Segregation of 'Hayward' kiwifruit for storage potential

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Jinquan Feng

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"Science is but an image of the truth."

Francis Bacon

Abstract

This study aimed to develop technologies to segregate 'Hayward' kiwifruit (*Actinidia deliciosa* (A.Chev) C.F. Liang et A.R. Ferguson) for storage potential. Such segregation will allow the industry to better match market opportunities with the storage potential of particular fruit thereby reducing fruit loss and assuring fruit quality in the market place.

The first part of the research rationalised methodologies to study storage potential of kiwifruit. These included firmness measurement, fruit temperature equilibration, calculation of storage life, sample preparation for mineral analysis and the feasibility of using compression force as an alternative to flesh firmness for firmness monitoring.

The second part of the research developed a model to segregate kiwifruit on a grower line basis. Based on data collected over 2 years from 108 grower lines, canonical discriminant analysis indicated that the first two canonical functions (CDF_1 and CDF_2) accounted for 95% variation of four softening-rate groups and correctly classified over 50% of the grower lines to the correct groups compared with a chance criterion of 25%. Grower lines with high CDF_1 (characterised by high Ca/N, high Mg, advanced maturity, late harvest and low lightness) softened at low rates and had long storage life. CDF_2 discriminated grower lines on prestorage delay. For grower lines with low CDF_1, extending prestorage delay improved storage potential. In contrast, extended prestorage delay reduced the storage potential of grower lines with high CDF_1.

The third part of the research developed a model to segregate kiwifruit on an individual fruit basis. NIR spectra taken at harvest were used to quantify many at-harvest fruit attributes, allowing for prediction of fruit firmness at the end of storage and discriminating disordered fruit from healthy fruit for fruit segregation on an individual fruit basis. Further work is needed to improve prediction and segregation accuracies by selecting appropriate NIR instrument and to incorporate the instrument with grading machines in a way that measurement error can be minimised. Factors affecting NIR measurement and possible improvements were also investigated.

The strategic application of segregation technologies and further research directions are discussed for the kiwifruit industry to develop cost-effective procedures to solve problems associated with fruit variation in storage potential.

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α	A coefficient between internal ethylene concentration and the concentration of a hypothetic enzyme catalysing fruit softening	
A	Surface area of a fruit	m^2
A	The upper asymptote of a Richards function	
AA	Ascorbic acid	
ABS	Absorbance	
ABS+NM	ABS normalised to minimum and maximum	
ABS+QBC	Quadratic baseline compensation of ABS	
ABS+SNV	Standard normal variate transformation of ABS	
ABS_{671}	VNIR absorbance at 671 nm	
ABS_{824}	VNIR absorbance at 824 nm	
ACC	1-aminocyclopropane-1-carboxylic acid	
ACO	ACC oxidase	
ASTD	Average standard deviation	
b	Positions parameter of a Richards function	
C	Fruit chroma	
CC_p	Ppercentage chance criterion	%
Ca	Calcium concentration	$mmol\ kg^{-1}$
CA	Controlled atmosphere	
Ca/N	The ratio of calcium and nitrogen concentrations	%
CDA	Canonical discriminant analysis	
CDF_1	The first canonical function	
CDF_2	The second canonical function	
CDF_3	The third canonical function	
CDF_{1i}	CDF_1 of observation $_i$	
CDF_{2i}	CDF_2 of observation $_i$	
$\overline{CDF}_{1j}$	The mean CDF_1 of group j	
$\overline{CDF}_{2j}$	The mean CDF_2 of group j	
CF	Compression force	N

CF_0	Initial compression force	N
CF_{end}	A CF value equivalent to a flesh firmness of 8.5 N at 20 °C	N
CFS	CF measured at simulated shelf life	N
CI	Chilling injury	
CT	Computed tomography	
d	Day	
D	Compression distance	mm
D_1	First order derivative	
D_2	Second derivative of ABS	
$D_{2(684)}$	The second derivative at 684 nm	
$D_{2(700)}$	The second derivative at 700 nm	
D_2+NM	D_2 normalised to minimum and maximum	
D_2+SNV	Standard normal variate transformation of D_2	
D_{ij}	Mahalanobis distance of observation *i* to the centre of group *j*	
DD	Cumulative degree-days	°C d
DEXA	Differential energy X-ray analysis	
df	degree of freedom	
DL	Postharvest delay	d
DM	Dry matter content	%
DW	Dry weight	kg
ECC	Eccentricity	
Enz	A hypothetic enzyme catalysing fruit softening	
Enz(t)	The concentration of Enz at time t	mol L^{-1}
Enz_0	Initial concentration of Enz	mol L^{-1}
Enz_{pre}	Precursor of Enz	
F	Fruit firmness (compression force or flesh firmness) as a substrate of a hypothetic fruit softening reaction	
F(t)	F value at time t	
F_0	Initial fruit firmness	N
FC	Flesh chroma	
FF	Flesh firmness	N
FF_0	Initial flesh firmness	N
FF_1	The first FF reading measured on a fruit	N
FF_2	The second FF reading measured on a fruit	N

FFS	FF measured at the simulated shelf life	N
FH	Flesh hue angle	
FL	Flesh lightness	
FR	Fungal rots	
FT	Fourier transformation	
FW	Fresh weight	kg
H	Fruit hue angle	
h	hour	
HD	Harvest date (days after 1 April)	d
HEM	High energy counts of middle part of a fruit	
HEO	High energy counts of outer part of a fruit	
HEW	High energy counts of the whole fruit	
I_d	VNIR spectrum of a dark reference	
I_f	VNIR spectrum of a fruit	
I_i	Spectral datum at point i	
I_s	VNIR spectrum of a chemical solution	
I_w	VNIR spectrum of a white reference	
I_{H_2O}	VNIR spectrum of water	
$\bar{I}_g$	Means of the spectral data of group g	
ΔI_g	Difference in spectral data between adjacent groups	
IEC	Internal ethylene concentration	mol L^{-1}
IP	Inner pericarp	
k	Increase rate of a Richards function	
k	The rate coefficient of fruit softening	
k_p	The rate constant of a hypothetic fruit softening reaction	
K	Potassium concentration	mmol kg^{-1}
K tray	Single-layered kiwifruit trays with a polyliner	
KG_1	The first group of grower lines that softened at the lowest rate	
KG_2	The second group of grower lines that softened at the second lowest rate	
KG_3	The third group of grower lines that softened at the second highest rate	
KG_4	The fourth group of grower lines that softened at the highest rate	
L	Fruit lightness	
LEO	Low energy counts of outer part of a fruit	

LEM	Low energy counts of middle part of a fruit	
LEW	Low energy counts of the whole fruit;	
$LSD_{0.05}$	Least significant difference at P=0.05	
LTB	Low temperature breakdown	
Lth	The length of a fruit in pixels	
M	The mass of a fruit	kg
M tray	Interleaving tray commercially used in 20 kg apple packages	
Mg	Magnesium concentration	mmol kg^{-1}
MLR	Multiple linear regression	
MPLS	Modified partial least square regression	
MSC	Multiplicative scatter correlation technique	
MSPR	The means of the squared prediction error	
N	Nitrogen concentration	mmol kg^{-1}
N	Newton	
NIR	Near-infrared	
NLIN	Non-linear regression	
NS	Not significant	
NSD	Normalised second derivative	
OP	Outer pericarp	
ρ	Power factor of a power function	
P	Phosphorus concentration	mmol kg^{-1}
p_e	Partial pressures of ethylene of the environment	Pa
p_i	Partial pressures of ethylene inside the fruit	Pa
$P^{*}_{C_2H_4}$	The permeance of the fruit skin to ethylene	mol s^{-1} m^{-2} Pa^{-1}
$\Delta p_{C_2H_4}$	The difference in partial pressures of ethylene between P_e and P_i	Pa
PC	Principal component	
PCA	Principal component analysis	
PCR	Principal component regression	
PG	Polygalacturonase	
PixM	Area of middle part of a fruit in pixels;	
PixO	Area of outer part of a fruit in pixels;	
PixW	Area of the whole fruit in pixels;	
PKG_I	Predicted KG_I	

PKG_2	Predicted KG_2	
PKG_3	Predicted KG_3	
PKG_4	Predicted KG_4	
PLS	Partial least square regression	
PME	Pectin methyl esterase	
PP	Purple patches	
PPT	Physiological pitting	
PRESS	Sum squares of predicted residual	
r	Correlation coefficient	
R^2	Coefficient of determination	
R^2_p	Coefficient of determination for validation data set	
$r_{C_2H_4}$	Ethylene production rate	mol kg^{-1} s^{-1}
$r'_{C_2H_4}$	Rate of ethylene transfer from fruit to the environment	mol s^{-1}
r_{CO_2}	Respiration rate	nmol kg^{-1} s^{-1}
REG	Regression	
RH	Relative humidity	%
RMSEP	Root mean square error of prediction	
s_g	Standard deviations of spectral data of group g at a wavelength	
SABS	Smoothed ABS	
SABS+NM	SABS normalised to minimum and maximum	
SABS+QBC	Quadratic baseline compensation of SABS	
SABS+SNV	Standard normal variate transformation of SABS	
SCC	Standardized canonical coefficient	
SDR	Data set standard deviation divided by RMSEP	
se	Standard error	
SEB	Stem end botrytis	
SEP	Standard error of prediction	
SL	Storage life	d
SP	Soft patches	
SR	Side rot	
SRD	The sum of relative difference between groups	
SSC	Soluble solids content	%
SSF	Soluble solids in whole fruit	%

SSFDM	The percentage of dry matter that have solubilized	%
STDP	Standard error of the mean predicted value	
STEPDISC	Stepwise discriminant analysis	
STP	Storage potential	
t	Storage time	d
t_{78}	Seven-eighth temperature equilibration time	h
TND	Trend	
ULO	Ultra low oxygen	
v	Inflexion point of a Richards function	
VNIR	Visible-near-infrared	
W_0	Initial fruit weight	kg
WL	Weight loss	%
WPI	Whey protein isolated	
Wth	The width of a fruit in pixels	
XET	Xyloglucan endotransglycosylase	

1.1 Background

Kiwifruit (*Actinidia deliciosa* (A.Chev) C.F. Liang et A.R. Ferguson and *Actinidia chinesis*) is the most important horticultural crop in New Zealand. In 2002-2003, the crop earned $539 million (FOB) in export receipts (HortResearch, 2003).

Variation in storage potential (STP) within and among grower lines (such as fruit from different production regions, orchard sites, seasons, preharvest and postharvest treatments) costs the kiwifruit industry millions of dollars each year because of the rejection of over soft (i.e. flesh firmness of a fruit falls below 10 N or the average of a grower line falls below 11.8 N) and disordered fruit (Hopkirk et al., 1996; NZKMB, 1996; Davie, 1997; Benge, 1999). Fruit softening is a major limiting factor for STP of 'Hayward' kiwifruit (Hewett et al, 1999). Kiwifruit soften rapidly when exposed to even minute (i.e. 0.01µl l^{-1}) concentrations of ethylene (Mitchell, 1990; Arpaia et al., 1994). The elevated production of ethylene by just a few ripe fruit in a shipment may trigger a chain reaction causing premature ripening of the whole load. Most segregation systems currently used in packhouses are based on fruit size and visual defects and do not reduce STP variation within packages. Some recent segregation systems are based on non-destructive measurements of dry matter content and soluble solids content and target eating quality (potential of developing desirable flavor) rather than STP (Richardson et al., 1997; McGlone and Kawano, 1998; Watt, 1999). Exporters risk sending lines of fruit that do not have the innate storage potential to arrive in good condition to foreign markets. Fruit segregation systems based on STP is urgently required (Banks, personal communication, 1999).

1.2 Research objectives and structure of the thesis

The current study on segregating kiwifruit for storage potential focuses on establishing STP models on both grower line and individual fruit bases. STP model on a grower line basis will enable the industry to better match market opportunities with storage capability of particular grower lines. STP model on an individual fruit basis will allow segregation on a per–fruit basis providing the industry with even greater management control of their harvested crop.

Chapter two reviews current understanding about STP of kiwifruit and two non-destructive technologies (visible-near-infrared spectroscopy and X-ray analysis) that have been evaluated in this thesis for inline measurement of fruit attributes.

Chapter three is a summary of rationalized methodologies for studying kiwifruit STP while chapter four further evaluates the feasibility of using compression force as an alternative to flesh firmness for STP study.

Chapter five presents a discriminant model for fruit softening rate during storage measured on a grower line basis. The model facilitates segregation of kiwifruit on a grower line basis according to fruit attributes measured at harvest on a sub-sample from each grower line. In chapters six and seven, the capabilities of visible-near-infrared spectroscopy (VNIR) on non-destructive measurement of at-harvest attributes and on discriminating fruit for firmness and disorders at the end of storage are evaluated. Factors affecting VNIR measurements are explored in chapter eight with an attempt to enhance the understanding of the measurement and to highlight some potential improvements of the technique. The ability of differential energy X-ray analysis to measure fruit attributes and STP is evaluated in chapter nine.

Chapter ten, a general discussion, draws together the results from this study and those found in the literature to propose a conceptual model for strategic application of fruit segregation technologies at an industry level.

Chapters three to nine were written as papers for publication in the Postharvest Biology and Technology.

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2.1 Introduction

Storage potential (STP) can be measured as storage life (SL), the time for a stored fruit to become unacceptable for a particular use. New Zealand kiwifruit becomes unacceptable for export when flesh firmness (FF) of a fruit falls below 10 N (1 kgf), or the FF of a grower line (fruit harvested from an orchard or a block of an orchard) falls below 11.8 N (1.2 kgf) (Hopkirk et al., 1996; Benge, 1999), or if fruit develop certain disorders, such as pitting, rots, low temperature breakdown (LTB) and shrivel (Lallu, 1997). Because all the disorders accelerate fruit softening, it is generally acceptable to use over-softening as an indication of the end of SL.

Due to the difficulty of measuring SL, only a few researchers (Pyke et al., 1996. Reid et al., 1996; Benge, 1999; Crisosto et al., 1999; Lallu et al., 1999) have actually presented SL data. Many researchers have compared flesh firmness (FF) and the incidence of fruit disorders measured after certain storage. Occasionally, the rate of fruit softening was compared between grower lines (Abdala et al., 1996).

The first part of this review summarizes existing knowledge about STP and related fruit attributes of kiwifruit with focuses on concepts, measurement, variability, and interrelationships. The second part of the review focuses on near-infrared (NIR) spectrometry and X-ray technologies that have been evaluated in this thesis.

2.2 Measurement of storage life

SL for a grower line of fruit is usually calculated from the softening curve generated from FF data measured at 2-4 week intervals during the storage on a subsample of 10-50 fruit at each measurement period (Pyke et al., 1996; Lallu et al., 1999; Benge et al., 2000).

2.2.1 Firmness measurement

Flesh firmness measured destructively using a penetrometer is the industry standard for monitoring kiwifruit firmness. This method measures the force required for a 7.9 mm diameter probe to penetrate into a fruit at a site where the skin tissue has been

removed using a fruit peeler. This method essentially measures the tensile strength of the outer pericarp tissue. The tensile strength represents the rigidities of the cell walls and the middle lamella (Harker and Hallett, 1994).

Flesh firmness measured by a penetrometer is subject to large variation caused by inconsistent operation and false readings by the operator from the dial of the pressure meter (Harker et al., 1996). Several studies have used motorized penetrometers, such as the Texture Analyser (TA.TX2, Stable Micro Systems, England) for firmness measurement of strawberries (Doving and Mage, 2002), tomatoes (Smith et al., 2002) and kiwifruit (Feng et al., 2002a) and reported improved accuracies.

Flesh firmness is temperature dependent (Bourne, 1982). FF measured upon removal from storage at 0°C (i.e. the industry practice; Hopkirk et al., 1996) varies considerably due to rapid changes in fruit temperature. It was suggested that FF should be measured either at 0°C or after two periods of seven-eighth equilibration time by which time fruit temperature has stabilized (Feng et al., 2002c). Such standardisation can lead to other benefits. For example, FF of 'Hayward' kiwifruit measured after 24 h equilibration at 20°C can be estimated from FF measured at 0 °C and vice versa, according to the firmness temperature coefficient (k_{fT}, % $^{o}C^{-1}$), a measure of the percentage change in firmness caused by a given temperature change (Jeffery and Banks, 1994).

There is increasing interest in the use of non-destructive firmness measures for kiwifruit as alternatives to the penetrometer method (Davie et al., 1996; Hopkirk et al., 1996; McGlone et al., 1997 & 1999; Muramatsu et al., 1997; McGlone and Kawano, 1998; Burdon et al., 1999; Hertog and Jeffery, 2000; McGlone and Jordan 2000; Terasaki et al., 2001a; Costa et. al, 2003a; Shmulevich et al., 2003; Valero et al., 2003). Unfortunately, some of these non-destructive techniques appear to measure different aspects of fruit firmness to that measured by penetrometers and thus may not be reliable or comparable. For example, the impact force analysis technique and parallel plate compression measure fruit stiffness which may either plateau or rise during storage. In contrast, FF consistently decreases with time. FF measurement remains the most reliable firmness test in both scientific studies and industry applications (McGlone et al., 1997).

2.2.2 Calculating SL from firmness monitoring data

The exact time when the fruit of a grower line softens to 11.8 N is difficult to estimate because of the triphasic nature of fruit softening curve (Figure 2.1). Pyke et al. (1996) used a linear regression of the logarithmic transformation of FF against storage time. This method oversimplifies the softening curve, and model parameters estimated from the entire storage period are not sensitive to FF data collected towards the end of storage when FF approaches 11.8 N. Benge (1999) used a quartic polynomial model to estimate storage life and arbitrarily set the criterion to 30 N because the fruit did not soften below 11.8 N (measured at 0 ^{o}C) during 180 days storage. The quartic polynomial model used involves 5 parameters, and requires weekly firmness monitoring to collect enough data for parameter estimation. Lallu and coworkers (1999) fitted a straight line between two FF means, one larger and another smaller than an arbitrary criterion. The precision of this method is limited by the variation of FF values between fruit that may contribute 6-13% error in grower line means towards the end of storage (Benge et al., 2000). The precision could be improved by measuring more fruit at each monitoring period and/or using firmness data from more than two monitoring periods. The former is limited by resources such as fruit, time and instrument while the latter is limited by the nonlinear nature of the softening curve (Fig 2.1). A compromise would be to fit FF data collected after a certain period of storage to a simple curvilinear model. Recent work by Feng et al. (2001) indicated that an exponential model based on data from the last four monitoring periods is reliable for the calculation of SL if FF is monitored monthly with 20 fruit per grower line at each monitoring period.

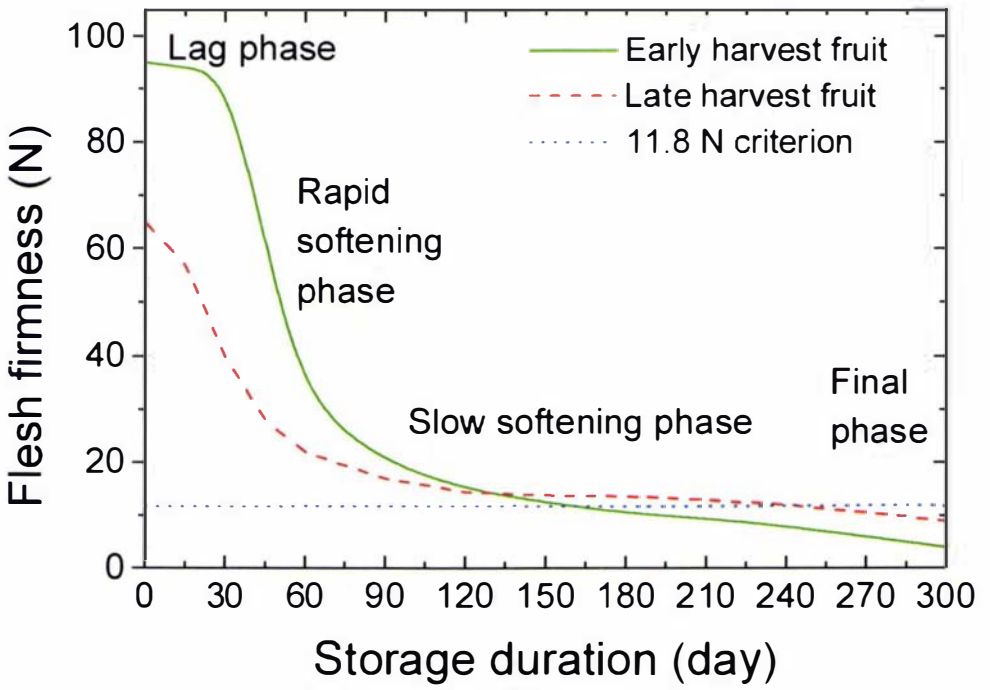

Figure 2.1 Diagrammatic representation of softening of kiwifruit (adapted from MacRae et al., 1990).

The different softening rates of different grower lines during storage (Fig 2.1) render FF, measured after a certain storage period, or SL, calculated using different FF criteria, unreliable for the estimation of STP. For example, when FF is assessed after 12 weeks' storage, late harvested fruit is likely to be softer than early harvested fruit, and the opposite might be true if FF is measured after 20 weeks' storage. Similarly, if firmness falls rapidly to 30 N (Benge, 1999) or 19.6 N (Lallu et al, 1999), such fruit may subsequently take longer to reach 11.8 N than fruit with a lower initial softening rate. Therefore, a constant FF criterion should be used for calculation of storage life.

2.3 Storage disorders affecting storage life

Storage life effectively finishes once a postharvest disorder develops to an unacceptable extent. However, time courses of the development of postharvest disorders have rarely been used to define SL of kiwifruit. Instead, incidences of disorders were

compared after a certain time of storage and susceptible grower lines have been marketed as soon as possible after repacking (Benge, 1999). This may result in considerable fruit loss and labour cost. Therefore, it is desirable that susceptibility be predicted at harvest so that inventory management could be introduced sooner rather than later.

2.3.1 Fruit rot

Fruit rots caused by *Botrytis cinerea* during storage is one of the most important postharvest disorders of kiwifruit in cool storage (Brook, 1992; Thanassoulopoulos and Yanna, 1997). Ripe rots caused by *Botryosphaeria, Fusicoccum, Diaporthe, Fusarium, Phoma, Glomerella, Colletotrichum* and *Cryptosporiopsis* usually develop only when the fruit is taken from cool storage and are less detrimental to the kiwifruit industry compared to *Botrytis* rots (Brook, 1992). The incidence of *Botrytis* rots could be predicted based on preharvest examination of *B. cinerea* colonizing the sepals or stem ends (Michailides and Morgan, 1997). While this method is practically feasible to predict the incidence of Botrytis rots of grower lines, it is too time consuming to manually assess every fruit for segregation purposes.

2.3.2 Soft Patches

Soft patches (SP) are localized soft and water soaked areas on the fruit surface (Davie, 1997). Compression and impact damages are the direct causes of SP (Davie, 1997). High incidence of SP are associated with severe compression and impact damages, low FF at the damage sites, low dry matter content, low Ca and Mg but high P concentrations as measured on a whole fruit basis (Davie, 1997; Benge, 1999).

2.3.3 Low temperature breakdown

Low temperature breakdown (LTB) is a physiological disorder that can affect 'Hayward' and some selections of *A. chinensis* fruit after several months of cold storage. One of its symptoms manifests as the grainy appearance of the outer pericarp, followed by water soaking associated with extreme softening at the stylar end of the

fruit (Bauchot et al., 1999). LTB was observed in fruit harvested below 6.2% SSC and then stored at 0°C, whereas the symptoms were absent in more mature fruit (Harman, 1981). LTB was also found to differ significantly from grower line to grower line harvested at the same time and the relationship between LTB susceptibility and factors other than harvest maturity are not clear (Lallu, 1997).

2.3.4 Purple patches

Purple patches (PP) are purple, scald-like areas on the skin of 'Hayward' kiwifruit. This disorder was thought to be a kind of chilling injury (CI, Lallu, 1997). However, more recent studies have suggested that the disorder is more likely to be caused by latent chemical burn resulting from preharvest sprays (Feng et al., 2002b). Fruit that developed PP also showed granular tissue in the outer pericarp, the granular tissue normally limited to the local area underneath the scald rather than forming a ring like that of CI. Feng et al. (2002b) found that fruit with a hue angle (measured externally at harvest) lower than 78 were susceptible to PP. This criterion could be used for fruit segregation as the measurement of hue angle is quick, easy, and non-destructive.

2.3.5 Physiological pitting

Physiological pitting is a storage disorder that causes sunken pits and discoloration in the tissue immediately below the fruit skin (Mowat et al., 2002). This disorder of 'Hayward' kiwifruit was associated with low Ca and high K and P concentrations of the fruit (Ferguson et al., 2001) and that in 'Hort16A' is associated with a late harvest (Mowat et al., 2002), a severe weight loss, a short delay prior to, or rapid CO_2 establishment in CA storage (Lallu et al., 2003). Because physiological pitting is one of the main factors causing fruit loss after storage (Ferguson et al., 2001; Mowat et al., 2002; Lallu et al., 2003), developing segregation technologies for this disorder would be of great value to the kiwifruit industry.

2.4 Mechanism of fruit softening

Microscopic examination of the fracture surface following tensile test revealed two mechanisms involved in measured FF during fruit ripening. At harvest (FF around 80 N), all cells at the fracture surfaces rupture, suggesting that adhesion between neighbouring cells through the middle lamella is far greater than cell wall rigidity. This situation is gradually reversed during fruit ripening: after as few as 2 weeks storage at 0^{o}C, most of the giant cells at the fracture surface remain whole and by 6 weeks (FF around 27 N), all the giant cells and many of the small cells that surround the giant cells detach from each other rather than breaking open. The proportion of the small cells that break further decrease as storage duration extends to 29 weeks with a decrease in FF from 27 to 5 N. No broken cells can be seen after 29 weeks cool storage, indicating that adhesion between neighbouring cells through the meddle lamella has become far weaker than the cell wall rigidity (Harker and Hallett, 1994).

2.4.1 Changes in cell wall

Many researchers have studied the mechanism of fruit softening with respect to physiochemical properties of cell wall. The cell wall accounts for about 1.5% of the fresh weight of the outer pericarp tissue of kiwifruit at harvest (Redgwell and Percy, 1992). This consists of 40-50% pectic substances, 15-25% hemicelluloses, 25-35% cellulose and 1-7% protein. Relative amounts of each type of polysaccharide vary between the different fruit tissues, as does the onset of cell wall breakdown during ripening (Redgwell et al., 1992a & b).

Morphometric analysis of the cell walls indicates that fruit softening is associated with cell wall swelling. Cell wall thickness of outer pericarp cells of 'Hayward' kiwifruit remains unchanged during the rapid phase of softening when the most significant changes in fruit firmness occur (75 to 31 N). The wall thickness then increases 3-4 fold during the slow softening phase and reaches its maximum when fruit soften to 15-5 N (Harker and Hallett, 1994).

Cell wall swelling is caused by solubilization of cell wall polysaccharides. Histochemical staining of kiwifruit cell walls for pectins and hemicelluloses showed a

marked decrease in intensity after fruit had partially softened to less than 30 N before the climacteric (Hallett et al. 1992). This might have resulted from *in situ* modifications to the polymers rather than from loss of the polymers themselves because the total amount of cell wall polysaccharide decreases only slightly (Redgwell et al. 1990; Hallett et al. 1992). However, more recent studies using sequential separation of different chemical components of kiwifruit tissue indicated that a substantial portion of the cell wall polysaccharide was hydrolyzed to soluble sugars in the early phase of ripening (Terasaki et al., 2001b). This work also indicated that a decrease in the molecular weight of xyloglucan was correlated with a loss in fruit tissue elasticity, while the changes in the molecular weight of pectin corresponded with changes in viscosity of fruit tissue. Cell wall swelling was also postulated to be the result of changes to the viscoelastic properties of the cell wall during pectin solubilisation, i.e. movement of water into voids left in the cellulose-hemicellulose network by the solubilised pectin (Redgwell et al., 1997a).

Plasmodesmatal regions of the cell wall may play an important role in maintaining fruit firmness after long-term storage. These regions were found to be different in composition to other wall areas (Sutherland et al., 1999). They did not swell and maintained staining intensity during fruit softening (Hallett et al. 1992).

2.4.1.1 Pectin

Changes to pectin during ripening involve at least three processes: solubilization of pectin, depolymerization of pectin, and loss of galactose side chains (Redgwell et al. 1997b). During the rapid softening phase, a large amount of pectin is solubilised in cell wall without being extensively changed or chemically altered. By the start of the slow softening phase, most of the pectin has been solubilized, but the degradation of solubilized pectin to smaller polymers continues (Redgwell et al, 1990).

Pectin methyl esterase (PME) and polygalacturonase (PG) act sequentially in pectin solubilization and depolymerization. In ethylene treated kiwifruit, PME activity increases briefly at the beginning of the rapid softening phase and then decreases as the fruit softens further through to the slow softening phase. PG activity, on the other hand, increases to reach a maximum by the slow softening phase (Redgwell et al., 1990). The rapid softening of kiwifruit in response to ethylene treatment was thought to be initiated

by an induction of PME activity, causing increased de-esterification of cell wall pectins, followed by degradation of solubilized pectin (Wegrzyn and MacRae, 1992). The rapid increase in PG activity observed in late storage period was associated with gradual dissolving and disappearing of the middle lamella. Kiwifruit clones having better storability exhibited a slower increase of PG activity (Wang et al., 2000).

Galactose loss appeared to be irrelevant to kiwifruit softening. When outer pericarp discs of 'Hayward' kiwifruit were treated with galactose (50 mM), galactose loss from the cell wall was completely inhibited for 24 h; reduced between 24 and 72 h while the rate of disc softening and pectin solubilization were not affected. On the other hand, discs treated with aminooxyacetic acid, an inhibitor of ethylene biosynthesis, did not soften or show any signs of pectin solubilization after 72 h, but did show a loss of galactose from the cell wall. It was suggested that the loss of cell wall-associated galactose and pectin solubilization in ripening kiwifruit are separate processes, and that galactose loss, in part, may be independent of ethylene (Redgwell and Harker, 1995).

2.4.1.2 Cellulose and hemicelluloses

Cellulose and hemicelluloses also have a significant role in kiwifruit softening. The size of hemicelluloses decreased during the rapid softening phase (Redgwell et al, 1990) and a decrease in the molecular weight of xyloglucan was observed during cell wall swelling (MacRae and Redgwell, 1992). It has been suggested that wall swelling may be caused by changes in the cellulose-hemicellulose interaction and that swelling or loosening of the wall may itself be a factor in the release or solubilization of pectic polysaccharides (Redgwell et al, 1991). Consequently, xyloglucan endotransglycosylase (XET), which can specifically modify the xyloglucan of cellulose and hemicellulose, may have a key role early in fruit ripening (Redgwell and Fry, 1993).

2.4.1.3 Cell wall synthesis

Cell wall degradation is a reversible biochemical process rather than an irreversible one. Recent studies indicate that softening 'Hayward' kiwifruit (10.8% soluble solids content, 88 N firmness) labeled with $^{14}CO_2$ and fruit discs labeled with ^{14}C-glucose incorporate some amount of radioactivity into cell wall materials during

ripening, indicating that mature and ripe kiwifruit are able to synthesize cell wall polymers (Redgwell, 1996). Therefore, the slowing down of the softening process after the rapid softening phase may result from equilibration between cell wall degradation and synthesis rather than a sole effect of enzyme activity. This knowledge also bridges the gap between starch metabolism and cell wall materials because they share many common materials, such as sucrose and fructose.

2.4.2 Starch

Starch degradation may play a crucial role in the early events of kiwifruit softening (Arpaia et al., 1987) because it occurs coincidently with the rapid softening phase when cell wall properties change very little. Starch degradation may affect fruit firmness through cell turgor changes (Arpaia et al., 1987; Redgwell et al., 1990; Redgwell and Percy, 1992; Bonghi et al., 1996). However, the relationships among fruit firmness, osmotic and water potentials, and cell turgor remain to be elucidated.

2.4.3 The role of ethylene

Ethylene has long been regarded as a hormone that initiates fruit ripening, a process which is usually thought to include fruit softening (Arpaia et al., 1994). Kiwifruit is a climacteric fruit which softens rapidly when exposed to even minute (i.e. 0.01ml l^{-1}) concentrations of ethylene (Mitchell, 1990; Arpaia et al., 1994). However, kiwifruit have been found to soften from about 90 N to 12 N without changes in endogenous ethylene production (below 0.2 $\mu l\ kg^{-1}\ h^{-1}$), 1-aminocyclopropane-1-carboxylic acid (ACC) concentration or ACC oxidase (ACO) activity. Autocatalytic ethylene production only occurred as fruit softened from 12 N to eating ripe (6-8 N; Kim et al., 1999).

The role of ethylene in kiwifruit softening is not fully understood. 'Hayward' kiwifruit allowed to soften on the vine softened in the same manner as harvested fruit in storage that had softened after an ethylene treatment. Pectin solubilization, galactose loss and cell wall swelling were major cell wall events which accompanied softening in both situations, although the relative timing of each process differed from those occurring in the ethylene-treated fruit (Redgwell and Percy, 1992). Genetic studies

revealed that polygalacturonase (PG) is coded by three genes (CkPGA, B and C). Expression of CkPGA and B was detected only in fruit producing detectable endogenous ethylene while CkPGC gene expression was readily detected during fruit development and in fruit harvested prior to the onset of softening when endogenous ethylene was not detected. However, CkPGC gene expression increased dramatically when fruit passed through the climacteric phase (Wang et al., 2000). It is still not clear whether CkPGC gene expression was promoted by ethylene at undetectable levels or it was an ethylene independent process. It was also noticed that kiwifruit becomes more sensitive to ethylene with time during maturation and storage at 0°C, possibly because ethylene receptors become more sensitive or more numerous (Kim et al., 1999).

2.5 Mathematical description of fruit softening

2.5.1 Empirical models

Various empirical models have been used to describe the softening curve (Pyke et al, 1996; Crisosto et al., 1999; Lallu et al, 1999; Benge, 1999; Benge et al., 2000). Simple models, such as complementary Michaelis-Menten type, exponential and complementary Gompertz models were unable to characterize firmness changes with sufficient accuracy. More complex models, such as segmented jointed Michaelis-Menten type, inverse exponential and quartic polynomial models can characterize firmness changes with good accuracy, but these models involve 4-5 parameters that are difficult to estimate accurately when the number of data points is limited. Therefore, none of the above models provided a standard curve that could be useful as a predictive model for firmness in storage (Benge, 1999; Benge et al., 2000).

2.5.2 Mechanistic models

Although changes in cell wall ultra-structure, cell wall materials and enzymes associated with kiwifruit softening are well documented (MacRae and Redgwell, 1992; Harker and Hallett, 1994), the kinetics of these physical and chemical changes have rarely been incorporated into a mechanistic model to describe the softening process until recently when a mechanistic model based on a conceptual enzymatic breakdown of

fruit firmness was proposed. Hertog and Jeffery (2000) assumed that fruit softening is a breakdown process catalysed by an active enzyme (Enz), which is formed from its precursor (Enz_{pre}) through ethylene-catalyzed activation. The mechanistic fruit-softening model was deduced as an analytical solution of the differential equations describing the assumed reactions. It was also hypothesized that the reactions were the same for all kiwifruit. Consequently, the rate constants and their activation energies could be estimated in common for all grower lines (i.e. fruit from different growers and/or treatments) and the differences in softening behaviours of different grower lines was determined by initial fruit firmness (F_0), and initial concentration of Enz (Enz_0). As F_0 can be measured at harvest, Enz_0 became the only parameter to be estimated for each grower line.

