 d	26.7 d	25.5 c
3.1	Peeled	3.0 (fully-ripe)	44.8 d	19.9 e	62.4 cd	12.7 b	5.7 c	0.140 a	96.3 c	51 f	7.6 f	15.0 d
4	Whole	7.9 (mature)	45.8 c	21.0 d	65.6 b	12.1 ef	5.6 d	0.030 c	99.5 a	840 a	129.0 a	70.4 a
5	Whole	9.1 (mature-green)	47.7 a	22.7 bc	65.2 b	12.5 bc	5.8 b	0.050 b	98.9 a	539 b	95.1 b	64.4 ab
5.1	Peeled	9.1 (mature-green)	47.0 b	15.9 f	70.9 a	12.2 de	5.9 a	0.040 bc	99.0 a	407 c	68.0 c	59.0 b
$LSD_{0.05}$			0.3	0.5	1.1	0.2	0.1	0.012	0.6	33	6.1	6.8

Means with the different letters within columns are significantly different ($P < 0.05$, $n = 4$).
[(a)] Water holding capacity is capacity of purée to hold water over a particular time.
[(b)] G′, G″ were measured at 1 Hz.
[(c)] Viscosity was evaluated at shear rate of 1.256 s^{-1}.

4.3.2.2 The influence of peeling on purée colour

Batches 3.1 and 5.1 were created from peeled persimmon at 3.0 and 9.1 kg_f maturities respectively. Lightness and chroma of peeled-purée batches were lower in comparison to whole-purée batches while there was an increasing hue angle (Table 4.2, Figure 4.1). The more ripe peeled-purée batch (3.1) showed lower lightness and hue angle with higher chroma, compared to less ripe peeled-purée (batch 5.1).

Enzymatic browning reactions occurred as a result of peeling. Cutting and purée creation damages cells, resulting in the release and mixing of enzymes (polyphenol oxidase) and substrates (Wright & Kader, 1997; Brewer, 2013). Browning in fruit is caused by the oxidation and polymerisation of phenolic compounds (Khademi et al., 2012). Total phenolic content in persimmon is highest in the peel, whole and pulp respectively (Gorinstein et al., 2001).

4.3.2.3 Titratable acidity (malic acid), total soluble solids and pH of purée as affected by maturity and peeling

Physicochemical qualities of different batches of purée were influenced by persimmon maturity and peeling (Table 4.2). This study observed an unexpected increase in TSS during ripening at 20 °C (see Section 3.3.4). This TSS increase was expected to carry through to the purée. TSS across all batches was significantly different with an unclear trend. However, Hua (2010) observed that fully-ripe and overripe 'Fuyu' persimmons produced higher TSS purée. It was noticed that batches 1, 2 and 4 were manufactured on a later day in comparison to batches 3, 3.1, 5 and 5.1, and after a different period of cool storage (1 month). It is possible that the lack of trend of TSS change was influenced by the storage time of the fruit prior to processing. The inconsistent changes in TSS during storage was similar to those observed by Cia et al., (2006) who reported inconsistency in TSS changes during 'Fuyu' fruit storage in MAP for 84 days at 1 °C. However, Turk (1993) found TSS gradually decreased in 'Fuyu' fruit during storage for 2 months at 0 °C, RH 90 %.

Across the seven batches of purée some pH differences were observed with the overall range being between 5.4-5.9. Interestingly, later processing batches (batch 1, 2 and 4) showed lower pH than the other batches. Batch 5.1 had a higher pH in comparison to softer fruit in a fairly consistent pattern across all batches. The study observed that there was trend in reduced pH as fruit ripen (see Section 3.3.4) and hence the observed reduced pH in riper fruit was expected to carry through to purée.

Titratable acid contents across the seven batches of purée showed differences with maturity while peeling had no effect. Titratable acid peaking at batch 3.1 was as discussed in section 3.3.4. The results were however similar to the changes in malic acid of whole persimmon fruit after harvest found by Senter et al. (1991). They revealed that 'Fuyu' persimmon high titratable acid content at fully-ripe stage (3.8 kg_f). However, Cia et al. (2006) reported inconsistent changes in titratable acidity of 'Fuyu' persimmon fruit stored in 50 μm LDPE and 58 μm PO bags for 90 days at 1 °C / 90 % RH plus five more days at 25 °C / 70 % RH.

4.3.2.4 Water holding capacity of purée as impacted by maturity and peeling

Maturity affected the WHC (Table 4.2) as expected. In particular the WHC of batches 1 and 2 made from soft fruit, were significantly lower than other batches. However, a significant difference was observed from batches 3 and 3.1. It can be suggested that persimmon skin appears to have effect on WHC. Higher WHC observed in batch 3 in comparison to batch 3.1 could be possible due to partially degradation of skin tissue in advance stage (Salvador et al., 2006; Salvador et al., 2007) resulting in further migration of the liquid fraction presented in the purée. The effects of maturity on WHC may be due to ripening causing tissue cell wall (mainly pectin) break down, resulting in more pectin being solubilised (Wills et al., 1998) which leads to a weakened matrix structure. In the more advance stage persimmon, cell walls are extensively degraded and hence have lost their integrity and hence their ability to hold solutes. Subsequently, the ability of the resulting purée structure to hold the soluble fraction is reduced resulting in a measured decline of WHC. However, Hua (2010) found that purées made from fully-ripe and overripe

persimmons had higher WHC than purées made by mature and mature-green fruit. Water separation created by syneresis in persimmon gelling was observed (Figure 4.5). Kunitz (1928) and Mao et al. (2001) stated that syneresis is generally found in polysaccharide gels and jellies.

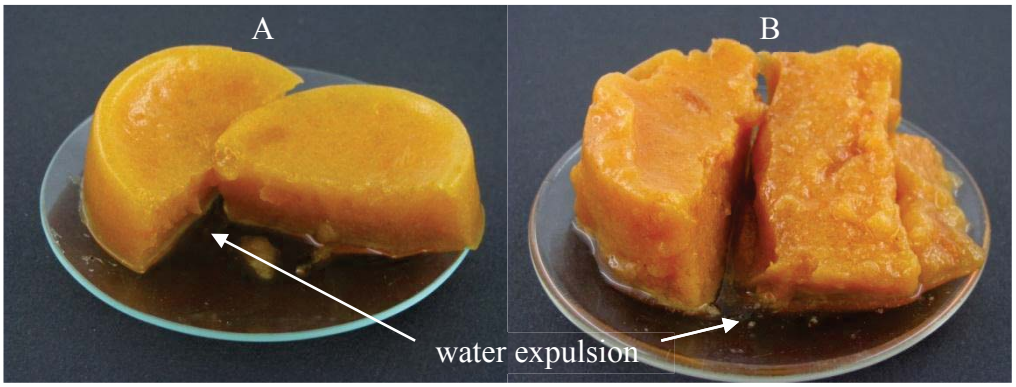


Figure 4.5 Visual appearances of persimmon gels made by using persimmons at; A-mature persimmon (7 kg_f) and B-overripe (1.7 kg_f) which present a gel structure. Syneresis was observed indicated by water expulsion. Mature persimmon purée shows high water expulsion than overripe persimmon purée.

4.3.3 Viscoelastic characteristics of purée from different batches

4.3.3.1 Effects of maturity and peeling on the viscoelastic characteristics (G′ and G″) of purée

Purées made from mature and mature-green persimmons (batches 4 and 5) resulted in a stronger structure (as indicated by higher G′) in comparison to overripe and fully-ripe persimmons (batches 1, 2, and 3). Peel was observed to increase the strength of purée in term of G′ and G″. G′ and G″ of batch 5 were 25 % and 30 % higher respectively in comparison to batch 5.1. For batch 3, increases of approximately 40 % and 28 % for G′ and G″ in comparison to batch 3.1 were observed.

After harvest, fruit remain physiologically active, and hence can ripen progressively (Fischer & Bennett, 1991). As a result, fruit become soft due to tissue degradation and the loss of cell-to-cell adhesion (Brummell & Harpster, 2001). This study

observed that G′ and G″ of the ripe purée were significantly lower than mature batches as expected. The reduction of G′ and G″ were likely to be caused by pectolytic enzymes particularly pectinmethylesterase (PME) and polygalacturonase (PG) (Waldron, 2004). PME de-esterifies methylesters from polygalacturonic acid (Pilnik & Rombouts, 1981) on the pectin chain during fruit ripening. This results in methyl free polygalacturonic acid regions which are sensitive to calcium cross linking. During purée manufacture tissues are damaged leading to contact between enzymes, substrates, coenzymes, activators and inhibitors (Oey, 2010). The free PME can then more readily catalyse the methyl-ester removal from polygalacturonic acid on the pectin substances resulting in more methyl free GalA residues. Hence, the calcium gel crosslinks between calcium ion and two methyl free GalA sites can be formed resulting in a high strength of purée (Figure 4.5A).

The PG activity during fruit ripening is the hydrolytic cleavage of α-1,4 linked galacturonan linkages (galacturonic acid) (Fischer & Bennett, 1991). PG hydrolysis causes solubilized and shortened pectin chains (Fischer & Bennett, 1991) resulting in tissue softening associated ripening. Processing damages cell wall tissue and will accelerate cell wall degradation by PG (Van Buggenhout et al., 2009). Low G′ and G″ in fully-ripe and overripe batches were observed. Lowering of G′ and G″ in fully-ripe and overripe batches suggests that pectin chains in fully-ripe and overripe persimmons were more hydrolysed by PG than mature-green and mature persimmons. Therefore, weaker purée structures in fully-ripe and overripe purées resulted (Figure 4.5B).

A gel state is said to occur if the solid-like behaviour (G′) prevails over viscous flow (G″) (Chronakis & Kasapis, 1995). This study found that across all purées G′ was greater than G″ (Table 4.1). This quantitatively implies that persimmon purées naturally form gel networks expressed as a semi-solid material at 20 °C. This finding needs to be addressed more in terms of persimmon gel formation mechanisms.

4.3.3.2 Flow properties of purée impacted by maturity and peeling

Typical flow properties of seven batches of purée are showed in Figure 4.6. Increasing slope with increasing shear rate indicates reducing viscosity which suggests a pseudoplastic fluid (shear thinning) with yield stress behaviour. Flow behaviour demonstrated that the slope of batches made from riper fruit showed lower curves than batch made from firmer fruit. The influence of peeling on the resulting flow properties of the batches at different maturities was unclear and hence needs further work.

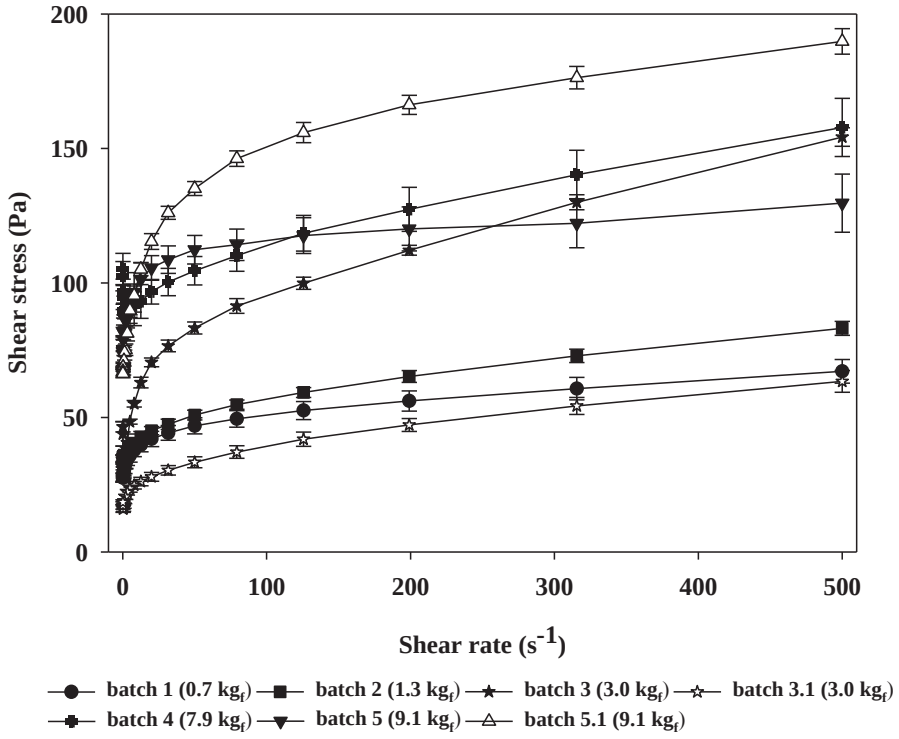


Figure 4.6 Shear diagram of shear rate against shear stress with yield stress for flow behaviour of persimmon purées made from different batches with five different maturities. Data presented in means of four replicates with standard deviation. Data were collected at shear rate between 0.1-500 s^{-1}.

The flow behaviour index (n) in which indicates the behaviour of each purée is shown in Figure 4.7. An average $n < 0.5$ was observed in batches that were made from persimmon of firmness 0.7, 1.3, 3.0 and 9.1 kg_f. However a $n > 0.5$ was found

for the batch made from persimmon at firmness 7.9 kg_f. Generally, the *n* indicates the behaviour of the fluid, if $n < 1$, that fluid has a pseudoplastic or shear-thinning behaviour (Barbosa-Canovas & Ibarz, 2002; Ditchfield et al., 2004; Ritzoulis, 2013). Flow behaviour index of purée made from peeled persimmon were higher than batches made from unpeeled persimmon at the same firmness. Purée in batches made from persimmon firmness at 7.9 kg_f were close to a Newtonian behaviour indicated by *n* being close to 1. In contrast, purées made from persimmon firmness at 9.1 kg_f were far away from 1 which indicated the behaviour becoming more and more non-Newtonian (Ditchfield et al., 2004).

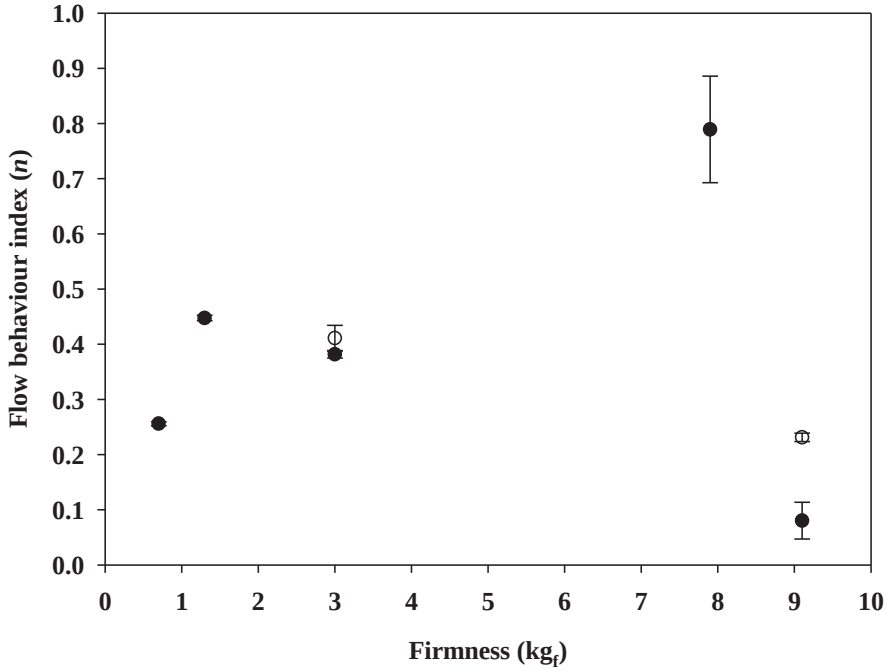


Figure 4.7 Flow behaviour index (n) of persimmon purée made from persimmon at different firmness. Purées made from persimmon at 0.7, 1.3 and 7.9 kg_f were made by using unpeeled (filled circle) persimmons. Purées made from persimmon at 3.0 and 9.1 kg_f were made by using peeled (unfilled circle) and unpeeled persimmons. Data presented is means of four replicates with standard errors (SE).

Persimmon purée made from soft fruit had a significantly lower viscosity in comparison to purée made from harder fruit (Table 4.2). This can be explained by the cell wall being modified by PME and PG in particular (Waldron, 2004). During fruit ripening, PME demethylated methyl-ester group from pectin molecules

resulting in methyl free carboxyl groups which change the pH and charge in the cell wall (Brummell & Harpster, 2001). Methyl free polygalacturonic acid molecules can then be more readily hydrolysed by PG resulting in more solubilised and short pectin chains. Hence, purées made from ripe fruit had a significantly lower viscosity. Moreover, peeling caused reductions in viscosity of mature-green and fully-ripe purées. The reduction of viscosity was due to removing skin (dermal tissue). The effects of peeling were observed more in fully-ripe purée which was the lowest viscosity in comparison to other batches.

Rao (1999a) explained that fluid foods are generally non-Newtonian foods which exhibit shear-thinning behaviour. Additionally, non-Newtonian foods might show yield stress – a stress that is required to be exceeded for the flow to occur. The behaviour could be described by using the Herschel-Bulkley model. Tomato concentrates, tomato ketchup, mustard, mayonnaise (Rao, 1999a), banana purée (Guerrero & Alzamora, 1997; Ditchfield et al., 2004) and modified solid content persimmon purée (Fraeye et al., 2010a) are examples showing shear thinning with yield stress behaviour.

4.4 Conclusions

The effects of persimmon maturity and peeling on the physicochemical characteristics and viscoelastic properties of purée were evaluated. This study found fruit maturity and inclusion of persimmon peel effects the quality attributes of purée (Figure 4.8).

Firmer fruit had higher purée yields in comparison to softer fruit. Including persimmon peel increases purée yield. Changes in the physicochemical properties of persimmon fruit during ripening carry through to influence purée properties. Fruit maturity influences colour (lightness, chroma and hue angle). Fully-ripe persimmon (3 kg_f) produced a slightly redder (lower hue angle) purée than overripe persimmon (0.7 and 1.3 kg_f) and mature persimmon (7.9 and 9.1 kg_f).

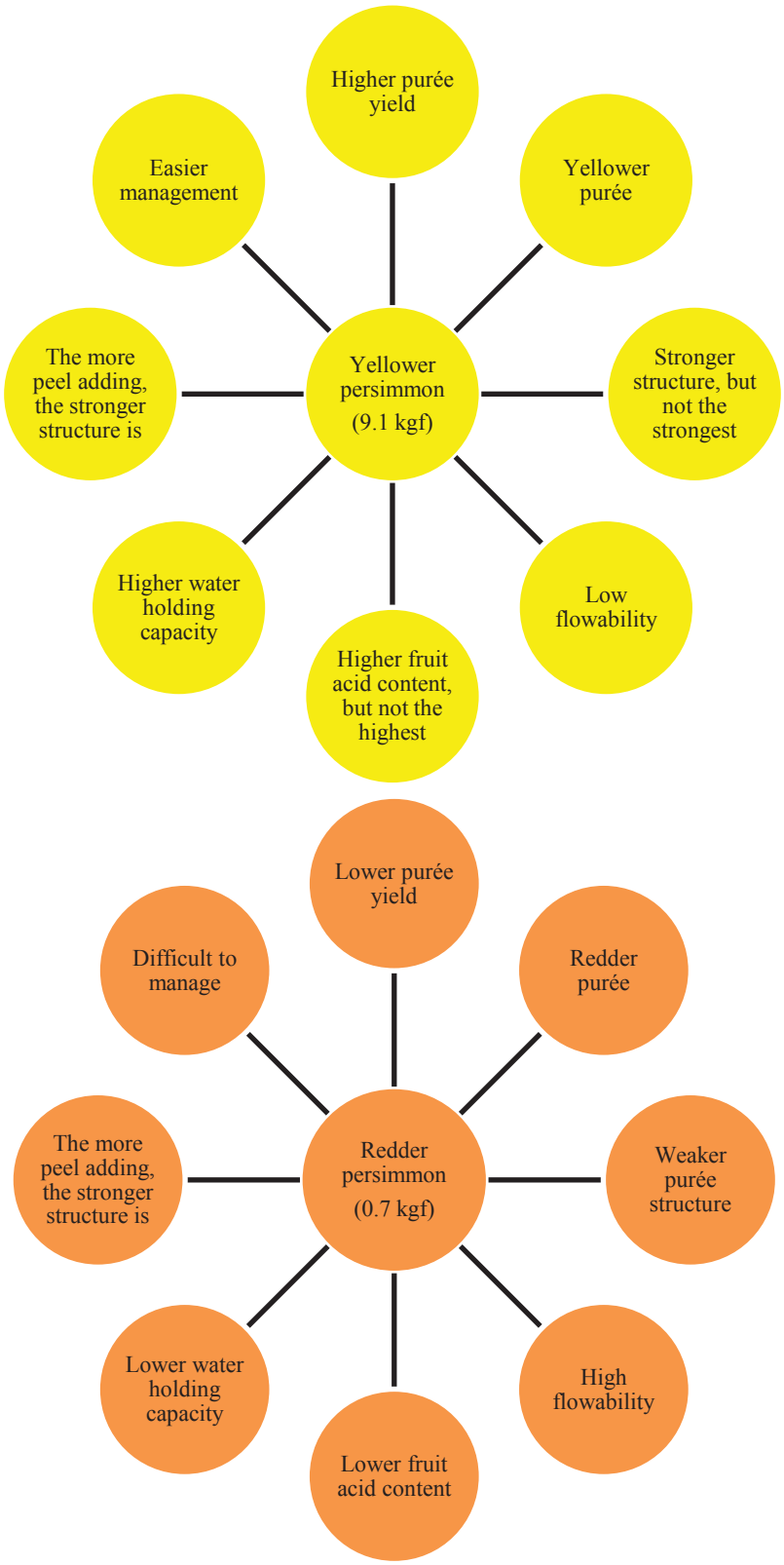


Figure 4.8 Summary of the influence of persimmon maturity on persimmon purée physicochemical and viscoelastic characteristics.

Variation in TSS during fruit ripening was found to transfer through to purée with peeling having a decrease on TSS. A reduction of pH in more ripe fruit was observed to carry through to purée while peeling may increase pH of mature-green purée. A peak in titratable acidity was found in batch 3 (firmness 3.0 kg_f) which was similar to observed by whole fruit that stored at 20 °C for 10 days (firmness 3.6 kg_f) while peeling showed no effect on titratable acid. The WHC of more ripe purées were found to be lower compared to less ripe batches. Water separation occurred due to syneresis. Persimmon skin appears to reduce the WHC of more ripe fruit purées.

Purées made from mature and mature-green persimmons resulted in a stronger structure (as indicated by higher G′) in comparison to overripe and fully-ripe persimmons. Peel also increased the strength of purées. G′ and G″ of ripe purée were significantly lower than mature batches. Persimmon purée made from soft fruit had a significantly lower viscosity in comparison to purée made from harder fruit. The predomination of G′ over G″ in persimmon purée suggested gelling properties. Observed flow properties suggest a shear thinning behaviour. Persimmon purée can then be classified as a non-Newtonian food (semi-solid material type) with gel forming behaviour.

This study found persimmon purées form gel networks spontaneously at 20 °C. This finding needs to be addressed more in terms of persimmon gel formation mechanisms. Should the mechanisms of the observed gelation be elucidated, the manipulation of the gel structure through processing and control of fruit maturity to achieve desired and constant textural properties may be possible. Further investigations of the factors that influence persimmon gel formation are explored in chapter 5.

CHAPTER 5

Factors Influencing Persimmon Gel Characteristics

5.1 Introduction

During fruit ripening, there are physical and chemical processes which lead to changes in the structure and composition of the tissue. For example the conversion of starch into free sugars, modification of the cell wall structure and tissue softening are common processes (Nath et al., 2006). Pectin is part of the primary cell wall and the main component of the middle-lamella (Van Buren, 1991). Persimmon pectin is enzymatically modified with ripening (Cutillas-Iturralde et al., 1993) by pectinmethylesterase (PME) and polygalacturonase (PG) (Fischer & Bennett, 1991).

The role of PME during fruit ripening is to de-esterify pectin chains, specifically methylester groups from polygalacturonic acid (Pilnik & Rombouts, 1981). This results in more methyl free galacturonic acid (GalA) regions which are extremely sensitive to calcium. The de-esterified polygalacturonic acid chains are more accessible for PG hydrolysis (Fischer & Bennett, 1991) causing a reduction of pectin length as well as increased solubilisation.

PME influences the final quality of processed fruit products as has been shown by a number of studies. Food processing damages cells and leaves them prone to contact between enzymes, substrates, coenzymes, activators and inhibitors (Oey, 2010). PME can be inactivated using various temperature, pressure and pH treatments in order to optimise viscosity and texture of tomato juice (Stoforos et al., 2002) and guava pulp (Leite et al., 2009). Activating PME in trimmed carrot by blanching at 76.6 °C for 20-30 minutes prior to being canned was reported to increase the firmness of canned carrot (Lee et al., 1979). The maximum firmness for cooked

onion was achieved when blanching at 50 and 70 °C for 20 minutes (Kim, 2006). Vacuum infusion with PME and calcium increased firmness of pasteurized fruit (Javeri et al., 1991; Degraeve et al., 2003; Guillemin et al., 2006) and minimized texture loss of frozen strawberries (Van Buggenhout et al., 2006). Food processing conditions can facilitate or slow down enzyme activity (Brummell & Labavitch, 1997; Van Buggenhout et al., 2009). For these reasons control of the extent of PG activity will be important in persimmon processing.

During fruit ripening PG catalyses the hydrolytic cleavage of α-1,4 linked galacturonan (polygalacturonic acid) linkages of the pectin structure (Fischer & Bennett, 1991). Consequently, PG hydrolysis causes more solubilized and shortened pectin chains (Fischer & Bennett, 1991) resulting in tissue softening associated with fruit ripening.

As discussed above, food processing damages cell wall tissue and can accelerate cell wall degradation by releasing PG which effects product quality (Van Buggenhout et al., 2009). Tomato processing conditions were reported to strongly influence the chain length of pectin, resulting in altering the viscoelastic properties of tomato paste (Chou & Kokini, 1987; Sánchez et al., 2002), tomato juice (Xu et al., 1986; Hsu, 2008) and tomato-based products (Verlent et al., 2006).

A gel is defined as a composition of polymeric molecules crosslinked to form a tangled interconnected network immersed in a liquid medium (Flory, 1953). In food production, polysaccharide gels are important ingredients to provide desired texture (Lopes da Silva & Rao, 1999). Textural properties such as brittleness, elasticity and rubberyness of polysaccharide gels are influenced by the number and length of the interacting chains. Gel types have been classified according to their degree of methylesterification (DMe) (Voragen et al., 1995; deMan, 1999). The DMe refers to the percentage of carboxyl groups esterified with a methyl group. Two types of pectin are high methoxyl pectin (HMP) (DMe ≈ 50-80 %) and low methoxyl pectin (LMP) (DMe < 50 %). HMP usually forms hydrogen-bridge bonded networks with low pH (2.5-3.5) and high concentration of sucrose (55-75%) (Lopes da Silva & Rao, 2006), whereas LMP forms ionic (calcium) crosslinked networks (Bacic et al.,

1988). LMP gels are thermo-reversible and exhibit higher gelation rates whilst HMP gels are thermo-stable with lower gelation rate (2012).

Polysaccharide and gelatin are polymer-like chain macromolecules (Lefever, 2003). Characterisation of the rheological behaviour of polymer-like chain macromolecules demands the study of the viscoelasticity. The interaction between molecules can be measured by low magnitude oscillatory measurement of complex modules. The storage modulus, G′, measures the solid-like properties while the loss modulus, G″, determines its liquid-like properties (Chronakis & Kasapis, 1995).

Previously (Section 4.3.3), persimmon purée was found to gel at room temperature irrespective of persimmon maturity (Figure 4.5). Purée made from mature persimmons (≈ 7 kg_f) produced the strongest gel (highest G′ and G″). Additionally, persimmon gels presented water expulsion (i.e. syneresis) which is a common issue with polysaccharide gels (Mao et al., 2001) and jellies (Kunitz, 1928). The viscoelastic characteristics of persimmon gel (i.e. G′ predominating over G″) appeared to follow LMP gelling properties as described by Chronakis et al., (1995).

Calcium gel formation occurs between two carboxylic groups which are linked with a calcium ion (Flutto & Caballero, 2003). Ethylenediaminetetraacetic acid (EDTA) is used to sequester metal ions likes calcium. EDTA has been used in the food industry for flavour preservation, prevention of odours, maintaining nutrient content and shelf life extension (Economou et al., 2009; Mastromatteo et al., 2010; Ntzimani et al., 2010). Applying EDTA in LMP systems can possibly eliminate calcium gel formation, resulting in significantly altering the gel properties.

Thermal processes cause cell wall softening and degradation. In particular the middle lamella changes and there is a loss of turgor pressure (Edwards, 1999; Nielsen et al., 2004). Heat treatments are also used to activate and/or inactivate enzymes. Heating at 60 °C (Lopez et al., 1997) and 65.5 °C (Van Buggenhout et al., 2009) are used in the cold break procedure for tomato juice and tomato purée respectively. During cold break processing, PME is activated and used to de-esterify methyl ester groups from

the pectin. PG then splits the α-1,4 hydrolytic bonds in polygalacturonic acid chains. The cold break procedure results in low consistency of final product.

In the temperature range of 82-104 °C, inactivation of PME and PG in the hot break tomato process occurs (Lopez et al., 1997; Van Buggenhout et al., 2009). High temperature inactivates PME, resulting in the retention of methylester groups in the pectin. As a consequence, there is higher resistance to PG attack on the pectin, and therefore higher viscosity of the final product is preserved.

Long methyl free galacturonic acid chains are extremely sensitive to calcium crosslinks (Bacic et al., 1988). Ripening caused pectin and cell wall solubilisation are indicated by the reduction in molecular weight (Nogata et al., 1996; Wang et al., 2002). Less ripe persimmon tissue is likely to be composed of longer pectin chains. The longer pectin chains can possibly have more methyl free GalA sites to form calcium crosslinks resulting in strong gels. In order to manipulate persimmon gel strength, therefore, adding mature-green persimmon tissue into overripe persimmon tissue can possibly alter gel viscoelastic characteristics.

This chapter aims to further investigate persimmon gel characteristics imparted by gelling time and persimmon maturities. Additionally, persimmon gel characteristics were manipulated with ethylenediaminetetraacetic acid (EDTA) chelation, heat treatments and persimmon tissue combination to further elucidate the characteristics of persimmon gels and the mechanisms involved in their formation.

5.2 Materials and methods

Factors influencing the characteristics of persimmon gel were observed via five experiments investigating the effects of gelling time, persimmon maturity, EDTA application, heat treatment and combining tissues of different ripeness.

5.2.1 Gelling time

A mature persimmon was peeled, decalyxed, cut into small irregular shapes (approximate size 10 x 50 x 10 mm), and blended for 20 s using a grinder (CG2B, Breville, Breville Ply. Ltd., Sydney, Australia). Nine 10 g purée samples were weighed and filled in a plastic tube ($\varnothing_{in}$ = 28 mm, height = 30 mm) and placed on glass watch plates. The total preparation time was 5 minutes. Gel formation was monitored on a single sample by photographs at 5 minutes intervals for the first 30 minutes and after 30, 60 and 180 min at 20 °C. Prior to samples being photographed, the tubes were removed and the gel was allowed to flow or relax for 5 s. All samples were prepared from the same fruit.

5.2.2 Maturity effect

Persimmon purée made from different fruit maturities were assessed with time sweep and frequency sweep rheological tests. Three individual purée samples were prepared using mature, fully-ripe and overripe persimmons for time sweep – small strain oscillatory tests. Frequency sweep tests were conducted using mature-green and fully-ripe persimmons.

Persimmons were peeled, decalyxed and cut into irregular shapes (approximately 10 x 20 x 10 mm), and then blended for 20 s using a grinder (CG2B, Breville, Breville Ply. Ltd., Sydney, Australia). Soon after, samples were examined with time sweep tests using the rheometer (Rheology Advantage, V5.7.0, TA Instruments-Waters LCC, Newcastle, USA). Storage modulus (G′), loss modulus (G″) and loss tangent (° δ) were determined using a 4-blade vaned rotor ($\varnothing$ = 28 mm, H_{blade} = 42 mm). Time sweep tests were performed at 1 % strain with a frequency of 0.16 Hz for 12 hours at 20 °C. Frequency sweep tests were assessed at 1 % strain with the frequency ranging from 0.1-10 Hz at 20 °C.

5.2.3 Sample preparation for EDTA and heat treatments and tissue combination

Persimmon maturity was manipulated by storing at room temperature (20 °C) for different extents. Consequently, a number of persimmons of different maturity were created with firmness ranging between 0.1 to 9 kg_f. Experiments were conducted following individual persimmon to account for fruit firmness effects on the results, which was measured prior to purée preparation (Section 4.2.1). Three sets (mature-green, ripe and overripe) of purée were prepared using five persimmon fruit to result in a final sample weight of 145 g (Figure 5.1). During preparation, all persimmon fruit were kept in an ice slurry to minimise enzymatic reactions. After samples received EDTA, heating and combination treatments, they were stored in a 125 mL clear wide mouth short jar and kept at 5 °C (open to the air) for 17 hours before viscoelastic measurements (Section 4.2.5). All experiments were done using duplicate samples.

5.2.4 EDTA applications

EDTA ('ultraPURE™', Disodium salt, Dihydrate-Na_2 EDTA.$2H_2O$, M.W. = 372.24 $g.mole^{-1}$, purity ≥ 99 %, insoluble ≤ 0.005 %, Gibcobrl) was added to persimmon purée at 0.001, 0.01, 0.1 and 1 % (w/w) and compared to purée with no added EDTA. EDTA was received as a fine white powder which was easy to dissolve.

EDTA was added into a single mixed persimmon tissue sample. After adding EDTA, persimmon tissue samples were blended for 20 s using a grinder (CG2B, Breville, Breville Ply. Ltd., Sydney, Australia). Purée was stored in clear wide mouth short jar at 5 °C for 17 hours before viscoelastic property measurement (Section 4.2.5). Purée with no EDTA added was subject to the same process. Experiments were run on duplicate samples.

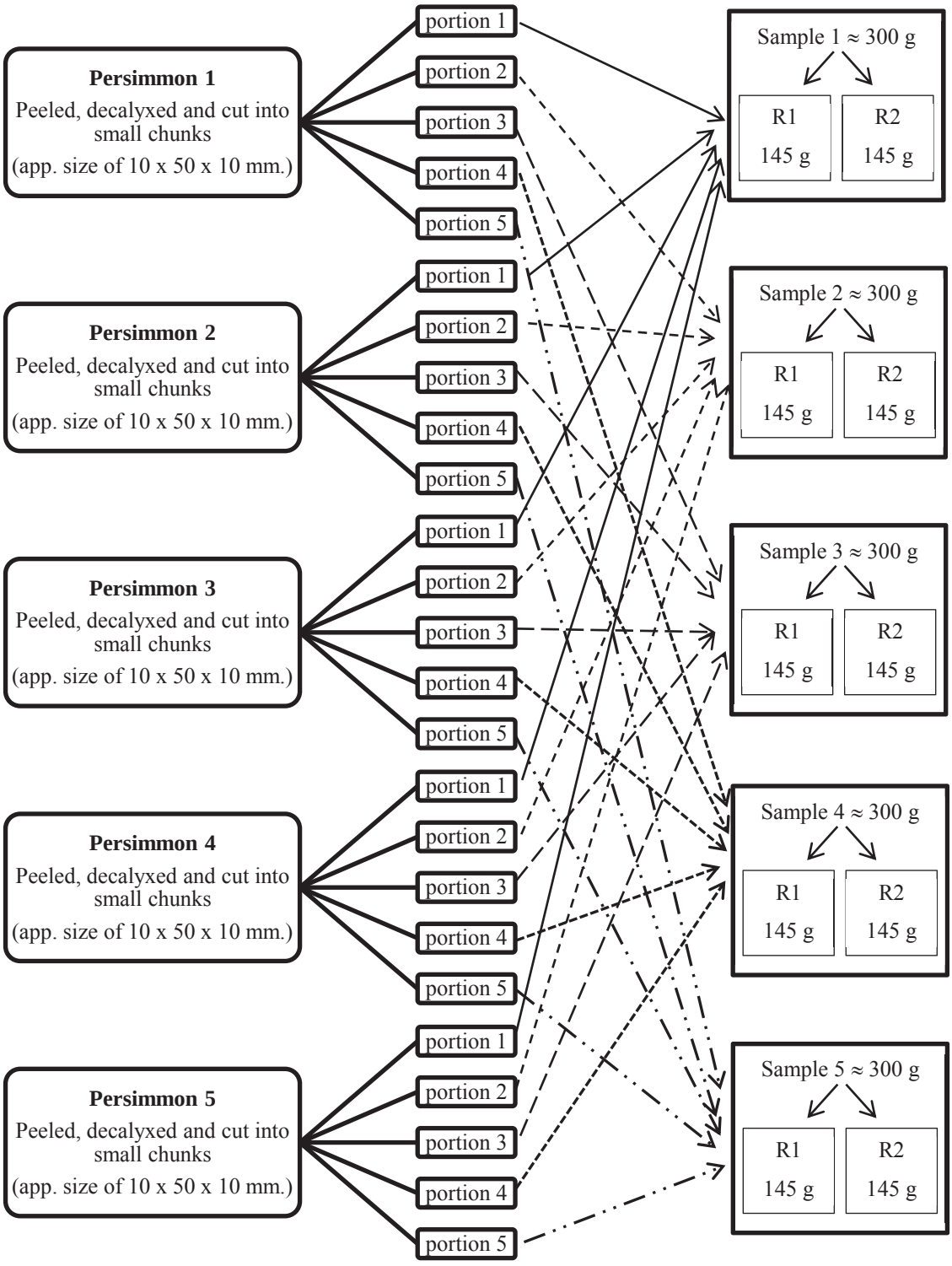


Figure 5.1 Diagrammatic representation of persimmon tissue sample preparation. Five persimmons were prepared into five portions each, and then combined to produce identical purée samples for experiments.

5.2.5 Temperature treatments

Two sets (mature-green and overripe) of persimmon tissue were prepared in duplicates as shown in Figure 5.1. Aluminium foil bags were filled with 145 g persimmon chunks. In addition, each sample bag included a temperature data logger (DS1922L Thermochron iButton, Embedded data systems, Kentucky, USA). Each iButton was placed in the centre of the sample bag and in the middle of persimmon chunks. Soon after installing the data logger, sample bags were vacuum sealed at 250 mbar (C200, Multivac, Sepp Haggenmüller GmbH & Co., Wolfertschwenden, Germany). Temperature treatments of 30, 50, 73 and 96 °C for 30 minutes were applied using a heated water bath (FTE10AE, Techne (Cambridge) Ltd., England). While heating treating samples, a control received no heating and was kept in an ice slurry. After heating, the sample bags and control were cooled down with an ice slurry for 15 minutes. After cooling, the samples were blended for 20 s using a grinder (CG2B, Breville, Breville Ply. Ltd., Sydney, Australia) to create a purée and stored in clear wide mouth short jar at 5 °C for 17 hours before viscoelastic property measurement (Section 4.2.5).

5.2.6 Combination of tissue

Duplicate samples were prepared as shown in Figure 5.1 using mature-green and overripe persimmons. Combining overripe at 0, 25, 50, 75, and 100 % respectively with mature-green persimmon tissue at 100, 75, 50, 25 and 0 % respectively were carried out prior to blending to create 5 treatments. Blending was again conducted for 20 s using a grinder (CG2B, Breville, Breville Ply. Ltd., Sydney, Australia). Purée was stored in clear wide mouth short jar at 5 °C for 17 hours before viscoelastic properties were assessed (Section 4.2.5).

5.2.7 Statistical analysis

All statistical analyses were performed using the Statistical Analysis System (version 9.2, SAS Institute Inc., Cary, NC, USA). Data were analysed by analysis of variance

(ANOVA). Comparison of means was performed by using standard error values (SE) at P = 0.05.

5.3 Results and discussions

5.3.1 Influences of gelling time

Mature persimmon purée was visually observed to develop a gel-like structure rapidly after puréeing (Figure 5.2) which continued to strengthen with time. Syneresis was observed after 15 minutes (Figure 5.2D) and gradually increased with time as expected. At 60 minutes, a strong gel had formed with the base, surrounded by liquid from water expulsion (Figure 5.2H). At 180 minutes, even more liquid around the persimmon gel was evident (Figure 5.2I). This indicates that persimmon purée gel possibly increase in polymeric network crosslinks, becoming more rigid with time resulting with more liquid expulsion. This result demonstrates that purée from mature fruit naturally forms a gel-like structure in low acid pH conditions suggesting it is a low methoxyl pectin.