Based on data collected by Lallu et al., (1999) from 27 growers, Hertog and Jeffery (2000) found that 62 % of the observed variation in Enz_0 could be explained by five at-harvest attributes (initial firmness, nitrogen, potassium, magnesium and reducing sugar concentrations). However, data collected in year 2001 on fruit from 10 growers failed to confirm such a relationship. However, the methodology of relating at-harvest attributes to a parameter (i.e. Enz_0) of a mechanistic model of fruit softening is of great value for future STP study.

2.6 Factors affecting STP

2.6.1 Soluble solids content

Soluble solids content (SSC, %), measured by a refractometer increases with maturity and has long been used as an index for harvest maturity of 'Hayward' kiwifruit (Asami et al., 1988). In New Zealand, 6.2% SSC (mean of an orchard or a block of an orchard) is the minimum maturity at which kiwifruit destined for export can be harvested (Richardson et al., 1997a & b; Watt, 1999). Fruit from an orchard may be harvested over several days at a SSC higher than 6.2% because of staff availability, packinghouse requirements or delays by wet weather.

The SSC measured at harvest does not necessarily represent the "age" of the fruit because the timing of increases in SSC differs between years and orchard locations (Sawanobori and Shimura, 1990). Temperature is the most important preharvest factor

affecting SSC of kiwifruit (Pailly, 1996; Hall and Mcpherson, 1997). SSC at harvest is determined by temperature conditions shortly before harvest rather than that of the whole fruit development period since flowering (Hall and Mcpherson, 1997).

2.6.1.1 Variation of at-harvest SSC

Average SSC of grower lines measured at harvest vary between orchards, blocks and harvest dates (Hopkirk et al., 1986). Considerable variation in SSC occurs in fruit from different positions on a kiwifruit vine. This leads to SSC variation within fruit harvested from the same orchard or block at the same day (Hopkirk et al., 1986; Smith et al., 1994; Pyke et al., 1996). The within-vine variation can be attributed to flowering time (Hopping, 1990; Cruz-Castillo et al., 1992), microclimatic conditions (Suzaki and Aoki, 1986; Tombesi et al., 1993; Smith et al., 1994), and differences in local carbon acquisition caused by variable crop load and leaf-fruit ratio (Costa et al., 1993; Richardson et al., 1994; Costa et al., 1996; Samanci, 1997; Famiani et al., 1997).

2.6.1.2 Changes in SSC during the postharvest stage

Soluble solid content of kiwifruit continues to increase after harvest in the same manner as it does on the vine (Kempler et al., 1992). The rate of change in soluble solids during storage at 0 ^{o}C follows a simple exponential function with a time constant of 20 days (Richardson et al., 1997b). SSC at ripe-to eat stage (when FF falls to 5-10N) is positively related to SSC at harvest (Pringle et al., 1991). But the relationship is not strong enough to be a predictor of SSC at consumption. Instead, fruit density and DM at harvest are reliable predictors of SSC at ripe-to eat stage. The standard errors of these predictions are at the order of 1% (Richardson et. al., 1997a). This implies that the percentage of DM not solubilized at eating ripe is almost constant across different fruit.

Increase in SSC results mainly from the breakdown of starch and pectin (Asami et al., 1988). SSC measured with fruit juice must be adjusted to a whole fruit basis to allow comparison with other fruit constituents (Anon, 1995). Jordan et al. (2000) found that soluble solids in whole fruit (SSF) was greater than the sum of measured sugars by about 4-5 % of fresh weight in unripe fruit, and 5 –6 % in ripe fruit. The magnitude of the difference between SSF and the sum of measured sugars depends on fruit density

(indication of DM) with larger differences associated with higher fruit density (Jordan et al., 2000). Some of the difference was attributed to three fruit acids (citric, quinic and malic acids), typically 1-2 % in total (Beever and Hopkirk, 1990). This left 2-4 % of the SSF unidentified (Fuke and Matsuoka, 1982; Jordan et al., 2000). Other soluble components such as potassium, nitrate or soluble pectin that increase during fruit ripening could account for some of the unidentified SSF (Jordan et al., 2000). For example, published values of total pectic substances in kiwifruit ranged from 0.17 to 0.98% fresh weight, depending on the source of the fruit sample and fruit ripeness which affects pectic constituents and the ease at which they can be extracted (Beever and Hopkirk, 1990).

2.6.1.3 SSC and STP

Harvest maturity is a major factor affecting fruit firmness both at harvest and after storage. Early harvested fruit with low SSC are generally firmer at harvest but softer after long-term storage compared to late harvested fruit with high SSC (Weet, 1979; Harman, 1981; Harman et al., 1982; Crisosto et al., 1984; Mitchell et al., 1992; Abdala et al., 1996; Costa et al., 1997a). However, harvesting too late can enhance fruit softening (to less than 50-60 N) to the detriment of storage life and quality (Ravaglia et al., 1995). The soft fruit problem of early harvested fruit may be attributed to development of LTB. LTB symptoms were observed in fruit harvested below 6.2% SSC and then stored at 0°C, whereas the symptoms were absent in more mature fruit (Harman, 1981). However, it could be argued that chilly nights during the late in the harvest season might have induced the resistance to LTB of late harvested fruit. This argument is supported by recent work in Greece that shows evaporative cooling with an over-tree mist system before harvest reduced the severity of LTB (Sfakiotakis et al., 2002).

The optimal SSC for the best STP is not clear. Most researchers found that fruit with SSC 6.2-6.5% stored well (Weet, 1979; Harman, 1981) while a few recommended higher values of just below 7% (Asami et al., 1988) and 7.5-9.5 % (Ravaglia et al., 1995). Given that SSC varies considerably between and within grower lines (Hopkirk et al., 1986; Smith et al., 1994; Pyke et al., 1996), the variation in SSC would contribute to STP variation. Further work is needed to clarify the relationship between SSC and STP.

2.6.2 Dry matter

Dry matter refers to fruit contents other than water. It includes soluble solids and insoluble solids, such as starch, cell wall materials and membranes. The proportion of insoluble solids changes dramatically after harvest due to the degradation of starch and cell wall materials while the total amount of dry matter remains almost constant. Loss of dry matter due to respiration and production of volatile materials such as ethylene and ethanol is negligible unless fruit is stored at high temperatures and becomes over-soft and/or rotten (Spraggon, 1988; Beever and Hopkirk, 1990).

Dry matter content is an important quality attribute of kiwifruit. Dry matter content at harvest minus 3% is the expected SSC at eating ripe stage. Fruit with DM above 19% (equivalent to 16% SSC) met consumer ideals for flavour, sweetness, and acid intensity and sweetness to acidic balance. Therefore, DM is now widely accepted as an index for harvest maturity (Watt, 1999) and is the target parameter for the near infrared (NIR) and density grading equipment currently under development (Jordan et al., 1997; Jordan et al., 2000).

2.6.2.1 Measurement of DM

Oven drying is a generally accepted method for determining the DM of kiwifruit. The disadvantage of this method is the long time (about 20 h) taken to complete the test. DM values using the microwave oven showed a good agreement with those of oven drying method. Microwave drying only required 25-30 minutes per sample and is recommended as a routine test for fruit quality (Spraggon, 1988; Ragaozza and Colelli, 1990). Infrared drying of kiwifruit also gave the same level of accuracy as oven drying, but with a reduced determination time. This method was comparable with microwave drying in terms of time, but had the advantage of continuous recording of the drying progress through to the final dry weight (Fenton and Kennedy, 1998). Many scientific studies have used the freeze-dry method to measure DM. This method tends to result in slightly (0.6% FW on average) higher DM than the oven-dry method (Jordan et al., 2000) due possibly to the loss of volatile materials at high temperatures (Dulphy et al., 1975).

2.6.2.2 Variation in DM

DM varies considerably between and within grower lines (Watt, 1999; Jordan et al., 2000; Amos and Mowatt, 2002). A survey carried out in New Zealand covering approximately 1700 grower lines produced in 1998 indicated that orchard means for DM were in range 13.0-22.5% and even greater variation were observed between individual fruit within each grower line (Watt, 1999). Similar results were observed in a recent survey involving 75 grower lines of 'Hayward' kiwifruit from 10 growing regions in New Zealand during the 2001 postharvest season (Amos and Mowatt, 2002).

DM increases during fruit development on the vine. The increase in DM during the late stages of growth, especially for fruit from the upper canopy, could largely be accounted for by the large build-up of starch during that period. Harvest time and factors affecting carbon acquisition are major causes for DM variation (Smith et al., 1995).

2.6.2.3 DM and storage potential

A direct link between DM and SL or fruit firmness at the end of storage is not seen from the literature. Given that DM increases with late harvest (Smith et al., 1995), it is logical to believe DM is important to STP through harvest maturity. Tagliavini et al. (1995) reported that firmer fruit at the end of storage had higher SSC than softer fruit. This implies that fruit with a high DM would last longer than a low DM fruit because a high SSC at the end of storage indicates a high DM at harvest (Watt, 1999). Furthermore, fruit with storage disorders such as soft patches were found to have a low DM (Davie, 1997).

2.6.3 Fruit size

Fruit size is an important attribute of all fruit crops, particularly for kiwifruit where fruit are packed by size and payment of export fruit is based on size (Richardson et al., 1997a; Snelgar et al., 1992; Hall et al., 1996). Both weight and volume are valid expressions of fruit size for 'Hayward' kiwifruit because they are interchangeable for most purposes (Hall et al., 1996). Fruit dimensions can also be used because they are

closely related to weight and volume (Green et al., 1990; Snelgar et al., 1992; Tombesi et al., 1994).

2.6.3.1 Variation in fruit size

Mean fruit volumes for 'Hayward' observed in the field survey in New Zealand during 1987-1989 ranged from less than 85 cm^3 to more than 130 cm^3. The variation was not consistent across years or sites (Hall et al., 1996). Individual fruit size varies considerably within a vine due to the position of cane along the cordon and the shoot position along the cane. Fruit size also differs within a shoot, and to a lesser extent between fruit borne on different shoots within a cane (Desilva and Balli, 1997).

Most of the variation in fruit size is established within 50 days after flowering (Hall et al., 1996). The variation is largely associated with seed number or seed dry weight per fruit (Volz et al., 1992). Insufficient pollination (Pan et al., 1994; Lescourret et al., 1997; Park and Park, 1997) and overloading of the vines with fruit (Vasilakakis et al., 1997) are the most important factors related to small fruit size. Other possible contributory factors include flowering time (Smith et al., 1994), irrigation (Reid et al., 1996), application of fertilizers (Testoni et al., 1990; Vasilakakis et al., 1997; Tagliavini et al., 1995) and plant growth regulators (Sive and Resnizky, 1987; Lotter, 1992; Antognozzi et al., 1993; Patterson et al., 1993; Costa et al., 1997b; Fang et al., 1996; Ohara et al., 1997), mulching (Costo et. al, 2003b), training and pruning (Manson et al., 1991; Smith et al., 1994; Miller et al., 2001). Therefore, large fruit are produced when all or most of the above conditions are favourable, particularly during the early fruit development stage. The distribution of fruit weight can be estimated based on state variables related to orchard design (including plant spacing and male:female plant ratio) and pruning strategy under various pollination practice (Testolin and Costa, 1992).

2.6.3.2 Fruit size and STP

Contradictory effects of fruit size on STP were found in the literature. Crisosto et al. (1999) reported that large 'Hayward' kiwifruit softened at slower rate compared to smaller fruit in cool storage. However, Reid et al. (1996) observed that smaller fruit resulting from regulated deficit irrigation remained firmer during storage compared

to larger fruit produced under irrigated conditions. Similarly, large fruit resulting from nitrogen fertilizer had a negative effect on fruit firmness measured during storage (Testoni et al., 1990). Given that high DM normally occurs in medium sized fruit while low DM normally occur in either small or large fruit (Bollen et al., unpublished), the association between fruit size and fruit firmness may reflect the effect of DM rather than a causal effect between fruit size and firmness.

2.6.4 Fruit colour and flesh colour

Colour is an important consideration in assessing the quality of horticultural commodities (McGuire, 1992). The Minolta Chroma Meter CR-200 is most commonly used to provide an objective colour determination. It gives measurements of L, a, b or in some other colour parameters (colour space) such as L value (L, lightness or luminosity), hue angle (H=arctangent of b/a), and chroma ($C = \sqrt{a^2 + b^2}$; Iglesias et al., 1999). Recent machine vision systems with colour image processing capability and a multi layered neural network-based software system offer great potential for online fruit sorting based on surface colour (Shibata et al., 1996; Abbott, 1999).

The fruit colour of apple, pear and cherries were found to be related to harvest maturity and many preharvest conditions such as physiological state of the tree, canopy position, light quality (particularly UV), temperature, fertilizer, irrigation and application of ethylene, abscisic acid, and gibberellins (Saure, 1990; Lancaster, 1992; Bible and Singha, 1993; Kootstra et al., 1994; Viljoen and Huysamer, 1995; Reay and Lancaster, 2001). Consequently, it is reasonable to believe colorimeter values measured at harvest can be related to storage behaviours because storage behaviours are related to harvest maturity and the preharvest conditions. This is highlighted by D'Souza and coworkers (1994) who found close correlation between superficial scald intensity of 'Rome Beauty' apples after storage and fruit chromaticity values measured at harvest.

'Hayward' kiwifruit has brown skin and green flesh at harvest. The green colour of kiwifruit flesh is due to the presence of chlorophyll in the ripe fruit (Ferguson, 1990). Vines with dense canopies produced fruit with lighter, more vivid, more yellow/brown skin but lighter, less vivid and less green flesh (Snelgar et al., 1998). However, shading vines does not affect either fruit colour or chlorophyll concentration of the fruit (Snelgar

and Hopkirk, 1988). This indicates that local shade rather than overall vine shade is responsible to variation in both fruit colour and flesh colour.

Flesh colour could be used in STP studies as an indication of fruit ripeness. Kiwifruit flesh partly loses its green colour and becomes dull green during fruit ripening in storage (Asami et al., 1988). Storage treatments such as controlled atmosphere (CA) and ultra low oxygen (ULO) that retard fruit ripening also retard flesh degreening compared with air storage (Antunes and Sfakiotakis, 1997).

2.6.5 Minerals

2.6.5.1 Assimilation of minerals to kiwifruit

The content of all minerals in the fruit increase throughout the season, particularly during the first 8 weeks of growth, corresponding to the cell division phase of fruit development (Clark and Smith, 1988).

The concentration of minerals declines during fruit development and maturation due to the dilution associated with growth in fruit size and accumulation of carbohydrates. Cu, Fe, N, P, K, S and Zn concentrations in kiwifruit flesh (seeds inclusive) declined sharply during the first 8 weeks of growth, reaching values that remained relatively constant, or declined only gradually, until harvest. B, Ca, Mg and Mn concentrations in the flesh declined steadily throughout the season (Clark and Smith, 1988).

2.6.5.2 Within fruit distribution of minerals

Minerals are not evenly distributed among different tissues of a fruit. Calcium (Ferguson, 1980; Clark and Smith, 1991) and boron (Sotiropoulos et al., 1998) concentrations are higher at the basal end than that at the distal end of the fruit while K and Mg did not show such a lateral distribution pattern (Ferguson, 1980). The skin and seeds (with surrounding flesh) had the highest concentrations of Ca and Mg (Ferguson, 1980) while N, P and K concentrations in the flesh were generally higher than corresponding concentrations in skin (Ferguson, 1980; Clark and Smith, 1988).

The within-fruit gradient of minerals is important for the study of STP

in two ways. First, some storage disorders occur more frequently in particular part of a fruit. For example, LTB (Harman, 1981; Lallu, 1997; Bauchot et al., 1999) and PP (Feng et al., 2002b) usually occur in distal end of the fruit where mineral concentrations are low. Second, the within-fruit gradient of minerals should be considered when preparing fruit samples for mineral analysis (Feng et al., 2002a).

2.6.5.3 Minerals and STP

Some mineral ions, particularly Ca (Huang et. al, 1999) and B (Hu and Brown, 1994) have a major effect on the structure of the pectin matrix of cell walls. Fruit low in Ca soften more quickly than those that have high Ca concentrations (Poovaiah et al. 1988). Preharvest Ca sprays and postharvest Ca dips can raise the Ca concentration in apples from 1.5 mg per 100 g fresh weight (control) to 2.2 mg per 100 g fresh weight ($CaCl_2$ spray) and 2.4 mg per 100 g fresh weight (post harvest Ca vacuum infiltration), leading to reduced bitter pit, a retention of firmness, a delay in the onset of senescence and a general improvement in fruit quality after storage (Poovaiah et al., 1988; Hewett and Watkin 1991).

Variation in STP of kiwifruit have also been shown to be related to mineral nutrition, especially N and Ca. Generally, fruit high in N and/or low in Ca have been found to store less well than fruit low in N and / or high in Ca (Prasad and Spiers, 1991; Banks et al., 1995; Lallu and Yearsley, 1995; Benge 1999; 2000). After storage, firm fruit were frequently found to have had significantly higher Ca and Mg concentrations than soft fruit (Tagliavini et al., 1995). Calcium treatment inhibited pectin solublization through inhibiting PG and PME activities (Lee et al., 2001). However, total endogenous Ca levels in kiwifruit from several orchard sources in Auckland were not correlated with fruit firmness during storage, and grower lines of fruit that became particularly soft during storage had Ca concentrations similar to or even higher than those in grower lines that had remained firmer (Hopkirk et al., 1990). This indicates that the effect of Ca on STP might be masked by other influential factors.

Smith et al. (1994) used principal component analysis (PCA) on the mineral concentrations and postharvest attributes of kiwifruit and concluded that individual elements could not be considered in isolation but rather in groups of elements. N was grouped strongly with P, S, K, and Cu, while Ca was linked with a second group that

included Mg and Zn. These two groups were negatively related to one another. However, these results failed to confirm the effects of N and Ca concentrations on fruit firmness after storage as observed by other researchers (Prasad and Spiers, 1991). Smith et al. (1994) suggested that the relationship between mineral concentrations and storage behaviours may be a reflection of vine positional effects rather than causative effects.

Mineral concentrations also affect the incidence of storage disorders. Benge (1999) found strong relationships between incidence of soft patches after storage and a predictor combining SSC, FF, Ca, Mg and N measured at harvest when fruit were sampled directly from orchards with fruit position and size standardized. But the relationship was not validated by subsequent experiment where fruit was sampled from bins at packinghouses. A study on physiological pitting (PPT) in 'Hayward' kiwifruit indicated that orchards growing fruit with a high risk of PPT tend to have soil with relatively low PH, Ca, cation exchange capacity (CEC) and base saturation values, but high P (Ferguson et al., 2001). Fruit with high risk of PPT tended to have relatively low Ca and Mg, but high P concentrations in the flesh. Fruit from small indeterminate fruiting laterals with few leaves and low leaf:fruit ratio had significantly lower Ca, higher P and higher PPT levels. However, it is not clear whether these nutrient conditions were a cause of PPT, or indicators of other physiological characteristics that predisposed the fruit to PPT (Ferguson et al., 2001).

2.6.6 Respiration and ethylene production

2.6.6.1 Respiration

Respiration is an important metabolic process that extracts energy and intermediate substrates required by living tissues from carbohydrates.

In preharvest stage, respiration rate is the highest in young kiwifruit and declines to a minimum around 20 weeks after flowering, followed by a slight increase before harvest (Sawanobori and Shimura, 1990). At postharvest stage, it is desirable to have respiration rate at the lowest possible level to preserve carbohydrates and freshness (Biale and Young, 1981). Kiwifruit is a special climacteric fruit with climacteric rise of respiration occurring at very late fruit ripening stage (Beever and Hopkirk, 1990;

Kim, 1999; Ritenour et al., 1999; Wang et al., 2000). The critical threshold firmness at which the climacteric commences appears to vary between 7 N and 20 N, depending on where the experiments were undertaken. This indicates that preharvest factors and/or growing conditions might influence in some way the firmness threshold where ethylene production rate increases rapidly. This also implies that respiration and fruit softening are independent processes because most of the fruit softening occurs before the climacteric rise in respiration. However, kiwifruit clones that stored best had a lower respiration rate (Wang et al., 2000) and cumulative CO_2 production was quantitatively related to quality deteriorations of several fruit, including kiwifruit (Hertog and Nicholson, 2001). Therefore, the relationship between respiration and STP merits further investigation.

2.6.6.2 Ethylene

Kiwifruit soften markedly when exposed to even minute (i.e. 0.01 μl L^{-1}) concentrations of ethylene (Arpaia et al., 1987; Mitchell, 1990), but the fruit itself produces little ethylene until it softens to a FF less than 14 N (Hyodo and Fukasawa, 1985; Bonghi et al., 1996; Kim, 1999; Ritenour et. al 1999; Feng et al., 2003b). Ethylene production increases during post-harvest ripening (Asami et al., 1988), and the ethylene climacteric is a late event occurring at a FF about 10 N (Bonghi et al., 1996). The increased rate of ethylene production was accompanied by increased internal ethylene concentration, a rise in respiration, increased soluble solids content, and flesh softening (Hyodo and Fukasawa, 1985).

The ethylene production rate of kiwifruit also depends on cultivar (Chen et al., 1997), harvest maturity (Kim, 1999; Ritenour et. al 1999), storage temperatures (Field, 1985) and postharvest treatment (Stavroulakis and Sfakiotakis, 1993). The ethylene climacteric occurred 14 days after harvest at 20°C in 'Hayward' kiwifruit, but much earlier for cultivars with shorter storage life, such as 'Zaoxian' (Chen et al., 1999). Fruit harvested at advanced maturity tend to have a higher ethylene production rate than fruit harvested less mature (Kim, 1999; Ritenour et. al 1999). Ethylene production increases as temperature increases with a Q_{10} value of about 2 between 20°C and 40°C. Further increases in temperature above 40°C resulted in a decline in ethylene production rate (Field, 1985). Auto-catalytic ethylene production induced by propylene increased in

'Hayward' kiwifruit as temperature increased from 17 to 35°C (Stavroulakis and Sfakiotakis, 1993). Exposing kiwifruit to low temperatures (0-10 °C) for 12 days increased ethylene biosynthesis and ripening compared with fruit held continuously at 20°C. The enhanced ethylene biosynthesis was due to increased 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) and ACC oxidase (ACO) activity immediately upon re-warming of the fruit (Antunes and Sfakiotakis, 2002a).

Individual fruit of the same cultivar harvested at the same time and stored under the same conditions vary considerably in amount of time before the onset of ethylene production following removal from storage (Hyodo and Fukasawa, 1985; Kim, 1999; Ritenour et. al 1999). Time to ethylene production and variability within the batch decreased as the time of storage increased (Hyodo and Fukasawa, 1985; Kim, 1999), but work by Feng et al. (2003b) suggests that the development of storage disorders (e.g. fungal rots and LTB) can also influence fruit to fruit variation in the onset of ethylene production.

Ethylene enhances fruit softening by activating enzymes involved in cell wall and starch degradation (Redgwell et al., 1990; Bonghi et al., 1996; Redgwell, 1996). The effects of ethylene treatment on kiwifruit softening are well documented (Stavroulakis and Sfakiotakis, 1993; Ritenour et. al 1999), but have rarely been used to predict storage life of the fruit until recently when Hertog and Jeffery (2000) included the concentration of ethylene treatment as one of the input variables to predict fruit firmness using their newly developed mechanistic fruit softening model. This model facilitates a quantitative link between external ethylene concentration and STP of 'Hayward' kiwifruit, although it ignores the variability of internal ethylene concentration.

Ethylene produced by fruit has a significant influence on fruit ripening. 'Bruno' kiwifruit stored at -1°C in sealed polyethylene bags of different thickness generated 0.07-0.35 $\mu l\ L^{-1}$ ethylene and 0.8–4% CO_2 inside the packages during 6 months of storage. The beneficial effect of the increased CO_2 was not observed unless ethylene was removed from the storage atmosphere by an ethylene absorbent (Ben-Arie and Sonego, 1985). New Zealand kiwifruit are commercially packed in polylined trays, the possibility of ethylene accumulation inside the package should be considered in STP studies.

2.7 Non-destructive measurement of fruit attributes

There are many non-destructive methods developed for measuring various attributes of intact fruit (Watada, 1989; Costa et al, 2003a). This review focuses on the near-infrared (NIR) spectrometry and X-ray technologies that have been evaluated in this thesis.

2.7.1 Near-infrared spectroscopy

Typical NIR spectroscopy studies the spectral property at a wavelength region of 780-2,500 nm (Williams & Norris, 2001). NIR spectroscopy has long been used in chemistry to study characteristics of chemical compounds and has recently been extended to measure various attributes of intact fruit (Richard and Ozanich, 1999). This method is the most developed non-destructive assessment of internal composition and texture of intact fruit with regard to instrumentation, applications, accessories and software packages (Guthrie and Walsh, 1997). As in many cases, NIR spectrometers also measure spectral property of visible wavelength ranges (i.e. 400-700 nm), it should therefore be described as visible-near infrared spectroscopy (VNIR; Williams & Norris, 2001).

2.7.1.1 Principle of VNIR

The principle of this method is that different chemicals have different absorption spectra in the VNIR region and VNIR spectra of a fruit can be affected by both the chemical components and the physical properties of the fruit (McGlone and Kawano, 1998). Fruit tissue consists of water, carbohydrates and proteins which have large numbers of NIR-active chemical groups such as CH, OH, NH, and C=O, each of which contributes its own set of overtone and combination bands (Williams & Norris, 2001). Spectral bands selected by previous studies for measuring different components and attributes are listed (Table 2.1).

Table 2.1 Important VNIR wavelengths used for measuring different attributes

Attributes	Victor peak positions	Matrix	Reference
Water	830-840*, 938, 958**, 970-990,1010-1030;	Water	Williams & Norris, 1987
	940-970, 970	Kiwifruit	Mowat & Poole, 1997
			McGlone and Kawano, 1998
	970, 1180, 1444	Apple	Lammertyn et al., 1998
Dry matter	838±20, 886±12, 924±10, 942±2, 960±10, 986±11, 1052±8, 1048±12	Water solution	Williams & Norris, 1987
	8 bands from 800-1100	Kiwifruit	McGlone & Kawano, 1998
			Guthrie & Walsh, 1997
Soluble solids content	842±10, 878±15, 936±10, 958±7, 984±12, 1016±4, 1052±15	Water solution	Williams & Norris, 1987
	5 bands from 800 to 1100	Kiwifruit	McGlone & Kawano, 1998
	872, 910	Peach	Slaughter, 1995
	6-12 bands from 380-1650	Apple	Lammertyn et al., 1998
	760, 788, 800-832, 866, 1232	Pineapple Mango	Guthrie & Walsh, 1997
	819, 835, 859, 867, 875, 883, 891, 899, 939, 947, 971, 987	Apple	Ventura et al., 1998
	2 bands range from 870 to 910	Peach	Peiris et al., 1998a
	780-980	Tomato	Peiris et al., 1998b
Carbohydrates	830-840, 870-890*, 900-930**, 970-990***,1010-1030,1053.	Water solution	Williams & Norris, 1987
Starch	901, 918	Water solution	Williams & Norris, 1987
Pectin	1900	Apple	Cho et al., 1992;
	980	Kiwifruit	McGlone and Kawano, 1998
Sucrose	838, 888, 913, 906, 878, 870 and 990	Water solution	Williams & Norris, 1987
	834, 884	Peach	Slaughter, 1995
	760, 918	Pineapple	Guthrie & Walsh, 1997

Sorbital	880, 894	Peach	Slaughter, 1995
Cellulose	905, 920	Water	Williams & Norris, 1987
Chlorophyll	670	Kiwifruit	Mowat & Poole, 1997
	680	Kiwifruit	McGlone and Kawano, 1998
	652, 774	Peach	Slaughter, 1995
Protein	1441, 1510, 1655, 1728, 1810	Beef & pork	Togersen et al., 1999
Lipids	1441, 1510, 1655, 1728, 1810	Beef & pork	Togersen et al., 1999
	850-1100	Salmon	Downey, 1996
Ca	1722, 1734, 1778, 2100, 2139, 2336, 2348	Animal feeds	Atanassova and Ilchev, 1997
P	1445, 1680, 1722, 1940, 1982, 2230, 2336	Animal feeds	Atanassova and Ilchev, 1997
pH	1607, 1127, 1402, 1437, and water bands	Apple	Lammertyn et al., 1998
Firmness	1900 (pectin band)	Apple	Cho et al., 1992
	14 bands from 800-1100	Kiwifruit	McGlone and Kawano, 1998
	3-13 bands (950-1650)	Apple	Lammertyn et al., 1998
Section drying	768, 960	Tangerine	Peiris et al.,1998c

* Indicative of the relative strength of an absorber with *** being the strongest.

± Indicative of absorbance bandwidth measured at half height.

2.7.1.2 Instrumentation and measurement procedure

The instrument for VNIR measurement includes two basic components: a detector (including a computer for controlling the detector and recording the spectra) and a light source. In addition, materials such as a $BaSO_4$-disc, Teflon block or white Halon tile are needed to take reference spectra for calibration purposes.

VNIR spectroscopy used for the measurement of intact fruit has three different modes of measurement (Fig 2.2; Schaare and Fraser, 2000). In the reflectance mode, the field of view of the detector includes parts of the fruit surface directly illuminated by the source (Mowat and Poole, 1997); in the transmission mode, the fruit surface viewed by the detector is diametrically opposite to the illuminated surface (Kawano, 1994; Miyamoto and Yoshinobu, 1995); while in the interactance mode, the field of view of

the detector is separated from the illuminated surface by a light seal in contact with the fruit surface (McGlone and Kawano, 1998; Osborne et al., 1993).

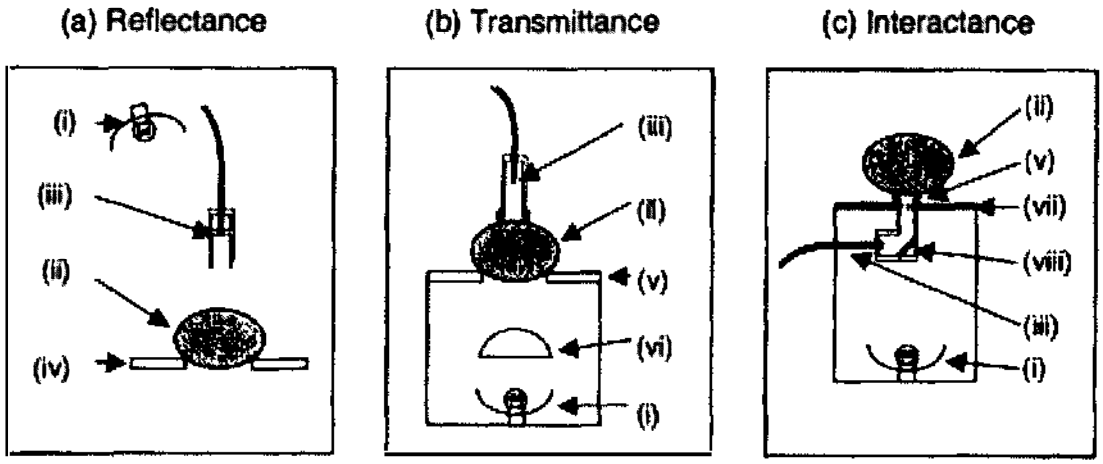

Figure 2.2 The apparatus used for measuring (a) reflectance, (b) transmittance, and (c) interactance spectra of kiwifruit, showing (i) the light source, (ii) fruit, (iii) fibre bundle leading to detector, (iv) black foam holder, (v) light seal, (vi) condensing lens, (vii) glass top, and (viii) mirror (Schaare and Fraser, 2000).

Reflectance mode measurements are the easiest to obtain because they require no contact with the fruit and light levels are relatively high. However, calibration may be susceptible to variations in superficial or surface properties of the fruit. Transmission mode measurements may also be made without contacting the fruit and may be less susceptible to surface properties and better for detecting internal disorders than reflectance mode measurements. However, the amount of light penetrating the fruit is often very small, making it difficult to obtain accurate transmission measurements at grading line speeds, particularly in conditions of high ambient light levels. Interactance mode provides a compromise between reflection and transmission modes in each of these characteristics, but obtaining a light seal may be problematic at the high conveyor speeds used in modern fruit grading systems (Schaare and Fraser, 2000).

Comparison between different measurement modes for estimating properties of the yellow-fleshed kiwifruit (*Actinidia chinensis)* suggests that interactance spectra provide the most accurate estimates of SSC, density and flesh colour (Schaare and Fraser, 2000).

2.7.1.3 Data analysis

Raw spectra contain background noise and are subject to instrument drift and baseline changes. Many pre-treatments have been developed to reduce background noise and to remove linear baseline changes between spectra (Williams & Norris, 2001). Log transformation of raw spectra against a baseline to generate absorbance spectra is an essential pre-treatment for VNIR data, while other pre-treatments, such as smoothing, differentiation (first derivative D_1 and second derivative D_2), standard normal variate transformation (SNV), multiplicative scatter correction (MSC) and Fourier transformation (FT) have also been useful (Geladi et al., 1985; Barnes et al. 1989; Schaare and Fraser, 2000; Mowat and Poole, 1997; Norris and Workman, 1997; Williams & Norris, 2001).

Relationships between targeted fruit attributes and derivative spectra could be established using the following procedures (Osborne et al., 1993; Schaare and Fraser, 2000; Williams and Norris, 2001):

- principal component analysis (PCA)
- multiple linear regression (MLR)
- multiplicative scatter correlation technique (MSC)
- partial least square method (PLS) and modified PLS method (MPLS)
- canonical discriminant analysis (CDA)

Finally, the established prediction model has to be assessed and validated by data from measurements of some other fruit. Criteria used for model assessment include (Osborne et al., 1993; McGlone and Kawano, 1998; Williams and Norris, 2001):

- coefficient of determination (R^2)
- standard error of prediction (SEP)
- root mean square error of prediction (RMSEP)
- bias (average difference between predicted and actual values)
- SDR (data set standard deviation divided by RMSEP)

2.7.1.4 Applications

NIR has been used successfully to measure several compositional attributes of intact horticultural produce including SSC (Richard and Ozanich, 1999), dry matter content (DMC), starch content (Weber et al., 1996), chlorophyll content (Slaughter, 1995; Zude-Sasse et al, 2002) and pH (Lammertyn et al., 1998). NIR has also been used to detect internal disorders of intact fruit (Peiris et al., 1998c). Mineral content (i.e. Ca and P contents) of poultry and pig feeds has also been estimated using NIR (Atanassova and Ilchev, 1997). However, the measurement of the mineral content of intact horticultural produce using NIR is yet to be explored.

In kiwifruit, NIR has been used successfully to estimate SSC (McGlone and Kawano, 1998; Schaare and Fraser, 2000) and DM (McGlone and Kawano, 1998) on intact kiwifruit with overall R^2 above 0.90. The estimation of firmness has been less accurate (the best $R^2 = 0.76$) possibly because there is insufficient pectin in kiwifruit (<1% by weight) for NIR to detect (McGlone and Kawano, 1998). Whole-fruit density and internal flesh hue angle of yellow-fleshed kiwifruit could be estimated from interactance spectra with R^2 of 0.74 and 0.82 respectively (Schaare and Fraser, 2000).

Instead of relating specific NIR spectral bands to compositional attributes, Mowat and Poole (1997) made direct use of canonical discrimination analyses (JMP software, SAS Institute Cary, NC, USA) based on 12 principal components (PCs) of VNIR reflectance at 550-990 nm to discriminate between kiwifruit berries with properties altered by preharvest treatments. Ninety nine percent of the fruit was correctly classified at harvest and 87% after storage. This is result is superior to that achieved using combinations of fruit weight, skin colour, DM and SSC, indicating that NIR spectra contain more information than sugar concentration and residual starch.

Despite the successes of the laboratory experiments mentioned above, application of NIR at commercial level has been less successful (McGlone et al., 2002). For example, when NIR spectrometric measurements were made with a fast low cost polychromatic spectrometer operating over the range 500-1100 nm, the predictive models for background colour, starch pattern index, SSC, FF, quantitative starch and titratable acidity of apples were significant in regression terms, typically explaining between 50 and 80% of the data set variance, but they were, nonetheless, very poor in prediction terms (McGlone et al., 2002). Most of the prediction models appear to be

primarily dependent on changes in the chlorophyll absorbance peak, which dramatically reduces in intensity during the progression of the fruit through the harvest period, rather than on the constituent or property of direct interest (McGlone et al., 2002).

In kiwifruit, when NIR spectra were taken with a commercial single beam NIR spectrometer (Ocean Optics S-2000, Giotto High Technology, Italy) in a reduced wavelength range (650-1200nm), R^2 of only 0.65 and 0.42 were achieved for SSC and FF respectively (Costa et al., 1999). These poor results may be attributed to the quality of commercial machinery and/or the ability of data analysis to deal with the high variation in commercial measurement.

2.7.2 X-ray technologies

X-ray radiography has long been used to detect internal split pits, insect-damage, mechanical damage, or diseases of seeds (Kamra, 1974 & 1976; Chavagnat, 1984 & 1985; Chavagnat and Lezec, 1985, Kireeva et al., 1988; Crochon et al., 1993) and fruit (Bowers et al., 1988; Han et al., 1992; Thomas et al., 1993 & 1995; Schatzki, et al., 1997; Shahin and Tollner, 1998).

Ten years ago, X-ray computed tomography (CT) was used to image interior regions of Red Delicious apples in varying moisture and, to a limited extent, density states. This enabled the quantification of the X-ray absorption coefficient associated with the dry solids portion of the fruit and the X-ray absorption coefficient associated with moisture (Tollner et al., 1992).

Recently, Yantarasri et al. (1998) showed that X-ray CT is suitable for detecting differences between immature, mature and overripe durians (cv. Murray) and detecting damage, internal disorders and rotten pulp in both durian and mangosteen fruit. The applications of X-ray CT for measuring physicochemical properties of peach (Barcelon et al., 1999a), mango (Barcelon et al., 1999b) and kiwifruit (Barcelon et al., 2000) were reported. CT number was found to be positively correlated with density, moisture content and titratable acidity, and negatively related to soluble solids and pH (Barcelon et al., 1999b). It was suggested that CT number measurements on intact fruit could be used as an indicator of fruit quality, with the potential to be adapted for on-line sorting and quality monitoring (Barcelon et al., 2000).

2.7.2.1 Principles of CT measurement

CT number, the standard unit for measuring X-ray absorption with the CT system represents the relative density of the scanned material compared to the density of water. X-ray absorbance is normalized to zero for water and –1000 for air. Materials with a density less than that of water have CT numbers less than zero; materials more dense than water would have CT numbers greater than zero (Tollner et al., 1989).

The density of a fruit tissue is determined by physicochemical properties which are subject to changes during maturation and ripening processes (Beever and Hopkirk, 1990; Barcelon et al., 2000). These changes may take place at different times in different manners among different tissues within a fruit (Jackson and Harker, 1997; Barcelon et al., 2000). The distribution of CT numbers in different regions of a fruit could be correlated to many fruit attributes, such as maturity, mechanical damage and the development of disorders. The correlations might be useful for the assessment of fruit quality. However, application of X-ray to fruit are subjected to safety concerns, therefore could be restricted by regulatory requirements.

2.7.2.2 Instrumentations for X-ray measurements

Three types of instruments have been used in previous studies: (1) X-ray image systems similar to those designed for medical usage. Results are recorded on film. (2) X-ray CT scanning systems similar to those designed for medical usage. Results are recorded as an image, CT number and its frequency. (3) X-ray inspection systems similar to those used at airports for luggage inspection. The models and operating conditions for X-ray scans are listed in table 2.2.

Table 2.2 Instruments and operating conditions used determination of fruit attributes in previous studies

Instruments	Types	Operating conditions	Measurement target	Reference
Genetic engineering 300 MA X-ray system	Medical X-ray image	50 kV	Split-pit of peaches	Han et al., 1992
Siemens Tridors 6R X-ray machine	Medical X-ray image	36 kV, 10 mA	Spongy tissue of mango	Thomas et al., 1993
Siemens Polymat 501 X-ray machine	Medical X-ray image	40kV, 12 mA 40kV, 800 mA	Seed weevil-infected mango	Thomas et al., 1995
Georgia Station EMI 5005 scanner	Medical X-ray CT	120 kV, 700 mA	Density and water content of apples	Tollner et al., 1992
X-ray CT scanner TOSCANER-20000	Agricultural and industrial X-ray CT	150 kV, 3 mA	Maturity of peach and mango	Barcelon et al., 1999 a & b
X-ray inspecting system	Airport security	140 kV, 0.87 mA and 80 kV, 0.5 mA	Spongy tissue of mango	Thomas et al., 1993

2.7.2.3 X-ray data processing

Analytical methods used for X-ray data include neural classifier, Bayesian classifier, regression analysis and analysis of variance. Neural classifier was found to be better than Bayesian classifier in classifying maturity levels of green tomatoes (Thai et al., 1991), sorting apples with bruises (Shahin and Tollner, 1998) and identifying onions with internal defects (Tollner et al., 1999). Regression analysis was used to determine the relationships between X-ray CT numbers and the physicochemical characteristics of mango (Bacelon et al., 1999b). Analysis of variance was used to determine the effect of

ripening time on CT number and physicochemical qualities of peach (Bacelon et al., 1999a) and mango (Bacelon et al., 1999b).

2.8 Discussion

Storage life is a complicated entity that involves fruit softening and the development of storage disorders. FF is essentially a measurement of tensile strength of the outer pericarp tissue. Tensile strength is a measure of the rigidity of cell walls and middle lamella. At harvest, the middle lamella is more rigid than the cell walls; the cell walls break during FF measurement and the measured FF represents the strength of the cell walls. At the end of storage, the middle lamella becomes less rigid than the cell walls; cells break in between and the measured FF represents the strength of the middle lamella. FF measured during storage can be affected by the decrease in proportion of cells that break from cell walls and the increase in the proportion of cells that break from middle lamella (Harker and Hallett, 1994). These two mechanisms involved in FF measurement merit consideration when developing a mechanistic fruit-softening model.

The triphasic nature of the kiwifruit softening curve makes the estimation of SL complicated. The lag phase of fruit softening is obvious for early-harvested fruit, but virtually disappears for late-harvested fruit. Rapid fruit softening lasts for only about one month at early storage time while the subsequent slow softening phase continues for several months (MacRae et al., 1990; Benge et al., 2000). The slow softening phase is more important in determination of SL than the first two softening phases. Therefore, further study on STP should focus on the slow softening phase.

Molecular studies of kiwifruit softening have enhanced understanding of the mechanisms of the softening process (Redgwell et al., 1990; Redgwell et al, 1991; Redgwell et al., 1992a; Wegrzyn and MacRae, 1992; Redgwell and Percy, 1992; Redgwell and Fry, 1993; Redgwell and Harker, 1995; Bonghi et al., 1996; Redgwell, 1996; Kim et al., 1999; Wang et al., 2000; Fan and Zhang, 2001; Kim et al., 2001; Antunes and Sfakiotakis, 2002a & b). Integrating this knowledge together with mechanistic modelling procedures (Hertog and Jeffery 2002) will ensure that STP models be established not only on statistical analysis of experimental data, but also on sound biochemical and physiological theories.

The limiting factors for SL of each fruit can be quite different. Some may be related to premature fruit softening and others may be associated with certain disorders. Premature softening and storage disorders may involve different mechanisms; it is preferable to predict them separately and then combine the models for fruit segregation.

Kiwifruit is sensitive to trace amounts of ethylene (Arpaia et al., 1987; Mitchell, 1990). The elevated ethylene produced by just a few premature or disordered fruit may trigger a chain reaction, causing premature softening of the whole load. Preventing fruit to fruit interactions during storage through ethylene would allow the full expression of the STP differences among fruit.

Many fruit attributes are measurable at harvest. It is impossible to measure all the attributes in either scientific research or industry applications. Careful selection of at-harvest fruit attributes most relevant to SL would simplify measurement work. Some fruit attributes, such as SSC, brown index and sugar content are highly correlated (Lallu et al., 1999); measurement of one (i.e. SSC) would be adequate. Some fruit attributes, such as fruit colour, which have rarely been measured in previous STP studies on kiwifruit, merit more attention.

Segregation on an individual fruit basis is preferred rather than segregation on grower line basis because of within-grower line fruit variability (Hopkirk et al., 1986; Smith et al., 1994; Pyke et al., 1996; Benge, 1999; Watt, 1999; Amos and Mowatt, 2002). While segregation of grower lines can be based on destructive measurement of at-harvest fruit attributes on a small fruit population randomly sampled from the grower line, segregation of individual fruit requires non-destructive measurements on every fruit at high speed and low cost.

Of the various non-destructive methods used to measure fruit attributes, VNIR is the most developed while X-ray technologies are also promising. Evaluation of these technologies would have great value for the kiwifruit industry.

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Suggestions on rationalized methodologies to investigate kiwifruit storage life

3.1 Introduction

Variation in storage life (SL) within and among grower lines (fruit from an orchard or a block in an orchard) costs kiwifruit producers throughout the world millions of dollars each year (Benge, 1999). Many studies have compared SL of fruit from different grower lines and have reported inconsistent results (Hopkirk et al., 1990; Prasad and Spiers, 1991; Smith et al., 1994; Benge, 1999; Lallu et al., 1999). For example, fruit with higher Ca concentrations have been found to store better than those with lower Ca concentrations (Prasad and Spiers, 1991; Banks et al., 1995; Lallu and Yearsley, 1995; Benge 1999). However, Ca levels in kiwifruit from several orchard sources in Auckland were not correlated with fruit firmness during storage, grower lines that had become particularly soft during storage having Ca concentrations similar to or even higher than those in grower lines that had remained firmer (Hopkirk et al., 1990).

The inconsistent results reflect the complexity of SL as well as the lack of rationalized methodologies. For example, calculation of SL is based on flesh firmness (FF; N) data collected during storage. The kiwifruit industry and most researchers measure FF by a drill-mounted Effegi penetrometer that records the force required for a 7.9 mm diameter plunger to penetrate into peeled outer pericarp tissue. This method is subject to large variation caused by inconsistent operation and false reading by the operator from the dial of the pressure meter (Harker et al., 1996). The methods to reduce measurement error of FF are yet to be investigated.

Measurement of FF is normally carried out at ambient temperature with or without temperature equilibration. FF of 'Hayward' kiwifruit measured after 24 h equilibration at 20°C can be estimated from FF measured immediately upon removal from storage at 0°C and vice versa (Jeffery and Banks, 1994). However it is not clear whether 24 h is appropriate for temperature equilibration, or how the equilibration time would be affected by package configurations or how different fruit attributes respond to equilibration time and package configurations.

Due to the triphasic nature of fruit softening curve (MacRae et al., 1990), the empirical models that describe the whole softening curve (Benge, 1999; Benge et al., 2000) involve four parameters that are difficult to estimate accurately when the number of data points is limited. The mechanistic model developed by Hertog and Jeffery (2000) requires fewer parameters to be estimated, but the model is not sensitive to FF measured towards the end of storage. Simplified models, such as an exponential (Pyke et al., 1996) fit the softening curve during late storage time, but it is not clear how many times of FF monitoring are required and how many fruit should be sampled for a reliable estimation of SL using this simplified model.

Many researchers have attempted to link fruit mineral concentrations, particularly Ca, to SL. Analytical methods for minerals are well established and standardized (AOAC International, 1995), but sample preparation methods vary considerably. Some researchers macerate the whole fruit for mineral analysis (wet sample method; Benge, 1999) while others grind a dried fruit section cut from the equator zone of a fruit (dry sample method; Clark and Smith, 1988; Smith et al., 1994). Occasionally, fruit juice has been used for mineral analysis (Lallu et al., 1999), but is arguably inappropriate because minerals also exist and function in the solid part of the fruit, i.e. cell wall and membrane. Current work compared mineral analysis results from wet and dry sample methods to select a more reliable sample preparation method.

3.2 Materials and methods

3.2.1 Improvement on firmness measurement

Flesh firmness of 180 kiwifruit was measured using a 7.9 mm plunger attached to the texture analyser (TA.TX2, Stable Micro Systems, Surrey, England). The plunger penetrated 9 mm into a fruit at a constant speed of 10 mm s^{-1}. The measurement was carried out at two opposite sites along the equator zone of each fruit where the skin tissue had been removed using a fruit peeler. Another 180 kiwifruit berries of similar firmness were measured using a drill-mounted Effegi penetrometer. Results from the two methods were compared to see if one method gave more accurate readings than the other.

3.2.2 Temperature equilibration for measurement of various fruit attributes

Fruit temperature, weight (W), external colour (L, C, H colour space measured using a Minolta chroma meter CR-200 at the equatorial zone of each fruit), respiration rate (r_{CO_2}, measured by accumulation of and CO_2 in closed system using an infra-red CO_2/O_2 analyser), FF and soluble solids content (SSC, measured using a refractometer) of fruit packed in moulded pulp trays (M tray; interleaving tray commercially used in 20 kg apple packages) or single-layered kiwifruit trays with a polyliner (K tray) were measured before and after shifting from a cool store (0.5°C, 80-90% *RH*) to an air-conditioned laboratory (20°C, 50-60% *RH*). Measured fruit values were plotted against equilibration time. The time when measured values of a fruit attribute became stable was selected as optimum equilibration time for the measurement of this attribute (Feng et al., 2002).

3.2.3 Improvement on calculation of storage life

Ten trays of count 36 kiwifruit from each of the nine growers from Bay of Plenty (New Zealand) were randomly sampled from a packhouse during the last three weeks in May 2000 and cool stored at 0°C. Flesh firmness was monitored monthly after September 2000 using 20 fruit per grower line at each monitoring time. Data were analysed using the REG (regression) and NLIN (non-linear regression) procedures in SAS (SAS Institute Inc., 1990) to select a model that best described the fruit-softening curve towards the end of storage. The number of monitoring periods required to give reliable estimation of the model parameters were selected based on the standard error of the mean predicted value (STDP) and the means of the squared prediction error (MSPR) for FF at the end of storage (Feng et al., 2001).

3.2.4 Comparison between mineral analysis results from wet and dry samples

Wet samples were obtained from 100 kiwifruit. An individual fruit (includes skin, flesh, seeds and core) was macerated using a blender and about 25 ml of the macerated sample was kept at –20°C as a wet sample.

Dry samples were obtained from another 100 kiwifruit. A section (about 1 cm in thickness) cut from the middle of each fruit was cut into two halves (each half includes

skin, flesh, seeds and core). Cut fruit pieces were freeze dried and ground to a fine powder. Fresh wet and dry weight were measured before and after freeze drying and dry matter contents (DM) were calculated.

Nitrogen (N) and phosphorus (P) concentrations were measured colorimetrically after Kjedahl digestion of 1 g wet or 0.2 g dry sample. Potassium (K), calcium (Ca) and magnesium (Mg) concentrations were measured by atomic absorption spectroscopy after nitric digestion of 0.5 g wet or 0.1 g dry sample. Measurements of all minerals were repeated twice for each fruit (started from weighing 1 g wet or 0.2 g dry sample).

An additional 5 fruit were cut into core, inner pericarp (seeds inclusive), outer pericarp and skin from 2 cm thick middle sections. Five fruit hair samples were collected from packages of five grower lines. All these samples were freeze dried and ground to a fine powder for mineral analysis.

3.3 Results and discussion

3.3.1 Improvement on firmness measurement

The plot of two FF readings (FF_1 and FF_2) measured using the Effegi penetrometer from each of the 180 fruit (Fig 3.1A) was more scattered than the plot for TX2 (Fig 3.1B). The regression between FF_1 and FF_2 of the Effegi penetrometer method has a coefficient of variance of 15.5% while that of the TX2 method has a coefficient of variance of 11.1%, while the regression lines of FF_1 and FF_2 were similar for both methods. These results indicate that the TX2 method is more precise than the Effegi penetrometer method. The advantages of attaching the plunger to the texture analyser might result from the constant speed at which the plunger penetrated into the fruit. The rupture force recorded by a computer connected to the TX2 is more objective than the Effegi penetrometer readings read by the operator from the dial of the pressure meter.

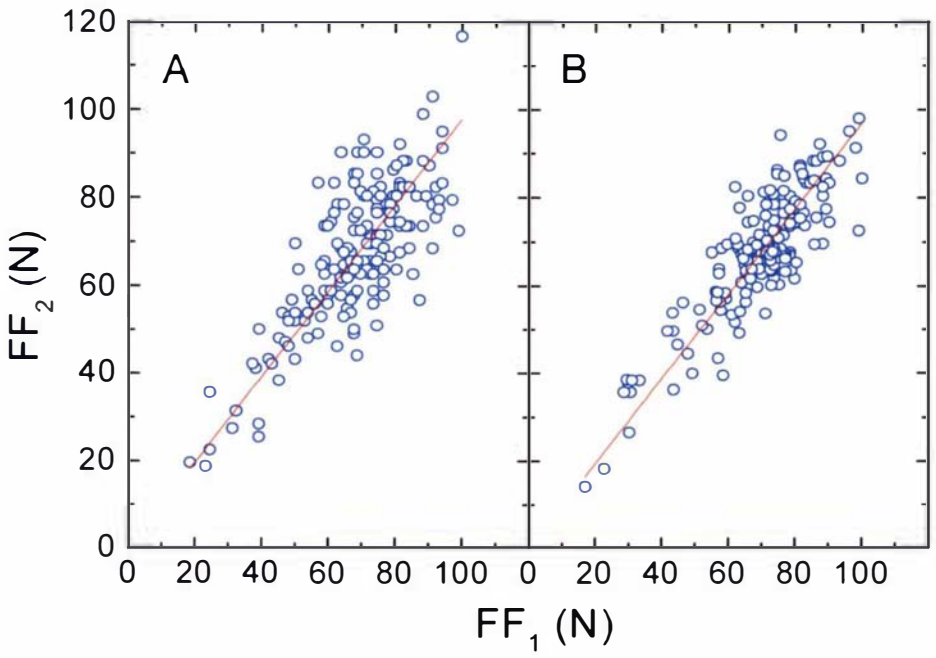

Figure 3.1 Relationship between two firmness readings measured on two sides of fruit by a drill-mounted Effegi penetrometer (A) and a texture analyser (B). FF_1 is the firmness value measured on side 1 and FF_2 is the value for side 2. Solid lines represent the fitted linear relationships between FF_1 and FF_2 with intercepts fixed to 0 ($FF_2=0.977\pm0.011\cdot FF_1$, n=180, $R^2=0.98$ in A; $FF_2=0.969\pm0.008\cdot FF_1$, n=180, $R^2=0.99$ in B).

3.3.2 Temperature equilibration for measurement of fruit attributes

Count 36 kiwifruit packed in M trays and K trays took 2.6 and 10.0 hours respectively to achieve seven-eighth temperature equilibration ($t_{7/8}$). Fruit temperature levelled off after two times $t_{7/8}$ (i.e. 5.2 and 20.0 h respectively for kiwifruit packed in M trays and K trays). Fruit packed in M trays gained up to 1.3 g kg^{-1} weight within the first 30 minutes of equilibration due to water condensation. Fruit were dry after 2 hours equilibration. Fruit in K trays gained twice as much weight (2.6 g kg^{-1}) as those in M trays and condensation and subsequent evaporation was slower. Fruit was still wet after 34 h equilibration in K trays (Fig 3.2).

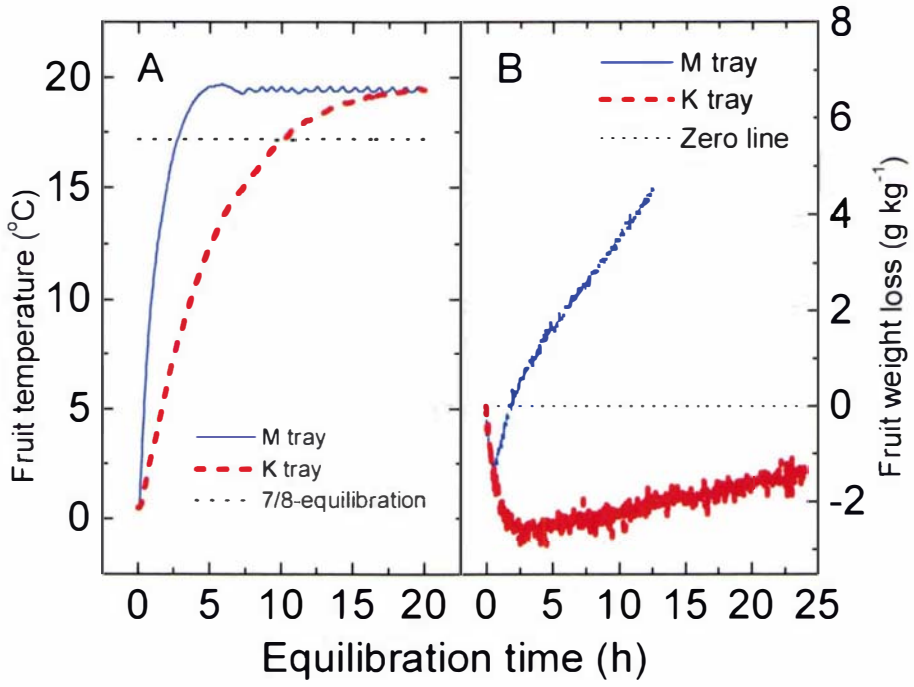

Figure 3.2 Changes in fruit temperature (A) and weight loss (B) of count 36 kiwifruit recorded at 2-minute intervals during equilibration in different packages. Each temperature point is the mean of the 9 plungers inserted into the core, flesh and under skin positions of 3 fruit in each package. Weight loss data were measured on a package basis.

Fruit firmness declined during equilibration and levelled off after 5.2 and 20 h (two times $t_{7,8}$) for M tray and K tray, respectively (Fig. 3.3). The current industry practice of measuring FF immediately after fruit have been taken out of storage tends to result in higher FF readings and larger variance because of the rapid change in fruit temperature during the measurement. If fruit from one treatment are measured immediately after fruit have been taken out of storage, and fruit from another treatment are measured after a delay as short as 30 minutes, comparison between the means of these treatments will be misleading (Feng et al., 2002).

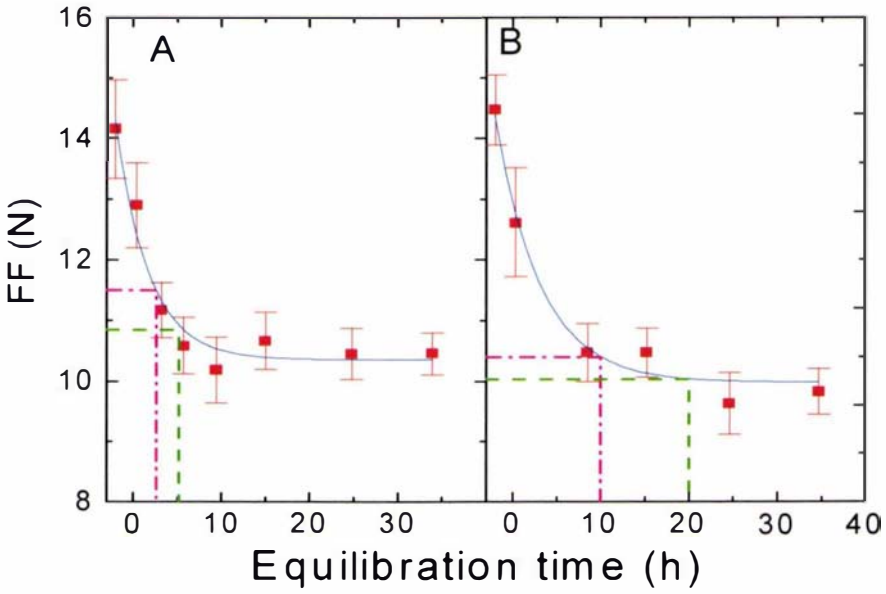

Figure 3.3 Changes in flesh firmness (FF) of count 36 kiwifruit during equilibration in moulded pulp trays (M tray, A) or single-layered kiwifruit trays with polyliner (K tray, B). Each point is the mean of 20 (A) or 24 (B) fruit. Vertical bars are standard errors of the means. Solid lines are fitted exponential curves. Dash–dot and dash lines indicate FF measured after one and two seven-eighth-equilibration times respectively.

In M trays, L, C and H changed considerably within the first $t_{7/8}$ but subsequently stabled. In K trays, L and C values remained stable within 24 h and increased afterward, while H increased within the first $t_{7/8}$, but subsequently levelled off (Fig. 3.4).

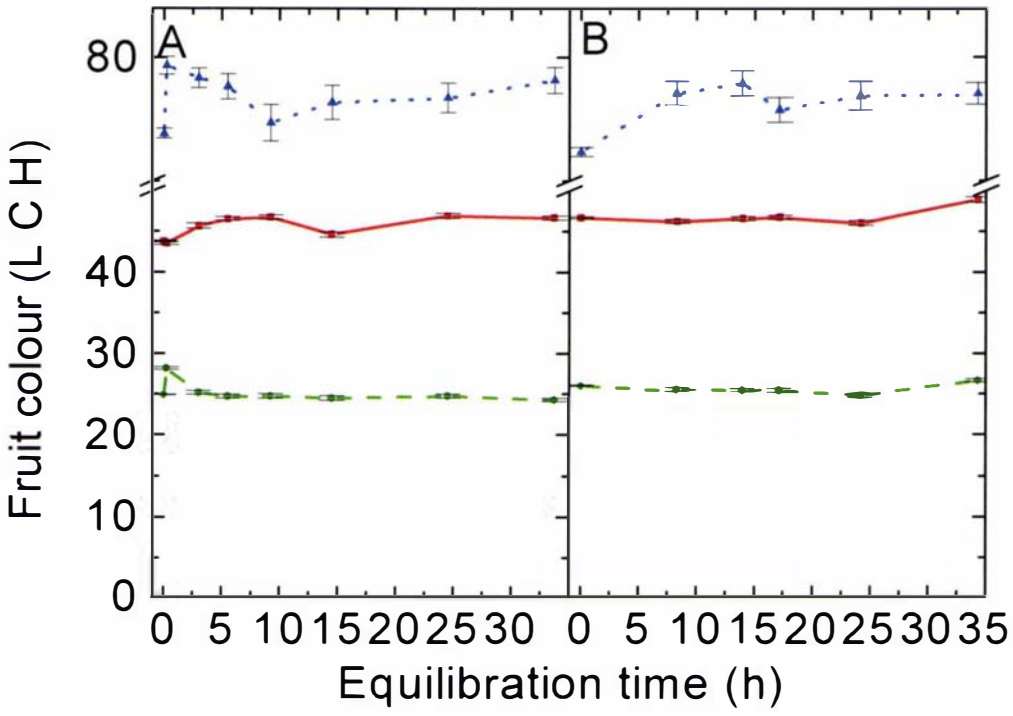

Figure 3.4 Changes in fruit colour (L, solid; C, dash; H, dot) during temperature equilibration in moulded pulp trays (M tray, A) or single-layered kiwifruit trays with polyliner (K tray, B). Each point is the mean of 20 (A) or 30 (B) fruit. Vertical bars are standard errors of the means.

In M trays, r_{CO_2} increased rapidly before peaking after 10 h equilibration (Fig. 3.5A), while that in K trays levelled off after 24 h equilibration (Fig. 3.5B). The respiration rates measured for fruit equilibrated in K trays were significantly higher than those of the fruit equilibrated in M trays. This difference is probably a result of the high CO_2 concentration (0.5-1.0%) inside the package that in turn resulted in higher fruit internal CO_2 concentration when fruit were shifted from K trays to the respiration jars. This accumulated CO_2 would have been released to the respiration jar and contributed to higher CO_2 concentration at gas sampling. The respiration rate of K tray fruit measured after additional 10 h in an M tray were at the same level as that of M tray fruit (Fig. 3.5B). This indicates that the measured respiration rate is the balance between the actual respiration rate and the rate of CO_2 diffusion from the fruit to external space.

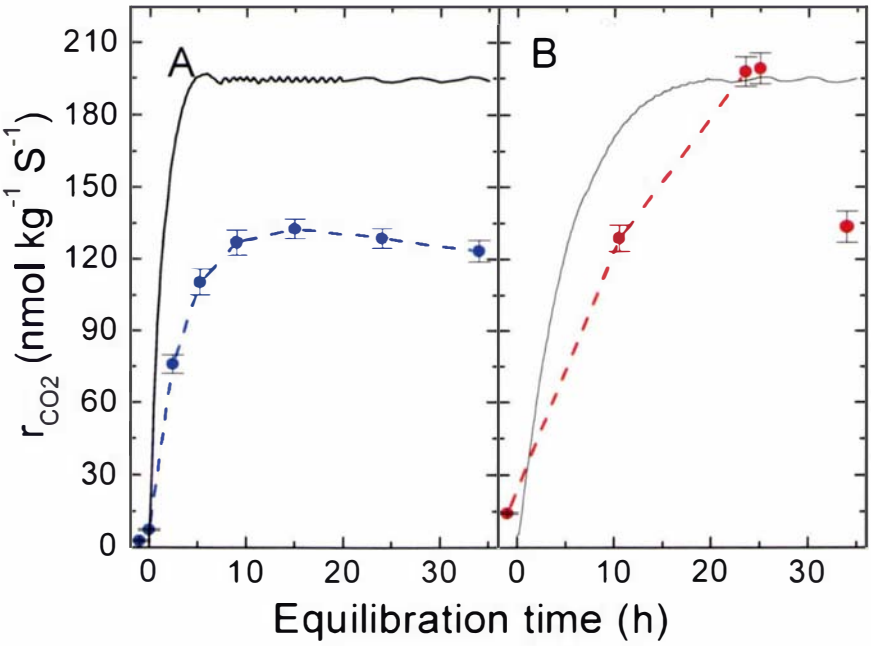

Figure 3.5 Changes in measured respiration rate during temperature equilibration in moulded pulp trays (M trays; A) or single-layered kiwifruit trays with a polyliner (K trays; B). Each point is the mean of 6 fruit. Vertical bars are standard errors of the means. The final point in B was measured after 10 h equilibration in an M tray in addition to 25 h equilibration in a K tray. Solid lines indicate fruit temperature (not in scale).

Soluble solids content measured at different equilibration times were similar for fruit in both packages (data not shown).

The long equilibration time, wet fruit surface and distorted respiration rate together render K trays unsuitable for temperature equilibration of kiwifruit. M trays, on the other hand, showed some advantages in these aspects, although they may result in excessive weight loss during prolonged equilibration period.

3.3.3 Improvement on calculation of storage life

Firmness data collected on fruit from 9 different growers at monthly intervals indicated that FF measured for storage durations exceeding 3 months was exponentially related to storage duration (Feng et al., 2001). Because of the variation in FF among fruit from the same grower line, the two FF means at the last two monitoring periods were not significantly different for seven out of the nine grower lines (data not shown).

Consequently, the calculation of SL based on FF data collected on 20 fruit each time at the last two FF monitoring periods was not valid.

When data from three or more monitoring periods were involved in the estimation of model parameters, the standard error of the mean predicted value (STDP; SAS Institute Inc. 1990) decreased. Conversely the mean of the squared prediction error (MSPR[a]) for FF at the end of storage increased (Fig. 3.6).

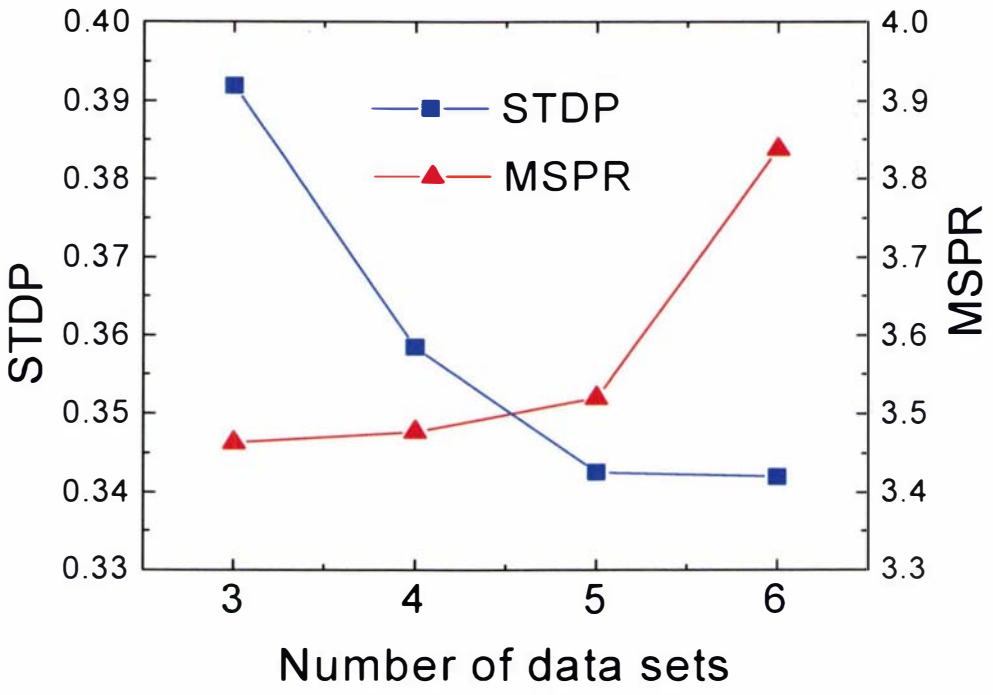

Figure 3.6 Effects of the number of monitoring periods on standard error of the mean predicted value (STDP) and the means of the squared prediction error (MSPR) for flesh firmness (FF) at the end of storage (each point represents an averaged value for fruit of the six growers whose FF had been monitored for six or more periods)

To minimise both STDP (which increased sharply when data from less than five monitoring periods were used) and MSPR (which increased sharply when data from more than five monitoring periods were used), data from either four or five monitoring periods should be used. For practical use, a procedure using fewer monitoring periods is

[a] $MSPR = \frac{\sum_{i=1}^{n}(Yi - \hat{Y}i)^2}{n}$ where Yi is the measured FF value for the ith fruit, $\hat{Y}i$ is the predicted FF value using the exponential model for the ith fruit, n is the number of fruit in each grower line that have been measured at the end of storage.

preferred, and therefore, an exponential model fitted to data from last four monitoring periods is suggested for SL calculation (Feng et al., 2001).