After 180 minutes, the persimmon purées gelled completely (Figure 5.2I) and it was very firm. This result indicates that more calcium crosslinks were developed over time. The persimmon gelling time was compared with other types of pectin gel. A gelling time of 3.5 minutes was found in calcium induced gel formation of chickpea pectin (Urias-Orona et al., 2011). Gel formed after 8 minutes during cooling in 0.8 % high methoxyl pectin added to 60 % sucrose (Löfgren & Hermansson, 2007). Whilst gel set times at about 120 minutes and 276 minutes were found in high methoxyl (71.1 % DMe) and low methoxyl (29.6 % DMe) pectin respectively (Walkenström et al., 2003). Persimmon gelling time was comparable to other studies.

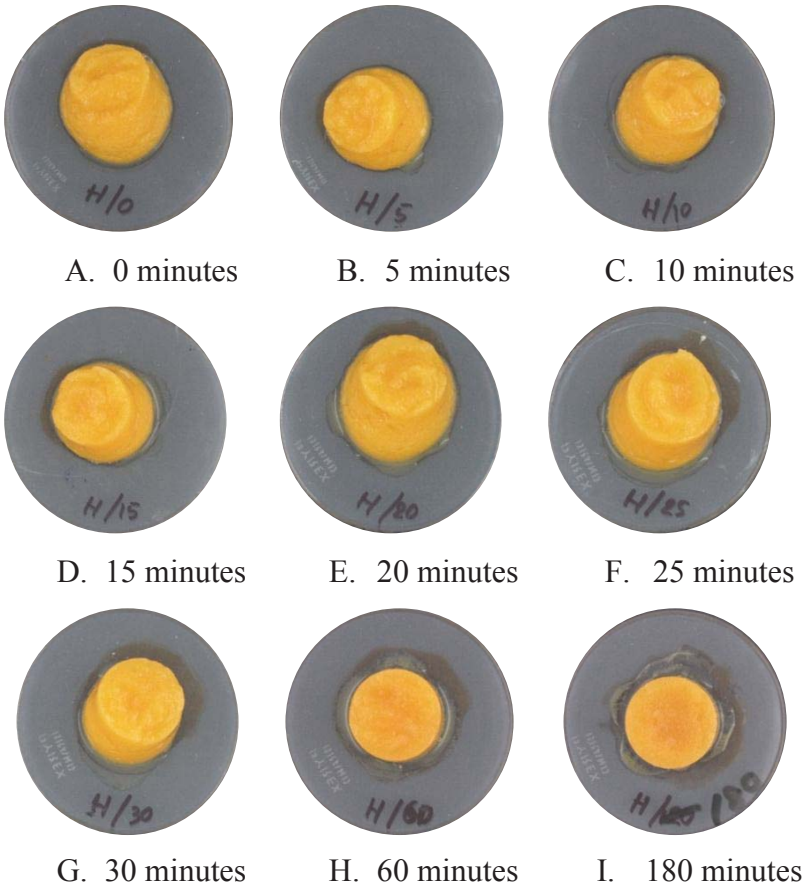


Figure 5.2 Gel formation in persimmon purées made from a mature persimmon (7.5 kg_f) at 20 °C. Representative individual sample photos were taken at identical times 5 s after the tubes were removed.

During this study an undergraduate project by Ong Suan Choo (Wang Xuan Zhu) was supervised by the author. This project conducted water-based extraction and characterization of pectin from mature persimmon. Extracted pectin was re-dispersed by fresh Milli-Q water at 1 % concentration. The pectin solution was continuously stirred at 70 °C for 1.5 hours followed by pouring into a small tube and keeping at 5 °C for 13 hours. This project observed that mature persimmon pectin formed a gel-like structure (Figure 5.3) with water expulsion.

Figure 5.3 Gel-like structure of 1 % water-extracted mature persimmon pectin stored at 5 °C for 13 hours (Choo, 2011).

To conclude, mature persimmon purées formed gels naturally at room temperature after 1 hour while extracted mature persimmon pectin gelled at high temperature.

5.3.2 Influences of maturity on persimmon gel formation

Changes in viscoelastic characteristics of mature, fully-ripe and overripe gels were observed with time sweep tests for 12 hours at 20 °C (Figure 5.4A-D). Gel strength was strongly dependent on fruit maturity with gels from mature fruit being approximately 2.5 times higher than from fully-ripe, approximately 10 times higher that overripe fruit. Overall, the trends of G′, G″ and G* for mature and fully-ripe gels were increased consistently with time. Overripe gel showed a constant G′, G″ and G* with no dynamic increases in gel strength over time.

During time sweep tests, the G′ and G″ of mature gel were higher than fully-ripe and overripe gels. Meanwhile G′ and G″ in overripe gels were flat. The high G′ and G″ found in gels made from less ripe persimmon was expected due to the presence of more intact pectin causing a stronger structure than gel made from more ripe persimmon.

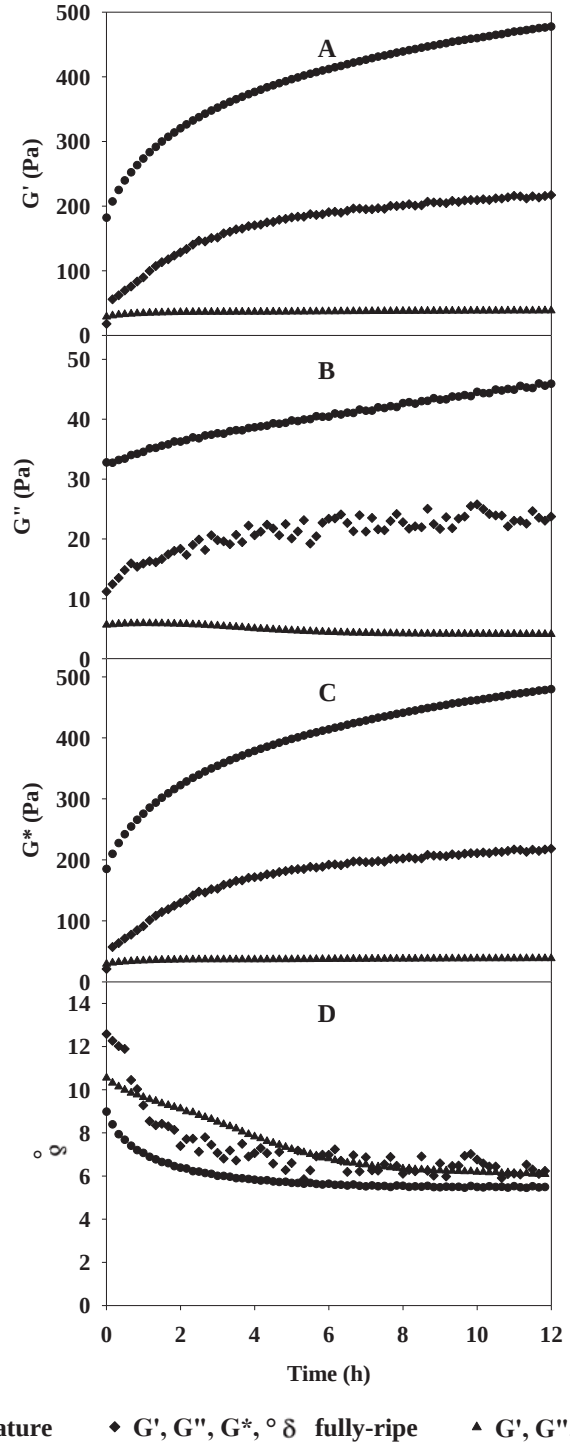


Figure 5.4 Time sweep tests of persimmon gel made from mature (avg. firmness at 7 kg_f), fully-ripe (avg. firmness at 2.5 kg_f) and overripe (avg. firmness at 0.1 kg_f) persimmons. Figure A = storage modulus (G′), B = loss modulus (G″), C = complex modulus (G*) and D = loss tangent (° δ). Experiments were done at 1 % strain with a frequency of 0.16 Hz for 12 hours at 20 °C.

The observed increase of G′ in persimmon gels during time sweep tests were similar to results reported for 'Rojo Brillante' persimmon, in which G′ increased approximately 47 % after 58 minutes from mashing (Tárrega et al., 2012). Likewise, the observed results were similar to results reported for yellow passionfruit rind (Yapo & Koffi, 2006) and extracted pectin gels (Lopes da Silva & Gonçalves, 1994; Löfgren et al., 2005; Löfgren & Hermansson, 2007) and alginate-pectin mixed gel (Walkenström et al., 2003). Figure 5.2 shows that the persimmon gel gradually formed soon after puréeing. Accordingly, persimmon gel strength (G′) increased by 66 % during the first hour of the time sweep test (Figure 5.4A). Results from visual observation and the time sweep tests were in agreement and demonstrated that persimmon gel could form a rigid structure after 1 hour from processing at 20 °C.

G′ of persimmon gel was consistently higher than G″ in all persimmon purées. When G′ is much greater than G″, a material will present an elastic solid property (Rao, 1999b), where the deformations of material under applying force are partially recoverable. This indicates that any persimmon purée forms a gel structure as indicated by G′ > G″ (Ross-Murphy, 1994; Chronakis & Kasapis, 1995). Persimmon gels from every sweep data made from mature-green and fully-ripe persimmons (Figure 5.5B) clearly match the behaviour of a gel as defined by Chronakis et al. (1995) (Figure 5.5A). It should be noted that the magnitude of scales for Figure 5.5A do not affect this definition.

The complex modulus (G*) of all samples were similar to those of G′ (Figure 5.4C). Persimmon gel displayed a drop of loss tangent in gel samples which was expected (Figure 5.4D) indicating formation of a firm gel (increasing G′) structure with time. For a perfect elastic gel and liquid, the loss tangent would be 0 and 90 ° respectively (Chronakis & Kasapis, 1995). The loss tangent of less ripe gel was lower than those in the more ripe gel. This lower loss tangent could suggest a more elastic gel property. The loss tangent of mature and overripe gels gradually decreased whilst an inconsistent decrease was observed for fully-ripe gel. The reduction of loss tangent of mature, fully-ripe and overripe gels were from about 9 to 5.5 °, 12.5 to 6.5 ° and 10.5 to 6 ° respectively.

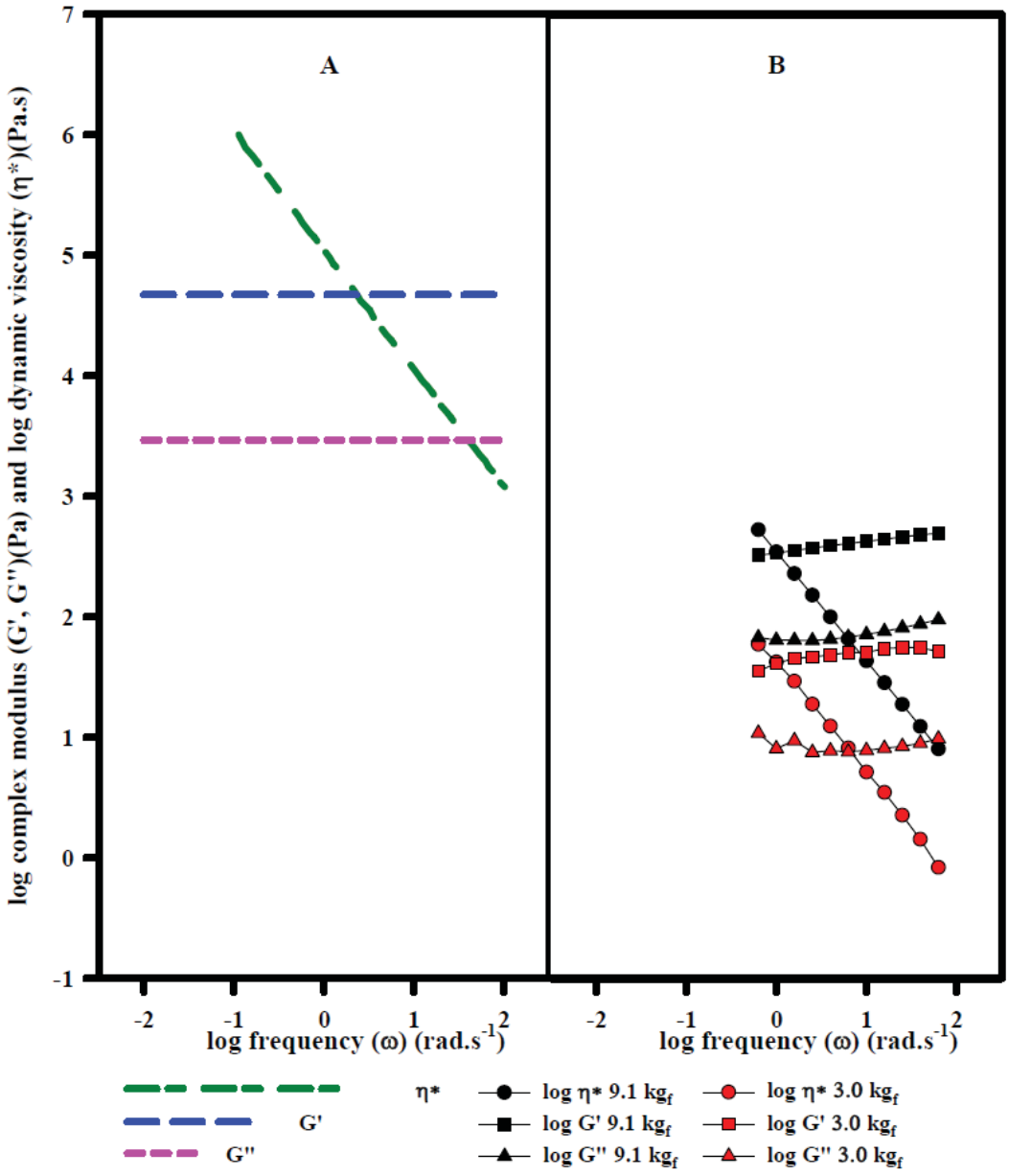


Figure 5.5 Comparison of mechanical spectra; A food thickening gel (adapted from Chronakis, et al. (1995)) and B persimmon gel. Persimmon gels were manufactured by mature-green (avg. firmness at 9.1 kg_f) and fully-ripe (avg. firmness at 3.0 kg_f) persimmons.

These loss tangents were found to be similar to those of vegetable purée-based baby foods (between 5.7 to 17.4 °) and fruit purée-based baby foods (between 8 to 17 °) (Ahmed & Ramaswamy, 2007).

The magnitudes of G′, G″ and G* of fruit purée vary depending on the type of fruit as well as the methodologies used (Table 2.2). Persimmon gel viscoelastic properties in this work were found similar to apple purée (G′ = 600, G″ = 180 Pa) (Espinosa et al., 2011), Jabiticaba pulp (G′ ≅ 700, G″ ≅ 80 Pa) (Sato & Cunha, 2009), apricot purée (G′ ≅ 150, G″ ≅ 50 Pa) and banana purée (G′ ≅ 125, G″ ≅ 50 Pa) (Ahmed & Ramaswamy, 2007). Differences in persimmon gel strength depend on pectin properties as influenced by maturity. Pectin property differences are caused by PME removing methylester groups from pectin chains resulting in methyl free GalA regions (Pilnik & Rombouts, 1981; Fischer & Bennett, 1991). PG is then able to depolymerise polygalacturonic acid, and cause pectin solubilisation (Fischer & Bennett, 1991).

The strong persimmon gel structure is possibly the result of more calcium gel crosslinks between two GalA chains and calcium ions. More methyl free GalA chains results in more calcium gel crosslinks and hence more a rigid gel structure. This also implies that the long pectin chains with many methyl free GalA residues can result in greater chain association and more rigid properties. Textural properties (e.g. brittle, elastic or rubber-like) of polysaccharide gels are influenced by the number and length of junction zones (Bacic et al., 1988). The higher the number and length of calcium crosslinks indicate more rigidity of the gel structure (Ngouémazong et al., 2012).

Apart from pectin characteristics at ripeness influencing gel strength, the total solid content of gel is another factor imparting gel strength. An honours project carried out by Hua (2010) demonstrated the effect of total solid content on gel strength. Typically, ripe persimmon (average firmness 5.5 kg_f) has total soluble solids content of 13.3 % (Section 3.3.4, Table 3.1). This study freeze dried persimmon flesh then grinded into powder and indicated as a natural strength. In order to make a double

strength, a same amount of freeze dried persimmon power was added into natural strength. For half strength, a half of natural strength was removed. This study found that gel strength increased by 25 % in double strength sample but reduced by 7.5 % in half strength sample.

In summary, this study demonstrated that persimmon purée formed a gel which expresses viscoelastic behaviour after puréeing. Water expulsion caused by syneresis was also observed. A more rigid gel was produced from less ripe persimmon.

5.3.3 EDTA chelation effects on persimmon gel characteristics

Previously, 'Fuyu' persimmon was found to contain calcium at 6-7 mg.100 g^{-1} FW (Table 3.2). This data was used to estimate the amount of EDTA to be added into the gel. Calculating the minimum requirement to inhibit gel formation was estimated by using Eq 5.1.

Ca^{2+}_{ripe} = amount of Ca^{2+} in ripe persimmon (7 mg.100 g^{-1} FW)

$M.W._{EDTA}$ = molecular weight of EDTA (372.24 $g.mole^{-1}$)

$A.W._{Ca^{2+}}$ = atomic weight of Ca^{2+} (40.078 $g.mole^{-1}$)

Then EDTA required to chelate Ca^{2+} in ripe persimmon gel was

$$\text{EDTA}\ (\text{g}.100\text{g}^{-1}\ \text{FW}) = \frac{(\text{M.W.}_{\text{EDTA}})\left(Ca^{2+}_{ripe}\right)}{(\text{A.W.}_{\text{Ca}^{2+}})} = \frac{(372.24\ \text{g.mol}^{-1})(0.007\ \text{g})}{(40.078\ \text{g.mol}^{-1})} = 0.065 \quad \textbf{Eq. 5.1}$$

This means to chelate all calcium ions in 100 g of ripe persimmon gel, 0.065 g of EDTA needs to be added. Further calculation found that there were 0.055 g of EDTA required to be added into mature-green and overripe persimmon gel for calcium chelation. Meanwhile, this study was conducted by increasing 10-fold of EDTA concentration which covered all range of required EDTA calculated, with some extra binding capacity to account for other metals. This study observed progressive EDTA effects at higher concentrations.

Overall the trend of G′ and G″ decreased after increasing EDTA concentration and clearly demonstrated inhibition of gel formation (Figure 5.6 and 5.7). Generally, the G′ of gels declined over the entire range of increased concentration of EDTA. This trend followed the expectation that EDTA sequesters calcium ions resulting in reducing gel strength. The reduction of G′ with increasing EDTA addition supports finding of Choo (2011) who found no gel formation after adding 1 M EDTA into 1 % pectin solution. EDTA sequesters calcium cations in purées (Wang & Regenstein, 2009) causing loss of calcium ion affinity as cations are converted to anions (Hart, 1985) as a consequence, formation of gel structure is inhibited.

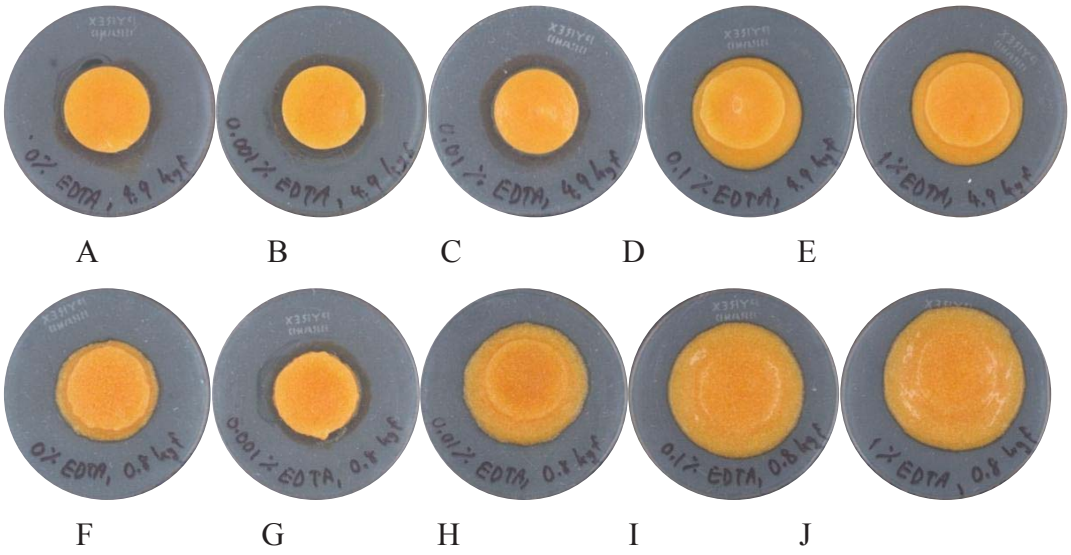


Figure 5.6 Visual observations in changes of persimmon gel structure as influenced by EDTA. Top and bottom rows represent gels made from ripe (avg. firmness at 4.9 kg_f) and overripe (avg. firmness at 0.8 kg_f) persimmons respectively. There were five concentrations of EDTA added; A, F at 0 %, B, G at 0.001 %, C, H at 0.01 %, D, I at 0.1 % and E, J at 1 %. Experiments were done at 20 °C. Pictures were taken after adding EDTA and incubating at 5 °C for 17 hours in 28 mm diameter tubes. Prior samples to being photographed, tubes were taken off.