3.3.4 Comparison between mineral analysis results from wet and dry samples

Repeat mineral analyses using dry samples gave more consistent results than wet samples (Fig. 3.7 A-E). Dry matter content measured on two halves of the same fruit showed high agreement (R^2 = 0.9; Fig. 3.7 F). This indicates that additional measurement of DM did not cause significant variation in mineral concentration on fresh weight basis (mmol kg^{-1} FW) calculated from concentrations measured on dry samples. The discrepancies of the results from repeated mineral analysis using wet samples (Fig. 3.7 A-E, triangles) was possibly caused by the mineral gradients within fruit (Fig. 3.8) and insufficient sample homogenisation.

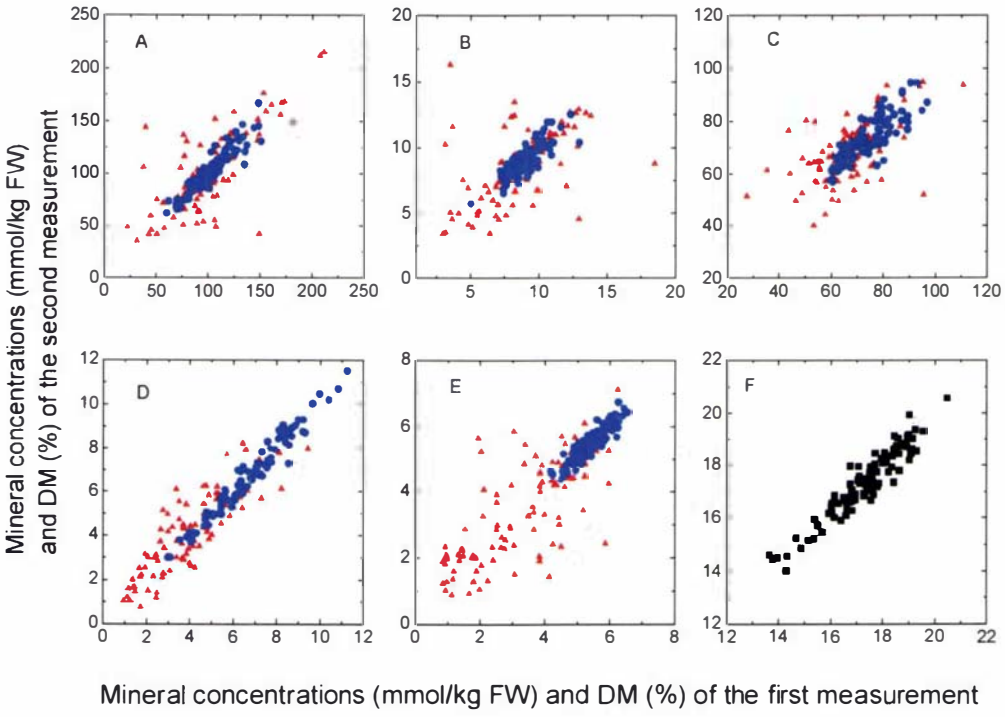

Figure 3.7 Relationship between repeated mineral (A - N, B - P, C – K, D – Ca, E – Mg) analysis results measured on wet (triangle) or dry (circle) samples. Dry matter content (DM, %) measured on two halves of the same fruit for calculating mineral concentrations on fresh weight basis for the dry sample method is shown in F.

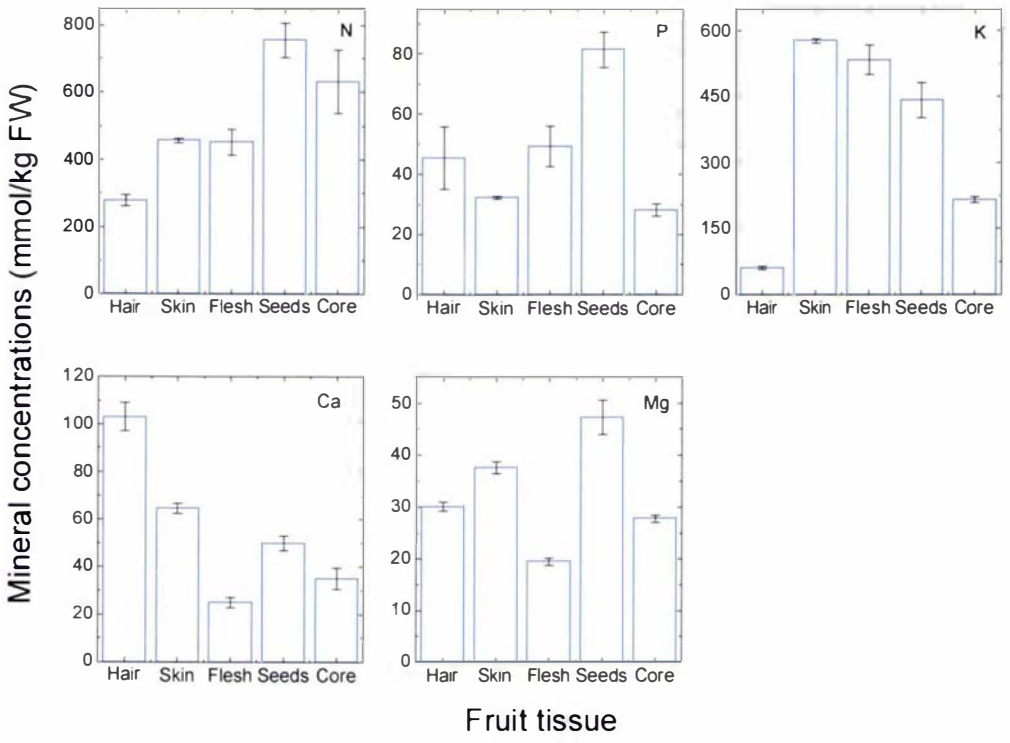

Figure 3.8 Difference in mineral concentrations among fruit tissues of 'Hayward' kiwifruit. Each column represents the means of 5 repeat measurements; vertical bars are standard errors of the means.

3.4 Conclusions

The reliability of storage life calculated from flesh firmness monitoring data depends on the method of flesh firmness measurement and the methods of data processing. Flesh firmness measured using a texture meter is significantly less variable than that measured using a drill-mounted Effegi penetrometer because of the controlled plunger speed and the computerised data recording.

Package and equilibration time affect fruit temperature and water condensation over the fruit surface, which in turn affects measured flesh firmness, fruit colour and respiration rate. Moulded-pulp trays are the preferred package for temperature equilibration. In this package, fruit colour and flesh firmness can be measured respectively after 2 and 5 h equilibration while respiration rate should be measured after 10 h equilibration. Soluble solid content can be measured at any time during the equilibration. Following these recommendations will ensure a more accurate and repeatable characterisation of 'Hayward' kiwifruit.

If firmness monitoring is carried out monthly on 20 fruit per grower line each time, an exponential model fitted to firmness data from the last four firmness monitoring periods towards the end of storage is recommended for calculation of storage life.

When storage life is to be related to mineral concentrations of the fruit, fruit sample should be dried and grinded to fine powder for mineral analysis. Mineral analysis using wet sample tends to have poorer repeatability.

Recommended methodologies should be used in both research and industry applications for more reliable, repeatable and comparable results.

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Application of compression force in the study of kiwifruit storage life

4.1 Introduction

Fruit to fruit variability is a major problem in studying kiwifruit softening behaviour. Using a penetrometer to measure flesh firmness (FF) is the industry standard method for firmness assessment (McGlone et al., 1997). This measurement punctures the fruit, and thus only a subsample, selected to represent all fruit, can be measured (Hopkirk et al., 1996). A non-destructive technique for firmness measurement would allow testing of every individual fruit and repeated testing of the same fruit over time, thus providing many advantages for researchers and the industry alike (Davie et al., 1996; Hopkirk et al., 1996).

However, to date no satisfactory non-destructive method has been found. Applications of various non-destructive techniques, such as SoftSense (McGlone et al., 1997), Softness Meter (Davie et al., 1996; Hopkirk et al., 1996), Kiwipoke (Hopkirk et al., 1996), Kiwifirm and Handy Hit have been limited by anomalous firmness changes (McGlone et al., 1997; Kim, 1999), slow speed (Davie et al., 1996), insufficient sensitivity, or poorer ability to separate fruit batches compared with FF measured using a penetrometer (Hopkirk et al., 1996). Near infrared spectroscopy (McGlone and Kawano, 1998), laser air-puff method (McGlone and Jordan, 2000), laser Doppler vibrometer (Terasaki et al., 2001), and acoustic methods (Muramatsu et al., 1997) are all at developing stages and are not available for practical use.

Compression force (CF), measured by compressing fruit for 1.5 mm with an 11 mm diameter round-tip plunger attached to a texture analyser, has been used to monitor kiwifruit softening from hard (FF about 90N) to soft (FF < 10 N) fruit. The relationship between CF and FF was postulated to be an asymmetric sigmoid function. However, the relationship was variable (Hertog and Jeffery, 2000). Compared with the flesh rupture force measured using a penetrometer, CF is basically a measurement of fruit stiffness similar to that of the parellel plate compression of whole fruit (McGlone et al., 1997) and the laser air-puff method (McGlone and Jordan, 2000). Fruit stiffness is a fruit property quite different from the flesh rupture force measured using the penetrometer (McGlone et al., 1997). Increases in cell turgor pressure lead to stiffer flesh but can also mean a decreased

force for cell rupture due to the cell walls being under greater stress at the higher pressures (Pitt, 1982). It is possible that the scattering relationship between CF and FF be caused by variable cell turgor pressure among fruits. Because postharvest weight loss (mainly water loss from transpiration and carbon loss due to respiration) is associated with changes in cell turgor pressure (De Smedt et al., 2002), the relationship between CF and FF could be affected by postharvest fruit weight loss.

The common reason for measuring firmness of kiwifruit is to assist in the calculation and prediction of storage life (SL; Hopkirk et al., 1996; Hertog and Jeffery, 2001; Feng et al., 2001). Although the relationship between CF and FF of kiwifruit is non-linear over the whole firmness range, a linear relationship could be approximated within the low firmness range of FF 0-30 N (Hertog and Jeffery, 2001), which is required for calculation of SL (Feng et al., 2001). Similarly, a linear relationship between FF and stiffness of kiwifruit measured using the air-puff method was shown at the same firmness range (McGlone and Jordan, 2000). If this linear relationship could be confirmed, then CF would decline exponentially towards the end of storage as FF does, and the exponential model procedure proposed for calculating storage life of kiwifruit from FF monitoring data (Feng et al., 2001) could also be adapted for calculating SL from CF data.

Experiments were set up to test the consistency of the CF-FF relationship over different years and to test the effect of fruit weight loss on the relationship. Data collected on different grower lines were used to evaluate the feasibility of using CF monitoring as an alternative to FF monitoring for the study of storage life of 'Hayward' kiwifruit.

4.2 Materials and methods

4.2.1 Year 2000 experiment

Ten trays of count 36 (93-103 g) 'Hayward' kiwifruit from each of nine Bay of Plenty (New Zealand) growers were randomly sampled from a packhouse during the last three weeks of May 2000 and sent to an air-conditioned laboratory (20°C, 50-60 % *RH*) within 24 hours of packing. Fruit weight (W) was measured for all fruit on arrival at the lab using a balance (0.001 g, Model PM1206, Mettler Toledo, Switzerland). Compression force and FF were measured on 20 fruits per grower line. Compression force was measured by

compressing fruit for 1.5 mm using a round-tip plunger (diameter = 11 mm) attached to a Texture Analyser (TA.TX2, Stable Micro Systems, England). The maximum force was recorded electronically. A force threshold (trigger force) was set to 0.2 N to make sure the probe had reached the fruit surface when the texture meter started counting the 1.5 mm compression distance. FF was measured using a 7.9 mm diameter plunger attached to the Texture Analyser. The skin tissue of about 15 mm in diameter was removed using a fruit peeler immediately before the measurement of FF. The plunger penetrated 9 mm into a fruit at a constant speed of 10 mm s^{-1}. Both CF and FF were measured at opposite positions along the equatorial zone of a fruit, and the two readings were averaged to give CF or FF value of the fruit.

The remaining fruit was cool stored at 0°C in polylined single-layered trays with 5 g $tray^{-1}$ of ethylene absorbent (Purafil, Papworth Engineering Ltd. New Zealand). Starting from September 2000 (after 3 months storage), CF and FF were monitored monthly with 20 fruit per grower line sampled at each monitoring period. W, CF, FF were measured after 12, 15 and 20 hours of equilibration time in moulded pulp trays (interleaving tray commercially used in 20 kg apple packages) at 20°C. The monitoring continued until the average FF of each grower's fruit sample reached 8.5 N. This value, according to the firmness-temperature coefficient of kiwifruit (Jeffery and Banks, 1994), is equivalent to 11.8 N if measured immediately upon removal from coolstore (i.e. the commercial condition check method). All monitoring was completed by April 2001 (11 months after harvest), even though the average FF values for some grower lines were still above 8.5 N.

4.2.2 Year 2001 experiment

Ten trays of count 36 (93-103 g) 'Hayward' kiwifruit from each of six Bay of Plenty (New Zealand) growers were randomly sampled from a packhouse on 28 May 2001 and cool stored for nine months. Compression force and FF were measured monthly on 20 fruits per grower line. Storage condition and measurement methods were similar to those used in the year 2000 experiment.

4.2.3 Influence of weight loss on the relationship between CF and FF

One hundred freshly harvested fruit of similar size (90-105g) were sampled from field bins of a Bay of Plenty orchard at 10 am 9 June 2001 and brought to the laboratory in a polylined kiwifruit box (count 100) within 24 hours. The fruit were randomly assigned to 4 groups for the following treatments to generate differences in weight loss.

- Packed in a polylined single-layer kiwifruit tray located 150 cm away from fans (WL treatment 1).
- Packed in an open single-layer kiwifruit tray without polyliner located 120 cm away from fans (WL treatment 2).
- Packed in a moulded pulp tray located 90 cm away from fans (WL treatment 3).
- Packed in a moulded pulp tray located 30 cm away from fans (WL treatment 4).

Weight loss treatments were applied for 10 days and CF and FF were measured after the treatments. Fruit weight was measured before and after the treatment. Weight loss (WL) was calculated as the percentage of initial fruit weight using equation 4.1.

$$WL = \frac{W_0 - W}{W_0} \cdot 100\ \% \qquad (4.1)$$

where W_0 is initial fruit weight measured before storage and W is fruit weight measured during storage.

4.2.4 Data analysis

Data analysis was carried out using the GLM, REG and NLIN procedures in SAS (SAS Institute Inc., 1990).

4.3 Results

4.3.1 Comparison of the CF- FF relationships observed over two years

The Richards function (equation 4.2) was used to quantify the relationship between CF and FF over the whole firmness range.

$$FF = A \cdot (1 + e^{(b-k \cdot CF)})^{-\frac{1}{v}} \qquad (4.2)$$

where A is the upper asymptote, b positions the curve on the CF axis, k is an increase rate of FF in response to an increase of CF, and v controls the inflexion point.

The fitted Richards function accounted for more than 95% of the FF variation measured throughout the storage in both years (Fig 4.1). Parameters estimated for one year were not consistent to those of the other year except for the position of the inflection point that were similar for both years (Table 4.1). The fitted curve for year 2001 experiment had a 3.5 N higher upper asymptote, 20% smaller increase rate, and the curve positioned 3 N to the left of the CF axis compared with the curve for year 2000 experiment. Scattering was more pronounced in the high firmness range than in the low firmness range for both years (Fig 4.1).

Table 4.1 Estimated parameters of the Richards functions that describe the relationship between compression force (CF) and flesh firmness (FF) in different years

Parameter	Estimated value (se)		Difference
	Year 2000 (N=1240)	Year 2001 (n=960)	Between years
A	76.3 (0.41)	80.0 (0.64)	**
b	13.0 (0.19)	10.0 (0.20)	**
k	0.5 (0.01)	0.4 (0.02)	**
v	4.0 (1.14)	3.0 (1.03)	NS

NS and ** Non significant or significant t test at P<0.01 respectively

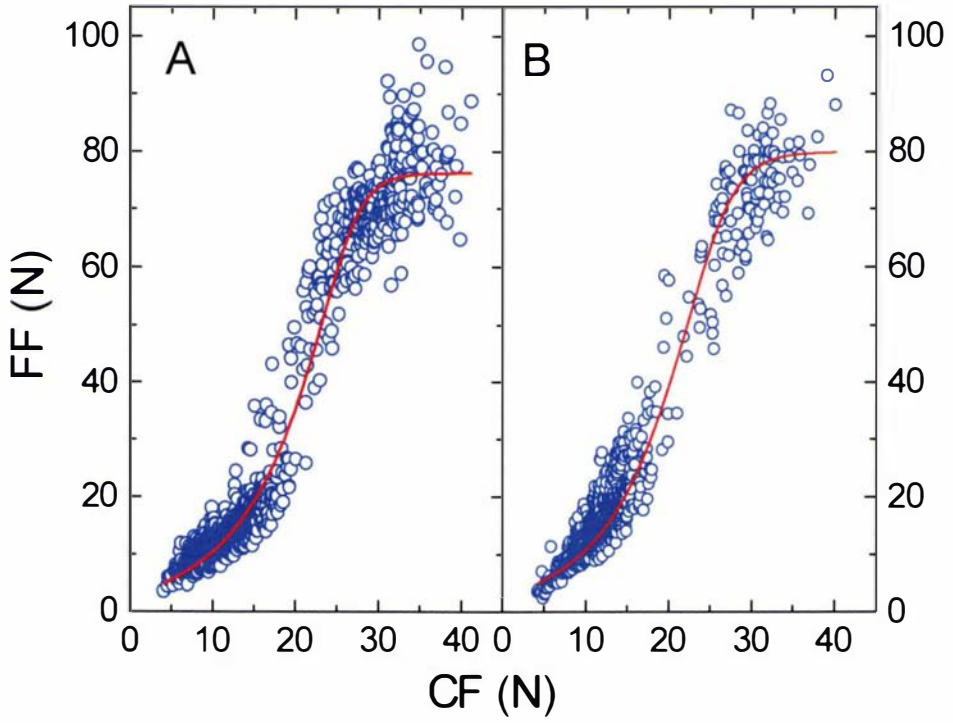

Figure 4.1 A Richards function (solid lines) fitted to compression force (CF) and flesh firmness (FF) data (circles) collected in year 2000 (A) and 2001 (B)

4.3.2 Influence of weight loss on CF-FF relationship

Fruit packed in a polylined kiwifruit tray lost 1.3% weight while those packed in open kiwifruit trays had a three-fold greater weight loss during the 10 days. The weight losses of fruit in open trays located at different distances to the fan were similar (Table 4.2).

A power function (equation 4.3) was fitted to the data from each weight loss treatment (Fig 4.2). The parameter ρ estimated for different weight loss treatments showed a similar trend to that of fruit weight loss. The ρ estimated for fruit packed in polylined trays was significantly smaller than the ρ estimated for fruit packed in open trays. The differences in ρ between fruit packed in open trays located at different distance to fan were not significant (Table 4.2).

$$FF = CF^{\rho} \tag{4.3}$$

Table 4.2 Influence of weight loss (WL) on parameters of a power function describing the relationship between CF and FF

Treatment	WL (%)*	se	ρ*	se	n
1. Sealed tray 1.5 m from fan	1.29 a	0.04	1.15 a	0.014	25
2. Open tray 1.2 m from fan	4.22 b	0.11	1.40 b	0.011	24
3. Open tray 0.9 m from fan	4.12 b	0.06	1.42 b	0.012	25
4. Open tray 0.3 m from fan	4.11 b	0.06	1.42 b	0.007	25

*Values labelled with different letters are significantly different in pare-wise t test at P<0.05 level.

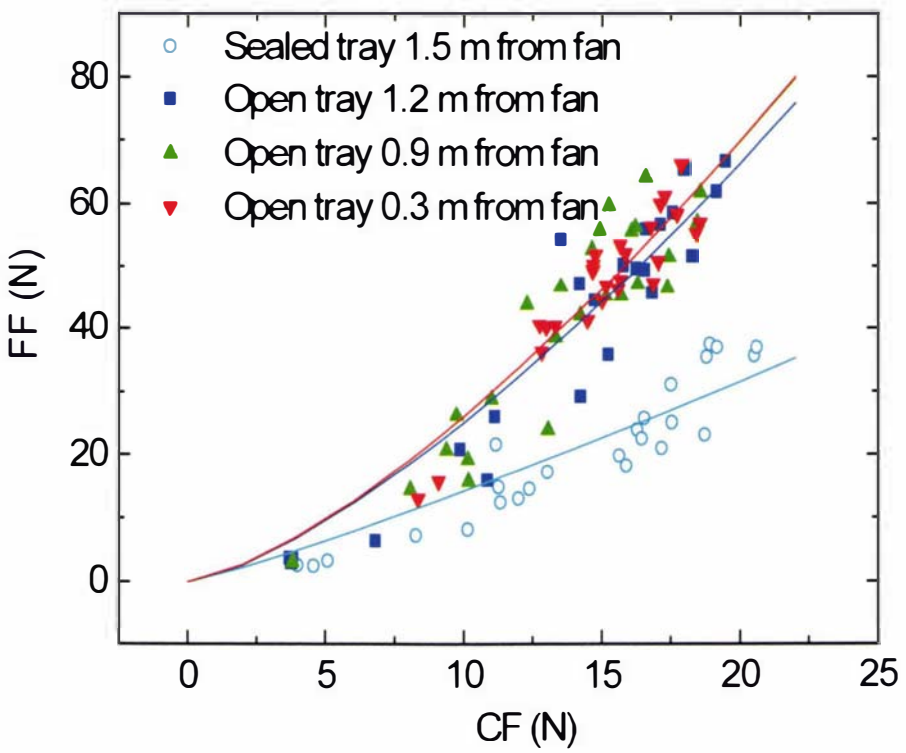

Figure 4.2 Effect of weight loss on the relationship between CF and FF. Each point represents a fruit. Solid lines represent predicted FF by power functions fitted to data in each treatment.

4.3.3 Changes in CF during storage

Compression force continued to decline exponentially (equation 4.4) after three months storage in 2000 (Fig 4.3) and 2001 (data not shown). This was similar to the relationship observed for FF (Feng et al., 2001). An exponential curve was fitted to CF data:

$$CF = CF_0 \cdot e^{-k \cdot t} \qquad (4.4)$$

where CF is compression force (N); t is the days in storage; CF_0 represents the initial CF value when t=0; *k* represents the rate coefficient of fruit softening.

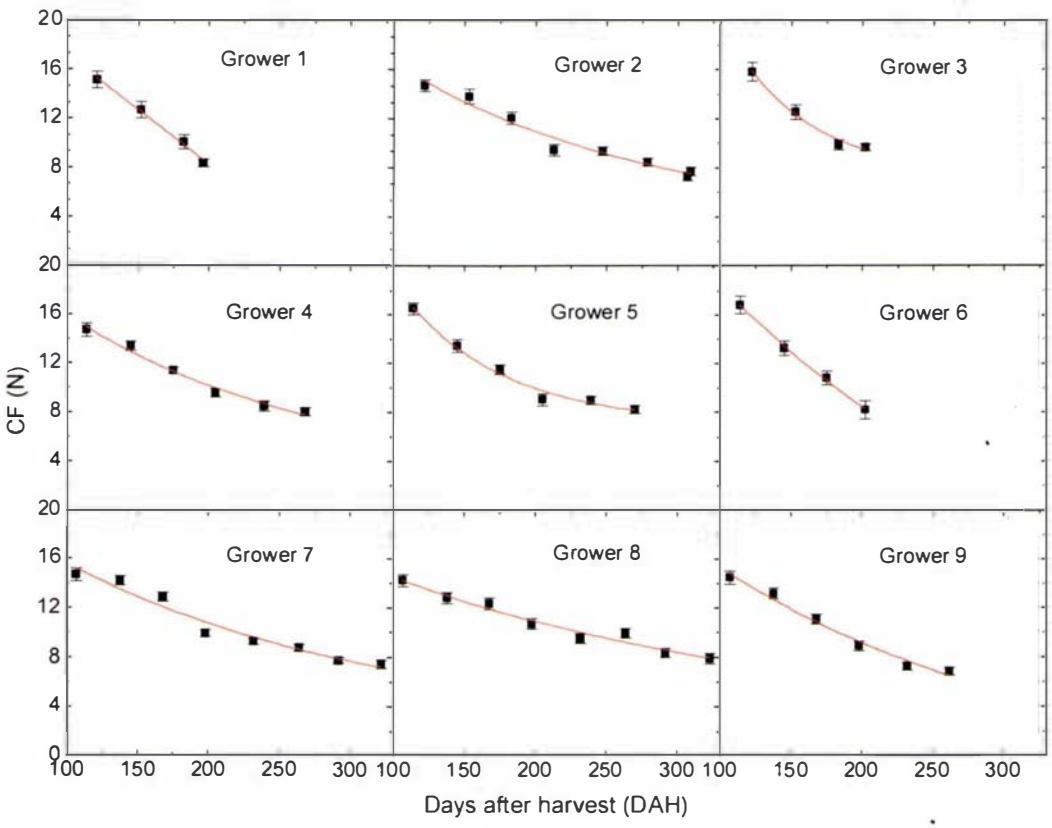

Figure 4.3 Plots and model fit for CF measured after three months storage versus DAH on grower line basis for fruit sampled in year 2000 (each point is the mean of 20 fruits. Vertical bars represent the standard errors of the means).

4.3.4 Ability of compression force to estimate storage life

Exponential models fitted to CF data collected at the last four firmness monitoring periods were rearranged (equation 4.5) to calculate SL on a grower line basis.

$$SL = \frac{\log_e(CF_0) - \log_e(CF_{end})}{k} \qquad (4.5)$$

where SL is storage life, CF_{end} is the minimum CF value (N) at the end of storage as required for export (CF value equivalent to FF 8.5 N), CF_0 and k are parameters of equation 4.4.

The plot of CF and FF measured at the end of storage indicated that the relationship between CF and FF is linear in the low firmness range (Fig 4.4 and table 4.3). This linear relationship could be used to convert the minimum FF required for export to an equivalent CF value (CF_{end}). Then the storage life could be calculated from equation 4.5.

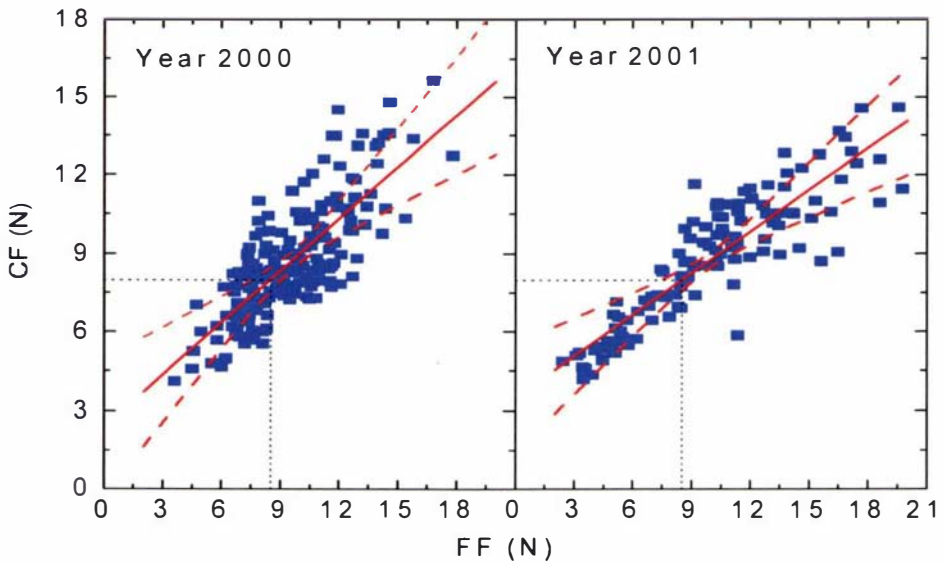

Figure 4.4 Plot of FF and CF measured at the end of storage. Each point represents a fruit. Solid lines represent linear relations between CF and FF. Dash lines represent 95% confidence intervals of predicted FF means. Dotted lines represent 8.5 N FF and equivalent CF values.

Table 4.3 Intercept and slope of linear relationships between FF and CF estimated for year 2000 and 2001 fruit. CF values equivalent to 8.5 N FF (CF_{end}) were calculated from the linear relationship for calculating storage life using CF data.

Parameter	Year 2000 (N=180)		Year 2001 (N=120)		Difference Between years
	Estimate	se	Estimate	se	
Intercept	2.40	0.40	3.50	0.27	*
Slope	0.66	0.04	0.53	0.03	**
CF_{end} (N)	8.03	0.19	8.00	0.18	NS

NS, *and ** indicate not significant, or significant in t test at $P<0.05$ and $P<0.01$ respectively.

According to the linear relationship between FF and CF, estimated values of CF_{end} were very close for year 2000 and 2001 fruit (Table 4.3) although the intercepts and slopes were significantly different between years. Storage life of the grower lines estimated from CF using a CF_{end} of 8.0 N explained over 86.0 % and 94.6 % of the variation in SL estimated from FF in year 2000 and 2001 respectively (Fig. 4.5).

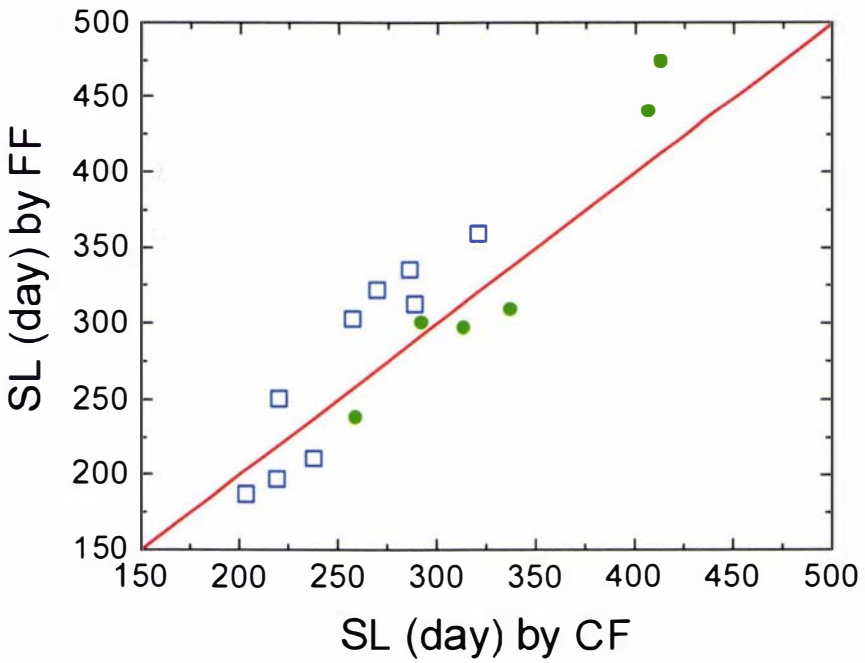

Figure 4.5 Storage life (SL) estimated from compression force (CF) matched to SL estimated from flesh firmness (FF) for grower lines of year 2000 (squares) and 2001 (round points).

4.4 Discussion

The relationship between CF and FF established in this study was similar to that observed previously (Hertog and Jeffery, 2000). Year to year differences in fitted curves describing the CF-FF relationship were shown in both studies. The reasons for the difference are yet to be elucidated. Fruit weight loss appeared to be an important factor affecting the CF-FF relationship. This implies that the conversion from CF to FF using a relationship established in a previous experiment is subject to systematic errors inherited from the difference between years and the difference between fruit of different weight loss. However, the relationships were similar for fruit sampled in the same year and stored in similar conditions with similar weight loss (Table 4.2 and Fig 4.2).

The effect of weight loss on the relationship between CF and FF (Table 4.2 and Fig 4.2) indicated that some scattering at a high CF (Fig 4.1) could have been caused by variable weight loss during the period between harvest and arrival at the laboratory. Fruit were cured in harvest bins in packhouses for up to 4 days before packing. Fruit located on the top would lose more weight than those located in the centre of the bin. Variable water vapour permeance of kiwifruit skin (Maguire et al., unpublished) may also contribute to the variation in fruit weight loss, and consequently the variation in CF-FF relationship. The scattering might have been reduced as water status of different fruit became more uniform during storage. Because package and time of temperature equilibration affect WL (Feng et al., 2002), it is advisable to equilibrate fruit for the same time using the same package before CF measurement.

The difference in the CF-FF relationship among weight loss treatments shown in Fig 4.2 may also reflect the positive effect of turgor pressure on whole fruit stiffness and the negative effect of turgor pressure on cell rupture force (Pitt, 1982). Fruit in the sealed tray with less weight loss and maintained higher turgor pressure would have lower cell rupture force compared with fruit of the same stiffness but lower turgor pressure due to excessive weight loss in the open trays. The convergence of the CF-FF relationships of fruit in different weight loss treatments at CF below 10 N (Fig 4.2) may indicate a reduction in turgor effects as fruit softened. However, it is not clear how fruit in the sealed tray did not show higher overall stiffness compared with those in open trays (Fig 4.2).

The close-to-linear relationship between FF and CF shown in Fig 4.4 and table 4.3 is consistent with previous studies where FF was found to be linearly related to firmness measured using the laser air-puff method (McGlone and Jordan, 2000) or compression force (Hertog and Jeffery, 2001) within the FF range of 0-30 N. This is the foundation of using CF data to calculate SL using the same procedure as the calculation using FF data. Because the CF-FF relationship at the low firmness range may also be affected by variable fruit weight loss (Fig 4.2 and Table 4.2) or year to year difference (Fig 4.4 and Table 4.3), it is important to keep storage conditions and temperature equilibration procedures consistent before each CF monitoring. When these conditions were met, storage life estimated from CF monitoring data was comparable to that estimated from FF monitoring data (Fig. 4.5). Therefore, it is suggested that CF measurement could be used as an alternative to FF measurement for firmness monitoring and the estimation of SL.

CF measurement is non-destructive and quicker (no skin peeling operation) than FF measurement. However, instruments capable of measuring CF, such as a texture meter, are very expensive. Therefore the feasibility of using CF as an alterative to FF depends on whether we could develop a less expensive device to measure CF. A further advantage of using CF is that the measurement can be conducted on the same fruit during storage, reducing the variation caused by sampling error. This procedure has been successfully used to compare the effect of storage treatments (i.e. storage temperature, ethylene, O_2 and CO_2 concentrations) on softening of kiwifruit (Hertog and Jeffery, 2000). Further work is needed to develop a less expensive instrument for CF measurement at industrial level and to elucidate factors other than fruit weight loss that affect the CF-FF relationship over different years.