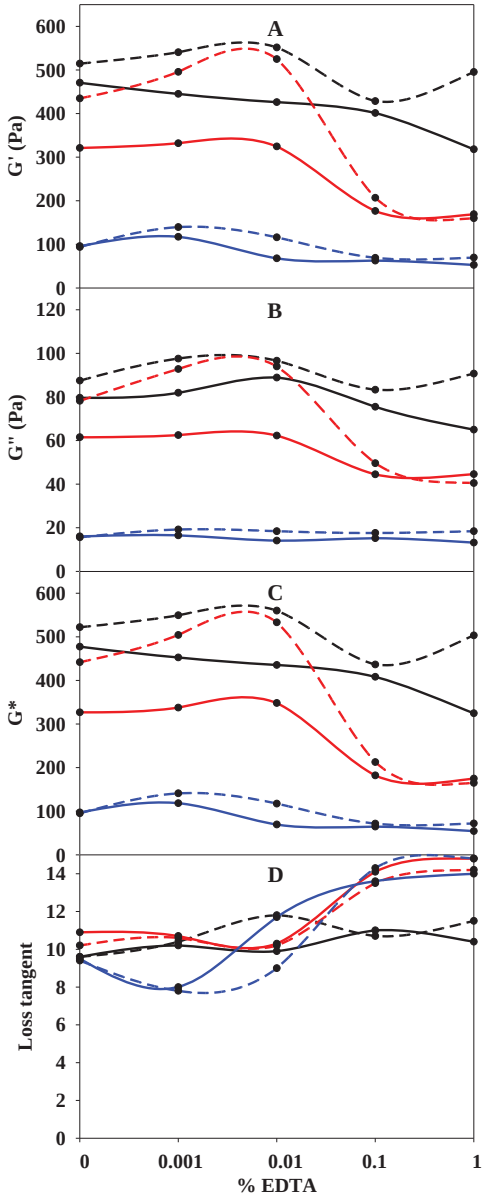


Figure 5.7 Gel viscoelastic characteristics at different fruit maturities as influenced by EDTA concentration. Unit of EDTA are expressed as percentage weight. Data presented in duplicates with each replicate produced from 5 persimmons (see Figure 5.1). Mature-green persimmons had a firmness of 9.8 ± 0.3 (±SE) kg_f (replicate 1, solid-black line) and 9.4 ± 0.1 (±SE) kg_f (replicate 2, broken-black line). Ripe persimmons had a firmness of 4.9 ± 0.2 (±SE) kg_f (replicate 1, solid-red line) and 4.6 ± 0.4 (±SE) kg_f (replicate 2, broken-red line). Overripe persimmons had a firmness of 0.7 ± 0.6 (±SE) kg_f (replicate 1, solid-blue line) and 0.8 ± 0.0 (±SE) kg_f (replicate 2, broken-blue line). G′ (A), G″ (B), G* (C) and loss tangent, °δ (D) were measured at 1 Hz.

This study also observed increasing G′ and G″ of gels at 0.01 % (gels made from mature-green and ripe persimmons) and 0.001 % (gels made from overripe persimmon) (Figure 5.6B, C, G). This result of increased gel strength at low EDTA concentration deviated from expectation. This result could be due to other ions such as magnesium and copper that are easily accessed and form negatively charged complexes. These complexes could possibly result in a stable complex that could potentially increase persimmon gel strength. The trends in G* of all samples were similar to those found for G′ which demonstrated a decline of gel rigidity. Loss tangent was observed to increase at higher concentrations particularly at 0.1 and 1 % EDTA.

Overall, EDTA caused a decline in persimmon gel viscoelastic properties. Increasing loss tangent was accompanied by reducing G′ and G″. This result possibly indicates that the presence of EDTA was sufficient to chelate calcium ions in persimmon gel resulting in loss in gel rigidity.

5.3.4 Influences of heating tissue prior to tissue puréeing on persimmon gel viscoelastic characteristics

A time-temperature record during heating of persimmon tissue at various temperatures is shown in Figure 5.8. The graphs present typical heating curves of the central temperatures of persimmon tissue chunks during heating to 30, 50, 73 and 96 °C for 30 minutes. Figure 5.8 also illustrates PME, PG and β-galactosidase activation and β-elimination temperature ranges as reported by a number of studies. According to previous data, it could be suggested that heating persimmon tissue to 30 and 50 could activate β-galactosidase while at 50 and 73 °C could cause PME and PG activation resulting in elevated PME demethylesterification, and β-galactosidase and PG hydrolysis. On the contrary, heating persimmon tissue to 96 °C could result in β-eliminative depolymerisation while inactivating PME, PG and β-galactosidase.

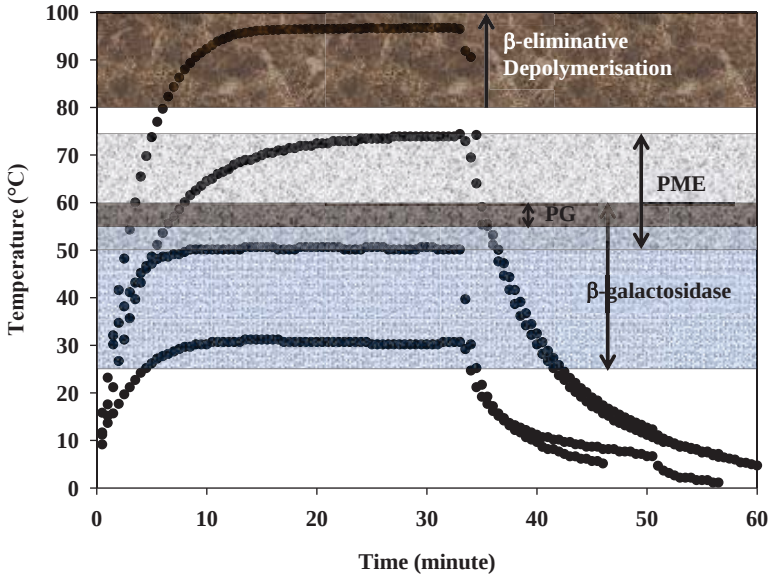


Figure 5.8 Time-temperature history during heating overripe persimmon tissue at 30, 50, 73 and 96 °C for 30 minutes. Previous data demonstrated that β-galactosidase was activated at temperature between 25-60 °C (Jurado et al., 2004), PG was activated at temperature between 55-60 °C (Verlent et al., 2004; Verlent et al., 2006) whilst PME was activated at 50-75 °C (Stoforos et al., 2002; Ni et al., 2005; Anthon & Barrett, 2006; Lemmens et al., 2009; Ümit Ünal & Bellur, 2009; Christiaens et al., 2012a). Meanwhile temperature >80 °C leads to β-eliminative depolymerisation to occur (Van Buren, 1979; Sila et al., 2009).

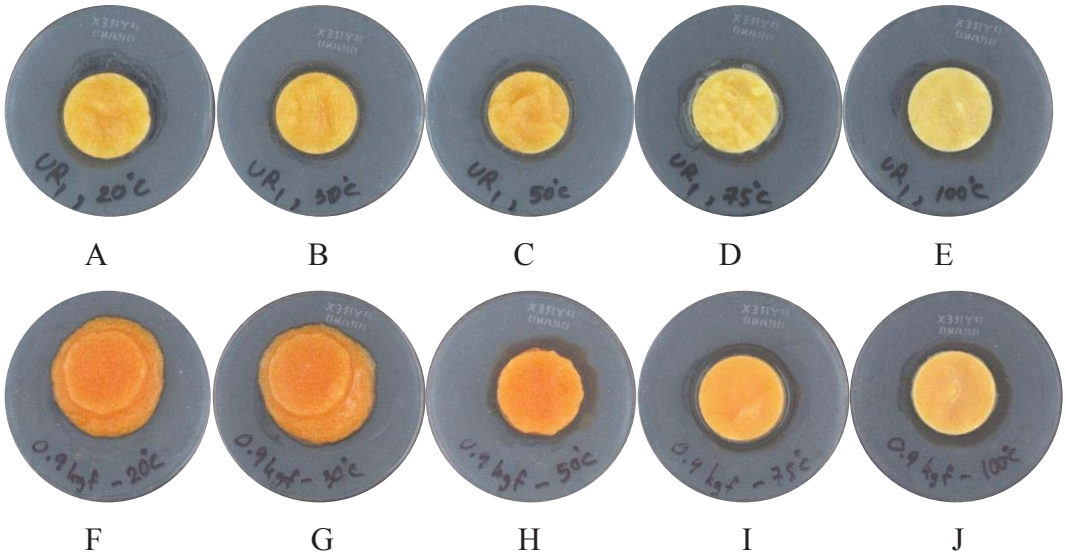


Figure 5.9 Visual observations in changes of persimmon gel structure as influenced by temperatures. Top and bottom rows represent gels made from mature-green (avg. firmness at 9.5 kg_f) and overripe (avg. firmness at 0.7 kg_f) persimmons respectively. There were five different temperatures. A, F at 20 °C; B, G at 30 °C; C, H at 50 °C; D, I at 73 °C; E, J at 96 °C. Experiments were done at 20 °C. Pictures were taken after incubated at 5 °C for 17 hours in 28 mm diameter tubes. Prior samples to being photographed, tubes were taken off.

Heating persimmon tissue at various temperatures prior to tissue puréeing resulted in altering viscoelastic characteristics (Figure 5.9 and 5.10) as expected. Heating tissue to 30, 50, and 73 °C in mature-green tissue resulted in gel formation with water expulsion (Figure 5.9A-D). Meanwhile, heating of overripe tissue at 50, 73 and 96 °C also formed gels (Figure 5.9H-J).

Heating mature-green tissue to 30 and 50 °C prior to puréeing caused a small reduction in G′ of 12 and 15 % respectively when compared to non-heating tissue. Heating to 96 °C caused a decline of approximately 13.5 % in comparison to non-heated tissue (Figure 5.10). Heating mature-green tissue to 73 °C resulted in similar G′ to non-heated tissue (Figure 5.9D). Heating overripe tissue to 50, 73 and 96 °C caused increases in G′ of approximately 43, 52 and 46 % respectively. The results demonstrated gels that completely formed (i.e. round-shape without any spread out purée) when overripe tissue was heated to 50, 73 and 96 °C. Heating overripe tissue to 30 °C increased G′ but did not result in complete gel formation (Figure 5.9G).

A change in G′ after heating to 73 °C was observed by Tárrega et al. (2012) who found gel formation in 'Rojo Brillante' persimmon at temperature treatments above 60 °C. However, a study revealed that soaking persimmon fresh in hot water at 50 and 70 °C for 30 min can inhibit PG activities and softening (Kim & Park, 1988). Additionally, Lee et al., (1979) reported that blanching trimmed carrots at 76.6 °C for 20-30 minutes prior to being canned increased carrot firmness which caused by the effects of PME activated by the low temperature blanch. A similar result was also reported in the firmness of sliced (1 x 2.5-3.75 cm) green bell pepper was improved when preheated at 70 °C for 15 minutes prior to a conventional blanch (Ni et al., 2005). Previous data showed that preheating carrot cylinders (∅ 12 mm x 10 mm thickness) from 60 to 70 °C for 30 to 40 minutes resulted in maintaining carrot texture (Vu et al., 2004).

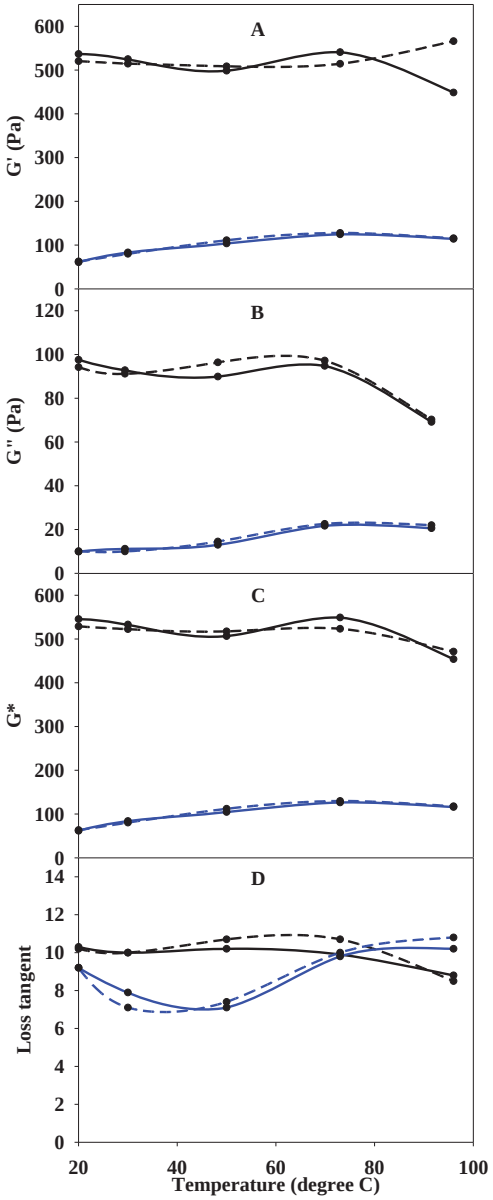


Figure 5.10 Gel viscoelastic characteristics at different fruit maturities as influenced by heating. Data presented in duplicates which each replicate was produced from 5 persimmons (see Figure 5.1). Mature-green persimmons had a firmness of 9.5 ± 0.1 (±SE) kg_f (replicate 1, solid-black line) and 8.6 ± 0.2 (±SE) kg_f (replicate 2, broken-black line). Overripe persimmons had a firmness of 0.7 ± 0.4 (±SE) kg_f (replicate 1, solid-blue line) and 0.9 ± 0.4 (±SE) kg_f (replicate 2, broken-blue line). G′ (A), G″ (B), G* (C) and loss tangent, °δ (D) were measured at 1 Hz.

As expected, firmer gels were observed when made from mature-green and overripe tissues heated at 30, 50 and 73 °C could be possibly due to PME was activation at optimum temperatures. Vanburen (1979) reported PME is activated at temperatures

between 50-75 °C (Figure 5.8). Subsequent puréeing could possibly free PME to demethylesterify resulting in more methyl free GalA residues which allows calcium gel crosslinks, resulting in firmer gels. Additionally, heating to 30, 50 and 73 °C could possible cause PG depolymerisation resulting in short GalA chain (i.e. shorter calcium gel crosslinks), and resulting in reducing G′. PME and PG activities caused by processing were expected to influence the pectin length and DMe.

Sánchez et al., (2002) found that heating tomato paste at 65 °C resulted in extensive degradation of cell wall structure by PME and PG and a subsequent reduction in viscoelastic characteristics due to increasing pectin depolymerisation. Similar results were reported for peach, pear and apple purées (Saravacos, 1970), banana purée (Ditchfield et al., 2004), vegetable purée-based baby foods (Ahmed & Ramaswamy, 2006) and fruit purée based-baby foods (Ahmed & Ramaswamy, 2007). On the contrary, Alvarez et al. (2004) showed no significant heat (between 25-65 °C) effects on G′ of fresh natural potato purée in comparison to frozen potato purée. However, PME and PG activities depend on temperature (Figure 5.8) which could result in differences in viscoelastic change.

The reduction of G′ and G″ in gels made from tissues heated at 96 °C (Figure 5.10) was possibly caused by β-eliminative depolymerisation which favoured by methyl esterified GalA residues and divalent cations (e.g. calcium) (Van Buren, 1979; Sajjaanantakul et al., 1989; Sajjaanantakul et al., 1993; Krall & McFeeters, 1998). Heating mature-green and overripe tissues at 96 °C prior to puréeing caused reduction in G′ of approximately 14 and 6 % respectively. Heating tissues at 96 °C could possible inactivate PME and PG enzymes and increase β-elimination (Figure 5.8). The results observed were similar to results reported by a number of researchers. Studies reported β-eliminative depolymerisation cause softening of broccoli purée (Christiaens et al., 2012a), diced carrot (Sila et al., 2005) and chemical pectin (de Roeck et al., 2009). β-eliminative depolymerisation can possibly result in tissue softening when heating to high temperature (Lee et al., 1979; Manganaris et al., 2005; Diaz et al., 2007) and degradation, particularly of the middle lamella (Edwards, 1999; Nielsen et al., 2004).

To explain the observed results for the heating of tissue prior to puréeing influencing gel viscoelastic characteristics, a conceptual model was constructed (Figure 5.11). Block A presents mature-green pectin chain whereas block B represents overripe pectin. Pectin characteristics (i.e. length and DMe) are indicated using colour bars (i.e. blue and orange bars respectively). Changes in bar length represent changes of pectin characteristic influenced by fruit ripening, and processing (i.e. heating and puréeing). Methyl free GalA residues are presented as a green box in which some are methylated.

Generally, long chain and high DMe pectin is present in mature-green persimmon. The observed results demonstrated that heating mature-green persimmon tissue to 30, 50 and 73 °C prior puréeing tissue resulted in hard gel ($G' \approx 500$-540 Pa). Hard gels produced from heated tissue could result from PME deesterification promoting more methyl free GalA residues for calcium bridge gel formation. Additionally, PME deesterification could cause a reduction in DMe (descending orange bar). Consequently, more methyl free GalA residues result which provides more regions available for calcium gel crosslinks and a hard gel ($G' \approx 500$-540 Pa) was observed. In contrast, heating mature-green persimmon tissue at 96 °C could cause β-eliminative depolymerisation but inactivate PME and PG. As a consequence, the reduction of pectin chain length and DMe would result and demonstrated by softer gel ($G' \approx 450$ Pa).

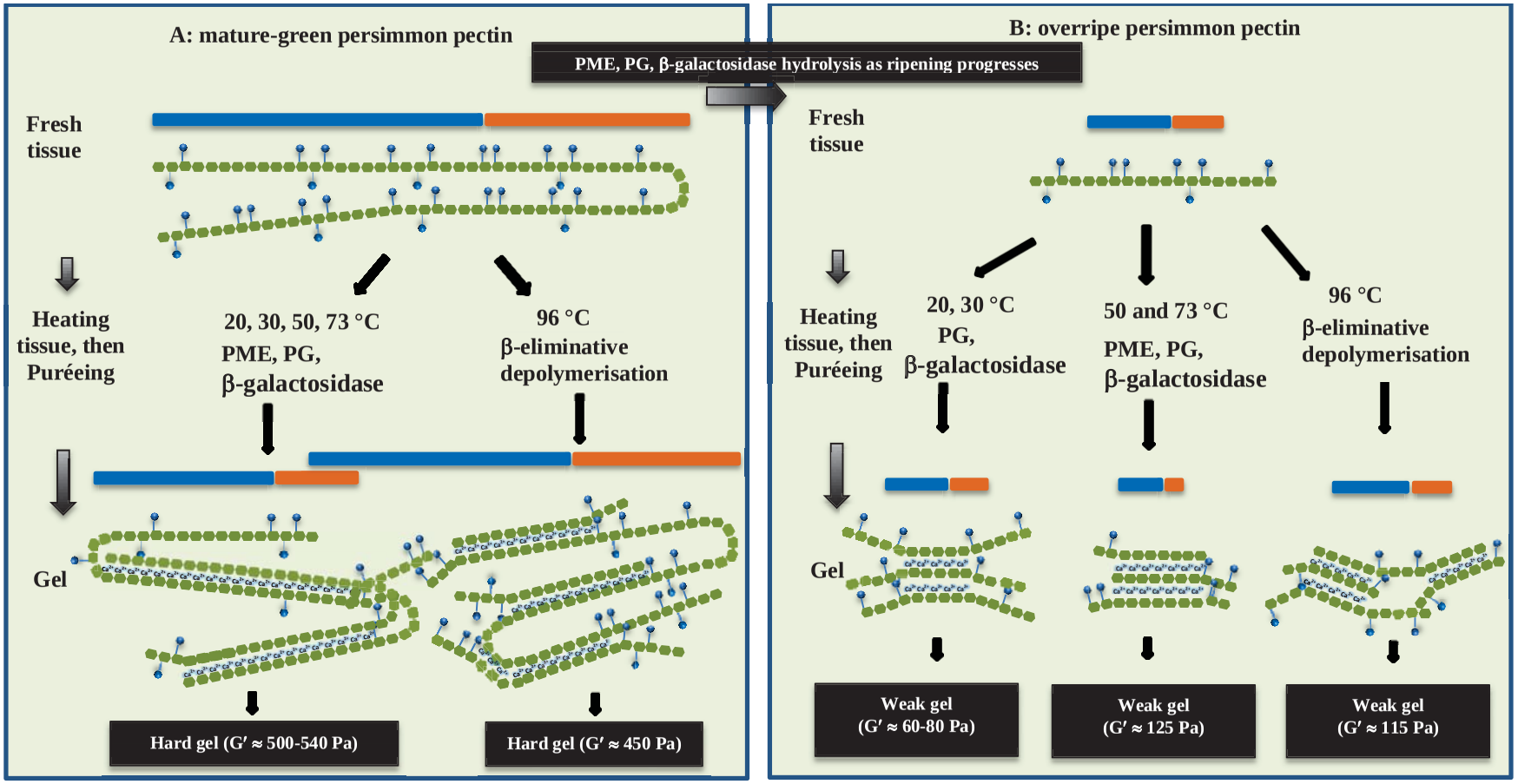


Figure 5.11 Conceptual models to explain observed results of how heat treatment influences gel viscoelastic characteristics.

Model B displays short pectin chains causes by enzymatic hydrolysis during the ripening progress, resulting in heavy reduction of pectin length and DMe (reducing blue and orange bars). Heating overripe persimmon tissue to 30 °C and puréeing could possibly cause minor enzymatic reactions resulting in a small reduction of GalA chain length and DMe. As a consequence, very weak gels (G′ ≈ 60-80 Pa) were observed. Heating to 50 and 73 °C could be the optimum temperatures for PME demethylesterification and PG depolymerisation resulting in short GalA residues and reducing DMe which is favoured by calcium crosslinks. Consequently, harder gel (G′ ≈ 125 Pa) in comparison to 30 °C was observed. Whereas, heating to 96 °C could cause β-eliminative depolymerisation causing shorter pectin chains and DMe reduction. Also, heating to 96 °C is suspected to inhibit all enzymatic reactions. Subsequently, intermediate gel (G′ ≈ 115 Pa) was then demonstrated.

This study also observed water expulsion from mature-green heated tissue (Figure 5.9A-D) and the gel made from overripe heated tissue (Figure 5.9I). Water expulsion was found in non-heated, 30, 50 and 73 °C heated overripe samples. The most water expulsion was observed from gels made when tissue was heated to 73 °C. High water expulsion indicates greater chain association and greater gel strength.

The complex modulus of all gels across the batches showed similar trends to the changes observed for G′ and G″ (Figure 5.10). Loss tangent in heated mature-green gels reduced from about 10 ° to about 8.5 °. The reduction of loss tangent in mature-green gels indicated that heating persimmon tissues resulted in a more rigid structure. Additionally, heating overripe persimmon tissues to 30 and 50 °C prior to tissue being puréed caused a constant reduction in loss tangent by 2 °, which indicates more rigid texture. Changes in loss tangent of heated overripe persimmon gels were similar to the trend observed for heated apple purée based baby foods (Ahmed & Ramaswamy, 2007). Increasing in loss tangent about 1 ° was found from 75 and 96 °C heated overripe samples. Increasing loss tangent was also observed in 96 °C heated mature-green samples. Changes of loss tangent of heated mature-green persimmon gels were similar to the trend of heated banana based baby foods (Ahmed & Ramaswamy, 2007).

Overall, heating persimmon tissues to 73 °C for 30 minutes could possibly activate PME de-esterifying resulting in harder gel in comparison to heating to 30, 50 and 96 °C. Heating to 30, 50 and 73 °C could possibly activate PME deesterification. Heating to very high temperature (96 °C) could cause β-elimination resulting in reducing gel strength and is likely to cause inactivation of PME and PG enzymes.

5.3.5 Tissue combination effects on gel viscoelastic characteristics

Combining overripe tissue with mature-green tissue in different ratios influenced persimmon gel characteristics (Figure 5.12). Water expulsion was observed in all mixes that contained mature-green fruit (Figure 5.13A-D) and had considerably higher G′ and G″ than the gels made from only overripe persimmon tissue. Strength of gel (G′ and G″) increased with increasing overripe tissue ratio up to 50 %, and decreased when adding overripe tissue > 50 %.

Harder gel (higher G′ and G″) in 0:100 (overripe:mature-green) in comparison to 100:0 (overripe:mature-green) was as expected and indicates that mature-green tissue could form a hard gel in comparison to overripe tissue (Figure 5.12). Treatment 25:75 (overripe:mature-green) resulted in a harder gel (increasing G′ and G″) in comparison to treatments 75:25 and 100:0 (overripe:mature-green). Treatment 75:25 (overripe:mature-green) showed similar G′ and G″ to treatment 0:100 (overripe:mature-green). The treatment with 50 % each of mature-green and overripe tissues demonstrated the strongest gel across all treatments. Gels of 50:50 showed high G′ associated with low loss tangent which indicated greater interchain association. High crosslinks could possibly be due to mature-green tissue containing more long blocks of methyl free GalA residues which are favoured by calcium for calcium gel formation. The other possible explanation was that more methyl free GalA exist in overripe tissue, an as a consequence, greater calcium crosslinking may occur. The softer gel (low G′ and G″) in treatment 75:25 (overripe:mature-green) could possibly be due to short pectin chains from overripe tissue resulting in short associated chains.

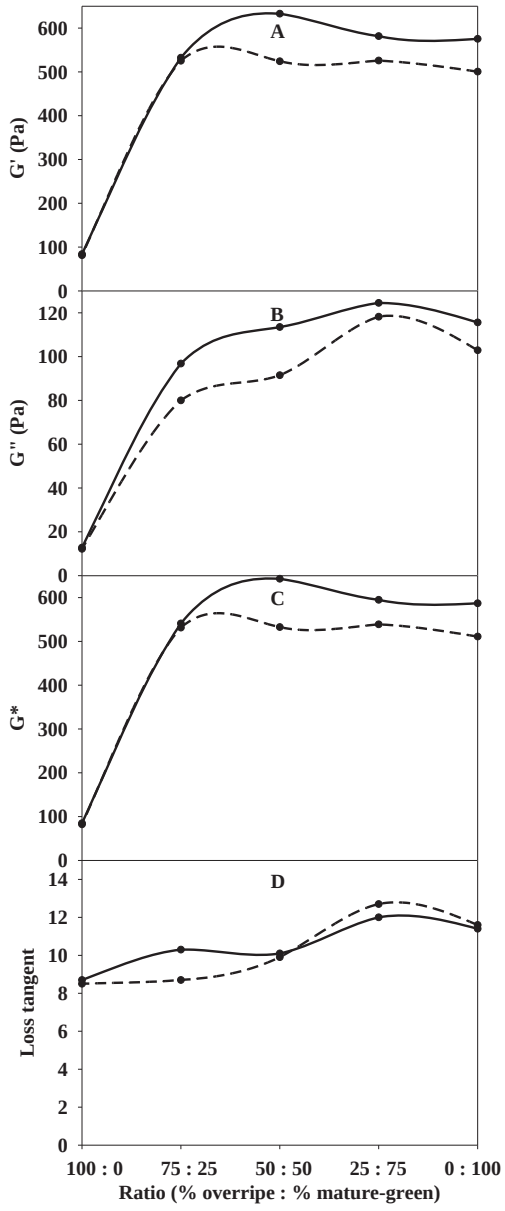


Figure 5.12 Gel viscoelastic characteristics when combining fractions of overripe tissue with mature-green tissue. Data presented in duplicates where each replicate was composed of 5 persimmons (see Figure 5.1). Overripe persimmons at firmness of 0.3 ± 0.1 (±SE) kg_f (replicate 1, solid line) and 0.2 ± 0.0 (±SE) kg_f (replicate 2, broken line). Mature-green persimmons at firmness of 9.8 ± 0.3 (±SE) kg_f (replicate 1, solid line) and 9.0 ± 0.4 (±SE) kg_f (replicate 2, broken line). G′ (A), G″ (B), G* (C) and loss tangent, °δ (D) were measured at 1 Hz.

Figure 5.13 Visual observations of changes in gel formation as influenced by combining tissues. There were five different ratios of combination of overripe tissue with mature-green tissue. The ratios were 0, 25, 50, 75 and 100 %. Experiments were done at 20 °C. Pictures were taken after combining tissues, then puréeing and incubated at 5 °C for 17 hours. Prior samples to being photographed, tubes were taken off.

A conceptual model was developed in order to explain observed results (Figure 5.14). This schematic presents mature-green and overripe pectin chains with different characteristics (i.e. length and DMe). The length and DMe of pectins are displayed by blue and orange bars respectively. Changes in bar length represent changes of pectin length and DMe influenced by fruit ripening and puréeing. GalA residues are demonstrated as green chains which are partially methylated.

This schematic demonstrates gel characteristics as influenced by proportion of mature-green and overripe tissue. Hard gel (G′ ≈ 530-580 Pa) could be created by 100 % mature-green or combining mature-green with overripe tissue. The hard gel at 100 % mature-green could be the result of long blocks of GalA in mature-green which favour calcium crosslinks. Increasing gel strength when combining mature-green with overripe could be due to calcium crosslinks created between the long blocks of GalA in mature-green and a short blocks of GalA in overripe fruit purée.

This model could explain why combining 25 % mature-green tissue with 75 % overripe tissue resulted in hard gel. The strength of this gel was dramatically increased by approximately 85 %. Whilst, a weak gel (G′ ≈ 80 Pa) was observed by overripe gel due to shorter pectin chains.

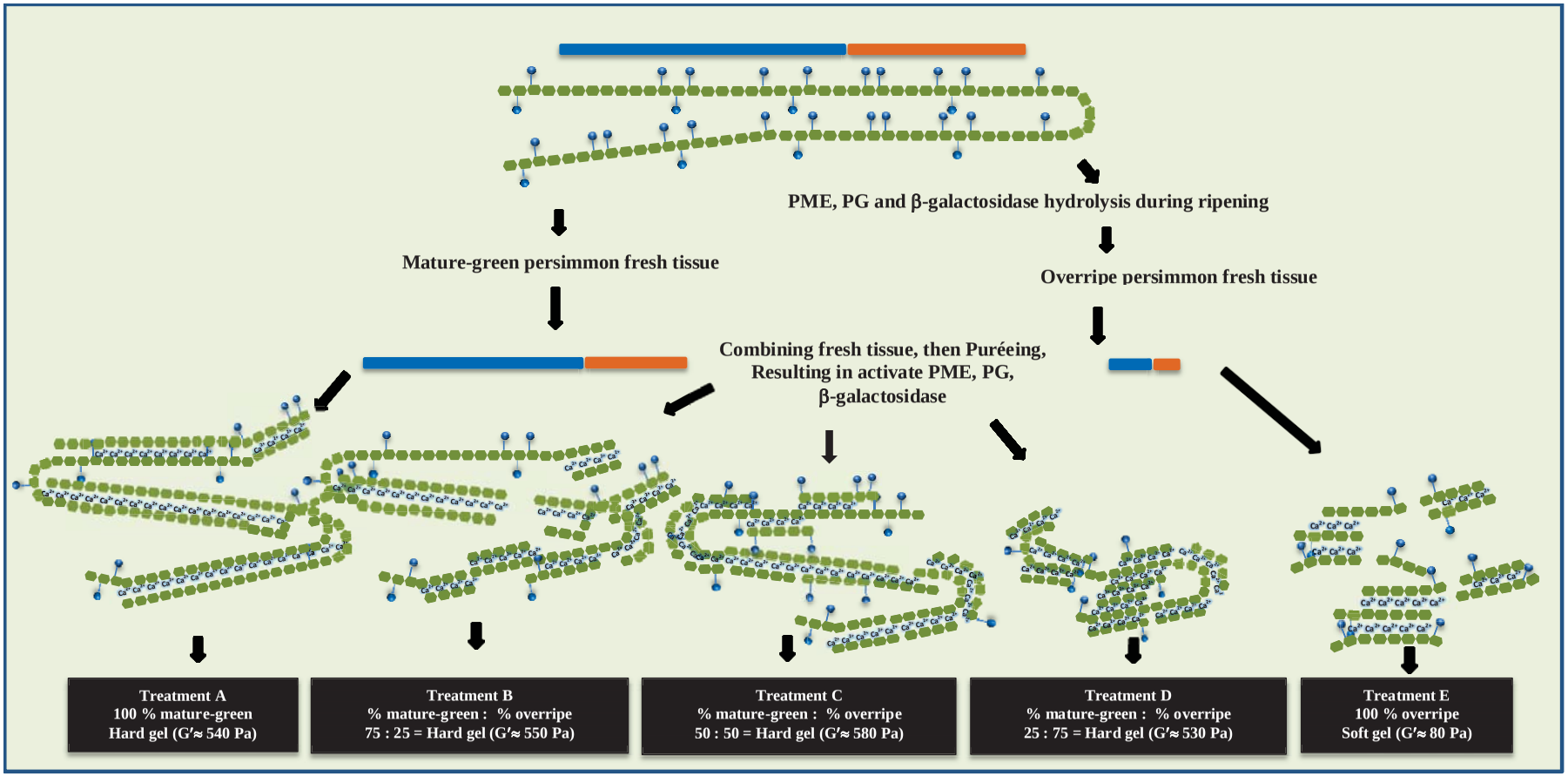


Figure 5.14 Conceptual models to explain observed results of combined tissue on gel viscoelastic characteristics.

To sum up, combining mature-green tissue with overripe tissue at different ratios then puréeing resulted in gels with different strength (G′ and G″). Strength of gel produced from 0:100 (overripe:mature-green) to 75:25 was >>> 100:0. Hard gels (higher G′ and G″) manufactured from 75:25 combination (overripe:mature-green) could have resulted by progressive calcium crosslinks created between the long blocks formed between the long methyl free GalA chains in mature-green and the short methyl free GalA chains in overripe tissues.

5.4 Conclusions

This chapter demonstrated persimmon gel characteristics are influenced by key factors – persimmon maturity and processing. Persimmon purées gelled after puréeing which was clearly observed after 1 h and presented G′ > G″. Persimmon gel exhibited calcium gel behaviour which is suspected to be classified as low methoxyl (LM) pectin that occurs at a pH. Water expulsion caused by syneresis was also observed and gradually increased with time. Strong gel (high G′) could be manufactured from less ripe persimmon whilst weak gels (low G′) could be made from more ripe persimmon.

EDTA chelation and processing (i.e. heating tissue and combining tissues) influenced gel characteristics. EDTA chelation eliminated gel matrix formation effectively resulting in a decline in persimmon gel strength. On the contrary, adding traces of EDTA, slightly strengthened gel structure. Heating tissue to 30 and 50 °C for 30 minutes prior to puréeing demonstrated decreasing gel strength in mature-green gels whereas increasing gel strength was observed by overripe gels. Heating to 73 °C for 30 minutes prior to puréeing resulted in a strong gel (high G′) in all samples. Meanwhile, heating to 96 °C for 30 minutes before puréeing resulted in reducing gel strength. Combining 50 % from mature-green and overripe tissues resulted in increasing gel strength. In contrast, gel strength declined when overripe tissues fractions greater than 50 % were added (treatment D and E). Interestingly, adding 25 % mature-green tissue into 75 % overripe tissue (treatment D), dramatically increased gel strength by approximately 85 %.

The changes to gel properties during heating and puréeing could relate to pectolytic enzyme activity during these processes. Additionally, combining persimmon tissues with different fractions altered gel structure which was possibly influenced by pectolytic enzymes. As a consequence, more study on the influences of pectolytic enzymes, DMe and GalA content of persimmon fruit were focused in the next chapter in order to investigate influences on persimmon gel formation.

CHAPTER 6

Persimmon Gelling Mechanisms

6.1 Introduction

The previous chapter investigated how fruit maturity and processing conditions influenced the gel formation in persimmon purées. The key factor effecting gel strength was fruit ripeness, with large differences observed for purées made from mature and overripe fruit. The gel formation at pH and at low osmolality, is consistent with gelling at low methyl pectin where calcium bridging occurs between adjacent methyl free areas. Mechanisms to explain why different fruit ripeness and processing conditions affected gel strength were proposed (Figure 5.9, 5.11). However further evidence is required to support these ideas.

Plant cell walls are composed of 60 % water and about 40 % solids. This solid portion consists of various polymeric and some minor small molecular weight compounds. About 20-35 % of the cell wall is pectin (Van Buren, 1991; Wang et al., 2002). Cell wall polymers are present in three major forms: cellulose, hemicellulose (xyloglucan and arabinogalactan) and pectin (homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II)) (Pérez et al., 2000; Gilbert, 2010). Fruit cell walls contain more pectin materials and less hemicellulose than other plant cell wall glycoprotein (Waldron, 2004). In the cell wall, HG is the most abundant pectin at about 65 % whilst RG I and RG II make up about 25-35 % and less than 10 % of the cell wall pectin, respectively (Harholt et al., 2010).

HG is composed of a polygalacturonic acid backbone (Mohnen, 2008) which is a linear chain of α-(1-4)-D-galacturonic acids with methyl ester groups (Pilnik & Rombouts, 1981) at the C-6 carboxyl position (Tucker & Grierson, 1987; Harholt et

al., 2010). A HG chain may contain about 100-200 galacturonic acid (GalA) residues (Willats et al., 2001). During fruit ripening, the cell wall undergoes degradation resulting in conversion, modification and degradation of cell wall constituents (Nath et al., 2006). Cutillas-Iturralde et al. (1993) reported that persimmon pectin degraded significantly from 46 % dry weight (DW) in young persimmon to 20 % DW in ripened fruit. Degradation of pectin can result in altering the chemical structure and properties of pectin molecules, thus affecting their gelation mechanisms upon processing due to changes in the amount of GalA.

During ripening, cell wall compositions are degraded by PME and polygalacturonase (PG) (Fischer & Bennett, 1991). The role of PME is to de-esterify specifically methylester groups of polygalacturonic acids on the pectin chain (Pilnik & Rombouts, 1981). The pectin chains containing GalA residues can then be depolymerised by PG (Fischer & Bennett, 1991; Wang et al., 2002). The function of PG during fruit ripening is to hydrolyse α-1,4 linked galacturonan linkages of the pectin structure (Fischer & Bennett, 1991). PG is usually known as an endo-acting enzyme (Valero & Serrano, 2010b) which depolymerises the pectin chain into its soluble form (Tong et al., 2000; Wang et al., 2002; Salvador et al., 2007). The more methylesters removed by PME, the more GalA residues are liberated by endo-PG (Daas et al., 1999). Consequently, pectin chains become short (Fischer & Bennett, 1991), resulting in tissue softening.

β-galactosidase is another enzyme which influences tissue softening in fruit, such as banana (Cheng et al., 2011), papaya (Othman et al., 2011), tomato (Wallner & Walker, 1975; Carrington & Pressey, 1996), mango (Ali et al., 1995), avocado (de Veau et al., 1993) and kiwifruit (Ogawa et al., 1990; Wegrzyn & Macrae, 1992; Ross et al., 1993; Tavarini et al., 2009). β-galactosidase is believed to contribute to the debranching (Ali et al., 1995) of galactose residues which is a constituent of side chains on RG backbone (Willats et al., 2001; Wang et al., 2002). β-galactosidase hydrolysis leads to significant decline in cell wall integrity (de Veau et al., 1993). Pectin lyase and pectate lyase catalyse the nonhydrolytic galacturonic acid which split by β-elimination at glycosidic linkage next to the methyl free GalA (Alkorta et

al., 1998; Muñoz & Barcelo, 2004). Cellulase randomly hydrolyzes the β-1,4-link between adjacent glucose residues and splits non-reducing ends of the polysaccharide chain to produce glucose or cellobiose which is broken down into glucose molecules (Brummell, 2006; Prasanna et al., 2007). Hemicellulase hydrolyses low molecular weight and short chains of exohydrolytic and endohydrolytic to monosaccharides and oligosaccharides that cannot be further split (Wakabayashi, 2000; Spiridon & Popa, 2004).