4.5 Conclusion

The relationship between CF and FF over the whole range can be described by a Richards function. Parameters of the function may differ from year to year and from fruit batch to fruit batch in the same year due to variable fruit weight loss associated with storage duration and storage conditions. Under similar storage conditions, the relationship between

CF and FF approaches linearity at the low firmness range and CF declines in a similar manner to FF. The lowest CF required for export could be estimated from the linear relationship between CF and FF measured at the end of storage. Storage life calculated from an exponential model fitted to CF monitoring data is comparable to that calculated from FF data. Therefore, CF can be used for storage management as well as scientific studies to allow repeated measurement on the same fruit.

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Discriminating grower lines of 'Hayward' kiwifruit for storage potential

5.1 Introduction

Storage life (SL) of kiwifruit measures the time (t, days) for a stored fruit to become unacceptable for a particular use. In this study, the use of New Zealand kiwifruit is defined as export and the minimum flesh firmness (FF) required of a grower line (fruit harvested from an orchard or a block in an orchard) average is 11.8 N (Hopkirk et al., 1996; Benge, 1999).

Factors affecting SL of kiwifruit was extensively reviewed by Hewett et al. (1999). Premature softening (MacRae et al., 1990; MacRae and Redgwell, 1992; Hopkirk et al. 1990; Pyke et al., 1996; Benge, 1999; Lallu et al., 1999; Hertog and Jeffery 2000; 2002); postharvest decay arising mainly from infection by *Botrytis cinerea*, *Cryptosporiopsis sp.* and other fungi (Brook, 1992; Michailides and Morgan, 1997; Thanassoulopoulos and Yanna, 1997); physiological disorders including 'soft patches' (Davie, 1997; Benge, 1999), low temperature breakdown (LTB; Harman, 1981; Lallu, 1997; Bauchot et al., 1999; Sfakiotakis et al., 2002; Lallu et al., 2003) and physiological pitting (Ferguson et al., 2001; Mowat et al., 2002; Lallu et al., 2003) are all limiting factors. The relative importance of these factors varies with cultivar, season and grower line. For 'Hayward' kiwifruit, premature softening and soft patches are the major concerns (Mulligan, personal comm., 2000). Millions of dollars are lost each year because of rejection and repacking of fruit for export (Benge, 1999). The challenge posed to postharvest physiologists is to develop a method whereby some inherent fruit parameter can be measured at harvest and related to subsequent softening or susceptibility to diseases or disorders (Hewett et al., 1999).

Softening of 'Hayward' kiwifruit was proposed to be the consequence of solubilization of insoluble materials such as cell wall materials and starch (Arpaia et al., 1987; Redgwell et al., 1990; Redgwell and Percy, 1992; Bonghi et al., 1996). Enzymes catalysing the degradation of starch and cell wall materials acted at different times during storage. Activities of amylase (catalysing starch degradation, Bonghi et al., 1996), β-

galactosidase (Wegrzyn and MacRae, 1992; Bonghi et al., 1996), pectin methyl esterase (PME, Redgwell et al., 1990), endo-1,4-β-glucanase (Bonghi et al., 1996) and xyloglucan endotransglycosylase (XET, Redgwell and Fry, 1993) increased shortly after harvest while the activities of enzymes, such as polygalacturonase (PG) increased much later when fruit had softened to less than 20 N (Redgwell et al., 1990; Bonghi et al., 1996). Therefore, two distinctive processes appear to be involved in fruit softening. One takes place at the beginning of storage causing starch degradation and loosening the cellulose-hemicellulose interaction to allow cell wall swelling (Redgwell and Fry, 1993), while the second takes place in the inner part of the cell wall causing the degradation of the middle lamella so that the individual fruit cells separate (Redgwell et al., 1990).

Several factors influence softening of 'Hayward' kiwifruit. These include harvest maturity (Weet, 1979; Harman, 1981; Harman et al., 1982; Crisosto et al., 1984; Asami et al., 1988; Mitchell et al., 1992; Abdala et al., 1996; Pan et al., 1996; Costa et al., 1997; Hertog and Jeffery 2000, 2002), mineral concentrations (Bangerth, 1979; Hopkirk et al., 1990; Prasad and Spiers, 1991; Banks et al, 1995; Lallu and Yearsley, 1995; Tagliavini et al., 1995; Dave, 1997; Lallu et al., 1999; Benge, 1999; Hertog and Jeffery 2000, 2002; Sale and Clark, 2002) and fruit size (Testoni et al., 1990; Reid et al., 1996; Crisosto et al, 1999). The results from previous studies have been inconsistent about the factors influencing the softening of 'Hayward' kiwifruit. For example, some researchers found that fruit with soluble solids content (SSC) of 6.2-6.5% stored well (Weet, 1979; Harman, 1981), while others recommended higher values of 7% (Asami et al., 1988), 7.5 - 9.5% (Ravaglia et al., 1995), or 10% (Pan et al., 1996). Fruit lower in N and/or higher in Ca have been found to store better than fruit higher in N and/or lower in Ca (Prasad and Spiers, 1991; Banks et al., 1995; Lallu and Yearsley, 1995; Benge 1999). However, Ca concentration in kiwifruit from several orchards in Auckland were not correlated with fruit firmness during storage, grower lines that had become particularly soft during storage having Ca concentrations similar to or even higher than the Ca concentrations of grower lines that had remained firm (Hopkirk et al., 1990). Benge (1999) found strong relationships between the incidence of soft patches after storage and a predictor combining SSC, FF, and Ca, Mg and N concentrations measured at harvest when fruit were sampled directly from orchards. However, the

relationship was not subsequently validated by experiments where fruit was sampled from bins at packhouses.

These observed inconsistencies may have resulted from the absence of other factors affecting SL, and a lack of rationalised methods (Feng et al., 2003c). This paper presents the storage potential model established from data containing many of these previously researched factors and other potentially important factors using rationalised measurement and data processing methods (Feng et al., 2003c).

5.2 Materials and methods

5.2.1 Experiment 1

Ten trays of commercially packed 'Hayward' kiwifruit (count 36) from each of nine grower lines were sampled during the main harvest seasons of year 2000 (9 May to 23 May) at weekly intervals (three grower lines per week) from pack houses in the Bay of Plenty. Another 28 grower lines were sampled during the main harvest season of year 2001 (28 May to 9 June) from pack houses in the same region. On the second day of sampling, fruit weight (W), fruit colour (L, C, H colour space), flesh colour (FL, FC, FH), FF, SSC, dry matter content (DM) and mineral concentrations (N, P, K, Ca and Mg) were measured on a sub-sample of 20 fruit per grower line. Harvest date, packing date, storage date and measurement date were recorded for each grower line. The remaining fruit were cool stored at 0.5°C in the polylined single-layer kiwifruit trays used by the New Zealand kiwifruit industry. Five grams of ethylene absorbent (Purafil, Papworth Engineering Ltd., New Zealand) was placed in each tray to prevent ethylene accumulation inside the polyliner. Between 3 and 6 months of storage, FF was monitored monthly using 20 fruit from each grower line at each monitoring period.

Fruit weight was measured with a balance (0.001 g; Model PM1206; Mettler Toledo, Switzerland). Fruit colour and flesh colour were measured using a Minolta chroma meter (CR-200, with a aperture of 8 mm in diameter) calibrated with a green calibration plate. Flesh firmness was measured using a 7.9 mm diameter plunger attached to a texture analyser (TA.TX2, Stable Micro Systems, Surrey, England) after fruit temperature had equilibrated to 20°C (Feng et al., 2003c). Soluble solid content was measured with a

refractometer (Atago, 0-20%) using the juice extracted when measuring FF. A 10 mm thick slice (including skin, flesh, seeds and core tissues) was cut along the equatorial line of each fruit and freeze dried. Fresh weight and dry weight were measured before and after freeze-drying. Freeze-dried samples were oven dried at 65°C for 12 hours and the samples reweighed to correct for the difference between these two drying methods. Dry matter content was calculated based on the oven-dried samples. Mineral concentrations were determined on the dried samples used for DM measurement. Dried samples were ground to a fine powder and oven dried at 65°C for 12 hours immediately before weighing samples for mineral analysis. Nitrogen and phosphorous concentrations were measured colorimetrically after Kjeldahl digestion of a 0.2 g sample. Potassium, calcium and magnesium concentrations were measured by atomic absorption spectroscopy after nitric acid digestion of a 0.1 g sample (Martin-Prevel et al., 1987).

Measurements for L, C, H, FL, FC, FH, FF and SSC were carried out at two opposite positions along the equator of each fruit. Readings from the two positions were averaged.

5.2.2 Experiment 2

This experiment was carried out by AgFirst Consultants on behalf of Zespri Innovation Ltd. Twenty seven trays of count 36 'Hayward' kiwifruit from each of 72 grower lines were sampled from the Bay of Plenty (57 grower lines), Gisborne (5 grower lines), Nelson (5 grower lines), Northland (5 grower lines) during the harvest season (25 May to 27 June) in 2001. Fruit weight, FL, FC, FH, FF, SSC and DM were measured on 60 fruit per grower line before storage. Dry samples from each grower line were combined for mineral analysis on a grower line basis. The remaining fruit was stored at 0.5°C for 24 weeks and their FF, SSC and DM were monitored fortnightly with 30 fruit per grower line at each monitoring period after 4 weeks storage. Measurement of FF during storage commenced within 5 minutes of being removed from 0.5°C storage. SSC measurements were taken at the two end caps of each fruit.

5.2.3 Data analysis

Fruit temperatures at FF measurement in experiment 2 were estimated to be 20°C for measurement before storage and 2°C for measurement during storage according to the changes in fruit temperature during equilibration (Feng et al., 2002). The FF value measured upon removal from 0.5°C storage was converted to FF measured at 20°C using the firmness-temperature coefficient of 'Hayward' kiwifruit (Jeffery and Banks, 1994).

The percentage of dry matter solubilized (SSFDM, %) was calculated using equation 5.1 (Jordan et al., 2000) to represent harvest maturity (Feng et al., 2003a).

$$\mathrm{SSFDM} = \frac{100 \cdot \mathrm{SSC} \cdot (100 - \mathrm{DM})}{\mathrm{DM} \cdot (100 - \mathrm{SSC})} \qquad (5.1)$$

Harvest date (HD) was converted to days after 1 April (the earliest possible harvest date). Storage duration (t, day) was calculated as days in storage before each firmness-monitoring period. Prestorage delay (DL) was calculated as days from harvest to the start of storage. The ratio of calcium and nitrogen concentrations (Ca/N, %) was also calculated because it was previously shown to affect storage behaviour of kiwifruit (Banks et al., 1995).

Fruit colour was not measured in experiment 2. L, C and H of grower lines in experiment 2 were calculated from FL, FC, FH, FF and DM using the relationships established from data collected in experiment 1 (data not shown).

Storage life was calculated from an exponential model (equation 5.2) fitted to FF monitoring data collected after three to six months' storage on a grower line basis. A bound condition of $k \geq 0.001$ was applied to avoid producing unrealistic SL. SL was calculated on the basis of the rearranged exponential model (equation 5.3)

$$FF = FF_0 \cdot e^{-k \cdot t} \qquad (5.2)$$

$$SL = \frac{\log_e(\mathrm{FF}_0) - \log_e(8.5)}{k} \qquad (5.3)$$

where FF is flesh firmness (N); t is storage duration; FF_0 represents an initial FF value when t=0; k represents the rate of fruit softening; SL is storage life; 8.5 is the minimum FF value at the end of storage as required for export (equivalent to 11.8 N if measured immediately after removal of fruit from cool storage).

All data from both experiment 1 and experiment 2 were pooled for analysis. Due to a missing value of nitrogen concentration for one grower line in experiment 2, only 108 grower lines were used for multivariate discriminate analysis. Four groups (KG_1 to KG_4) were formed according to the quartile distribution of k from low to high (Table 5.1). Stepwise discriminant analysis (Proc STEPDISC; SAS Institute, 1990) was used to select variables that had significant roles in discriminating grower lines into different groups. Two-thirds of the data were randomly assigned to the model building dataset with the remainder being allocated to the validation dataset (Table 5.1). Canonical discriminant analysis (Hair, et al., 1987; SAS Institute Inc., 1990; Cruz-Castillo et al., 1994) was performed on the model building dataset based on the selected variables. The standardized canonical coefficients (SCC) were used to evaluate the relative contribution of each independent variable to the discriminant functions (CDF_1, CDF_2 and CDF_3). Canonical functions having a significant role in separating grower lines were calculated for grower lines in the validation dataset and the Mahalanobis distances of each grower line to each of the four centroids (D_{ij}) were compared to assign a grower line to its closest softening-rate group. Classification accuracy (percentage of grower lines correctly classified) relative to the percentage chance criterion (C_p) was evaluated (Hair et al., 1987).

In cases where only CDF_1 and CDF_2 had a significant role in discriminating grower lines, D_{ij} was calculated using equation 5.4:

$$D_{ij} = \sqrt{(CDF_{1i} - \overline{CDF}_{1j})^2 + (CDF_{2i} - \overline{CDF}_{2j})^2} \qquad (5.4)$$

where D_{ij} is the Mahalanobis distance of grower line i to the centre of softening-rate group j; CDF_{1i} and CDF_{2i} are the first and the second discriminate functions of grower line i; $\overline{CDF}_{1j}$ and $\overline{CDF}_{2j}$ is the mean CDF_1 and CDF_2 of softening-rate group j.

In cases where multivariate analysis was used to assign observations into four groups, C_p was calculated using equation 5.5:

$$C_p = (\frac{n_1}{n})^2 + (\frac{n_2}{n})^2 + (\frac{n_3}{n})^2 + (\frac{n_4}{n})^2 \qquad (5.5)$$

where n_1, n_2, n_3 and n_4 are the number of grower lines in softening-rate group 1, 2, 3 or 4 respectively; n is the total number of grower lines.

Table 5.1 Softening-rate groups (KG) formed for multivariate discriminant analysis

KG	k (day^{-1})	Whole sample		Model building dataset		Validation dataset	
		n	Percentage	n	Percentage	n	Percentage
KG_1	=0.001	28	25.9	19	26.4	9	25.0
KG_2	0.001-0.0019	26	24.1	17	23.6	9	25.0
KG_3	0.0019-0.0034	27	25.0	18	25.0	9	25.0
KG_4	>0.0034	27	25.0	18	25.0	9	25.0

5.3 Results

5.3.1 Storage life

The value of FF_0 and k estimated for the 108 grower lines ranged from 13.8 to 51.5 N and 0.001 to 0.0096 day^{-1}, respectively. Estimated FF_0 values were highly correlated with estimated k values (Fig 5.1).

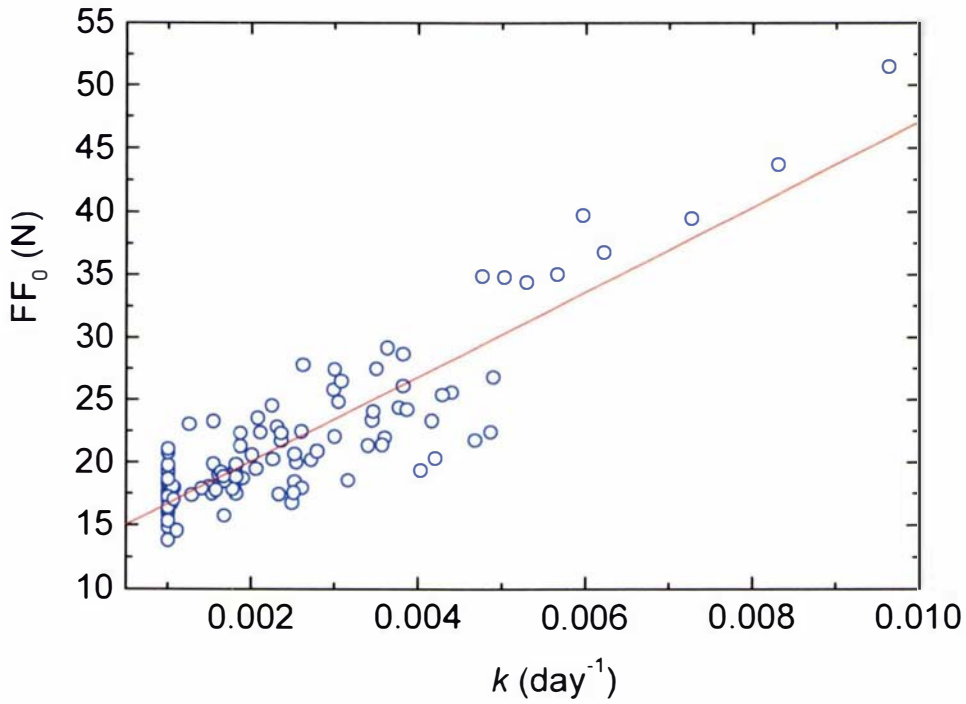

Figure 5.1 Relationship between fruit softening rate (k) and initial fruit firmness (FF_0) estimated for 108 grower lines based on flesh firmness data collected after three to six months storage. Each point represents a grower line. The solid line represents a linear model fitted to data ($FF_0 = 13.3 \pm 0.5 + 3378 \pm 168 \cdot k$, n=109, r=0.89)

Storage life calculated for the grower lines ranged from 187 to 908 days. There was a strong curvilinear relationship between k and SL (Fig 5.2).

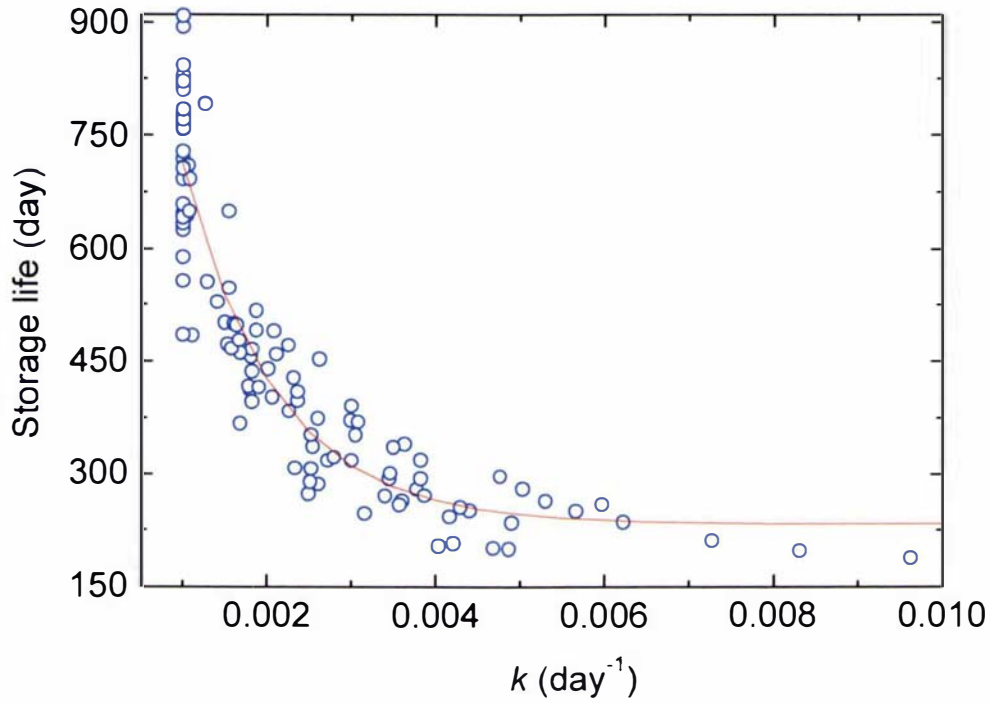

Figure 5.2 Relationship between storage life (SL) and softening rate (k). Each point represents a grower line. The solid line represents a curvilinear model fitted to the data ($SL = 1184 \pm 103 \cdot e^{-905 \pm 101 \cdot k} + 233 \pm 19$, n=109, R^2=0.87)

5.3.2 Multivariate discriminant analysis

Stepwise discriminant analysis indicated that SSFDM, Mg, HD, Ca/N, DL, and L had significant roles in discriminating grower lines for softening rate. Canonical discriminant analysis of the model building dataset based on the selected variables indicated that the first two canonical functions (CDF_1 and CDF_2) accounted for 95 % variation of the four softening-rate groups had significant roles in assigning grower lines into different groups (Fig 5.3).

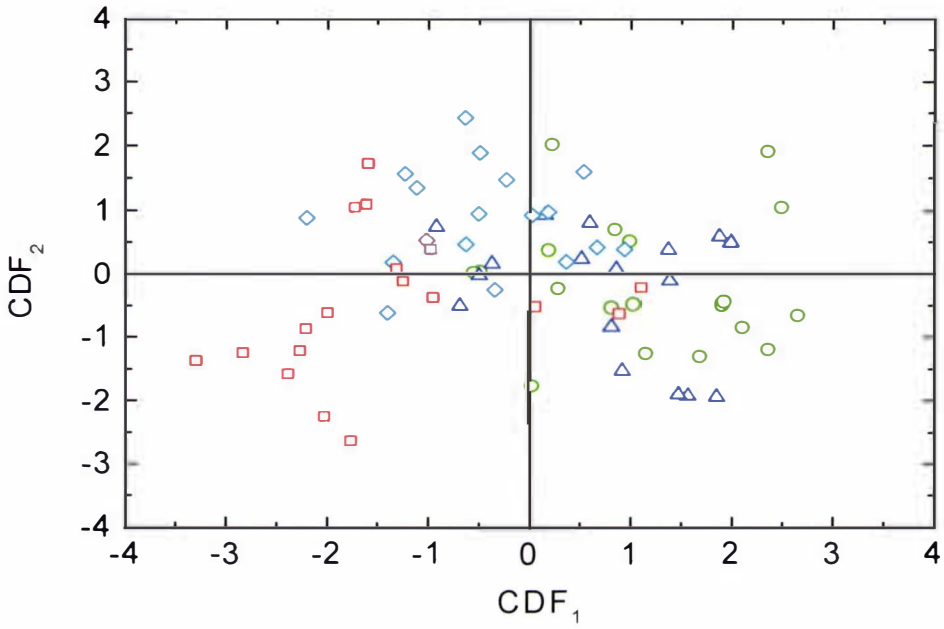

Figure 5.3 Separation of the fastest (□), second fastest (◇), second slowest (△) and the slowest (○) softening grower lines by the first two canonical functions (CDF_1 and CDF_2) for 72 grower lines in the model building dataset.

Analyses of variance of CDF_1 and CDF_2 indicated that grower lines that softened slowly (i.e. KG_1 and KG_2) had higher CDF_1 scores than grower lines that softened rapidly (i.e. KG_3 and KG_4). Grower lines in KG_3 also differed from KG_4, KG_1 and KG_2 in their CDF_2 scores (Table 5.2).

Table 5.2 Means of canonical scores of the first two canonical discriminant functions (CDF_1 and CDF_2) for four softening-rate groups (KG_1 softened at the lowest rate while KG_4 softened at the highest rate).

Softening-rate group	CDF_1		CDF_2		Number of grower lines
	Mean*	se	Mean*	se	
KG_1	1.15 a	0.24	-0.12 a	0.24	19
KG_2	0.75 a	0.23	-0.25 a	0.24	17
KG_3	-0.47 b	0.20	0.87 b	0.18	18
KG_4	-1.46 c	0.27	-0.50 a	0.27	18

* Values labelled with different letters are significantly different in pare-wise t test at $P<0.05$ level.

Standardized canonical coefficients (Table 5.3) indicated that CDF_1 discriminated grower lines on Mg, Ca/N, SSFDM and, to a lesser extent, HD and L. Grower lines harvested late with advanced maturity, high Mg, high Ca/N, low lightness and short prestorage delays were likely to soften slower, consequently having longer storage life. CDF_2 discriminated grower lines on harvest date and harvest maturity, and, to a lesser extent, prestorage delay. Grower lines with high CDF_2 scores were harvested late with low harvest maturity and long prestorage delays.

Table 5.3 The standardized canonical coefficients (SCC) and the correlation coefficients (r) between the canonical functions (CDF_1 and CDF_2) and the variables

Variable	CDF_1		CDF_2	
	SCC	r	SCC	r
Harvest maturity (SSFDM)	0.54	0.75	-1.40	-0.22
Mg	0.72	0.60	0.42	0.16
Harvest date	0.36	0.59	1.37	0.35
Ca/N	0.55	0.43	0.17	-0.03
Prestorage delay	-0.17	-0.27	0.57	0.47
Fruit lightness	-0.30	-0.33	-0.20	0.09

5.3.3 Discriminant accuracy

Discrimination based on CDF_1 and CDF_2 yielded correct classifications over twice that likely by chance (Table 5.4). The classification accuracies for the slowest and the fastest softening groups (i.e. PKG_1 and PKG_4) were 55.6% and 73.3% respectively compared with 33.3% and 45.8% of the moderate softening groups (i.e. PKG_2 and PKG_3).

Table 5.4 Discriminant accuracy based on the first two canonical functions for 72 grower lines in the model building dataset

Actual group	Grower line number (percentage) in each predicted group				Total
	PKG_1	PKG_2	PKG_3	PKG_4	
KG_1	10 (55.6%)	5 (33.3%)	4 (16.7%)	0 (0.0%)	19
KG_2	6 (33.3%)	5 (33.3%)	5 (20.8%)	1 (6.7%)	17
KG_3	1 (5.6%)	3 (20.0%)	11 (45.8%)	3 (20.0%)	18
KG_4	1 (5.6%)	2 (13.3%)	4 (16.7%)	11 (73.3%)	18
Total	18	15	24	15	72

Percentage of grower lines correctly classified: 51.4% (Percentage chance criterion = 25.04%)

5.3.4 Validation of the canonical discriminant model

Grower lines in the validation dataset had similar discrimination patterns along the CDF_1 and CDF_2 axis to those in the model building dataset (Fig 5.4).

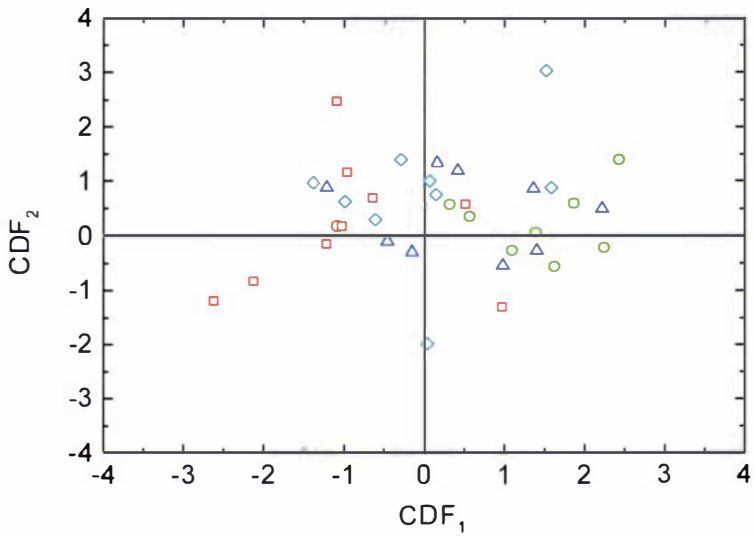

Figure 5.4 Separation of the fastest (□), second fastest (◇), second slowest (△) and the slowest (○) softening grower lines by the first two canonical functions (CDF_1 and CDF_2) for 36 grower lines in the validation dataset.

Discrimination of grower lines in the validation dataset based on CDF_1 and CDF_2 yielded 52.8% correct classification. This is more than twice that possible by chance (Table 5.5). The classification accuracies for the slowest and the fastest softening groups (i.e. PKG_1 and PKG_4) were 66.0% and 80.0% respectively compared with 33.3% and 46.7% of the moderate softening groups (i.e. PKG_2 and PKG_3).

Table 5.5 Discriminant accuracy based on the first two canonical functions for 36 grower lines in the validation dataset

Actual group	Grower line number (percentage) in each predicted group				Total
	PKG_1	PKG_2	PKG_3	PKG_4	
KG_1	6	1	1	1	9
	(60.0%)	(16.7%)	(6.7%)	(20.0%)	
KG_2	3	2	4	0	9
	(30.0%)	(33.3%)	(26.7%)	(0.0%)	
KG_3	1	1	7	0	9
	(10.0%)	(16.7%)	(46.7%)	(0.0%)	
KG_4	0	2	3	4	9
	(0.0%)	(33.3%)	(20.0%)	(80.0%)	
Total	10	6	15	5	36

Percent of grower lines correctly classified: 52.8% (percentage chance criterion = 25.0%)

5.3.5 Comparison between experiments

When the discriminant accuracies were analysed with respect to experiment, 64.9% grower lines in experiment 1 were correctly classified (Table 5.6) compared to 45.1% in experiment 2 (Table 5.7). Most of the grower lines in experiment 1 softened rapidly (17 grower lines were allocated to KG_4, accounting for 46% of the total of 37 grower lines and 12 grower lines were allocated to KG_3, accounting for 32.4%) while only a few grower lines softened slowly (2 grower lines were allocated to KG_1, accounting for 5.4% and 6 grower lines were allocated to KG_2, accounting for 16.2%). In this experiment, 12 out of the 17 grower lines in KG_4 and 11 out of the 12 grower lines in the KG_3 were correctly classified. However, all the 2 grower lines in KG_1 and 5 out of the 6 grower lines in KG_2 were incorrectly classified (Table 5.6). On the other hand, most grower lines in experiment 2 softened slowly (26 grower lines were allocated to KG_1, accounting for 36.6% of the total of 71 grower lines and 20 grower lines were allocated to KG_2, accounting for 28.2%) while

only about one-third of the grower lines softened rapidly (10 grower lines were allocated to KG_4, accounting for 14.1% and 15 grower lines were allocated to KG_3, accounting for 21.1%). In this experiment, 16 out of the 26 grower lines in KG_1 were correctly classified while the number of grower lines correctly classified in the other groups were all below half of the total number of grower lines in each group (Table 5.7).

Table 5.6 Discriminant accuracy based on the first two canonical functions for 37 grower lines in experiment 1.

Actual group	Grower line number (percentage) in each predicted group				Total
	PKG_1	PKG_2	PKG_3	PKG_4	
KG_1	0	0 (0.0%)	1 (4.5%)	1 (7.1%)	2
KG_2	0	1 (100.0%)	5 (22.7%)	0 (0.0%)	6
KG_3	0	0 (0.0%)	11 (50.0%)	1 (7.1%)	12
KG_4	0	0 (0.0%)	5 (22.7%)	12 (85.7%)	17
Total	0	1	22	14	37

Percent of grower lines correctly classified: 64.9%

Table 5.7 Discriminant accuracy based on the first two canonical functions for 71 grower lines in experiment 2.

Actual group	Grower line number (percentage) in each predicted group				Total
	PKG_1	PKG_2	PKG_3	PKG_4	
KG_1	16 (57.1%)	6 (30.0%)	4 (23.5%)	0 (0.0%)	26
KG_2	9 (32.1%)	6 (30.0%)	4 23.5%)	1 (16.7%)	20
KG_3	2 (7.1%)	4 (20.0%)	7 (41.2%)	2 (33.3%)	15
KG_4	1 (3.6%)	4 (20.0%)	2 (11.8%)	3 (50.0%)	10
Total	28	20	17	6	71

Percent of grower lines correctly classified: 45.1%

5.4 Discussion

Standardized canonical coefficients (Table 5.3) indicated that slow softening grower lines (i.e. KG_1 and KG_2) were associated with high Mg, Ca/N, advanced maturity, late harvest, low lightness (possible indication of fruit being exposed to wind and sunlight during the growing season) and short prestorage delays.

Grower lines in KG_3, a more rapidly softening group, had the highest CDF_2 scores compared with grower lines in KG_4, KG_1 and KG_2 (Table 5.2). Harvest date contributed positively to CDF_2 with the highest SCC value of 1.37 while SSFDM contributed to CDF_2 negatively with a comparable absolute SCC value of 1.4 (Table 5.3). Because SSFDM increased as harvest date increased ($r=0.77$), the contributions of harvest date and SSFDM to CDF_2 were minimised. Consequently, prestorage delay was actually the most important factor contributing to CDF_2 (prestorage delay had the highest correlation coefficient with CDF_2 compared with other variables; Table 5.3).

The positive effect of Mg on fruit softening identified in this study (Table 5.3) is consistent with the results of Benge (1999) who found fruit without soft patches, on average, contained more Mg that fruit with soft patches. The physiology of Mg in kiwifruit softening merits further investigation.

The selection of Ca/N by the stepwise canonical analysis indicated that the balance between Ca and N are more important than Ca and N individually. The association of high Ca/N with low softening rates (Table 5.3) is consistent with the literature where low Ca/N ratios were found to be associated with premature fruit softening and severe incidence of storage disorders (Prasad and Spiers, 1991; Banks et al., 1995; Benge 1999). The effect of Ca lies in its role in cell wall structure and ethylene production. Calcium acts to cross-link the pectin chains via calcium bridges (Stanley et al., 1991) and inhibits pectin solubilization by inhibiting PG and PME activities (Lee et al., 2001). Ca^{2+} ions are thought to be involved in regulating the activity of the ethylene-forming enzyme through calmodulin (Bailly et al., 1992). Calcium also protects fruit from ethylene-catalysed fruit softening by reducing such storage disorders as fungal rots and LTB (Wade, 1981; Poovaiah, et al., 1988; Wang, 1993) found to be the major causes of ethylene production (Wang, 1982, Feng et al., 2003b).

Nitrogen is the most important mineral for its role in many essential compounds, such as protein. Nitrogen deficiency result in reduced size of all plant organs, particularly leaf and fruit (Martin-Prevel et al., 1987). However, nitrogen fertilizers showed no significant effect on storage behaviour of kiwifruit (Buwalda et al., 1990). The current study used medium sized fruit and the orchards are in general well supplied with nitrogen fertilizer. The nitrogen concentration of the grower lines of the current study ranged from 399-901 mmol kg^{-1} DW, all fell within the normal range previously observed for 'Hayward' kiwifruit (Smith et al., 1994; Prasad and Spiers, 1991). Therefore, N alone is not a factor affecting fruit softening.

Principal component analysis on mineral composition of 'Hayward' kiwifruit indicated that individual elements could not be considered in isolation but rather in groups of elements: N, P, S, K, and Cu were classified into one group negatively related to FF after 12 weeks storage while Ca, Mg and Zn were in another group positively related to the FF. These two groups were negatively associated with each other (Smith et al., 1994). Therefore, Ca/N ratio may represent the balance of the two groups of elements rather than the balance of just Ca and N. The physiological reasons for the grouping of these elements and how the balance of these groups affects fruit softening are not immediately apparent except that Ca is a divalent element which is accumulated by the fruit largely during the very early stage of growth, possibly reflecting its lower mobility in the phloem (Clark and Smith, 1988). By contrast, the group of elements which includes nitrogen, are relatively more mobile in the phloem, and have been found to accumulate at a steady rate throughout the growth of the fruit (Clark and Smith, 1988). The physiological basis of the negative association between the two groups of elements is not clear. It is possible that N affects Ca accumulation in fruit through promoting vegetative growth (Johnson et al., 1997) that reduces xylem flow to fruit (Buwalda, 1991). Further work is required to clarify the physiological mechanism of the Ca/N ratio and its effect on fruit softening.

The association between high Mg concentration and slow fruit softening is consistent with previous observation where firm fruit (after 14 weeks of storage) had significantly higher Mg concentration than soft fruit (Tagliavini et al., 1995). 'Hayward' kiwifruit has green flesh due to the presence of chlorophyll (Ferguson, 1990). Magnesium concentration may also be an indication of fruit position in vine. Fruit exposed to direct

sunlight may have high chlorophyll concentration, consequently having high Mg concentration.

Grower lines harvested later at higher SSFDM tended to soften at lower rates than those harvested earlier at lower SSFDM (Table 5.3). This may relate to the high incidence of LTB associated with early harvested fruit at low maturity (Harman, 1981). Pre-conditioning fruit to cool temperatures is a technique that has been used to ameliorate LTB in crops such as cucumbers and zucchini (Wang, 1993; 1996). Indeed, recent work in Greece that shows evaporative cooling with an over-tree mist system before harvest has reduced LTB possibly through a chilling acclimation effect (Sfakiotakis et al., 2002). The effects of harvest date on fruit softening rate is possibly a reflection of a chilling acclimation during late autumn. Alternatively, the effect of harvest date may relate to a large increase in cytokinin concentration and content per fruit observed during the harvest season (Lewis et al., 1996). Increased cytokinin concentration may inhibit ethylene production in mature fruit (Lieberman, 1975), consequently reducing the softening rate.

The effect of prestorage delay on fruit softening (Table 5.3) may be a balance of two influences: First, prestorage delay may act as a postharvest curing to reduce the incidence of fruit rot caused by *Botrytis cinerea* (Poole and McLeod, 1994), consequently to prevent fruit softening caused by ethylene production of rotten fruit. Second, fruit's susceptibility to vibration damage during the grading and packing process increases as fruit becomes softer (Mitchell, 1990). The second influence may not be of significant importance for early harvested grower lines which display a lag phase in fruit softening shortly after harvest (MacRae et al., 1990). However, the influence may become increasingly significant as the lag phase disappears in the late harvested grower lines (MacRae et al., 1990; Benge, 1999). These two influences of prestorage delay were reflected by the negative SCC of prestorage delay in CDF_1 and its positive SCC in CDF_2 (Table 5.3). An extended prestorage delay may improve storage potential of grower lines harvested early at low maturity, but the same practice may reduce the storage potential of grower lines harvested late at advanced maturity.

The negative coefficient of fruit lightness in CDF_1 indicates that grower lines with a low lightness (measured on fruit skin) tend to soften at lower rate than those with higher lightness (Table 5.3). Kiwifruit vines with dense canopies produce fruit with lighter, more

vivid, more yellow/brown skin (Snelgar et al., 1998). This suggests that fruit produced in orchards with dense canopies will have high fruit lightness and consequently be more likely to soften rapidly during storage. Such a relationship between fruit lightness and softening rate may be explained by the dependence of Ca accumulation on water influxes driven by fruit transpiration (Clark and Smith, 1988). Fruit grown in an open canopy would be more exposed to light and wind, consequently have a high transpiration rate; the high transpiration rate in turn would enhance the driving force for Ca accumulation.

The percentage of grower lines correctly classified by the first two canonical functions for both the model building dataset (51.4%) and the validation dataset (52.8%) was twice that of the percentage chance criteria (Tables 5.4 and 5.5) and five times greater than the minimum improvement of 25% suggested by Hair et al. (1987) for justifying the significance of improvement using a discriminant model. The discriminant accuracy was higher for groups with softening rates at both ends of the softening-rate continuum (i.e. KG_1 and KG_4) than that of moderate groups (i.e. KG_2 and KG_3; Tables 5.4 and 5.5), suggesting that the model could be used to select grower lines with excellent storage potential for long-term storage and to identify grower lines with poor storage potential that need immediate attention.

Further work is needed to improve the discriminant model given that over 40% of the grower lines have been assigned to incorrect groups. Classification of grower lines was based on canonical functions (CDF_1 and CDF_2) calculated from fruit maturity (SSFDM), Mg, harvest date, Ca/N, prestorage delay and fruit lightness. Some of the incorrect classifications could be attributed to the measurement errors of these variables. Experiment 1 was carried out using rationalised methodologies (Feng et al., 2003c) and the minerals concentrations of each grower line were the average of 20–40 fruit measured separately. Measurement errors in this experiment were minimised and the discriminant accuracy (64.9%) for grower lines in this experiment is satisfactory. Mineral concentrations of experiment 2 were measured on a combined sample on grower line basis without internal replication and fruit lightness of this experiment was estimated from flesh colour (FL, FC and FH), FF and DM (the regression model explained only 64% of the variation in fruit lightness), this may explain the relatively low discriminant accuracy (45.1%) for grower lines in this experiment compared with that for grower lines in experiment 1.

Sample preparation and measurement method are two major sources of measurement error for fruit attributes (Feng et al., 2003c). For mineral analyses that use only 0.1-0.2 g dry sample or 0.5-1.0 g wet sample, thorough homogenisation is essential to make sure this small amount of sample represent the whole sample. Internal replication is another way to reduce measurement error. However, measuring minerals on an individual fruit basis (experiment 1) is too costly for industry application. Combining every 4-5 fruit of the total fruit sample from each grower line for mineral analysis (Hopkirk et al., 1990) could be a compromise method to obtain accurate mineral concentrations with affordable cost. Fruit colour should be measured on dry fruit after appropriate temperature equilibration because water condensation has significant effect on colour measurement (Feng et al., 2002).

The postulated effects of harvest date and prestorage delay (e.g. chilling acclimation and curing) not only depend on harvest date and the duration of the delay, but also the climate conditions (e.g. temperature, humidity and wind speed) that vary with orchard site and year. The use of harvest date and the days of prestorage delay regardless of climate conditions may introduce some uncertainties to the discrimination results. Further work is needed to clarify the effects of harvest date and prestorage delay on fruit softening rate and how these effects can be accessed more accurately by taking other relevant climatic factors such as temperature, humidity and wind speed into consideration.

The association of Mg, Ca/N, harvest maturity, harvest date, fruit lightness and prestorage delay with fruit softening rate reflected by the coefficients in CDF_1 and CDF_2 can also be used to identify problems with different grower lines. Growers producing fruit with low Mg, Ca/N, and high fruit lightness need to improve their soil and canopy management. Late harvested fruit of advanced maturity is not only preferred for better storage potential, but also for better eating quality (high dry matter content; Smith et al., 1995; Jordan et al., 2000). However, postponing harvest is limited by the threat of frost and the requirements of pack house management. For a given grower line arriving at a pack house, it would also be possible to determine the most appropriate prestorage delay based on the context of mineral concentrations, fruit maturity, harvest date and fruit colour. However, this may be limited practically by the mineral analysis that takes at least 3 days to complete. It would be advisable to sample fruit a few days before harvest for mineral

analysis or develop quick mineral analysis methods such as near infra-red spectroscopy (Williams & Norris, 2001) or X-ray fluorescence analysis (Schelle et al., 2002; Bohlen et al., 2003) for kiwifruit.

It is also noticed that two-thirds of the grower lines in experiment 1 softened rapidly (i.e. 29 out of the 37 grower lines were allocated to KG_4 and KG_3) while only a few grower lines softened slowly (i.e. 2 grower lines were allocated to KG_1 and 6 grower lines were allocated to KG_2). On the other hand, two-thirds of the grower lines in experiment 2 softened slowly (i.e. 46 out of the 71 grower lines were allocated to KG_1 and KG_2) while only about one-third of the grower lines softened rapidly. Comparison of fruit attributes of the grower lines in experiment 1 with those of the grower line in experiment 2 harvested from the same production region with similar harvest date indicated that grower lines in experiment 2 had shorter prestorage delay but higher SSFDM and higher Ca/N than those in experiment 1. It is difficult to clarify whether the differences in SSFDM and Ca/N represent the true difference between grower lines of the two experiments or was caused by measurement bias given that the data of the two experiments were collected by different people using different instruments.

Furthermore, the discriminant results for grower lines in experiment 1 indicate that both grower lines in KG_1 and 5 out of the 6 grower lines in KG_2 were incorrectly classified to rapidly softening groups (i.e. KG_3 and KG_4; Table 5.6). Due to the small observation number, it is hard to judge if the misclassification represents a tendency of classifying slow-softening grower lines to rapid-softening grower lines for experiment 1 or it happened by chance. Attempts to overcome experiment bias by standardizing all or selected variables on an experiment basis did not give meaningful results (data not shown). Therefore, further work is needed to validate the discriminant model data collected using standard measurement methodologies.

5.5 Conclusion

Storage potential of 'Hayward' kiwifruit is characterised by fruit softening rate estimated by fitting FF monitoring data collected after three to six months' storage to an exponential model. Two canonical functions calculated from SSFDM, Mg, harvest date,

Ca/N, prestorage delay, and fruit lightness classified over 50% grower lines to the correct groups compared with the percentage chance criterion of 25%. Grower lines harvested later at advanced fruit maturity with high Mg, Ca/N, low lightness and short prestorage delay are likely to soften slowly, consequently having high storage potential. For grower lines harvested early at low maturity, extending prestorage delay may improve their storage potential. However the delay could be detrimental to other grower lines harvested late at advanced maturity. The association of Mg, Ca/N, harvest maturity, harvest date, prestorage delay and fruit lightness with fruit softening rate reflected by the discriminant model can be used for postharvest management as well as orchard management for producing fruit of high storage potential. Reducing measurement errors of the fruit attributes using rationalised sample preparation and measurement methods is essential for accurate discrimination of grower lines using the discriminant model. Further work is needed to validate the discriminant model with data collected using standard measurement methodologies.

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Measurement of at-harvest fruit attributes using visible-near-infrared spectroscopy

6.1 Introduction

The quality and storage behaviour of kiwifruit are associated with such fruit attributes as harvest maturity (Weet, 1979; Harman, 1981; Harman et al., 1982; Crisosto et al., 1984; Asami et al., 1988; Mitchell et al., 1992; Abdala et al., 1996; Pan et al., 1996; Costa et al., 1997; Benge, 1999), fruit size (Hall et al., 1996; Crisosto et al., 1999), mineral concentration (Hopkirk et al., 1990; Prasad and Spiers, 1991; Banks et al, 1995; Lallu and Yearsley, 1995; Tagliavini et al., 1995; Davie, 1997; Lallu et al., 1999; Benge, 1999; Hertog and Jeffery 2000, 2002; Ferguson et al., 2001) and fruit colour (Feng, et al., unpublished). Developing non-destructive technologies to measure these attributes at low cost and high speed would facilitate fruit segregation in the packhouses. Fruit of different quality and storage potential could be packed separately and matched to appropriate storage strategies and marketing destinations.

Of the various non-destructive methods used to measure intact fruit attributes, near infra-red spectroscopy (NIR) is the most advanced in terms of instrumentation and application (Guthrie and Walsh, 1997). In many cases, NIR spectroscopy is extended to visible wavelengths, down to 400 nm, and therefore should be described as visible-near infrared spectroscopy (VNIR; Williams & Norris, 2001).

VNIR has been used successfully to estimate soluble solid content and dry matter content on intact kiwifruit (McGlone and Kawano, 1998; Osborne and Kunnemeyer, 1999; Schaare and Fraser, 2000; McGlone et al., 2002b) with an overall standard error of estimation (SEP) of about 0.5% and $R^2 > 0.80$. Estimation of flesh firmness has been less accurate (SEP=7.8 N, R^2=0.66) possibly because there is insufficient pectin in kiwifruit (<1% by weight) for VNIR to detect (McGlone and Kawano, 1998). Internal flesh hue angle of yellow-fleshed kiwifruit (*Actinidia chinensis*) could be estimated by interactance VNIR spectra with SEP of 1.6 degree and with a R^2 of 0.82 (Schaare and Fraser, 2000).

VNIR technologies for measuring attributes other than DM and SSC of green-fleshed 'Hayward' kiwifruit are yet to be evaluated.

VNIR measurement can be conducted in three different modes: reflectance, interactance and transmission. Reflectance mode measurements are the easiest to make on a grading line, while interactance mode spectra provided the most accurate estimates of SSC, density and flesh colour (Schaare and Fraser, 2000). Use of the transmission mode for measuring fruit quality is less common, possibly because of the difficulties in obtaining accurate transmission measurements at grading line speeds (Schaare and Fraser, 2000).

Raw VNIR spectra contain background noise and are subject to instrument drift. Many pre-calibration transformations exist to reduce background noise and to remove linear baseline changes between spectra (Williams & Norris, 2001). Log transformation of raw spectra against a baseline to generate absorbance spectra is an essential pre-treatment for VNIR data, while other pre-calibration transformations such as smoothing, differentiation (D_1 and D_2), standard normal variate transformation (SNV), multiplicative scatter correction (MSC), and Fourier transformation (FT) have also been useful (Geladi et al., 1985; Barnes et al. 1989; Schaare and Fraser, 2000; Williams & Norris, 2001).

Traditionally, the chemical species relating to an attribute of interest were identified and estimation models established using multiple regression on the basis of a small number of absorbance bands. This procedure is successful when determining attributes having a simple chemical basis, such as moisture content, protein content and lipid content of grains, meat, milk and their products (Osborne et al., 1993; Williams & Norris, 2001). However, when the attribute to be determined has a complex chemical basis, peaks of different chemical species overlap and estimates based on several peaks are insufficient. Therefore, full spectrum methods of data analysis such as principal component regression (PCR) and partial least squares regression (PLS) were developed. Principal component regression works by first reducing the dimensionality of the spectral data by principal component analysis (PCA), which extracts successive linear combinations of the spectral data, called principal components (PCs). The first few PCs are extracted to account for most of the variation of the spectral data. These PCs are then related to the attribute to be determined using multiple regression (Osborne et al., 1993).

Partial least squares (PLS) regression is the most common technique used to developed calibrations based on the full spectrum. Similar to PCR, PLS reduces the dimensionality of the spectral data by extracting successive linear combinations of the spectral data, called factors, and then regresses the factor scores against attributes to be determined. Factors are similar to principal components in that they are orthogonal and the first few factors extracted account for most of the variation of the spectral data. In addition, factor extraction also takes into account the correlation between factors and the response variable (i.e. fruit attributes to be predicted), so that the first few factors explain as much of the response variation as possible. PLS also provides cross validation options to protect against over fitting. Factors are added to the regression model one at a time until the mean squared estimation error of the validation data set reaches a minimum (Osborne et al., 1993; Williams & Norris, 2001).

A VNIR spectrum taken from a fruit may have 200-2000 or more data points. In contrast, the number of independently varying spectrally active constituents in a sample is usually rather small. Consequently, large data sets are collected with high degree of redundancy, which in turn render computerized data analysis extremely memory- and time-consuming. Compression of spectral data by boxcar averaging (average blocks of data points) not only speeds up computerized data analysis, but also produces superior models through the enhancement of signal to noise (Faber, 1999). As only some wavelength regions in a spectrum are actually related to a particular attribute, a reduced spectral range is essential for developing good predictive models. Wavelength selection can be achieved by progressively reducing and sliding a spectral window across the spectral range (McGlone and Kawano, 1998) or removing spectral data at regular or random intervals (Osborne et 1998; Carlini et al., 2000).

The premise for the current study is to evaluate existing VNIR technologies in interactance and reflectance modes for estimation of kiwifruit attributes relating to fruit quality and storage potential.

6.2 Materials and methods

6.2.1 Experiment 1

Four hundred 'Hayward' kiwifruit (count 36) from four grower lines in the Bay of Plenty were randomly sampled from harvest bins on 1 May 2000. Fruit were sent to Massey University by overnight courier and randomly assigned to five batches, each consisting of 20 fruit from each of the four grower lines. Two batches were kept in the laboratory (20°C, 50-60% RH) for measurement on 2 and 3 May while the other three batches were cool stored at 0.5°C for 1, 2, and 4 days before taking them out for temperature equilibration (12 h at ambient temperature before the start of measurement) and measurement. Fruit hair was brushed off using a piece of foam to simulate hair brushing on commercial packing lines.

NIR measurements were taken at two opposite points along the equator of each fruit. The measuring instrument was constructed around a TM300 monochromator (Bentham Instruments Limited, Reading, UK) fitted with a ruled grating (1200 lines mm^{-1}) and a 250 W quartz halogen lamp. The light from the exit slit of the monochromator was focussed onto one arm (6 mm in diameter) of a bifurcated fibre optic bundle (Oriel Instruments, Stratford, Connecticut, USA) and the light from this was focussed onto the fruit surface (this end of the fibre-optic bundle was a rectangle of 6 × 2 mm). Light from the fruit passed back through the other arm of the fibre optic bundle and was focussed onto a silicon photodiode (1.75 mm^2 active area, RS Components, Auckland, New Zealand). The voltage output of the photodiode and associated circuitry was acquired using a DAS-1600 A/D board (Keithley, Taunton, Massachusetts, USA). All the operations of the instrument were controlled using in-house software. Spectra were collected at 2 nm intervals and each point represents the average of 1000 acquisitions. NIR measurement was carried out in darkness, and fruit were covered by a piece of cloth during the measurement to avoid disturbance by light from the environment. Reference spectra were taken daily on a Teflon block.

Fruit colour (L, C, H), compression force (CF), flesh firmness (FF), flesh colour (FL, FC, FH) and soluble solid content (SSC) were measured within 4 hours of NIR measurement. A middle section approximately 1 cm thick was cut from each fruit along the

equatorial zone immediately next to the holes left from FF measurement and dried for dry matter (DM) and mineral (N, P, K, Ca, and Mg) measurement. Measurement methods for L, C, H, FL, FC, FH, CF, FF, SSC, DM and mineral concentrations were the same as described previously (Chapters 4 and 5).

6.2.2 Experiment 2

Ten trays of commercially packed 'Hayward' kiwifruit (count 36) from each of the nine grower lines were sampled during the main harvest seasons of year 2000 (9 May to 23 May) at weekly intervals (three grower lines per week) from pack houses in the Bay of Plenty. Fruit were cool stored at 0.5°C in polylined single-layered trays with 5 g per tray of ethylene absorbent (Purafil, Papworth Engineering Ltd., New Zealand) after two days for transportation and laboratory measurements. After 2-8 days cool storage and 12 hours temperature equilibration, 20 fruit from each grower line were taken out of storage for VNIR measurement in reflectance mode using a commercial NIR system (Compac Sorting Equipment Ltd, New Zealand). Fruit carried by a conveyer belt with stem-calyx axis horizontal passed through the NIR scanning chamber where a continuous beam of light generated from a halogen lamp was focused on moving fruit and the reflected light from the fruit measured ten times. Two separate spectral measurements were made on each kiwifruit, on opposite sides of the median equator. The 20 VNIR spectra recorded for each fruit were averaged after subtracting the dark reference and dividing by a white reference (materials and methods used to obtain reference spectra are confidential to the Compac Sorting Equipment Ltd). The averaged spectra were then smoothed using in-house software. Spectra were collected within a wavelength range of 300-1200 nm at 3.50-3.56 nm intervals (256 points). Fruit attributes such as L, C, H, FL, FC, FH, CF, FF, SSC, DM and mineral concentrations were measured on the following day.

6.2.3 Experiment 3

Ten trays of commercially packed 'Hayward' kiwifruit (count 36) from each of 22 grower lines were sampled during the main harvest season (28 May to 9 June) of year 2001 from pack houses in the Bay of Plenty. On the day of sampling, fruit in different trays of a

grower line were randomised and interactance VNIR measurements were taken on four trays (total of 144 fruit) of fruit from each grower line using an USB2000 fibre optic spectrometer (Ocean Optics Inc.) fitted with a fibre optic interactance probe. The probe was a R400-7 probe that consists of a tight bundle of 7 optical fibres of 400 μm in diameter (6 illumination fibres around 1 read fibre) in a stainless steel ferrule of 76 mm long and 0.6 mm diameter. The probe was pointed at the median equator of a fruit and a spectrum of 519-1156 nm was recorded at about 0.31 nm intervals (2048 points). Both white reference spectra (obtained when the probe was inserted into the RPH-1 reflection probe holder at 90° angle) and dark spectra (obtained by blocking the aperture to the optical fibre) were recorded regularly during the measurement period. Two separate spectra were taken on opposite sides of each fruit.

Fruit were then transferred to Massey University where L, C, H, FL, FC, FH, CF, FF, SSC, DM and minerals were measured on twenty VNIR scanned fruit from each grower line on the next day.

6.2.4 Experiment 4

This experiment was carried out by AgFirst Consultants on behalf of Zespri Innovation Company Ltd. Nine hundred and twenty fruit from each of 72 grower lines were sampled from the Bay of Plenty, Gisborne, Nelson and Northland during the main packing season (25 May to 27 June) in 2001. Interactance VNIR measurement was taken on all fruit using a USB2000 fiber optic spectrometer. Spectrum of 520-1159 nm was recorded at about 0.31 nm intervals (2048 points). Ten separate spectra were taken on each fruit and were averaged to produce one spectrum for each fruit. Six fruit attributes (FF, FL, FC, FH, SSC and DM) were measured on 60 fruit per grower line after the VNIR measurement. FF was measured by the same method as that of experiments 1-3 but used a different penetrometer (HortPlus Quick Measure Penetrometer System; HortPlus Ltd, New Zealand). Flesh colour was measured using three chroma meters of the same model as that used in experiment 1-3. The chroma meters were calibrated with white calibration plates rather than green calibration plate used in experiments 1-3. SSC was measured at two end caps of each fruit using refractometers of the same model as that used in experiments 1-3. DM was measured on equatorial slices 3-5 mm thick that had been dehydrated at 65°C for 20 hours.

6.2.5 Data analysis

6.2.5.1 Pre-treatment of spectral data

Spectral data from all experiments were baseline (reference spectra) corrected to produce absorbance spectra (ABS). Absorbance spectra were normalised in different ways (Table 6.1) before or after smoothing and second differentiation using a simplified least square procedure (Savitzky and Golay, 1964, Griffiths, 1972). Denominators for different normalizations were calculated within a further reduced wavelength range of 600-1050 nm where the spectra were reasonably smooth and meaningful across all experiments.

Table 6.1 VNIR data pre-calibration transformations used in this study

Pretreatment	Description	Transformation
ABS	Absorbance	$I_i = -\log(\frac{I_{f_i} - I_{d_i}}{I_{w_i} - I_{d_i}})$ *
ABS+NM	ABS normalised to minimum and maximum	$I_i = \frac{I_i - Min}{Max - Min}$
ABS+SNV	Standard normal variate transformation of ABS	$I_i = \frac{I_i - Mean}{Std}$
ABS+QBC	Quadratic baseline compensation of ABS	$I_i = I_i - (a_0 + a_1 \cdot \lambda + a_2 \cdot \lambda^2)$
SABS	Smoothed ABS	$I_i = \frac{\sum_{j=i-m}^{j=i+m} S_j \cdot I_j}{Norm}$
SABS+NM	SABS normalised to minimum and maximum	$I_i = \frac{I_i - Min}{Max - Min}$
SABS+SNV	Standard normal variate transformation of SABS	$I_i = \frac{I_i - Mean}{Std}$
SABS+QBC	Quadratic baseline compensation of SABS	$I_i = I_i - (a_0 + a_1 \cdot \lambda + a_2 \cdot \lambda^2)$
D_2	Second derivative of ABS	$I_i = \frac{\sum_{j=i-m}^{j=i+m} S_j \cdot I_j}{Norm \cdot (\Delta\lambda)^2}$
D_2+NM	D2 normalised to minimum and maximum	$I_i = \frac{I_i - Min}{Max - Min}$
D_2+SNV	Standard normal variate transformation of D2	$I_i = \frac{I_i - Mean}{Std}$
NSD	Normalised second derivative	$I_i = \frac{I_i}{\sum I_i}$

I_i is the spectral datum at point i (i=1, 2, 3, ...n); n is the total number of data points in a spectrum; I_{f_i} is the raw spectral datum at point i in a spectrum taken on a fruit; I_{d_i} is the raw spectral datum at point i in a dark reference spectrum; I_{w_i} is the raw spectral datum at point i in a white reference spectrum; *Mean*, *Max*, *Min* and *Std* represent, respectively, the average value, maximum value, minimum value and standard deviation of a spectrum within 600-1050 nm; m is the number of points before and after a central point i that was used to calculate SABS or D_2 for point i; a section of spectrum from i-m to i+m, including 2m+1 points is called the window size for smoothing and differentiation. S_j is the weight coefficient of data point j for smoothing by polynomial curve fitting (Savitzky and Golay, 1964); *Norm* is the denominator for smoothing or second derivative by polynomial curve fitting (Savitzky and Golay, 1964; Griffiths, 1972); λ is the wavelength. $\Delta\lambda$ is the wavelength interval between two adjacent data points of a spectrum. a_0, a_1 and a_2 represent coefficients of a quadratic baseline fitted to spectral data at 750, 880 and 1100 nm where the absorbance are expected to be zero.

* ABS of experiment 2 was calculated as $-\log(\frac{I_{f_i}-I_{d_i}}{I_{w_i}})$, ABS of experiment 3 was calculated as $-\log(\frac{I_{f_i}-I_{d_i}}{2\cdot(I_{w_i}-I_{d_i})})$ and ABS of experiment 4 was obtained from Zespri as $-\log(\frac{I_{f_i}-I_{d_i}}{1.5\cdot I_{w_i}})$. $I_{f_i}-I_{d_i}$ of experiment 3 and I_{w_i} of experiment 4 were multiplied by 2 and 1.5 respectively to prevent negative ABS values.

Boxcar averaging (Faber, 1999) at two levels of wavelength-band widths (1 nm and 2.5 nm) was applied to spectral data from experiment 3 and experiment 4 before smoothing and normalisation. Noisy spectral data at both ends of the spectra were truncated (Table 6.2).

Table 6.2 Wavelength range, truncation and boxcar-averaging of spectral data in different experiments

Experiment	Full wavelength range (nm)	Truncated wavelength range (nm)	Data points before/after boxcar averaging
1	400-1200	400-1200	400/not applicable
2	300-1200	400-1200	256/ not applicable
3	519.00-1156.01	535.52-1154.44	2048/619 or 251
4	520.14-1158.65	536.05-1154.6	2048/619 or 249

6.2.5.2 Development of calibrations on pre-treated spectra

Two-thirds of the data from each experiment were randomly assigned to the model-building data set with the remainder being allocated to the validation data set. Initially, predictive models were developed by PLS regression between fruit attributes and spectral data following different pre-calibration transformations. Wavelength selection was performed by progressively reducing and sliding a spectral window across the spectral range. A matrix of the root means of the sum squares of predicted residual (PRESS) for all combinations of pre-treatment and wavelength range was subsequently obtained for each of the four experiments. The minimum root mean PRESS achieved for each fruit attribute in each experiment was selected from the matrix and the predictive model with the minimum root mean PRESS was taken as a benchmark for further model development.

Further model development started by comparing the association of spectral data with fruit attributes. Fruit in each experiment were divided to 5 groups according to the fruit attribute to be predicted, with the first group having the lowest value and the fifth group having the highest value. The means ($\bar{I}_g$) and standard deviations (s_g) of the spectral data at each wavelength point were calculated for each group (g). The trend (*TND*) of the association between spectral data at each wavelength point with the reference variable was judged by the difference between adjacent groups (ΔI_g; equation 6.1).

$$\Delta I_g = \bar{I}_{g+1} - \bar{I}_g \qquad (6.1)$$

where g=1, 2, 3 and 4. If all the four ΔI_g were positive, then *TND* = 1, indicating a strong positive trend; if all the four ΔI_g were negative, then *TND* = -1, indicating a strong negative trend; else *TND* = 0, indicating no trend.

The sum of relative differences between groups (SRD) and the average standard deviation (ASTD) of the five groups were calculated using equations 6.2 and 6.3 respectively.

$$SRD = \sum_{g=1}^{g=4} \frac{I_{g+1} - I_g}{s_{g+1} - s_{g-1}} \quad (6.2)$$

$$ASTD = \frac{\sum_{g=1}^{g=4} s_g}{4} \quad (6.3)$$

When *TND*, *SRD* and *ASTD* were plotted against wavelength, wavelength regions with *TND* of 1 or –1, high *SRD* and low *ASTD* were highlighted as important regions for estimation of the fruit attribute. Highlighted regions were further removed group by group for PLS regression and stepwise regression until the PRESS reached a minimum.

Due to accidental factors, VNIR spectra from one grower line in experiment 3 and two grower lines in experiment 4 were deemed unreliable and were excluded from analysis.

Absorbance of experiment 1, 2 and 3 were calculated using SPECTRUM (Brown, unpublished) while that of experiment 4 were calculated using GRAMS/32 AI (V6; Galactic Industries Corporation, USA). Further data processing and analysis were performed using SAS (V8.2; SAS Institute Inc. USA).

6.3 Results

6.3.1 Predictive models established in experiment 1

Of the 15 at-harvest fruit attributes measured, fruit colour, flesh colour, CF, FF, SSC and N could be predicted by spectral data with $R^2_p > 0.5$; while the estimation for other attributes (DM, P, K, Ca and Mg) was poor ($R^2_p < 0.37$; Table 6.3 and Fig 6.1).

Table 6.3 Predictive models for at-harvest fruit attributes based on VNIR spectra of a monochromator in experiment 1 (n=268 for model-building data set and 132 for validation data set).

Variable	Pre-treatment	Wavelength ranges (nm)	R^2_p	SEP
L	ABS	550-1054	0.79	1.65
C	SABS	500-1058	0.84	0.82
H	ABS	454-702, 754-1148	0.71	2.93
FL	ABS	488-780, 894-1174	0.85	2.66
FC	ABS+SNV	488-730, 1042-1158	0.63	2.18
FH	ABS	442-778, 838-1136	0.58	0.63
CF	SABS+NM	604-1146	0.82	3.71 N
FF	SABS+NM	600-1126	0.59	10.78 N
SSC	SABS+SNV	584-832, 938-1188	0.64	0.65 %
DM	ABS+SNV	576-724, 1114-1196	0.37	0.99 %
N	SABS+SNV	480-708, 758-1114, 1146-1186	0.59	73.72 mmol kg^{-1}
P	SABS+SNV	404-768, 1128-1168	0.34	7.40 mmol kg^{-1}
K	ABS+SNV	502-708, 788-1180	0.31	50.98 mmol kg^{-1}
Ca	SABS+NM	410-748, 1042-1090	0.09	9.79 mmol kg^{-1}
Mg	SABS+NM	422-528, 998-1178	0.19	3.10 mmol kg^{-1}

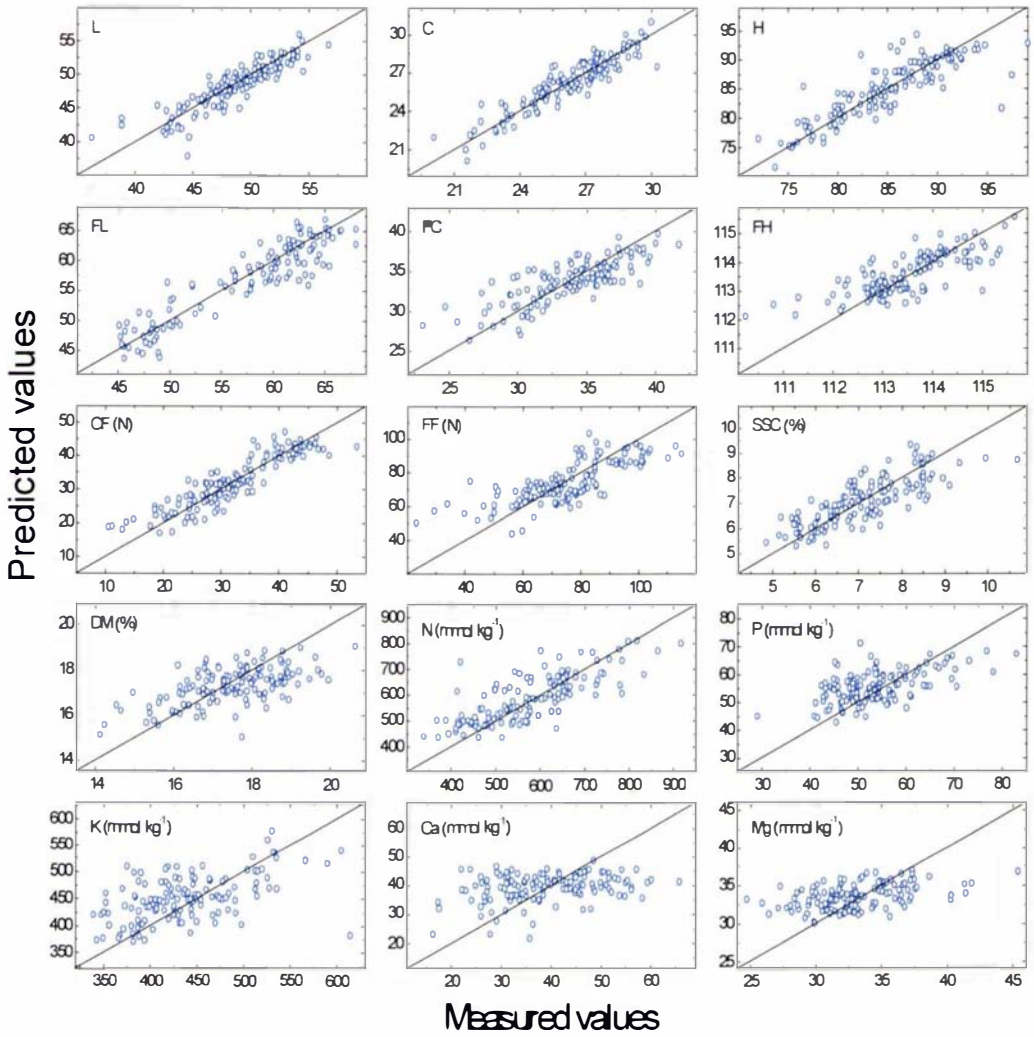

Figure 6.1 Measured and predicted values of fruit colour (L, C, H), flesh colour (FL, FC, FL), compression force (CF), flesh firmness (FF), soluble solid content (SSC), dry matter content (DM) and mineral concentrations (N, P, K, Ca, and Mg) using the predictive models (Table 3) established in experiment 1 for a validation data set.

6.3.2 Predictive models established in experiment 2

PLS between spectral data and the 15 fruit attributes measured at harvest revealed that fruit colour, flesh colour, SSC, DM, N, P, Ca and Mg could be predicted with $R^2_p > 0.5$ while the estimation for other fruit attributes were poor (R^2_p <0.46; Table 6.4 and Fig 6.2).

Table 6.4 Predictive models for at-harvest fruit attributes based on reflectance spectra of a commercial VNIR fruit sorting system in experiment 2 (fruit number in model data set was two times that of the validation data set listed).