Pectin is generally classified based on the degree of methylesterification (DMe) (Voragen et al., 1995; deMan, 1999), into high methoxyl pectin (HMP) and low methoxyl pectin (LMP). The DMe refers to the proportion of carboxyl groups de-esterified with methanol (Voragen et al., 1995). When > 50 % of carboxyl group of GalA are methylated, the pectin is considered to be a high methoxyl pectin (HMP) (Damodaran et al., 2008; de Roeck et al., 2009). DMe can be influenced by fruit ripening as pectin methylesterase (PME) esterifies the carboxyl groups of galacturonic acids on the pectin chain (Fischer & Bennett, 1991). During ripening of fruit such as strawberry, date, mango and guava, the DMe decreases significantly (El-Zoghbi, 1994). On the other hand, increasing DMe (≈ 25 %) of apple after 6 weeks of postharvest ripening have been reported (Fischer & Amadò, 1994).

In the presence of divalent cations (e.g. calcium) LMP molecular crosslink with the cation acting as a bridge between pairs of carboxyl groups on pectin adjacent molecules (Bacic et al., 1988; Thakur et al., 1997; Flutto & Caballero, 2003). These crosslinkages are often described with the egg box model (Thakur et al., 1997; Flutto & Caballero, 2003). The number of consecutive carboxyl groups with negative charges required for the calcium crosslink is at least 7 in each of the interacting chains (Thakur et al., 1997). It was reported that a threshold value of approximately 15-20 carboxyl residues would be required for a full strength of calcium crosslink in the egg box model (Kohn, 1975; Liners et al., 1992). At full strength, a few methylated carboxyl residues can be endured within this pectin length (Thakur et al., 1997).

The previous results demonstrated that persimmon gel characteristics are influenced by fruit maturity. Pectin, the key component for calcium gelation is changed by pectolytic enzymes. Therefore, the objectives of this chapter were to evaluate the effects of PME, PG and β-galactosidase on changes in pectins and DMe and to determine their impacts on persimmon gelation.

6.2 Materials and Methods

6.2.1 Materials

Persimmons were removed from cold storage randomly and kept at 20 °C for different periods of time, resulting in persimmon with different firmness. Persimmon flesh firmness was measured (Section 3.2.2) prior to the start of the experiment. First, the peel was removed, and the remaining tissue was cut into small chunks (approximately 10 x 50 x 10 mm). PME, PG, β-galactosidase activities were measured in addition of total GalA and the DMe.

6.2.2 PME activity

PME enzyme was extracted from tissue, and then titrated with 0.1 M NaOH. PME enzyme assay and preparation followed the methods of Awad (1985) and Salvador et al. (2007) with modification. Data were presented as individual measurements.

6.2.2.1 Enzyme preparation

Persimmon tissue samples (5 g) were frozen in liquid nitrogen and ground using a pestle and mortar. A negative control sample was also prepared by firstly denaturing the PME enzyme prior to sample preparation by boiling persimmon tissue for 30 minutes and cooling. Tissue was then mixed with 30 mL of cold extraction solution (1.5 M NaCl, pH 7.5 adjusted with 1 M NaOH) and homogenised at 1350 rpm using a homogenizer (DIAX 600, Heidolph, Schwabach, Germany) for 15 s. The homogenised persimmon tissue was allowed to stand at 4 °C for 1 h to enable desorption of water and then centrifuged at approximately 27000 x g at 4 °C for 10

min using a high speed centrifuge (F21-8 x 50 mL, Fiber lite centrifuge Inc, Califlornia, USA). The supernatant extracted was titrated with 0.1 M NaOH to determine the PME activity.

6.2.2.2 PME assay

A total of 30 mL of 0.5 % (w/v) citrus pectin substrate (Citrus pectin, DMe = 68-72 %, The Cooperative Group Herbstreith & Fox, Neuenbürg/Württ, Germany) was prepared by dissolving in 0.5 M NaCl at 30°C and adjusting to pH 7.5 with 1 M NaOH. This pectin concentration was ensured to be sufficient for enzyme reaction. Then, 2 mL of extracted enzyme solution (supernatant) was added into the pectin substrate. The PME assay was conducted at 30 °C for 15 minutes through continuous titration to maintain a pH 7.5 using 0.1 M NaOH in order to measure the carboxyl group released by PME when hydrolysing the pectin substrate. The PME activity measured was expressed in unit.g^{-1} fresh weight (FW) using Equation 6.1.

$$\text{PME activity (units.g}^{-1}\ \text{FW)} = \frac{(\text{mL}_{\text{NaOH}})(\text{molarity}_{\text{NaOH}})(\text{mL}_{\text{supernatant}})(10^6)}{(\text{Time, min})(\text{mL}_{\text{sample}})(\text{sample fresh weight, g})(10^3)} \qquad \textbf{Eq. 6.1}$$

The unit definition of PME activity was expressed as the amount of μmol of carboxyl groups released per minute at pH 7.5 and 30 °C.

6.2.3 PG activity

PG enzyme assay was carried out using the methods of Luh & Daoud (1971) and Srichantra (2002). PG activity was measured spectrophotometrically at 575 nm by measuring the amount of reducing sugar end-groups (D-galacturonic acid) released from a chain of polygalacturonic acid during hydrolysis. The determination of total reducing sugar level was estimated using dinitrosalicylic acid (DNS) reagent as a colouring agent. Data were presented as individual measurements.

6.2.3.1 PG standard curve

A standard curve was created with aqueous standard solutions of purified D-galacturonic acid (Sodium Polypactate, water soluble, Sigma Ltd.) at 6 concentrations ranging between 0.1 and 1.0 $mg.mL^{-1}$ in distilled water. A 1 mL sample of each of the standard solutions was placed in a glass test tube and mixed with 3 mL of DNS reagent. DNS reagent was prepared by dissolving 10 g of NaOH, 182 g potassium sodium tartrate (Rochelle salt), 10 g of dinitrosalicylic acid, 2 g of phenol and 0.5 g sodium sulphite in 600 mL distilled water. This mixture was transferred to a 1 L volumetric flask, and made up to 1 L with distilled water. Test tubes containing standard solutions were placed in a boiling water bath for 15 minutes and cooled down in cold water for 20 minutes. The absorbance of samples was measured using UV/VIS spectrophotometer (UV-Visible Recoding Spectrophotometer, UV-160A, SHIMADZU, Kyoto, Japan) at 575 nm against a blank which was prepared by using 3 mL of distilled water. A standard line was created by plotting the concentration of D-galacturonic acid versus absorbance given from different concentrations. The linear regression equation ($R^2 = 0.996$) obtained from this standard line was used to calculate the PG activity of persimmon samples. In this experiment, a standard line was newly prepared for each batch of samples.

6.2.3.2 PG assay

Persimmon chunks (Section 6.2.1) were blended using a grinder (CG2B, Breville Ply. Ltd., Sydney, Australia). Purée samples (2.5 g) were added into 100 mL of 1 % polygalacturonic acid solution at 30 °C. The polygalacturonic acid was prepared with 250 mL 1 % polygalacturonic acid (Sodium Polypactate, water soluble, Sigma Ltd.) at pH 4.5, 100 mL 0.5 M acetate buffer at pH 4.5, and 150 mL distilled water in a 500 mL volumetric flask. The sample mixture was incubated in a shaking water bath (Shak-R-Bath, Lab-Line Instruments, Inc., Illinois, USA) at 30 °C for 15 minutes. Then, 1 mL of incubated sample mixture was withdrawn at 0, 20, 40, 60 and 120 minutes and heat-treated by immersing the sample, which was in the 15 mL test tube, in boiling water for 2 minutes to stop the enzymatic reaction. Afterwards, samples were diluted 10-fold using distilled water and mixed with 3 mL of DNS

reagent. The solution was then boiled in a water bath for 15 minutes followed by cooling for 20 minutes at room temperature. The amount of D-galacturonic acid hydrolysed was determined by measuring absorbance at 575 nm using the UV/VIS spectrophotometer. The standard curve equation described in section 6.2.3.1 was used to calculate the amount of D-galacturonic acid. A negative control sample was also prepared by adding PG enzyme, which had been previously inactivated by boiling tissue for 5 minutes, and cooled down by tap water for 5 minutes. The PG activity was calculated using Equation 6.2.

$$\text{PG activity (units.g}^{-1}\text{ FW)} = \frac{[\text{D} - \text{galacturonic acid}](\text{df})(1000)}{(\text{MW}_{\text{D-galacturonic acid}})(\text{sample weight, g})} \qquad \textbf{Eq. 6. 2}$$

where D-galacturonic acid = D-galacturonic acid content ($mg.mL^{-1}$),
df = dilution factor 10,
conversion factor = 1000
$MW_{D\text{-galacturonic acid}}$ = molecular weight (194.139 $g.mol^{-1}$),
sample weight = 2.5 g

The definition of PG activity unit is 1.0 mM of released reducing sugar measured as D-galacturonic acid from polygalacturonic acid per minute at pH 4.5 at 30 °C.

6.2.4 β-galactosidase

This study was carried out to investigate the β-galactosidase activity present in persimmon at different maturities using a commercial standard method reported by Anon. (2001). Data were presented as individual measurement points.

6.2.4.1 Enzyme preparation

Flesh persimmon chunks (164 g, Section 6.2.1) were homogenized in 492 mL of a 10 mM sodium phosphate buffer (pH 7.2) containing 50 mM NaCl (Nakamura et al., 2003). The homogenate was centrifuged at 8000 x g at 4 °C for 40 minutes. The supernatant was collected and used for the enzyme assay. A negative control sample

was created by inactivating the enzyme by boiling for 30 minutes, and cooling with water for 15 minutes.

6.2.4.2 β-galactosidase assay

The assay mixture consisted of 0.7 mL of 20 mM phosphate-citrate buffer (pH 4.5) and 0.75 mL of 10 mM o-nitrophenyl β-D-galactopyranoside. The mixture was mixed by vortex, and then equilibrated at 30 °C for 10 minutes. A 0.3 mL sample of supernatant (from Section 6.2.4.1) was added and incubated at 30 °C for 10 minutes. To stop the reaction, 3 mL of 200 mM borate buffer (pH 9.8) at 30 °C was added to the reaction mixture. A blank sample of 0.3 mL of enzyme solution with no added β-D-galactopyranoside was also prepared under the same conditions. The o-nitrophenol formed was measured for absorbance at 410 nm using a UV/VIS spectrophotometer (UV-Visible Recoding Spectrophotometer, UV-160A, SHIMADZU, Kyoto, Japan). The activity was calculated using Equation 6.3 (Anon., 2001).

$$\beta - \text{galactosidase activity (units.g}^{-1}\ \text{FW)} = \frac{(A_{410nm})(ml_{assay})(ml_{supernatant}) * 1000}{(\text{Time, min})(4.6)(ml_{sample})(\text{sample fresh weight, g})} \quad \textbf{Eq. 6.3}$$

where A = absorbance and 4.6 = millimolar extinction coefficient of o-nitrophenol ($m.M^{-1}.cm^{-1}$) at 410 nm. The β-galactosidase activity was expressed as units per g of sample fresh weight. Enzyme unit is defined as 1.0 mol of o-nitrophenol β-D-galactoside hydrolysed to o-nitrophenol and D-galactoside per minute at pH 4.5 at 30 °C (Anon., 2001).

6.2.5 Total galacturonic acid (GalA) analysis

The amount of GalA in persimmon was determined according to the method of McFeeters & Armstrong (1984). Data were presented as individual measurement points.

6.2.5.1 Cell wall preparation

The extraction of the cell walls from persimmon was carried out based on the method of Ahmed & Labavitch (1978) with some modifications. Fruit were de-calyxed, peeled and deseeded and then chopped and blended for 20 s using a grinder (CG2B, Breville Ply. Ltd., Sydney, Australia). Pulp samples (10 g) were added to 20 mL aliquots of chilled distilled water and vortexed, followed by centrifugation at 23000 x g using a high speed centrifuge (F21-8 x 50 mL, Fiber lite centrifuge Inc, Califlornia, USA) for 10 minutes at 4 °C. The supernatant was discarded and the pallet washed with 20 mL of chilled distilled water, followed by 20 mL of chilled chloroform-methanol (1:1 v/v), and 20 mL of chilled acetone. Between washing, the mixture was centrifuged at 23000 x g using high speed centrifuge (F21-8 x 50 mL, Fiber lite centrifuge Inc, Califlornia, USA.) for 10 minutes at 4 °C and the supernatant was discarded. After all washing steps, the pellet was collected and dried for about 2 hours under a stream of nitrogen gas in a fume hood. The cell wall material prepared was kept at -18 °C with silica gel until used.

6.2.5.2 Total GalA determination

The total content of GalA was determined using the method of McFeeters & Armstrong (1984). A 3 mg sample of persimmon cell wall prepared as described in section 6.2.5.1 was placed in a 2 mL Expender tube and added with 750 µL of 95 % ethanol. The cell wall was sonicated for 3 minutes using a sonicator (Soniclean 160 HT, Soniclean Pty. Ltd. South Australia, Australia). Then, 1500 µL of chilled 72 % sulphuric acid (H_2SO_4) (prepared from concentrate H_2SO_4 (18.4 M) with distilled water) was added to the cell wall samples then mixed and chilled by putting the sample tubes in an ice bath to prevent charring of the cell wall which can result in a reduction of GalA.

The cell wall sample tubes were sonicated for 3 minutes, heated to 50 °C for 10 minutes, and then cooled down in an ice-bath. A 500 µL aliquot of the cell wall sample was taken and diluted with 2.5 mL of cold water. Then, 1.0 mL of chilled (5 °C) 6 M NaOH was added to 1.0 mL of the diluted sample to make an alkaline

condition. A 125 µL sample of diluted solution was mixed with 125 µL of 2 % NaCl and 2 mL of cold concentrated H_2SO_4 and kept in an ice bath. The mixture was then heated to 70 °C for 10 minutes. After cooling down under tap water, 100 µL of 3,5-dimethylphenol (1%) in glacial acetic acid was added and mixed into samples as a colouring agent. Samples were measured for absorbance at 450 and 400 nm after 15 minutes at room temperature. The content of GalA was expressed as the absorbance difference between these two wavelengths which was multiplied by a factor of 1.12 (Scott, 1979).

Commercial citrus pectin (68-72 % DMe, Herbstreith & Fox Corporate Group) was used as standard sample in order to validate the methodology for GalA measurement. The standard curve was created by using D-galacturonic acid ranging from 0 to 125 nmol. Citrus pectin sample was also prepared by dissolving 3.6 mg of pectin in 1 mL distilled water. A 1 mL sample of citrus pectin solution was then mixed with 3.5 mL of chilled (5 °C) concentrated H_2SO_4, chilled in an ice bath and then heated at 50 °C for 10 minutes followed by cooling down. Then, the steps as described above for cell wall samples were followed.

6.2.6 Degree of methylesterification (DMe) of unheated and heated persimmon tissues

The degree of methylesterification (DMe) of pectin was determined by measuring the content of methanol after saponification of pectin from persimmon cell walls. The method of McFeeters & Armstrong (1984) was used with some modifications (O'Donoghue, E. personal communication). Data were presented as individual measurements.

6.2.6.1 Cell wall saponification

Unheated and heated persimmon tissues were prepared by following the same methodology. Heated tissue samples were collected as described in section 5.2.5. A 3 mg sample of persimmon cell wall prepared as described in section 6.2.5.1 was placed in a tube and mixed with 25 µL of buffer (50 mM citric acid pH 5.0

containing 1 M NaCl). Then, 125 µL of chilled (5 °C) distilled water and 20 µL of 1 M NaOH were added into the tube and the sample was left overnight at 4 °C for saponification before measuring the methanol content.

6.2.6.2 Analysis of released methanol content

The analysis of methanol content was carried out using gas-liquid chromatography (GLC) with a BP20 column (SEG, 10 m), with a helium flow at 10.34 kPa with injection port at 240 °C, oven at 80 °C and FID at 240 °C. Prior to analysis, 30 µL of 82.5 mM citric acid solution was added into the sample to increase the pH to approximately 7. Then, 33.3 µL of sample of 25 mM *n*-propanol was added as an internal standard. The samples (1-5 µL) were withdrawn carefully using a syringe without taking suspended cell walls and injected into the GLC.

The content of methanol in samples was calculated from a standard curve created. Then, 33.3 µL of sample of 25 mM MeOH standard was mixed with 25 µL of buffer (50 mM citric acid, 1 M NaCl, pH 5.0) followed by 191.7 µL of distilled water and 20 µL of 1 M NaOH. Later, 30 µL of sample of 82.5 mM citric acid solution was added and different volumes of the standard solution of methanol were injected into the GLC and analysed under the same conditions.

The DMe was calculated on a percentage basis using Equation 6.4 which is based on the proportion of moles of methanol to moles of total GalA in a sample.

$$\% \text{ DMe} = \frac{\text{moles of methanol}}{\text{moles of GalA}} \text{ x } 100 \quad \textbf{Eq. 6. 4}$$

6.3 Results

The PME activity of persimmons varied between 3.5 and 7.5 units.g^{-1} FW (Figure 6.1) depending on maturity. The overall trend showed a peak of PME activity in ripe fruit. This result is similar to Salvador et al. (2007) who reported high PME activity (8.9 µeq-g.min^{-1}.g^{-1}) during 'Rojo Brillante' persimmon ripening (firmness

approximately 5-6 kg_f). Similarly, Awad (1985) demonstrated the highest PME activity at the ripe stage of 'Taubate' persimmon at about 8.5 $\mu eq.min^{-1}.g^{-1}$ FW. Low PME in firm persimmon was similar to results reported by Paull (1983) who found low PME in preclimateric mature-green papaya fruit.

PG activity was found to be between 0.2 and 1.6 $unit.g^{-1}$ FW (Figure 6.1). A relatively low level of PG activity was observed in persimmon purée at all maturities. Previously, Nakamura et al. (2003) reported similar changes in PG activities between 0.1 and 0.5 $unit.g^{-1}$ FW during 'Saijyo' persimmon ripening. Salvador et al. (2007) also found no significant changes of PG activity at different maturity stages in deastringent 'Rojo Brillante' persimmons.

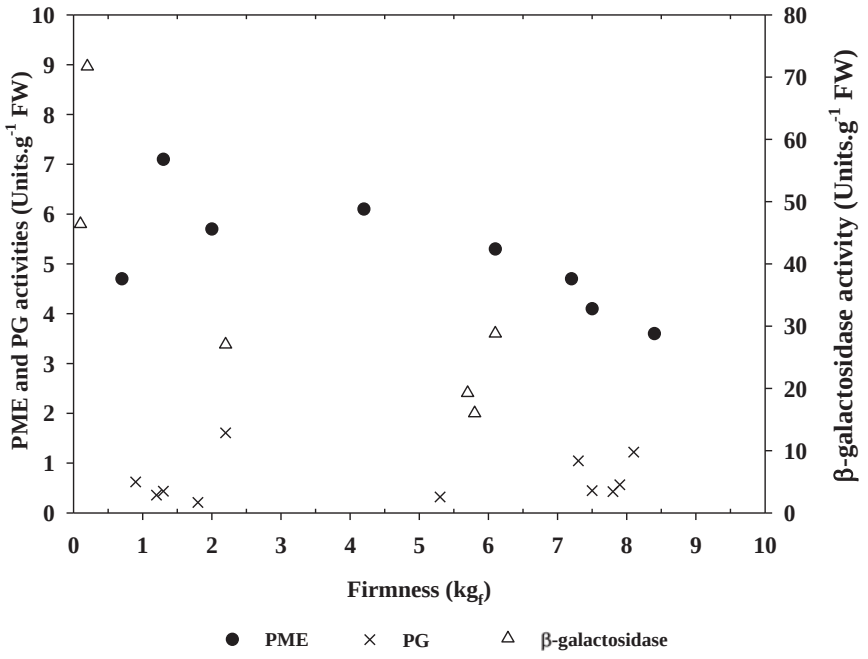


Figure 6.1 PME, PG and β-galactosidase activities of persimmon fruit at different stages of maturity. Persimmon maturities were defined as mature-green (> 9 kg_f), mature (7-9 kg_f), ripe (4-7 kg_f), fully-ripe (2-4 kg_f) and overripe (< 2 kg_f). Data points are single measurements.