Variable	Pre-treatment	Wavelength range (nm)	R^2_p	SEP	Validation fruit number
L	SABS+SNV	384-1196	0.60	1.41	357
C	ABS	489-941	0.71	0.98	357
H	ABS+NM	489-1065	0.80	1.85	357
FL	ABS+NM	493-1008	0.68	1.57	67
FC	ABS	598-1051	0.51	1.27	67
FH	SABS+SNV	486-1150	0.72	0.48	67
CF	SABS	500-1051	0.44	3.15 N	331
FF	SABS	630-913	0.38	8.55 N	67
SSC	ABS+NM	799-1136	0.73	0.50 %	67
DM	SABS+SNV	500-1044	0.85	0.36 %	67
N	ABS	384-1154	0.67	43.9 mmol kg^{-1}	67
P	ABS+SNV	528-1001	0.66	5.55 mmol kg^{-1}	67
K	SABS	616-1061	0.46	36.8 mmol kg^{-1}	67
Ca	SABS+SNV	346-1157	0.51	6.64 mmol kg^{-1}	67
Mg	SABS+NM	353-356, 402-1005	0.56	2.60 mmol kg^{-1}	67

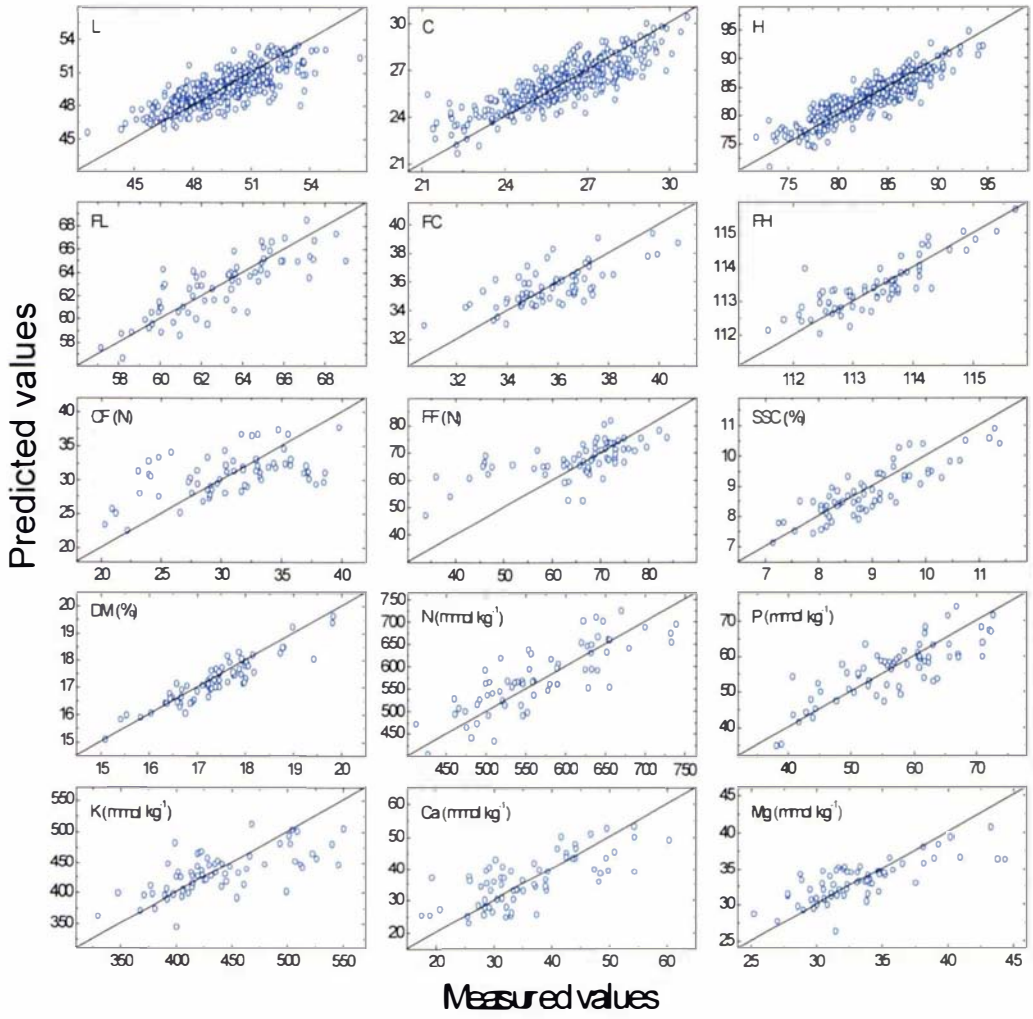

Figure 6.2 Measured and predicted values of fruit colour (L, C, H), flesh colour (FL, FC, FL), compression force (CF), flesh firmness (FF), soluble solid content (SSC), dry matter content (DM) and mineral concentrations (N, P, K, Ca and Mg) using the optimal predictive model (Table 6.4) established in experiment 2 for validation data set.

6.3.3 Predictive models established in experiment 3

PLS regression between at-harvest fruit attributes and the spectral data at the truncated spectrum range (535.52-1154.44 nm) before boxcar averaging or after the boxcar averaging over 1 nm wavelength intervals did not show any advantage over that averaged

over 2.5 nm intervals (data not shown). The spectral data averaged over 2.5 nm were used for further data analysis. The analysis indicated that L, C, H, FL, FC, FH, CF and N could be predicted with R^2_p above 0.5 while the predictive models for FF, SSC and DM only explained respectively 42 %, 47 % and 36 % variation of the measured data. The estimates for other fruit attributes (P, K, Ca and Mg) were even poorer ($R^2_p \leq 0.25$; Table 6.5 and Fig 6.3).

Table 6.5 Predictive models for at-harvest fruit attributes based on VNIR spectra of a USB2000 fibre optic spectrometer in experiment 3 (fruit number in the model data set was two times that of the validation data set listed).

Variable	Pre-treatment	Wavelength range (nm)	R^2_p	SEP	Validation fruit number
L	ABS	543-777, 841-891, 915-975	0.58	1.28	137
C	SABS	543-738, 891-968	0.69	1.06	137
H	SABS	535-751, 764-829, 1066-1153	0.64	2.23	137
FL	SABS	535-650, 671-1062	0.63	2.28	105
FC	ABS	537-563, 614-1106	0.53	1.67	105
FH	SABS+NM	555-679, 725-738, 928-968	0.60	0.53	105
CF	SABS+NM	537-600, 650-698, 757-998, 1026-1155	0.51	3.44 N	137
FF	ABS	603-821	0.42	9.87 N	137
SSC	SABS+SNV	600-793, 831-901, 1017-1040	0.47	1.03 %	137
DM	SABS+SNV	600-793, 856-903, 1010-1078	0.36	0.96 %	137
N	SABS	543-566, 679-693, 1058-1099	0.53	77.33 mmol kg^{-1}	137
P	SABS+NM	535-928	0.25	10.69 mmol kg^{-1}	137
K	ABS	535-1015	0.19	61.31 mmol kg^{-1}	137
Ca	ABS	549-968	0.14	8.74 mmol kg^{-1}	137
Mg	SABS	597-704	0.24	3.58 mmol kg^{-1}	137

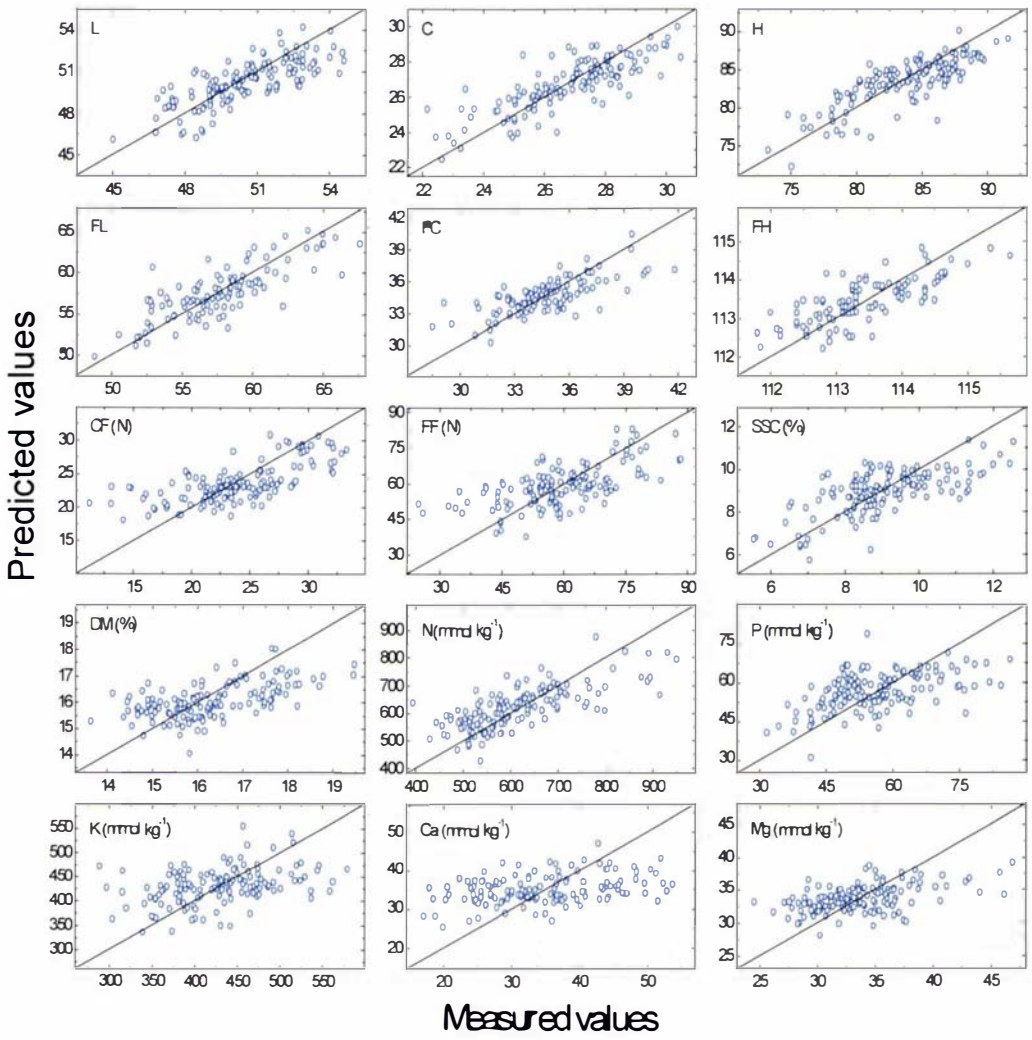

Figure 6.3 Measured and predicted values of fruit colour (L, C, H), flesh colour (FL, FC, FL), compression force (CF), flesh firmness (FF), soluble solid content (SSC), dry matter content (DM) and mineral concentrations (N, P, K, Ca and Mg) using the predictive model (Table 6.5) established in experiment 3 for validation data set.

6.3.4 Predictive models established in experiment 4

PLS between spectral data (averaged over 2.5 nm) and fruit attributes measured at harvest revealed that only SSC could be predicted with $R^2_p > 0.5$ while the predictive

models for FL, FC, FH, FF and DM only explained 30 %- 44 % variation of the measured data (Table 6.6 and Fig 6.4).

Table 6.6 Predictive models for at-harvest fruit attributes based on VNIR spectra of a USB2000 fibre optic spectrometer in experiment 4 (fruit number in the model data set was two times that of the validation data set listed).

Variable	Pre-treatment	Wavelength range (nm)	R^2_p	SEP	Validation fruit number
FL	ABS	584-1055	0.38	2.85	1339
FC	ABS	551-945, 1008-1058	0.44	1.88	1339
FH	ABS	551-1008	0.33	0.69	1339
FF	ABS	546-1058	0.38	9.07 N	1339
SSC	ABS	537-896,908-1070	0.55	1.15 %	1339
DM	ABS+SNV	537-1120	0.30	1.21 %	1325

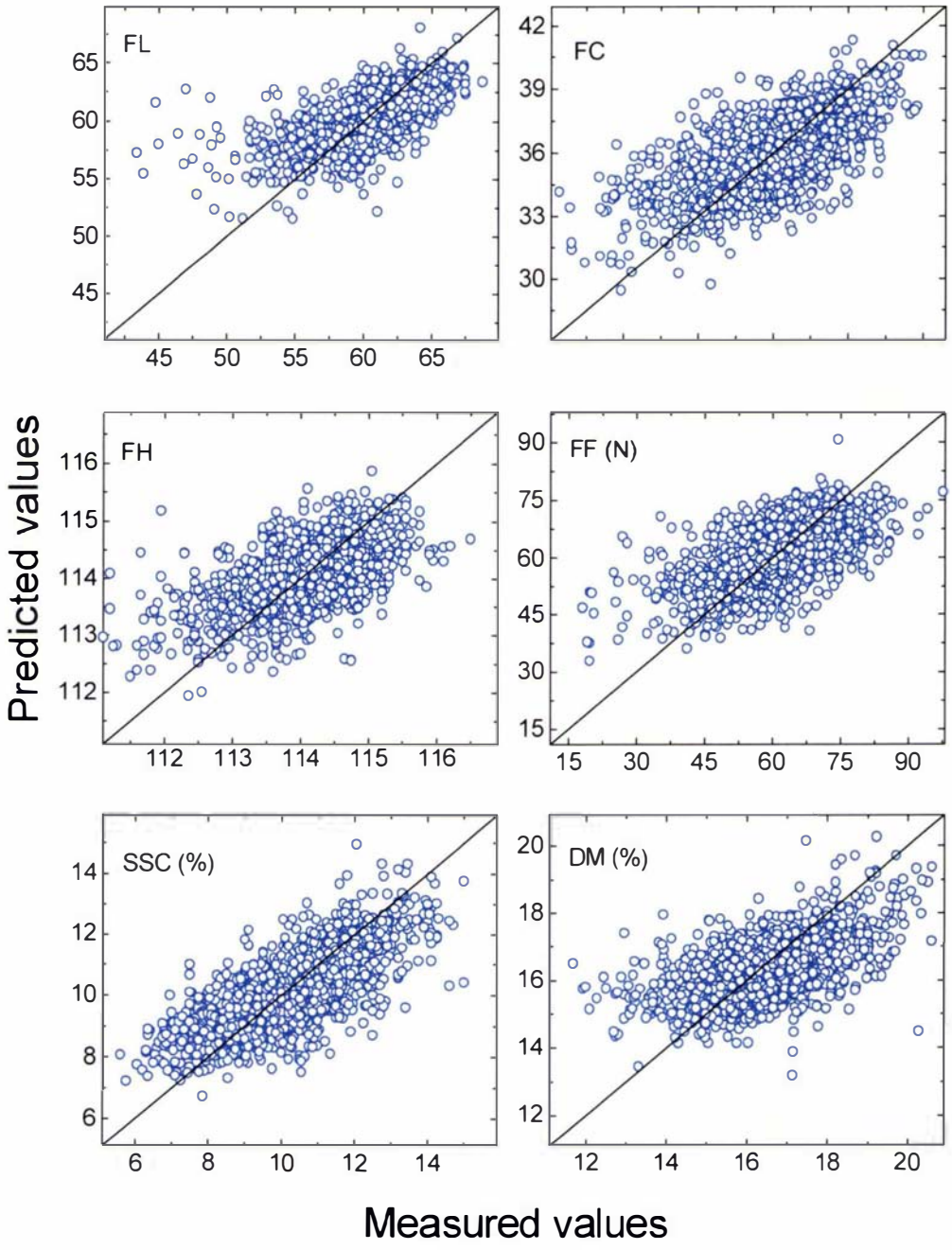

Figure 6.4 Measured and predicted values of flesh colour (FL, FC, FL), flesh firmness (FF), soluble solid content (SSC) and dry matter content (DM) using the predictive model (Table 6.6) established in experiment 4 for validation data set.

6.3.5 Comparison of VNIR spectra from different experiments

The average absorbance spectra from different experiments differed in shape (Fig 6.12) but did share the chlorophyll absorbance band at about 670 nm and some water-carbohydrate bands appearing between 900 – 1100 nm (Fig 6.5).

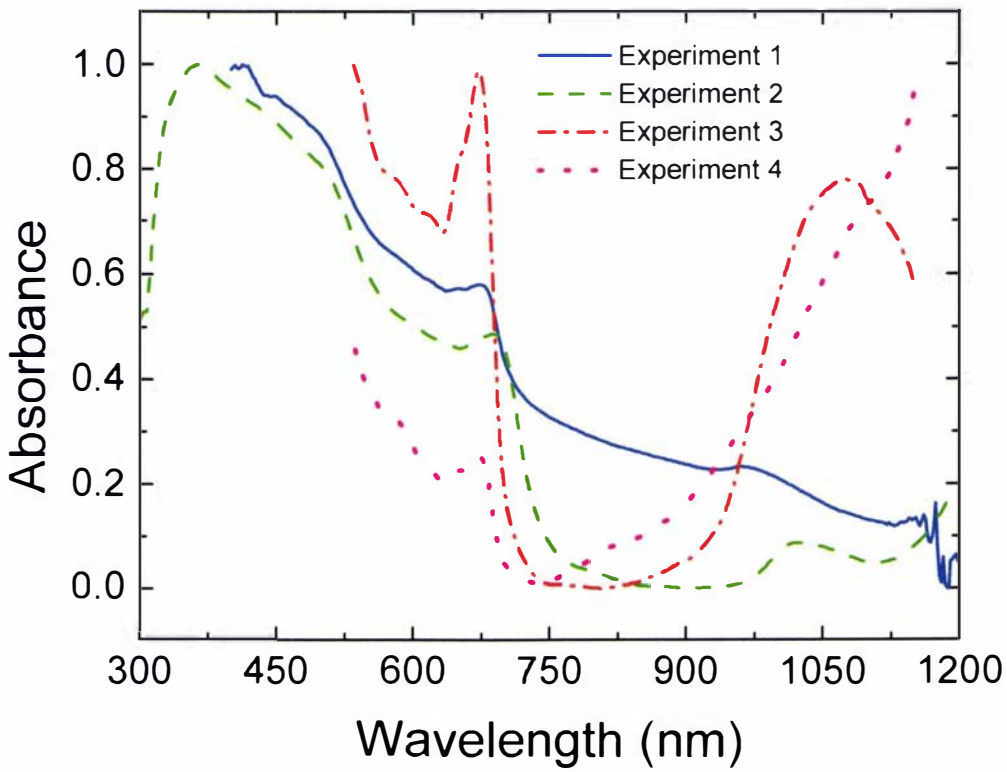

Figure 6.5 Comparison between VNIR absorbance spectra from the four experiments. Each line represents the mean of all fruit in the experiment. Absorbance spectra were normalised to absorbance range (maximum-minimum) per experiment for comparison.

PLS regression coefficients for estimation of most fruit attributes had the most pronounced peaks and troughs around the chlorophyll band and the water-carbohydrate bands. Comparison among coefficients of the same fruit attribute obtained in different experiments showed some common peaks and troughs among 2-3 experiments. However, there was hardly any peak or trough found to be common to all the 4 experiments (Fig 6.6).

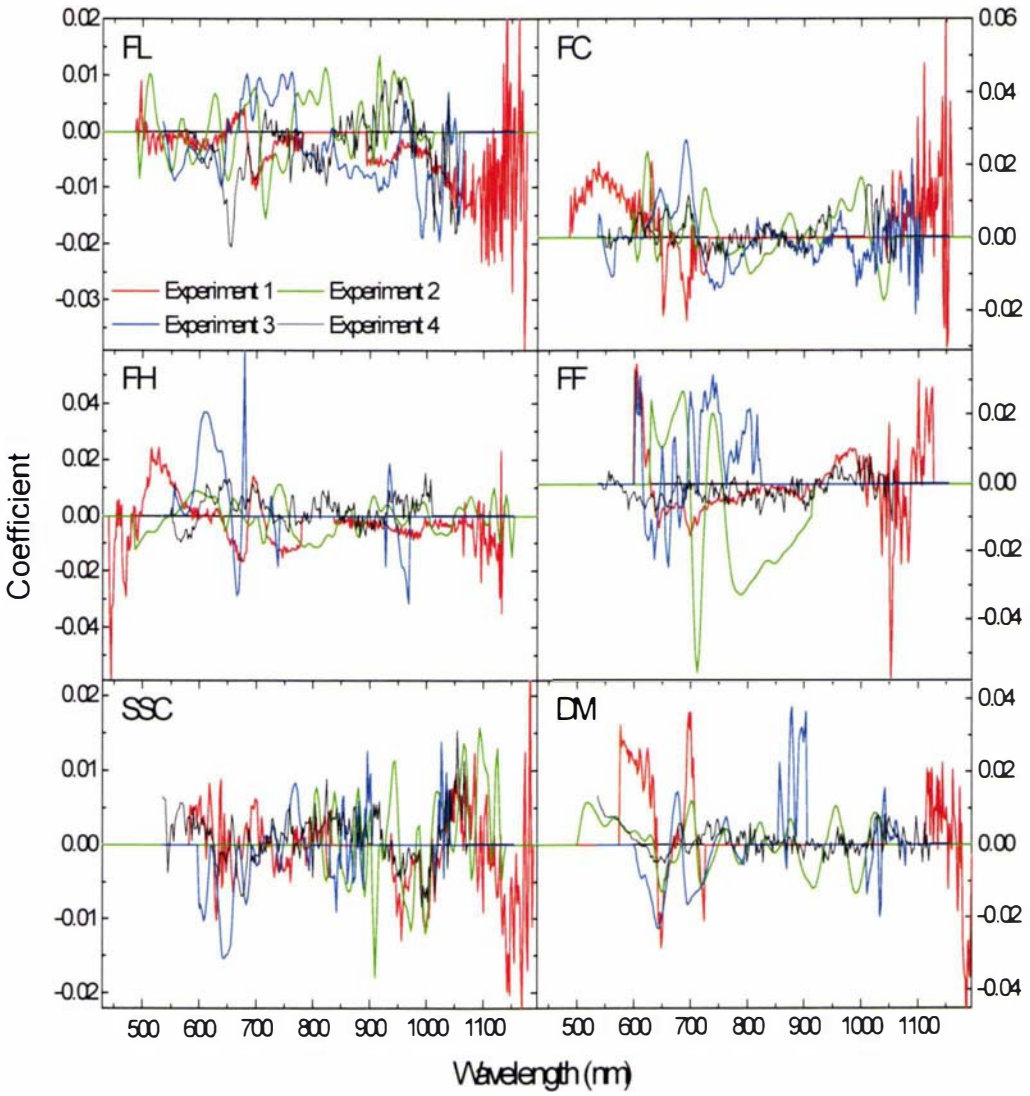

Figure 6.6 PLS regression coefficients for estimation of flesh lightness (FL), flesh chroma (FC), flesh hue (FH), flesh firmness (FF) soluble solid content (SSC) and dry matter content (DM) obtained in different experiments.

6.4 Discussion

6.4.1 Comparison between current study and the literature

The best predictive models for SSC and DM obtained in the current study (Table 6.4) were comparable to those reported by McGlone and Kawano (SEP 0.39 % for SSC and 0.42% for DM; 1998), Costa and co-workers (SEP 0.36 % for SSC; 1999), Osborne and co-workers (SEP 0.27 % for SSC and 0.32% for DM; 1999), Schaare and Fraser (SEP 0.8 % for SSC; 2000), McGlone and co-workers (SEP 0.52% for SSC and 0.46% for DM; 2002b). The best predictive model obtained for FF (Table 6.3) had higher SEP than that reported by McGlone and Kawano (SEP of 7.8 N; 1998). The superior estimation of FF in McGlone and Kawano's experiment might be attributed to more repeated VNIR scanning (50 scanning on each fruit; McGlone and Kawano, 1998) compared with 2 scans per fruit in experiment 1. The predictive models obtained for FH (Table 6.3, 6.4 6.5 and 6.6) had smaller SEP values than the SEP of 1.6 obtained by Schaare and Fraser (2000). The difference may have resulted from the small variability of 'Hayward' kiwifruit used in the current study (FH ranged from 112-116) compared with that of the yellow-fleshed kiwifruit used by Schaare and Fraser (FH ranged from 98-115). Estimates of CF, L, C, H, FL, FC and mineral concentrations of kiwifruit using VNIR technology are not seen in the literature.

6.4.2 Comparison of the four experiments

Experiment 1 was conducted in the same measurement mode (interactance) as that of experiment 3. Predictive models for at-harvest fruit attributes obtained in experiment 1 had higher R^2_p values than those obtained in experiment 3. However, the SEP values for most of the attributes were lower in experiment 3 than those in experiment 1 except for C and SSC (Tables 6.3, and 6.5). These differences were likely to be caused by the smaller fruit variability in experiment 3 (VNIR measurement carried out after approximately the same period of postharvest delays) compared with that of experiment 1 where fruit had been stored for up to 4 days before measurement.

Experiment 4 used the same kind of VNIR spectrometer as that in experiment 3. However, the normalised absorbance spectra from these two experiments showed different shapes. The spectra from experiment 3 were significantly higher at short wavelengths up to the chlorophyll absorbance band and showed a more pronounced chlorophyll band compared with that of experiment 4; the spectra from experiment 3 declined rapidly after the chlorophyll absorbance band, flattened at wavelengths from 700-900 nm, increased at wavelengths from 900-1050 nm and decreased thereafter while that of experiment 4 decreased slightly after chlorophyll absorbance band, flattened at wavelengths from 700-750 nm and increased continuously thereafter without showing a decline after 1050 nm (Fig 6.6). The difference at wavelengths above 1050 nm (where the raw spectra of the white reference and fruit were closed to that of the dark reference, i.e. both $\frac{I_{w_\lambda}}{I_{d_\lambda}}$ and $\frac{I_{f_\lambda}}{I_{d_\lambda}}$<5) can be attributed to the difference in methods used to calculate absorbance data (Table 6.1). The absorbance spectra from experiment 3 also increased continuously after 1050 nm if the dark reference was not subtracted from the white reference (data not shown). However, the magnitude of the dark reference spectra were negligible compared with the raw spectra of the white reference or that of fruit at wavelengths of 600-900 nm (both $\frac{I_{w_\lambda}}{I_{d_\lambda}}$ and $\frac{I_{f_\lambda}}{I_{d_\lambda}}$≥50), the method used to calculate absorbance data was not responsible for the differences at these wavelengths. The difference at wavelengths of 600-900 nm could be attributed to difference between individual spectrometers, difference in fruit population (fruit in experiment 3 were sampled from one production region within 2 weeks while those in experiment 4 were sampled from different production regions over 5 weeks) and changes in light source quality due to working in an environment with lots of kiwifruit hairs (Mowat, 2003, personal communication). The predictive models established in experiment 4 (Table 6.6) were poorer than that of experiment 3 (Table 6.5), except for SSC. This can be attributed to the greater variability of the fruit used in experiment 4 (i.e. fruit were sourced from different production areas and delayed for 2-8 days from harvest to VNIR measurement) while the fruit used in experiment 3 were sourced from one production area and delayed for 3-4 days from harvest to VNIR measurement.

Experiment 2 in reflectance mode gave the best estimates for SSC, DM, H, FH, N, P, Ca, Mg, CFS and FFS but the worst estimates for CF and FF compared with experiment 1 and experiment 3 in interactance mode. The reason for these differences is not clear because the details of the instrumentation used in experiment 2 are commercially sensitive and not available for inspection. The reflectance measurement mode used in experiment 2 was not likely to yield superior estimates, but it could be responsible for the poor estimates of CF and FF, because interactance was found to be superior to reflectance in the estimation of SSC (closely related to both CF and FF), density (closely related to DM) and FL of yellow-fleshed kiwifruit (Schaare and Fraser, 2000).

PLS analysis of data from experiment 2 with spectral data truncated to the same range as that of experiment 3 did not change the relative estimation accuracies (data not shown). Therefore, wavelength range does not seem to be a limiting factor either. The more repeated scanning of experiment 2 (20 scans per fruit) improved the signal to noise ratio and allowed for more relevant factors to enter into the PLS models. For example, the average PLS factor numbers for the estimation of the 11 at-harvest fruit attributes (L, C, H, FL, FC, FH, CF, FF, SSC, DM and N) were 19.4 for experiment 2 compared with 9.3 and 14.6 for experiment 1 and experiment 3, respectively. This indicates that the predictive models could be improved by increasing the number of VNIR scans taken on each fruit. However, it is not clear whether the instrument background noise or the variability of fruit surface was the major source of the noise in VNIR spectra. If the surface variability was the major source of the noise, it would be possible to increase the probe size to cover a larger area of the fruit surface for a better representation of the fruit by each VNIR spectrum. In other words, a few repeated scans with a large probe might produce an estimation accuracy comparable to that achieved by more repeated scanning with a smaller probe. Fewer repeated scans is preferable for inline measurement because it would allow more fruit to be measured per minute. Further work is needed to address this issue.

Estimation models obtained for the same fruit attributes in different experiments shared a few similarities within the common wavelength range. However, such matches could not be made for most of predictive models in the majority of the wavelength ranges (Fig 6.6). This implies that the PLS regressions in the different experiments extracted information from the spectra in different ways. This could have been affected by the

differences in measurement mode, instrumentation, reference spectra and wavelength range.

More systematic comparisons between the results from different experiments are not possible because the experiments were not designed for such comparison. The VNIR measurements made under different conditions using different VNIR instruments arose from limitation of availability rather than predetermined choices.

6.4.3 Chemical and spectroscopic analysis of predictive models

The best predictive model for SSC was based on absorbance spectra at wavelengths ranging from 799-1136 nm (Table 6.4). This range is similar to that of 800-1100 nm selected by McGlone and Kawano (1998). PLS regression coefficient vectors of SSC estimation had the most pronounced trough at 909 nm followed by smaller troughs at 973, 998, 891 and 866 nm (Fig 6.6). These troughs approximated the second derivative absorption peaks of carbohydrates at 900-930, 970-990, 870-890 nm (the second differentiation of the absorbance spectrum turn peaks in the absorbance spectrum to troughs in the second derivative spectrum; Williams and Norris, 1987). This indicates that the estimation model for SSC was based on its chemical species.

The best predictive model for DM was based on absorbance spectra at wavelength ranging from 500-1044 nm (Table 6.4), a range at shorter wavelength than the 800-1100 nm range selected by McGlone and Kawano (1998). PLS regression coefficient vectors of DM estimation had the most obvious trough at 990 nm followed by smaller troughs at 651, 920 and 722 nm (Fig 6.6). The first and the third troughs approximated the second derivative absorption peaks of carbohydrates at 970-990 and 900-930 nm (Williams and Norris, 1987). The second and the fourth troughs located respectively before and after a peak at 704 nm, the expected chlorophyll absorption peak (Mowat and Poole, 1997; McGlone and Kawano, 1998). This suggests that fruit with a high chlorophyll absorption peak would have high DM. The biochemical and physiological basis of this trend merits further investigation.

Estimation of flesh colour, particularly FH was expected to be based on chlorophyll absorption bands because the green colour of kiwifruit flesh is due to the presence of chlorophyll (Ferguson et al., 1990). However, coefficient vectors of the best predictive model for FH had the most pronounced trough at 806 nm followed by smaller troughs at 789 and 715 nm. None of these troughs were related to chlorophyll absorption band at about 670 nm. This indicates that the estimation of FH was not directly based on chlorophyll band.

Chemical and spectroscopic analysis of the predictive models for fruit attributes other than SSC, DM and FH did show unambiguous relationships to known absorbance bands. Absorbance spectra at short wavelength ranges (i.e. 350-650 nm) were useful to estimates of several fruit attributes (Tables 6.3, 6.4, 6.5 and 6.6), but there is limited knowledge about corresponding chemical species in this range.

VNIR spectra from experiment 2 yielded the best estimation models for minerals. In this experiment, N and P were predicted at R^2_p above 0.65 followed by Mg (R^2_p =0.56) and Ca (R^2_p =0.51). The estimation for K was surprisingly poor (R^2_p =0.46) given that K precent in fruit is at concentrations 5-10 times more than that of P, Ca and Mg. The majority of N, P, Ca and Mg in fruit bind to large organic molecules such as proteins, nucleotides, cell wall polymers and chlorophyll while K mostly exists as free ions (Martin-Prevel et al., 1987). It appears that the estimates of minerals are more likely to be based on the VNIR absorbance of the large organic molecules to which they were bound rather than the absorbance of the minerals themselves.

6.4.4 Effects of pre-calibration transformations of spectral data

Among the 12 pre-calibration transformations applied to spectral data, absorbance spectra in association with smoothing and SNV or NM appeared sufficient for the estimation of all the fruit attributes. Quadratic baseline compensation and the second derivation of absorbance spectra did not result in better estimation for any fruit attribute. This agrees with Schaare and Fraser (2000) who found that second derivation of

absorbance spectra did not produce better estimates for SSC, density and FL compared with normalization and smoothing.

It was also noticed that normalization was required in at least 3 out of the 4 experiments for the estimation of fruit attributes having clear chemical basis (e.g. SSC and DM) while the transformation was not required in most cases for estimating fruit attributes that do not have clear chemical basis, such as L, C, H, FF, FL and FC (Tables 6.3, 6.4, 6.5 and 6.6). This suggests that normalization is useful if the information relies on the absorbance bands.

Boxcar averaging of densely sampled spectral data in experiment 3 and experiment 4 over 2.5 nm reduced the dimensionality of the spectra data to 1/8 of the original dimension number without increasing the estimation error of the subsequent PLS regression analysis (data not shown). Instead, it reduced the size of the spectral data set and facilitated more efficient data storage and analysis.

6.4.5 Measurement error

It was also noticed that the estimates of CF was always better than the estimates of FF in the three experiments where CF was measured. Part of this may be attributed to the difference between the whole fruit stiffness and the flesh rupture force measured by CF and FF respectively (McGlone et al., 1997; Feng et al., unpublished). In addition, the variation in peeled skin area and thickness in FF measurement may have caused extra measurement error in FF data compared with the measurement error in CF that was measured on intact fruit. The measurement error would contribute to the error term of the predictive models (Osborne et al., 1993). This implies that the evaluation of VNIR on estimation of fruit attributes relies on the reliability of both the reference and the spectral data. The reliability of reference data could be improved by application of rationalised methodologies (Chapter 3) and factors affecting the reliability of spectral data are yet to be investigated. As spectral measurements are temperature-sensitive (Clark, personal communication, 2004), it is advisable that VNIR measurement should be made at a constant temperature.

6.5 Conclusion

VNIR is capable of estimating such at-harvest fruit attributes, as fruit colour, flesh colour, soluble solid content, dry matter content, fruit firmness and some mineral concentrations. However, the accuracy of the estimation varied considerably among fruit attributes and among experiments. Soluble solid content was predicted at $R^2_p \geq 0.5$ in all the four experiments while fruit colour, flesh colour and nitrogen concentration were predicted at $R^2_p \geq 0.5$ in three experiments. At harvest fruit firmness (CF and FF) could only be predicted in experiment 1 and experiment 3 using the interactance spectra. Dry matter content was predicted very well using the reflectance spectra from the commercial NIR instrument in experiment 2, but its estimates in other three experiments were poor (R^2_p <0.37). Mineral concentrations other than nitrogen were poorly predicted except for P, Ca and Mg which were predicted at $R^2_p \geq 0.5$ using the reflectance spectra in experiment 2. Further work is needed to select the most appropriate instrument and measurement method for more accurate estimation of fruit attributes using VNIR spectra.

6.6 References

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Segregation of 'Hayward' kiwifruit for storage potential using visible-near-infrared spectroscopy

7.1 Introduction

Storage life of an individual fruit ends when it softens to a flesh firmness (FF) less than 10 N (Hopkirk et al., 1996; Benge, 1999) or a disorder develops to certain extent (NZKMB, 1996). Over-soft fruit and fruit with storage disorders produce ethylene that promotes the softening of all fruit in the same package (Hyodo and Fukasawa, 1985; Arpaia et al., 1987; Mitchell, 1990; Bonghi et al., 1996; Kim, 1999; Ritenour et. al 1999; Feng et al., 2003). It is of great industry interest to predict fruit firmness at the end of storage and to differentiate fruit with respect to susceptibility to storage disorders based on non-destructive measurements on each fruit at harvest so that high-risk fruit can be separated and excluded for long-term storage.