β-galactosidase activity was observed to increase as persimmon softens (Figure 6.1). It was observed that ripe persimmon (firmness 4-7 kg_f) and fully-ripe persimmon (firmness 2-4 kg_f) persimmons showed approximately 2.5 times lower β-galactosidase activity in comparison to overripe persimmon (firmness 0.1-2 kg_f). β-

galactosidase activity during 'Saijyo' persimmon ripening were reported by Nakamura et al. (2003) which was much lower than for 'Fuyu' persimmon. The maximum activity in 'Saijyo' was reported at 0.8 unit.g^{-1} FW (firmness 1.8 kg_f) and reduced to 0.5 unit.g^{-1} FW (firmness 0.1 kg_f). Whereas β-galactosidase activity in 'Fuyu' persimmon were 27 unit.g^{-1} FW (firmness 2.2 kg_f) and increased to 72 unit.g^{-1} FW (firmness 0.2 kg_f) (Figure 6.1). The high β-galactosidase activity result was similar to studies reported for banana (Cheng et al., 2011), papaya (Othman et al., 2011), tomato (Wallner & Walker, 1975; Carrington & Pressey, 1996), mango (Ali et al., 1995), avocado (de Veau et al., 1993) and kiwifruit (Ogawa et al., 1990; Wegrzyn & Macrae, 1992; Ross et al., 1993; Tavarini et al., 2009).

A decrease in the total GalA content was observed as persimmon matured (Figure 6.2). Some fluctuation in GalA content during ripening was observed (Figure 6.2). Mature-green persimmon retained the highest total GalA at 43.5 mg.g^{-1} DW. The total GalA content reduced gradually during ripening to approximately 25 % of the mature-green content.

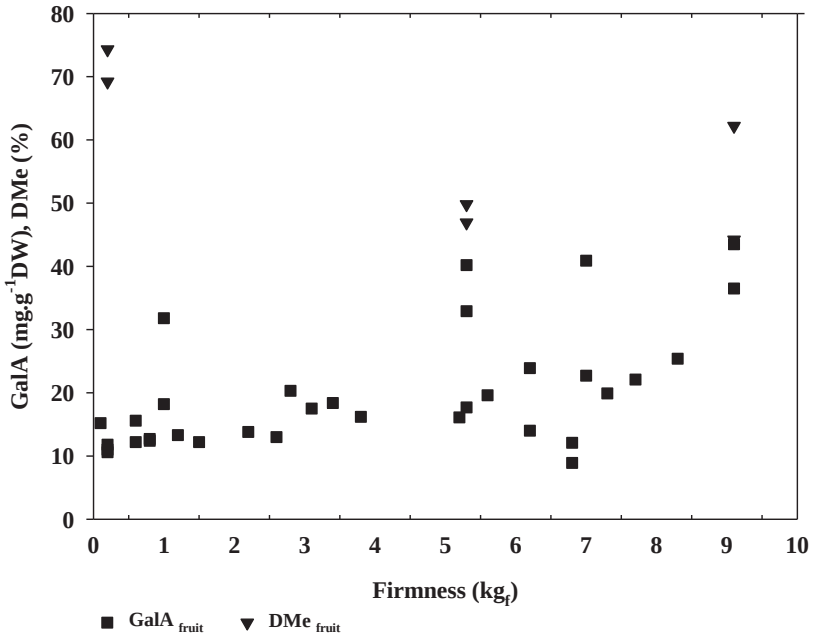


Figure 6.2 Changes in persimmon GalA content and %DMe. Data points are single measurements. Persimmon maturities were defined as mature-green (> 9 kg_f), mature (7-9 kg_f), ripe (4-7 kg_f), fully-ripe (2-4 kg_f) and overripe (< 2 kg_f).

The observed magnitude of the reduction of total GalA during ripening was comparable with Cutillas-Iturralde et al. (1993). In a previous report of 'Fuyu' softening, the water-insoluble polyuronide decreased (Megumi et al., 2002) due to the extensive solubilization and depolymerization by PME and PG (Wakabayashi, 2000) respectively. Subsequently, the total GalA content declined. However, it should be noted here that there were differences of methodologies used to determine the total GalA content which could possible influence the magnitude.

Total GalA content of tissue heated to 73 °C for 30 minutes prior to tissue puréeing increased in both unripe (firmness 9.1 kg_f) and overripe (firmness 0.2 kg_f) persimmon in comparison to unheated tissue (Figure 6.3). Increase in total GalA content of heated tissue were similar to increasing water insoluble pectin of heated carrot purée (Christiaens et al., 2012a).

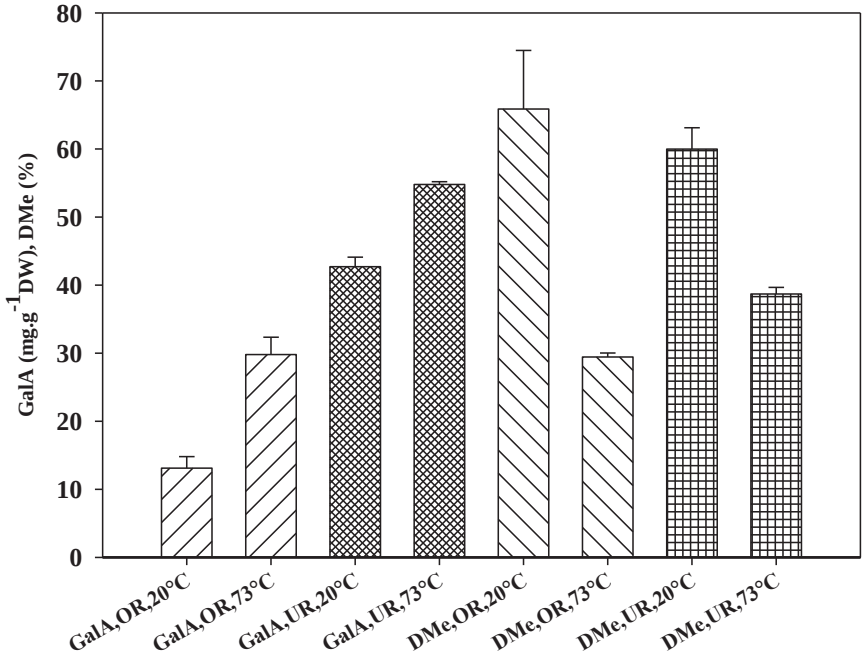


Figure 6.3 Effects of heating tissue to 73 °C prior tissue to puréeing on GalA content and DMe. Experiments were done using unripe persimmons (UR, firmness 9.1 kg_f) and overripe persimmons (OR, firmness 0.2 kg_f). Bar represents duplicate observed samples with ±SE.

DMe was found to be at an average of 53 % in persimmon with a firmness > 9 kg_f (Figure 6.2). DMe showed a reduction from mature-green to ripe stage with a later

increase at the overripe stage to around 72 %. The increasing DMe in overripe persimmon was similar to increasing DMe in overripe peach (Brummell et al., 2004). Meanwhile, a decrease in the DMe during fruit ripening, such as mango, guava, date and strawberry have previously been found (El-Zoghbi, 1994). In contrast, inconsistent increases and decreases of DMe at 73-120 % were reported during Golden Delicious apple ripening (Fischer & Amadò, 1994).

An observed increase in G′ was observed in tissue heated to 73 °C prior to tissue puréeing (Section 5.3.4). In order to clarify PME activity during heating to 73 °C, the DMe of gels made from tissue heated to 73 °C prior to tissue puréeing was evaluated. It was found that heating to 73 °C prior to tissue puréeing caused lower DMe in gels made from mature-green (firmness 9.1 kg_f, approximately 35 %) and overripe (firmness 0.2 kg_f, approximately 55%) fruit (Figure 6.3). Reducing DMe after heat treatment was similar to reports of a decline of DMe in heated carrot discs (Lemmens et al., 2009), of blanched carrot prior to tissue puréeing (Christiaens et al., 2012a), of heated broccoli stem (Christiaens et al., 2011), and of heated strawberry (Fraeye et al., 2009).

6.4 Discussion

In order to discuss the observed changes of PME, PG, β-galactosidase, GalA content and DMe in relation to persimmon gelation and mechanisms, Figure 6.4 was created to generalise all the observed results. Figure 6.1 and 6.2 were normalised to a relative scale to the maximum value of each set of data. Then these rescaled data were simplified and plotted by using a second order polynomial model to create Figure 6.4.

Overall PME activity peaked in ripe fruit (firmness 4 kg_f), while PG activity seemed to decrease as persimmon ripened. In contrast, β-galactosidase activity increased dramatically in overripe fruit (firmness < 3 kg_f). GalA content declined in a similar pattern to G′ and the DMe increased as the fruit ripened.

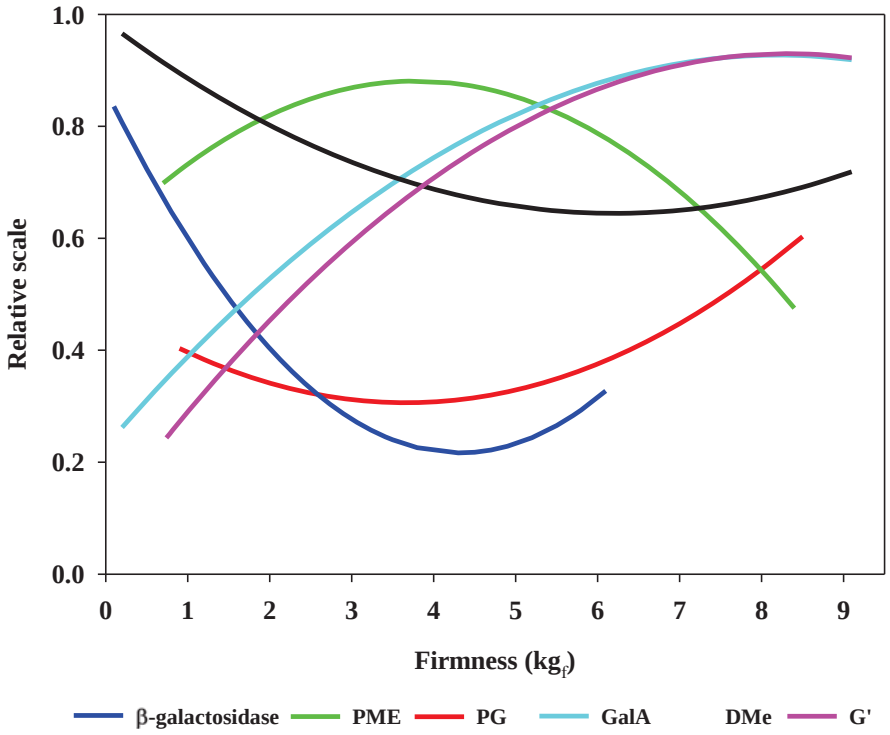


Figure 6.4 β-galactosidase, PME and PG activities in persimmon fruit at different stages of maturity to strength (G′) of persimmon gel including change in total GalA content and DMe. Persimmon maturities were defined as mature-green (> 9 kg_f), mature (7-9 kg_f), ripe (4-7 kg_f), fully-ripe (2-4 kg_f) and overripe (< 2 kg_f). Data were re-plotted and showed the overall trends on a normalised scale.

The G′ parameter is commonly used to characterise the elastic property of a pectin gel (Chronakis & Kasapis, 1995; Lopes da Silva & Rao, 1999). This research found persimmon purée formed a gel spontaneously after puréeing at 20 °C which was symptomatic of calcium gel behaviour (Section 5.3.1). The gelling mechanism can be explained by a series of reactions: enzymatic and ionic reactions.

During ripening, pectin was hydrolysed by PME de-esterifying specifically methylester groups of polygalacturonic acids (Pilnik & Rombouts, 1981; Fischer & Bennett, 1991), and resulting in long chains of GalA residues which were extremely sensitive to calcium ions for calcium crosslinkages (Van Buren, 1979; Bacic et al., 1988; Sajjaanantakul et al., 1989; Sajjaanantakul et al., 1993; Krall & McFeeters, 1998). Consequently, the GalA chain can be accessible for PG depolymerisation which causes reduction of pectin length (Fischer & Bennett, 1991; Wang et al., 2002) (measured as decreased GalA, Figure 6.4) and increased solubilisation. PG

hydrolysis results in short GalA chain leading to relatively short calcium interaction chains, resulting in G′ reduction progressively as persimmons ripening (Figure 6.4). Meanwhile, there was low PME activity in mature fruit (firmness 7-9 kg_f) which could possibly result in long chains of GalA, which could potentially create long calcium interaction chains, resulting in relatively strong gels (high G′). However, during persimmon ripening, other pectolytic enzymes such as pectin lyase, pectate lyase, cellulase and hemicellulase could break down persimmon cell wall polysaccharides resulting in other impacts on the GalA chain.

Generally, the DMe decreases during fruit ripening due to PME deesterification of the methyl groups from the pectin (Stoforos et al., 2002). In contrast, the present study observed increasing DMe in overripe persimmon (firmness 0.2 kg_f). Brummell et al. (2004) reported that increasing DMe in overripe peach may be caused by loss of HG fragments of low methylesterification from cell walls or loss of HG during cell wall preparation for measurement. This could possible imply that during cell wall preparation for measurement, there were high amount of pectin which was highly methylated and were not depolymerized by PG. Fischer & Amadò (1994) and Fischer et al. (1994) reported high DMe is caused by a weak ionic bonding between pectin molecules and divalent calcium ions which results in less coherence of the middle lamella. High DMe in overripe persimmon could possibly be due to the weak ionic bonding of pectin in the middle lamella which could be caused by PME and β-galactosidase hydrolysis.

This study found that β-galactosidase became more active in overripe persimmon and hence likely to result in further pectin degradation, particularly of highly branched areas of the pectin chains (de Veau et al., 1993; Ali et al., 1995; Willats et al., 2001; Wang et al., 2002). Consequently, the pectin chain was potentially more extensively shortened resulting in limiting the calcium inter-chain reaction, and hence, potentially contributing to the rapid reduction in observed G′ for gels made from overripe fruit (Figure 6.4).

GalA characteristic changes as fruit ripen have the potential to impact on gelation. The blockiness of GalA could influence gel characteristics (Winning et al., 2007). Further study on the degree of blockiness and GalA distribution patterns of persimmon pectin as fruit ripen could help to understand more of the influence of pectolytic enzymes on pectin characteristics and subsequent effects on gelling mechanism and gel characteristics.

Heating persimmon tissue to 73 °C for 30 minutes prior to tissue puréeing resulted in a more firm gel (Section 5.3.4). This more firm gel is possibly due to activation of PME deesterification during heating resulting in decreased DMe with higher methyl free GalA content than for purées made at 20 °C (Figure 6.3) which could result in increased length of GalA residue chain. Christiaens et al. (2012a) reported increasing water insoluble pectin that was caused by PME deesterification when blanching at 60 °C for 40 minutes prior to carrot blending resulting in increasing long block of methyl free GalA residues which is similar to previous reports (de Roeck et al., 2008; Fraeye et al., 2009; Christiaens et al., 2011). In addition, Angersbach et al. (1999) reported that heating increases cell wall permeability (Figure 6.6). High cell wall porosity could possibly increase PME accessibility to the substrate, which together with subsequent puréeing could potentially increase PME hydrolysis (Section 5.3.4). Heating to 73 °C for 30 minutes inactivates PG depolymerisation (Verlent et al., 2004; Verlent et al., 2006) (Section 5.3.4), therefore length of pectin chain would consequently degrade less.

Figure 6.5 was constructed to further explain the PME, PG and β-galactosidase activities, affected by temperature and processing in persimmon product. This figure was drawn by using observed results from this research and past data from literature. Time-temperature combinations were plotted using a semi-log scale.

PME inactivation was obtained from persimmon data extracted from Katsaros et al. (2006) for inactivation rate constant (k) of 0.029 min^{-1} at 80 °C and E_a at 207 $kJ.mol^{-1}$. The PG inactivation curve was plotted from data for tomato juice (Fachin et al., 2003) to be a k of 0.588 min^{-1} at 65 °C and E_a at 457.1 $kJ.mol^{-1}$. The β-

galactosidase inactivation curve was plotted from data for inactivation in tomato (Houben et al., 2013) to be a *k* of 0.119 min^{-1} at 47.5 °C and E_a at 208 $kJ.mol^{-1}$.

These enzyme inactivation kinetics suggest that temperatures above 70 °C are required to inactivate β-galactosidase and PG. This could explain the higher total pectin measurements made in samples heated to 73 °C prior to puréeing. PME, however is very heat stable and is likely to remain active and able to reduce the DMe and therefore gel strength. Processing at lower temperatures is likely to result in some pectin hydrolysis and debranching and lower gel strength would result (see Figure 6.7). These trends are consistent with those observed in chapter 5.

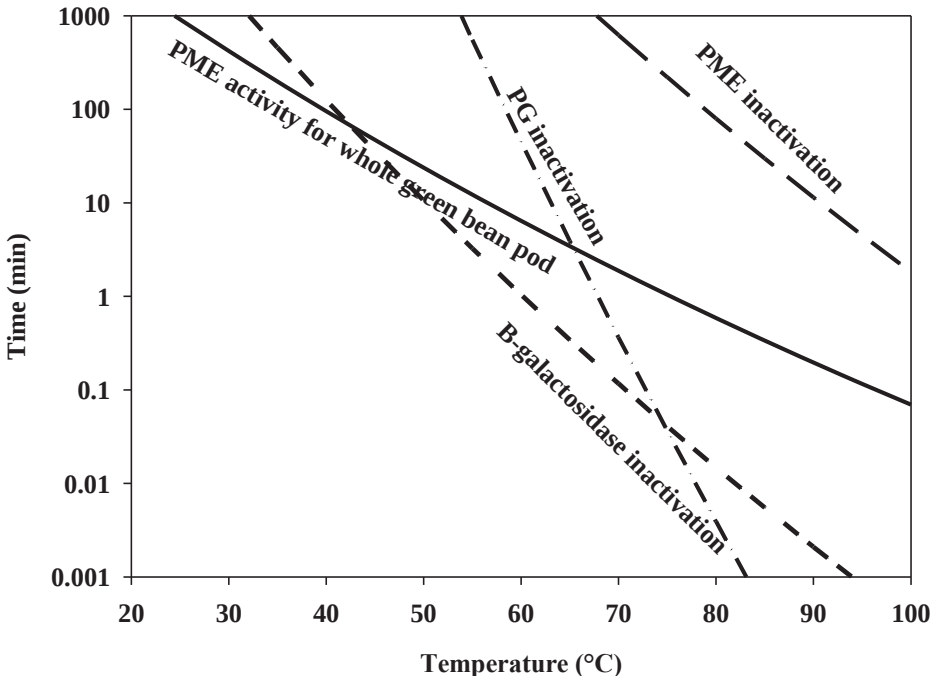


Figure 6.5 Plots showing pectolytic enzyme activity and inactivation for persimmon purée with temperature. This plotted by using observed past data for 90 % reduction in enzyme activity. PME, PG and β-galactosidase inactivation kinetics were plotted by using data extracted from Katsaros et al. (2006), Fachin et al. (2003) and Houben et al. (2013) respectively.

The enzyme activities reported in this chapter are relatively high and it might be expected that heating the tissue prior to puréeing would result in rapid pectin hydrolysis. This however was not observed in practice. This may be due to the natural compartmentation of enzymes from their substrate in the live fruit as part of

the physiological control of fruit softening. Anthon & Barrett (2006) showed that in whole green bean pod the PME activity was orders of magnitude slower than for extracted enzymes (see Figure 6.5). They explain that the activities are limited by the loss of cell membrane integrity that results from heating. This can be seen in Figure 6.6, taken from Angersbach et al. (1999) where the cell membrane integrity was lost at temperature above 60 °C.

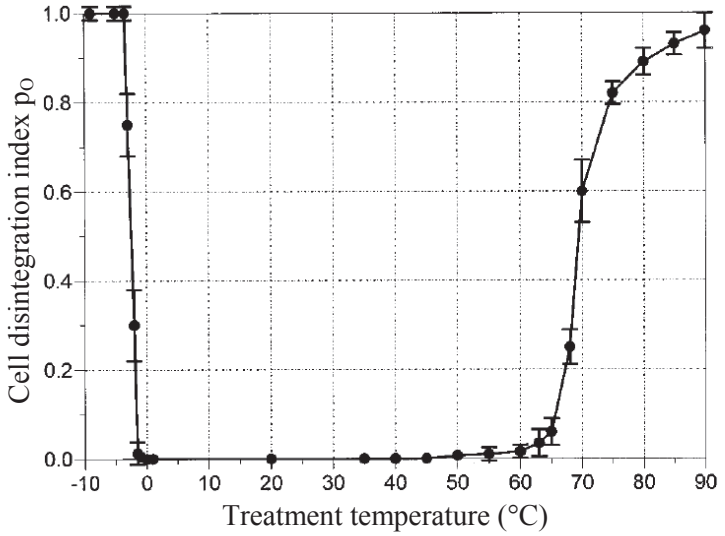


Figure 6.6 Effect of temperature on cell permeability (cell disintegration index, p_O) in potato tissue (Angersbach et al., 1999).

DMe reduced slightly as persimmon ripened, then increased when persimmon become overripe. This could be due to hydrolysis of the less methylated regions. This study also observed a lower DMe with higher increasing GalA content when persimmon tissue was heated to 73 °C for 30 minutes prior to tissue puréeing. This could indicate that heating activates PME. Increasing total GalA content could result in long methyl free GalA chains which are sensitive to calcium crosslinkages, resulting in a more firm gel. A further study on pectin blockiness and pectin distribution pattern is suggested in order to clarify the influences of pectolytic enzymes on pectin characteristics and subsequent effects on gelation mechanism.

6.5 Conclusions

The effects of PME, PG and β-galactosidase and temperature on the changes of total GalA content and DMe, and their impacts on persimmon gelling mechanism were evaluated. In order to clarify these influences, Figure 6.4 was created to generalise all the observed results.

As ripening progressed, β-galactosidase activity increased coupled with increased PME activity causing pectin degradation as observed by decreased total GalA content and reduced GalA chain length. A reduction of total GalA content and its chain length contributed to a weaken gel strength due to limiting calcium crosslinkages. This study found a small reduction of PG activity with decreasing total GalA content. To be able to understand more on how pectolytic enzyme influence pectin characteristics and subsequent influence gelling, further studies of the degree of blockiness of pectin and the distribution of GalA molecules is suggested.

Heating tissue to 73 °C for 30 minutes prior to tissue puréeing resulted in reducing DMe with increasing total GalA content in all persimmon gels. Possibly this heating activates PME deesterification and as a result, contributes to relatively long chains of methyl free GalA residues. Subsequently, these long GalA chains could possible potentially create greater calcium inter-chain associations resulting in a more firm gel.

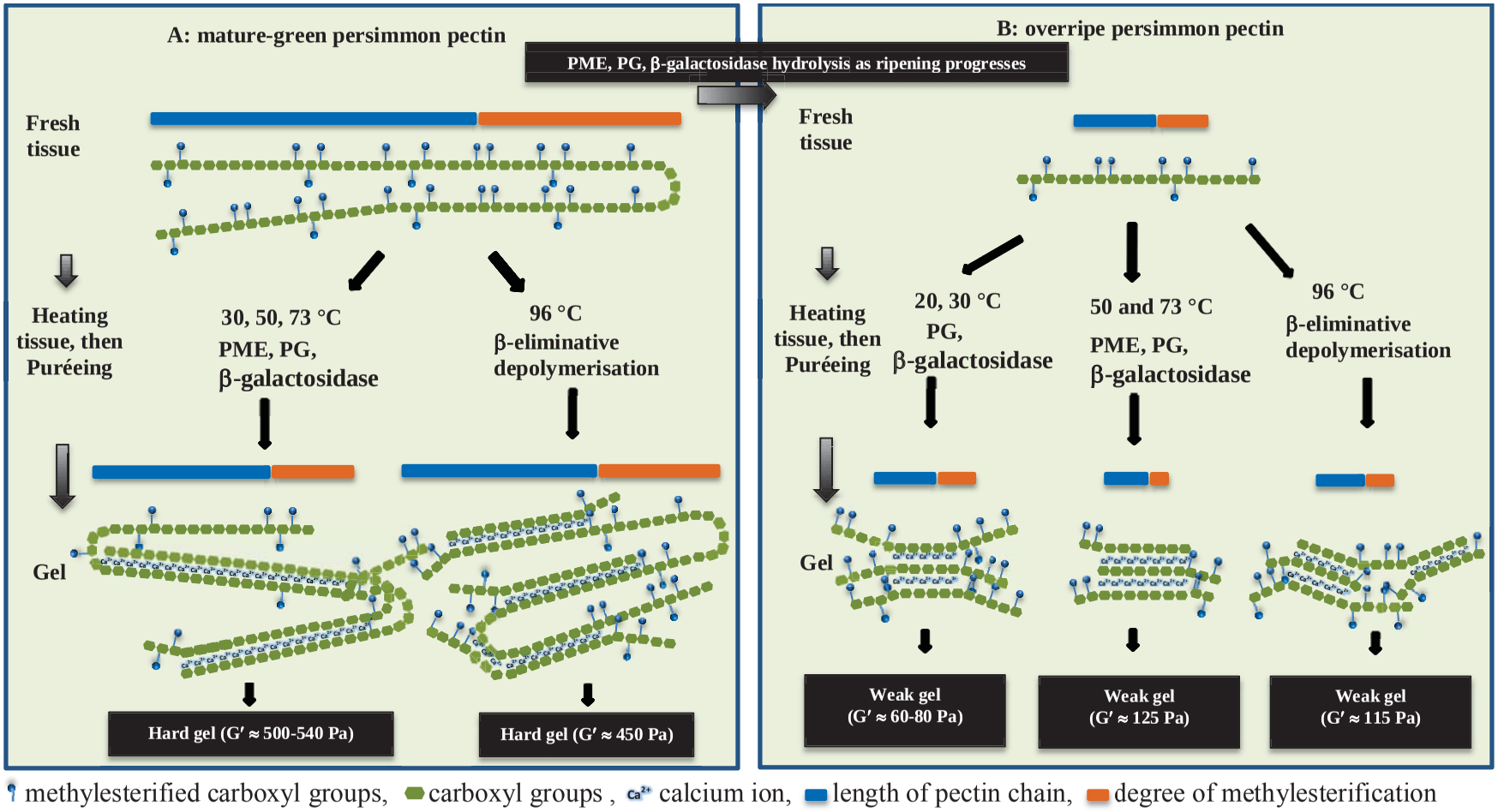


Figure 6.7 Conceptual models to explain observed results of how heat treatment influencing enzyme activity resulting in changes gel viscoelasticity.

CHAPTER 7

Application and Recommendations

7.1 Application

Processed fruit production from New Zealand has increased by nearly 30 % since 2009. Processing unmarketable persimmon into a product is a potential method to extract more value from this crop. This research has provided guidelines for assisting a processing possibility to the New Zealand persimmon industry. Persimmon as a processed ingredient has a number of inherent properties that may be advantageous. Persimmon fruit contains a high content of β-cryptoxanthin which is a precursor of vitamin A, has high vitamin C and dietary fibre that can all impart health benefits such as antiatherosclerotic diet (Bartley & Scolnik, 1995; Thakur et al., 1996 ; Cooper et al., 1999; Takekawa & Matsumoto, 2012). Additionally, persimmon fruit has a pH and a light flavour and colour which may be advantageous when processing with other ingredients, as is could be used as a carrier for more dominant flavours whilst still imparting textural or health benefits.

One of the most common fruit products is jam. A key ingredient of modern jam is pectin. Gelling mechanisms for jam differ depending on degree of methylesterification (DMe) of pectin. High methoxyl pectin (HMP, DMe > 50 %) forms a sugar-acid gel that usually forms with hydrogen-bridge bonding crosslinks. HMP gels can be produced in the presence of 55-75 % co-solute such as sucrose with low pH at 2.5-3.5. Low methoxyl pectin (LMP, DMe < 50 %) generally forms a calcium gel with calcium crosslinks with a soluble solid content between 10-70 % and pH 2.6-7.0. Calcium crosslinks are created between two methyl free galacturonic acid (GalA) residues on pectin chains by a calcium ion. Pectin properties (i.e. GalA content and DMe) are the key factor influencing textural quality of processed fruit products.

After harvest fruit are physiological active, altering postharvest quality, and leading to different properties of the final product. This study observed significant effects of input fruit maturity on final product properties. Processing less ripe fruit obtains a harder gel while processing more ripe fruit resulted in weaker gels. Processing less ripe persimmon resulted in a yellow product whilst more ripe persimmon created a red product. The production yield of processing more ripe fruit is lower in comparison to less ripe fruit as the soft nature of ripe persimmon makes handling difficult, resulting in high losses during peeling and calyx removal.

This study found self-gelling characteristics of persimmon purée that behaves like a LMP. A calcium gel forms under pH even though DMe ranges from 50-70 %. Pectin characteristics vary depending on fruit maturity. Gel strength differences were a result of different pectin characteristics caused by PME, PG and β-galactosidase hydrolysis during ripening. As ripening progressed, PME deesterification results in longer chains of methyl free GalA residue which favour calcium crosslinking resulting in harder gels. PME deesterification also facilitates PG depolymerisation causing shorter chains in ripe fruit and resulting in weaker gels.

Combining less ripe tissue with more ripe tissue can increase gel strength in comparison to more ripe tissue. This is due to the potential for greater calcium crosslinking between pectin chains. Gel strength can also be increased by increasing total solid content. Doubling the total solid content results in a 25 % increase in gel strength. After reducing the total solid content by half, the gel strength was decreased by 94 %. Adding skin can increase gel strength, potentially by competing for water and thereby increasing the apparent total solids of the gel.

Pre-heat treatment prior to tissue puréeing (blanching) could activate PME but inactivate PG. This pre-heating results in PME methylesterification resulting in longer methyl free GalA chains that are favoured for calcium crosslinking. Heat treatment can aim to eliminate microbial pathogen and to inactivate undesirable enzymes to achieve a shelf stable product (Lewis & Jun, 2012). Vegetative cells including yeast and mould spores are easily eliminated by temperatures > 75 °C (Smelt et al., 2002). Persimmon has a pH (5-6) which introduces a stability challenge

because in food with a pH > 4.6, spores of the pathogen *Clostridium botulinum* can germinate and grow producing a lethal neurotoxin (Gaman & Sherrington, 1990; Gould, 1997). Heat treatment for the foods with pH > 4.6 of requires a 12D reduction process, where D at 121.15 °C = 0.21 minutes (Silva & Gibbs, 2004). Lowering pH < 4.6 would be a potential solution to produce a safe and shelf stable food product. Consequently, the effect of pH change on gel strength will need to be considered. Calcium gel properties are pH independence when pH > 4.5. However, at pH < 4.5 the charge density of pectin decreases, reducing its affinity for calcium (Capel et al., 2006; Fraeye et al., 2010b). Treatment after processing could also reduce gel strength due to pectin degradation as a result of β-eliminative depolymerisation (Lootens et al., 2003; Fraeye et al., 2007; Cardenas et al., 2008; Sila et al., 2009). In addition, this study observed colour deterioration in persimmon gel made from tissue heated to 73 and 96 °C prior to tissue puréeing which is similar to other reports (Lozano & Ibarz, 1997; Anese et al., 2002; Shi et al., 2008; Patras et al., 2009).

Fruit maturity is the key factor influencing the textural characteristics of persimmon gel. Grading before processing in order to minimise the variation of raw material fruit, could assist in producing the consistent product quality. This study found skin colour change from pale green-yellow to orange-red (Section 3.3.2) can be used for practical application for grading fruit maturation.

Cold storage is also a useful tool to slow down physicochemical reaction during ripening and heating fruit can be used to accelerate the ripening to achieve the target fruit quality. Studies reported low temperature can extend shelf life. Persimmon can be stored for 3 months in air (0 ± 1 °C, RH 90-95 %) or for 5 months in controlled atmosphere (3-5 % O_2 with 5-8 % CO_2) (Crisosto et al., 1999). Cold storage with other applications such as modified packaging (Ben-Arie & Zutkhi, 1992; Chae et al., 2004; Cia et al., 2006; Jeong et al., 2013) and carnauba wax (Blum et al., 2008) can be also used to extend shelf life. Use of postharvest techniques can therefore be useful to extend the processing seasons and to achieve greater raw material uniformity.

Persimmon is a seasonal fruit which is available for approximately 3-4 months during April to June each year in the southern hemisphere. Freezing persimmon flesh is therefore an interesting practice in terms of increasing opportunity to produce persimmon product throughout the year. Freezing could also preserve the pectin component which would allow greater gel strength.

7.2 Recommendations for further study

As ripening progressed, pectin is degraded by pectolytic enzymes resulting in altered pectin characteristics. The textural property of the resulting persimmon gel can be influenced by modifying pectin during ripening. The mechanism for the observed texture differences was hypothesised to be related to the firm or weak gel depending on calcium crosslink differences. A greater calcium gel matrix can be formed with long chains of methyl free GalA residues. In developing the mechanism it was assumed that persimmon pectin has long blocks of methyl free GalA which favours calcium crosslinking. The degree of blockiness and the patterns of GalA distribution are reported to influence gelling properties of pectins (Ström et al., 2007; Winning et al., 2007). Hence, further study on the blockiness and the distribution of GalA residues on persimmon pectin chain will allow greater understanding of the self-gelling mechanism for persimmon purée.

The colour of persimmon fruit was found to significantly change from pale green-yellow to deep red as ripening progressed due to increasing of β-cryptoxanthin and zeaxanthin content (2007). These compounds can influence colour of any processed product, and since concentration is different between flesh and skin, the resulting colour will depend on whether the skin is removed or not. High temperature treatment causes loss of these colour pigments (Section 5.3.4, Figure 5.8). This is also reported in the literature (Chutintrasri & Noomhorm, 2007; Zepka et al., 2009; Zepka & Mercadante, 2009; Mertz et al., 2010). Therefore, further research should focus on the influence of fruit maturity and processing conditions on β-cryptoxanthin and zeaxanthin content in order to manipulate final product colour. The outcome of this study would benefit process design to produce the desired product colour.

Textural characteristics have an important impact on product acceptability. This research demonstrates that textural characteristics can be manipulated through combining different types of persimmon tissue. For more wide industrial application, further studies in combining persimmon tissue with other ingredients should be conducted. Interactions of persimmon pectin with other food texture manipulating agents such as alginate, agar, xanthan; protein (Voragen et al., 1995); other fruit pulps; or dairy material should be investigated. The results from those further studies could provide opportunities for explaining how persimmon purée may be used as a useful ingredient in the processed food industry.

If a free flowing purée was required, self-gelling characteristics can be stopped by applying food-grade calcium chelator. By removing calcium ions, the pectin crosslinking and gel formation can be prevented. Additionally, applying pectolytic enzymes can stop gel formation as pectin degradation (Tárrega et al., 2012). These techniques can be of benefit in cases where gel formation is not acceptable for further processing.

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Appendix I

Suntudprom, J., Andrew, R. E., John, E. B., & Sung, J. L. (2011). *Influence of fruit maturity on texture and colour of purée product.* Paper presented at the iCEF11 International Congress on Engineering and food, Athens, Greece.

Influence of Fruit Maturity on Texture and Colour of Puree Products

Jantana Suntudprom [a], Andrew R. East [a], John E. Bronlund [b, c], Sung Je Lee [d]

[a] *Institute of Food, Nutrition and Human Health, College of Sciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand*
[b] *School of Engineering and Advanced Technology, College of Sciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand*
[c] *Riddet Institute, Private Bag 11222 Palmerston North, New Zealand*
[d] *Institute of Food, Nutrition and Human Health, College of Sciences, Massey University, Private Bag 10 2904, Auckland, New Zealand*

INTRODUCTION

Fruit puree is an important ingredient for many food products such as jams, marmalades, spreads, fillings and toppings. Texture and colour are important quality indicators that influences food acceptance [1]. After harvest, fruit remain physiologically active, and hence can ripen, changing their physicochemical, textural and sensory properties from harvest. When persimmons ripen, the skin colour turns to an orange-red colour due to a rapid increase in carotenoid pigments (e.g. lycopene, β-cryptoxanthin). Softening of the fruit texture is largely attributed to degradation of the cell wall pectin of the internal tissues by the enzymes pectinmethylesterase (PME), polygalacturonase (PG) [2] and β-galactosidase [3,4]. Any fruit processing industry is required to understand the influence of fruit maturity and subsequent quality variability on the quality of the processed product. This study was carried out in order to evaluate the effects of fruit maturity on fruit puree characteristics using persimmon fruit as a case study.

MATERIALS & METHODS

Persimmon fruit (*Diospyros kaki,* cv. Fuyu) were stored at 0 ± 1 °C, 90-95 % RH [2-6] and later equilibrated to 20 °C for 24 h before use. Five (5) sets of fruit were placed at 20 °C in order to facilitate fruit ripening and hence create a range of fruit maturities as measured by fruit firmness. Each set of persimmon was made into a separate batch of puree. In total 7 batches of puree were manufactured: 5 batches of puree from unpeeled fruit and another 2 batches of puree made from peeled fruit of 3.0 and 9.1 kg_f average firmness. Batches were packed in a 125 ml clear wide mouth short jar and kept at 6°C overnight. On the following day after processing physical qualities (colour, water holding capacity (WHC), and textural properties) were analysed at 20°C.

RESULTS & DISCUSSIONS

Skin colour of whole persimmon fruit at different firmness showed significant differences. All three CIELAB colour parameters (lightness, chroma, and hue angle) decreased with reducing fruit firmness. Colour differences between the purees made from different average firmness fruit were not as dramatic as observed for the whole fruit. Unpeeled and peeled batches show significant differences in colour (Table 1).

Puree batches made by fruit at an average firmness of 3.0 and 9.1 kg_f displayed the highest water holding capacity.

Table 1. Properties of persimmon puree made by fruit of different maturity (firmness).

Batches	Average firmness (kg_f)	Puree colour parameters*			% WHC	Storage Modulus* G′, (Pa)	Loss Modulus* (G″, Pa)	Viscosity* (Pa.s)
		Lightness	Chroma	Hue angle				
Unpeeled	0.7	43.7 ^{c}	22.9 ^{b}	61.4 ^{c}	97.6 ^{b}	86.4 ^{e}	17.5 ^{e}	0.42 ^{d,e}
	1.3	44.0 ^{c}	22.4 ^{b}	63.4 ^{d}	97.4 ^{b}	84.9 ^{e}	17.8 ^{e}	0.47 ^{d}
	3.0	45.5 ^{c}	24.5 ^{a}	57.8 ^{f}	99.1 ^{a}	199.7 ^{d}	26.7 ^{d}	0.80 ^{c}
	7.9	45.8 ^{c}	21.0 ^{c}	65.6 ^{cb}	96.0 ^{c}	840.0 ^{a}	129.0 ^{a}	0.94 ^{b}
	9.1	47.7 ^{a}	22.7 ^{b}	65.2 ^{c}	99.5 ^{a}	539.2 ^{b}	95.1 ^{b}	0.94 ^{b}
Peeled	3.0	44.9 ^{d}	19.9 ^{d}	62.4 ^{dc}	99.0 ^{a}	50.7 ^{f}	7.6 ^{f}	0.33 ^{e}
	9.1	40.7 ^{b}	15.9 ^{e}	71.0 ^{a}	99.1 ^{a}	406.7 ^{c}	68.0 ^{c}	1.24 ^{a}
	$LSD_{0.05}$	0.4	0.5	1.2	0.6	34.1	6.5	0.12

*Means with the different letters within columns are significantly different ($P < 0.05$, $n = 4$).

Persimmon puree could be described as a semi-solid sample (G′ > G″) [5]. Unpeeled purees made from unpeeled persimmons were found to have significantly higher G′ and G″ values. Purees made from soft fruit (unpeeled and peeled batches) showed a much lower G′ and G″ than those made from firm fruit. Puree made from high firmness fruit produced puree with high viscosity, whereas puree made from soft fruit showed the lowest viscosity. Peeling fruit did not produce a consistent response to viscosity change, in comparison to the unpeeled equivalent purees.

CONCLUSIONS

Soft persimmon produced redder puree due to high carotenoid contents of the fruit. Additionally soft fruit produce puree with a lower water holding capacity. The rheological properties of persimmon puree could be described as a characteristic semi-solid sample. Hard persimmon could be used to produce stronger structure puree. This puree made from firm fruit is less flowable than those produced by soft persimmon. This study produces an indication of the range of properties that can be produced in persimmon puree as influenced by fruit maturity.

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