Visible-near-infrared spectroscopy (VNIR) is capable of measuring many fruit attributes relating to fruit softening and susceptibilities to storage disorders (McGlone and Kawano, 1998; Osborne and Kunnemeyer, 1999; Schaare and Fraser, 2000; McGlone et al., 2002; Feng et al., unpublished). This chapter evaluates the possibility of using VNIR to predict fruit firmness at the end of storage and fruit susceptibility to storage disorders for fruit segregation purposes.

7.2 Materials and methods

7.2.1 Experiment 1

Ten trays of commercially packed count 36 'Hayward' kiwifruit from each of nine grower lines were sampled during the 2000 harvest season (9 May to 23 May) at weekly intervals (three grower lines per week) from pack houses in the Bay of Plenty. Fruit were cool stored at 0.5°C in polylined single-layered trays after two days for transportation and laboratory measurements. Ethylene absorbent (5 g per tray of Purafil, Papworth

Engineering Ltd., New Zealand) was placed inside the trays to prevent accumulation of ethylene inside the package. After 2-8 days cool storage, 120 fruit from each grower line were equilibrated to ambient temperature for VNIR measurement in reflectance mode using a commercial NIR system (Compac Sorting Equipment Ltd, New Zealand). Fruit were carried with stem-calyx axis horizontal by a conveyer belt through the NIR scanning chamber where a continuous beam of light from a halogen lamp was focused on the moving fruit and the reflected light sampled ten times. The fruit passed through the NIR scanning chamber twice with opposite sides facing the detector and twenty VNIR spectra were recorded for each fruit. All spectra from each fruit were averaged after subtracting the dark reference and dividing by the white reference (materials and methods used to obtain reference spectra are confidential to Compac Sorting Equipment Ltd). The averaged spectra were then smoothed using in-house software. Spectra were collected within a wavelength range of 300-1200 nm at 3.50-3.56 nm intervals (256 points). One hundred fruit from each grower line were returned to cool storage after the VNIR measurement.

After three months storage, CF and FF were monitored monthly on a subsample of 20 fruits (10 from VNIR scanned fruit and another 10 from un-scanned fruit) from each grower at each monitoring period. Fruit were equilibrated to 20°C before measurement. Fruit were removed from storage for shelf life testing when the average FF of the grower line reached 8.5 N. Based on the firmness-temperature coefficient of kiwifruit (Jeffery and Banks, 1994), 8.5 N measured at 20°C is equivalent to 11.8 N measured immediately upon removal from cool storage (i.e. the measurement method of New Zealand kiwifruit industry). The storage duration for different grower lines ranged from 6 to 11 months. Fruit attributes measured during the shelf life test included compression force (CFS) on all remaining fruit, flesh firmness (FFS) on 20 fruit (10 from VNIR scanned fruit and another 10 from un-scanned fruit) per grower line and storage disorders such as soft patches (SP, local soft, water soaked areas; Davie, 1997; Benge, 1999), fungal rots (FR, including stem end rot, distal end rot and body rots; NZKMB, 1996), low temperature breakdown (LTB, large soft area associated with water soaked appearance at the stylar end of the fruit; Lallu, 1997; Bauchot et al., 1999), and purple patches (PP, purple scald-like patches; Feng et al., 2002) on all remaining fruit. CFS and FFS were measured on the second day after fruit

been removed from cool storage to simulated shelf life conditions when fruit temperature was equilibrated to 20°C. Storage disorders were assessed on the fifth day of shelf life.

7.2.2 Experiment 2

Four trays of commercially packed 'Hayward' kiwifruit (count 36) from each of the 22 grower lines were sampled during 2001 harvest season (28 May to 9 June) from pack houses in the Bay of Plenty. On the day of sampling, interactance VNIR measurements were taken on each fruit using an USB2000 fibre optic spectrometer (Ocean Optics Inc.) fitted with a fibre optic interactance probe. The probe was a R400-7 reflection probe consisting of a tight bundle of 7 optical fibres of 400 μm in diameter (6 illumination fibres around 1 read fibre) in a stainless steel ferrule of 3.0" long and 0.25" diameter. The probe was pointed at the median equator of a fruit and a spectrum (519-1156 nm) was recorded at about 0.31 nm intervals (2048 points). Both white reference spectra (obtained when the probe was inserted into the RPH-1 reflection probe holder at 90° angle) and dark spectra (obtained by blocking the aperture to the optical fibre) were recorded for each grower line. Two separate spectra were taken on opposite sides of each fruit.

Fruit were then transferred to Massey University where 20 VNIR-scanned fruit from each grower line were used for the measurement of at-harvest attributes (Chapter 6) and the remaining fruit were cool stored in the same way as described in experiment 1. Fruit from each grower line were taken out of storage for shelf life test after 9 months storage. Fruit attributes measured during the shelf life were the same as described in experiment 1.

7.2.3 Experiment 3

This experiment was carried out by AgFirst Consultants on behalf of Zespri Innovation Ltd. Nine hundred and twenty fruit from each of 72 grower lines were sampled from the Bay of Plenty, Gisborne, Nelson and Northland during the main packing season (25 May to 27 June) in 2001. Interactance VNIR measurement was taken on all fruit using a USB2000 fibre optic spectrometer. Spectra (520-1159 nm) were recorded at about 0.31

nm intervals (2048 points). Ten separate spectra were taken on each fruit and they were averaged to produce one spectrum for each fruit.

Fruit from each grower line were then separated as repeating fruit (520) and library fruit (400). Repeating fruit were used for destructive measurements before and during storage at 0.5°C for 24 weeks. Fruit disorders were recorded for repeating fruit at destructive measurements and those of the library fruit were recorded every 4 weeks after 8 weeks storage. At the end of storage, FFS was measured on 60 repeating fruit and all the remaining fruit were removed from storage and kept at ambient temperatures until each individual fruit became soft or had developed a disorder. Fruit that had developed disorders were removed once identified. Fruit disorders and the dates when the disorders were found were recorded. Disorders assessed included stem end botrytis (SEB), SP, side rots (SR), shrivel, pitting, sunken pit, wound botrytis, blossom end rot, botrytis, nested botrytis, discoloration, firm wound rots, soft wound rot, LTB and stem end rot.

7.2.4 Data analysis

Spectral data from all experiments were baseline-corrected and log transformed to produce absorbance spectra (ABS) as described in section 6.2.5. Absorbance spectra were normalised in different ways (same as those described in section 6.2.5) before or after smoothing and second differentiation using a simplified least square procedure (Savitzky and Golay, 1964, Steinier et al., 1972).

Boxcar averaging (Faber, 1999) over 2.5 nm was applied to spectral data from experiment 2 and experiment 3 before smoothing and normalisation. Noisy spectral data at both ends of the spectra were truncated (the same as that described in section 6.2.5).

Predictive models for CFS and FFS were developed using partial least squares (PLS) regression as described in section 6.2.5.

Fruit in each experiment were categorised into several groups for multivariate discriminate analysis according to the types of storage disorder observed or the time when the disorders were found. When several disorders were observed on the same fruit, purple patches group had the first priority followed by fungal rot group, LTB group and soft patch group.

Stepwise discriminant analysis (Proc STEPDISC; SAS Institute, 2003) was used to select VNIR wavelengths that had significant roles in discriminating fruit into different groups. Two-thirds of the data were randomly assigned to the model building dataset with the remainder being allocated to the validation dataset. Canonical discriminant analysis (Hair, et al., 1987; SAS Institute, 2003; Cruz-Castillo et al., 1994) was performed on the model building dataset based on the selected wavelengths. Canonical functions having a significant role in separating fruit were calculated for fruit in the validation dataset and the Mahalanobis distances of each fruit to the centroid of each group were compared to assign a fruit to its closest group. Classification accuracy (percentage of fruit correctly classified) relative to the percentage chance criterion was evaluated (Hair et al., 1987).

For experiment 1, an exponential model was fitted to both CF and FF data collected for VNIR scanned fruit of each grower line at the last four monitoring periods (Feng et al., 2001). CFS and FFS measured after variable storage time (ranged from 6 to 11 months) were adjusted to values after 6 months storage using equations 7.1 and equation 7.2 respectively before PLS regression. CFS and FFS in experiment 2 and 3 were not adjusted because the storage durations for all grower lines in each experiment were the same.

$$CFS_{ig} = CFS_{igt} + CF_{0g} \cdot (e^{(-k_{CFg} \cdot t_{ig})} - e^{(-k_{CFg} \cdot 180)}) \qquad (7.1)$$

where i denotes fruit number, g denotes grower line, t is the storage time (days) before CFS measurement. CFS_{ig} is the CFS value of the ith fruit of grower line g after 180 days of storage. CFS_{igt} is the CFS value of the ith fruit of grower line g measured after t_{ig} days of storage. CF_{0g} and k_{CFg} are the intercept and softening rate constant of grower line g respectively.

$$FFS_{ig} = FFS_{igt} + FF_{0g} \cdot (e^{(-k_{FFg} \cdot t_{ig})} - e^{(-k_{FFg} \cdot 180)}) \qquad (7.2)$$

where FFS_{ig} is the FFS value of the ith fruit of grower line g after 180 days of storage. FFS_{igt} is the FFS value of the ith fruit of grower line g measured after t_{ig} days of storage. FF_{0g} and k_{FFg} are the intercept and softening rate constant of grower line g respectively.

Due to accidental factors, VNIR spectra from one grower line in experiment 2 and two grower lines in experiment 3 were deemed unreliable and were excluded from analysis. VNIR spectra of 1,521 fruit in experiment 3 were missing. Therefore, VNIR spectra on a total of 1,080 fruit in experiment 1, 3,024 fruit in experiment 2 and 62,879 fruit in experiment 3 were used for analysis.

Absorbance of experiment 1 and 2 were calculated using SPECTRUM (Brown, unpublished) while that of experiment 3 were calculated using GRAMS/32 AI (V6; Galactic Industries Corporation, USA). Further data processing and analysis were performed using SAS (V8.2; SAS Institute Inc. USA).

7.3 Results

7.3.1 Predictive models for fruit firmness measured at the end of storage

The best predictive models for CFS and FFS were established in experiment 1 followed by experiment 2 and experiment 3. The predictions were in general not satisfactory, predicted firmness only explained 14-35% of the measured firmness variation (Table 7.1).

Table 7.1 Predictive models for fruit firmness after 6 (Experiment 1 and Experiment 3) or 9 months (Experiment 2) storage at 0.5°C based on VNIR spectra (fruit number in model data set was two times that of the validation data set listed).

Experiment	Variable	Pre-treatment	Wavelength range (nm)	R^2_p	SEP	Validation fruit number
1	CFS	SABS	437-990	0.32	1.59	223
	FFS	ABS+NM	500-1104	0.35	2.25	83
2	CFS	SABS	566-666, 698-725, 783-994	0.24	1.71	859
	FFS	ABS	622-630, 923-973	0.22	2.99	146
3	FFS	ABS+SNV	546-1153	0.14	2.87	1571

Fruit in the validation data set were ranked according to predicted CFS from high values to low values. The top third of fruit were taken as the predicted firm group, the middle third as the predicted medium group and the lower third as the predicted soft group. The measured firmness of the predicted firm group was significantly higher than those of the predicted medium and soft groups. The percentage of soft fruit (measured firmness less than export requirement) in the predicted firm group was significantly lower than those of the predicted medium and soft groups. Similar results were obtained when fruit in the validation data set were ranked and divided into three equal sized groups according to predicted FFS (Table 7.2).

Table 7.2 Segregation effects for fruit in the validation data set according to predicted CFS or FFS after 6 (experiment 1 and experiment 3) or 9 months (experiment 2) storage at 0.5°C using the predictive models listed in table 7.1.

Experiment	Segregation basis	Measured characteristics*	Predicted groups		
			Soft	Medium	Hard
1	Predicted CFS	CFS (Mean ± se, N)	9.4±0.19	11.0±0.18	11.8±0.18
		Number (%) of soft fruit	17 (21.8%)	2 (2.6%)	1 (1.3%)
		Total fruit number	78	78	77
	Predicted FFS	FFS (Mean ± se, N)	10.0±0.45	12.0±0.44	13.8±0.43
		Number (%) of soft fruit	9 (32.1%)	1 (3.6%)	0 (0.0%)
		Total fruit number	28	28	27
2	Predicted CFS	CFS (Mean ± se, N)	7.4±0.1	8.3±0.1	9.4±0.1
		Number (%) of soft fruit	190 (66.4%)	130 (45.3%)	58 (20.3%)
		Total fruit number	286	287	286
	Predicted FFS	FFS (Mean ± se, N)	7.6±0.4	9.2±0.4	11.3±0.5
		Number (%) of soft fruit	32 (65.3%)	19 (39.6%)	10 (20.4%)
		Total fruit number	49	48	49
3	Predicted FFS	FFS (Mean ± se, N)	13.89±0.10	14.99±0.12	16.46±0.15
		Number (%) of soft fruit	0 (0%)	0 (0%)	0 (0%)
		Total fruit number	524	524	523

*Soft fruit means fruit with CFS ≤8.0 N or FFS ≤8.5 N, as measured at 20°C. These criteria equivalent to the minimum fruit firmness required for 'Hayward' kiwifruit for export, i.e. FF 11.8 N if measured immediately after removal from cool storage.

7.3.2 Discrimination of disordered fruit

7.3.2.1 Discrimination of disordered fruit in experiment 1

Of the 1,080 VNIR scanned fruit, 468 fruit remained for measurement of storage disorders during simulated shelf life while the others were used for destructive measurements during storage. Among the 468 fruit, 388 fruit remained healthy (healthy group), 36 fruit developed soft patches (SP group), 17 fruit developed low temperature breakdown (LTB group), 23 fruit developed purple patches (PP group), and 4 fruit developed fungal rots (excluded for analysis because there were too few of them to form a group). Fruit in each group were further divided into a model-building data set (310 fruit) and a validation data set (154 fruit).

Standard normal variate transformation of absorbance data (ABS+SNV) gave the best discriminant results compared to other pre-calibration transformations. Stepwise CDA indicated that nine out of the 256 wavelength data points had a significant contribution to the discrimination. CDA based on the spectral data at the nine wavelengths in the model-building data set revealed that the first two canonical functions (CDF_1 and CDF_2), accounting for 93% variation of the four fruit groups, had significant roles in assigning fruit to different groups. CDF_1 separated fruit with purple patches and LTB from healthy fruit and fruit with soft patches while CDF_2 further separated healthy fruit from fruit with soft patches. Fruit with purple patches were associated with the lowest CDF_1 value while fruit with LTB had the highest CDF_1 value. Both the healthy fruit and fruit with soft patches had moderate CDF_1, however, the healthy fruit had the lowest while the fruit with soft patches had the highest CDF_2 values (Table 7.3).

Table 7.3 The means of the first two canonical discriminant functions (CDF_1 and CDF_2) of the four groups of fruit in the model building data set of experiment 1.

Group	CDF_1		CDF_2		n
	Mean*	se	Mean*	se	
Healthy	0.06b	0.06	-0.11a	0.06	260
Soft patches	0.17b	0.19	0.70b	0.23	24
Low temperature breakdown	1.03c	0.27	0.61b	0.29	11
Purple patches	-2.12a	0.32	0.26a	0.34	15

* Values labelled with different letters are significantly different in pare-wise t test at $P<0.05$ level.

CDF_1 separated fruit on spectral data at 605 nm, and to a lesser extent, on spectral data at 595, 465, 468 and 609 nm (Table.7.4). Fruit with high absorbance at 605 and 465 nm, but low absorbance at 595, 468 and 609 nm had high risk of developing purple patches while fruit with low absorbance at 605, 465 and 465 nm, but high absorbance at 595, 468 and 609 nm had high risk of developing LTB. CDF_2 separated fruit on spectral data at 605 nm, and to a lesser extent, on spectral data at 609 and 595 nm (Table.7.4). Fruit with high absorbance at 605 nm, but low absorbance at 609 and 595 nm had high risk of developing soft patches while fruit with low absorbance at 605 nm, but high absorbance at 609 and 595 nm were likely to be healthy.

Table 7.4 The standardized canonical coefficients (SCC) and the correlation coefficients (r) between spectral data and the first two canonical functions (CDF_1 and CDF_2) of experiment 1.

Wavelength	CDF1		CDF2	
(nm)	SCC	r	SCC	r
300	0.40	0.19	0.13	0.11
465	-45.27	-0.11	-8.44	0.33
468	45.23	-0.10	8.79	0.34
542	-4.40	0.55	2.23	0.62
595	55.63	0.43	-41.77	0.64
605	-80.59	0.39	116.13	0.65
609	28.59	0.37	-75.08	0.65
651	1.74	-0.41	0.13	0.10
708	-0.70	-0.23	1.23	-0.47

Fruit in the validation data set had similar distribution patterns along the CDF_1 and CDF_2 axes as those in model-building data set (Fig 7.1 A and B).

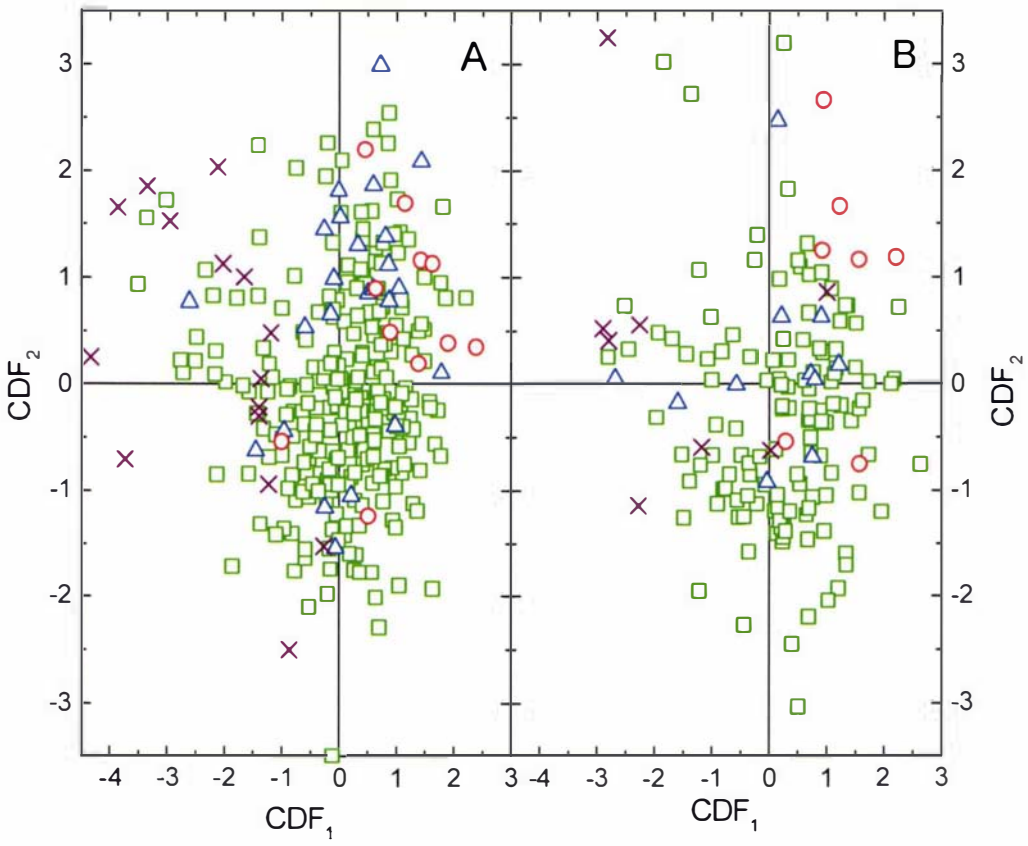

Figure 7.1 Separation of healthy fruit (□), fruit with soft patches (△), low temperature breakdown (○) and purple patches (x) by the first two canonical functions (CDF_1 and CDF_2) for fruit in the model building data set (A) and the validation data set (B) in experiment 1.

Discrimination based on the first two canonical functions yielded 93.4% healthy fruit in the predicted healthy group compared with 83.1% by chance (i.e. the percentage of healthy fruit in the population). The percentage of SP, LTB and PP fruit in the predicted SP, LTB and PP groups were 15.4%, 12.2% and 25.0% respectively compared with 7.8%, 3.2% and 5.9% respectively by chance (Table 7.5). The cumulative percentage of disordered fruit (fruit with SP, LTB or PP) in the predicted healthy group (6.6%) was less than half of the percentage in the predicted SP group (15.4%), predicted LTB groups (26.8%) or predicted PP group (33.3%).

Table 7.5 Effect of a four-group segregation based on the first two canonical functions for 154 fruit in the validation data set of experiment 1

Actual group	Fruit number (percentage) in each predicted group				Total fruit number (percentage)
	Healthy	Soft Patch	LTB	Purple patches	
Healthy	71 (93.4%)	11 (84.6%)	30 (73.2%)	16 (66.7%)	128 (83.1%)
Soft Patch	3 (3.9%)	2 (15.4%)	5 (12.2%)	2 (8.3%)	12 (7.8%)
LTB	1 (1.3%)	0 (0.0%)	5 (12.2%)	0 (0.0%)	6 (3.9%)
Purple patches	1 (1.3%)	0 (0.0%)	1 (2.4%)	6 (25.0%)	8 (5.2%)
Total fruit number	76	13	41	24	154

7.3.2.2 Discrimination of disordered fruit in experiment 2

Of the 3,024 VNIR scanned fruit, 420 fruit were used for destructive measurement immediately after the VNIR measurement, 2,604 fruit remained to test shelf life after 9 months storage. Among these fruit, 1,242 fruit were healthy (healthy group), 690 fruit showed LTB symptoms (LTB group), 613 fruit developed soft patches (soft-patch group), 51 had purple patches and 8 fruit developed fungal rots. Fruit that developed fungal rots and purple patches were excluded for discriminant analysis because there were too few of them to form their own groups. Therefore a total of 2,545 fruit were used for a three-group (Healthy, soft patch and LTB) multivariate discriminant analysis with 1,705 fruit in the model-building data set and 840 fruit in the validation data set.

Smoothed absorbance (SABS) data gave the best discriminant results compared to spectral data of other pre-calibration transformations. Stepwise CDA indicated that 37 out of the 248 wavelength data points had a significant contribution to the discrimination. CDA based on the spectral data at the 37 wavelengths in the model-building data set revealed that both CDF_1 and CDF_2 had significant roles in assigning fruit to different groups. The healthy group had the highest CDF_1 score followed by soft-patch group and LTB group. The soft-patch group also differ from the healthy and the LTB groups in CDF_2 while the CDF_2 scores were similar for healthy and the LTB groups (Table 7.6).

Table 7.6 The means of the first two canonical discriminant functions (CDF_1 and CDF_2) of the three groups of fruit in the model building data set of experiment 2.

Group	CDF_1		CDF_2		n
	Mean*	se	Mean*	se	
Healthy	0.44 a	0.04	-0.14 b	0.04	832
Soft patches	-0.08 b	0.05	0.50 a	0.05	411
Low temperature breakdown	-0.73 c	0.04	-0.20 b	0.05	462

* Values labelled with different letters are significantly different in pare-wise t test at $P<0.05$ level.

CDF_1 separated fruit on the contrast between spectra data at two sets of wavelengths: one set included 586, 614, 706, 720, 816, 841, 854, 915 and 959 nm with positive SCC in CDF_1, and another set include 608, 709, 811, 819, 834, 871, 918, 925 and 944 nm with negative SCC in CDF_1 (Table.7.7). This implies that fruit with high absorbance at 586, 614, 706, 720, 816, 841, 854, 915 and 959 nm but low absorbance at 608, 709, 811, 819, 834, 871, 918, 925 and 944 nm are likely to be healthy fruit, otherwise would be susceptible to LTB. Fruit with moderate absorbance at these wavelengths are susceptible to soft patches.

CDF_2 separated fruit on the contrast between spectra data at another two sets of wavelengths: one set include 586, 614, 658, 709, 816, 834, 915 and 920 nm with positive SCC in CDF_2, and another set include 608, 660, 706, 720, 811, 841, 918 and 925 nm with negative SCC in CDF_2 (Table.7.7). This indicates that fruit with high absorbance at 586, 614, 658, 709, 816, 834, 915 and 920 nm but low absorbance at 608, 660, 706, 720, 811, 841, 918 and 925 nm are susceptible to soft patches, otherwise would likely to be healthy or susceptible to LTB.

It was also noticed that the wavelengths important to the discrimination (absolute SCC larger that 34) were those from 586-959 nm. Wavelengths beyond this range are of minor importance (absolute SCC ranged from 0.5-26). However, spectral data at wavelengths from 1075 nm to 1149 nm had high correlation coefficients with both CDF_1 ($r \geq 0.5$) and CDF_2 ($r \geq 0.15$; Table 7.7). This implies that the spectral information at long wavelengths was not fully used due possibly to large background noise.

Table 7.7 The standardized canonical coefficients (SCC) and the correlation coefficients (r) between spectral data and the first two canonical functions (CDF_1 and CDF_2) of experiment 2.

Wavelength (nm)	CDF_1		CDF_2	
	SCC	r	SCC	r
572	-11.06	0.48	-2.73	0.26
586	44.23	0.47	34.17	0.28
597	-18.75	0.46	-27.77	0.29
608	-81.02	0.45	-107.66	0.30
614	66.93	0.44	105.61	0.31
644	18.78	0.35	-22.05	0.32
658	-10.00	0.27	161.57	0.32
660	-13.64	0.26	-160.51	0.32
671	6.68	0.19	17.19	0.33
706	55.98	0.48	-127.07	0.21
709	-95.11	0.49	173.76	0.20
720	36.80	0.50	-51.25	0.18
811	-34.62	0.48	-86.57	0.10
816	156.58	0.48	127.78	0.10
819	-121.93	0.48	-27.13	0.10
834	-53.71	0.48	54.90	0.09
841	35.86	0.48	-49.72	0.08
854	72.84	0.48	-14.51	0.07
871	-53.89	0.47	-0.52	0.06
915	61.30	0.47	125.85	0.03
918	-50.05	0.47	-236.38	0.03
920	15.53	0.47	154.26	0.02
925	-35.48	0.47	-48.65	0.02
932	20.81	0.46	-0.65	0.01
944	-41.55	0.46	-5.51	-0.01
954	-14.26	0.45	-14.02	-0.03
959	49.63	0.44	9.31	-0.04
968	-0.92	0.43	25.66	-0.07
998	-8.75	0.38	-15.98	-0.14
1031	6.15	0.43	16.62	-0.04
1035	-0.45	0.44	-12.11	-0.02
1075	-2.42	0.50	1.19	0.15
1097	4.35	0.51	-7.55	0.19
1104	-8.13	0.50	5.93	0.20
1130	1.08	0.53	3.04	0.22
1140	2.00	0.54	2.72	0.23
1149	0.62	0.53	-1.70	0.21

Fruit in the validation data set had similar distribution patterns along the CDF_1 and CDF_2 axes as those in model-building data set (Fig 7.2 A and B).

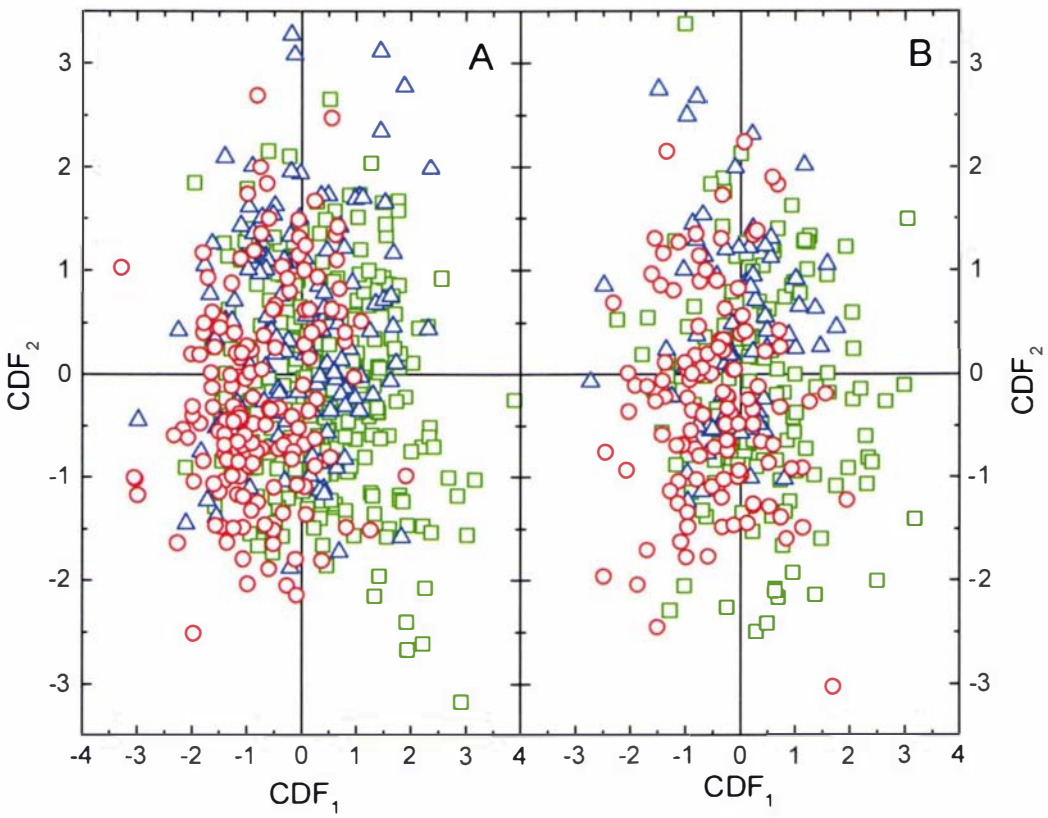

Figure 7.2 Separation of healthy fruit (□), fruit with soft patches (△) and low temperature breakdown (○) based on the first two canonical functions (CDF_1 and CDF_2) for fruit in the model building data set (A) and the validation data set (B) in experiment 2. Three quarters of the data points were omitted for clarity.

Discrimination based on the first two canonical functions yielded 66.9% healthy fruit in the predicted healthy group compared with 48.8% by chance. The percentage of SP and LTB fruit in the predicted SP and LTB groups were 35.1% and 46.3%, respectively, compared with 24.1% and 27.1%, respectively, by chance (Table 7.8). The cumulative percentage of disordered fruit (fruit with SP or LTB) in the predicted healthy group

(33.1%) was about half of the percentage of disordered fruit in the predicted SP group (57.5%) or in the predicted LTB group (67.1%).

Table 7.8 Effect of a four-group segregation based on the first two canonical functions for 840 fruit in the validation data set of experiment 2

Actual group	Fruit number (percentage) in each predicted group			Total fruit number
	Healthy	Soft Patch	LTB	(percentage)
Healthy	220	97	93	410
	(66.9%)	(42.5%)	(32.9%)	(48.8%)
Soft Patch	63	80	59	202
	(19.1%)	(35.1%)	(20.8%)	(24.1%)
LTB	46	51	131	228
	(14.0%)	(22.4%)	(46.3%)	(27.1%)
Total fruit number	329	228	283	840

7.3.2.3 Discrimination of disordered fruit in experiment 3

Of the 62,879 fruit in this experiment with valid VNIR spectra, 29,990 fruit were assessed for disorders while the rest of the fruit were used for destructive measurements during storage. Disorders were detected during storage and within 2 weeks after removal from storage to ambient temperature on 2,445 fruit. Fruit that had not shown any disorders until 2 weeks after removal from storage to ambient temperature were regarded as healthy fruit. Three disorders, stem end botrytis (SEB), soft patches (SP) and side rots (SR) that accounted for respectively 26.7, 25.5 and 12.7 % of the disordered fruit were used for CDA. Fruit with other disorders were excluded for discriminant analysis because there were too few of them to form their own groups. Therefore, the total number of fruit used for a four-group CDA was 29,130 fruit (27,545 healthy fruit, 624 SP fruit, 651 SEB fruit and 310 SR fruit). Two thirds of the fruit in each group were randomly assigned to the model-building data set (19,518 fruit) with the reminder being allocated to the validation data set (9,612 fruit).

The best discriminant results were produced from absorbance (ABS) spectra compared with the results produced from the spectra of other pre-calibration transformations. Stepwise CDA indicated that 150 out of the 249 wavelength data points had a significant contribution to discrimination of the fruit groups. CDA on the model-

building data set revealed that the first, second and the third canonical functions (CDF_1, CDF_2, and CDF_3) accounted for respectively 71.9%, 15.9% and 12.2% variation of the four fruit groups all have significant role in assigning fruit into different groups.

Healthy fruit had the lowest CDF_1 score, moderate CDF_2 score and moderate CDF_3 score. Fruit with SP had the highest CDF_1 score, the lowest CDF_2 score and moderate CDF_3 score. Fruit with SEB had moderate CDF_1 and CDF_2 scores and the highest CDF_3 score. Fruit with SR had moderate CDF_1 score, the highest CDF_2 score and the lowest CDF_3 score (Fig 7.3A).

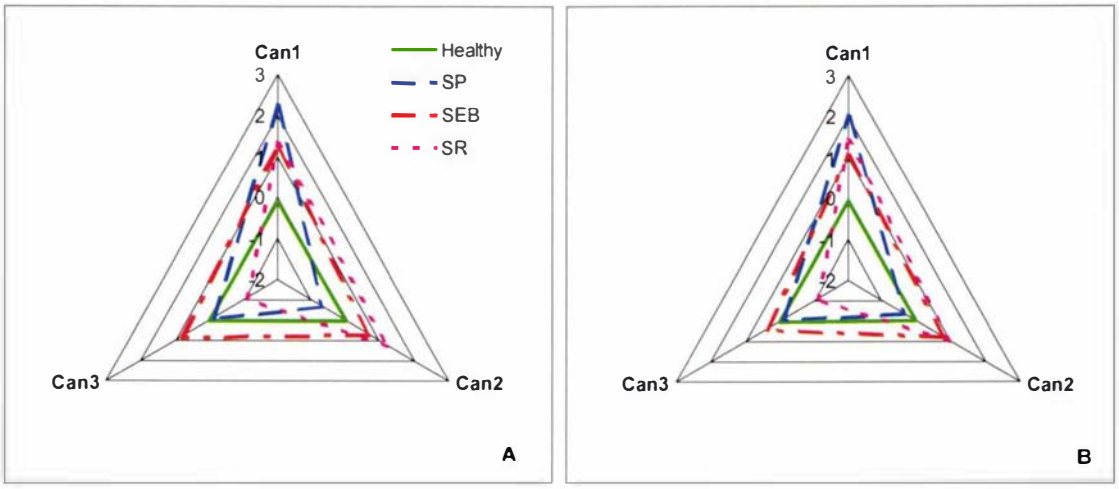

Figure 7.3 Means of the three canonical functions (CDF_1, CDF_2 and CDF_3) of healthy fruit group and groups of fruit with soft patch (SP), stem end botrytis (SEB) and side rots (SR) in the model-building data set (A) and the validation data set (B) of experiment 3.

The standardized canonical coefficients (SCC) in CDF_1, CDF_2 and CDF_3 displayed many sharp peaks and troughs at wavelengths ranged between 550 and 1000 nm while the SCC values for spectra data beyond this range were small (Fig 7.4).

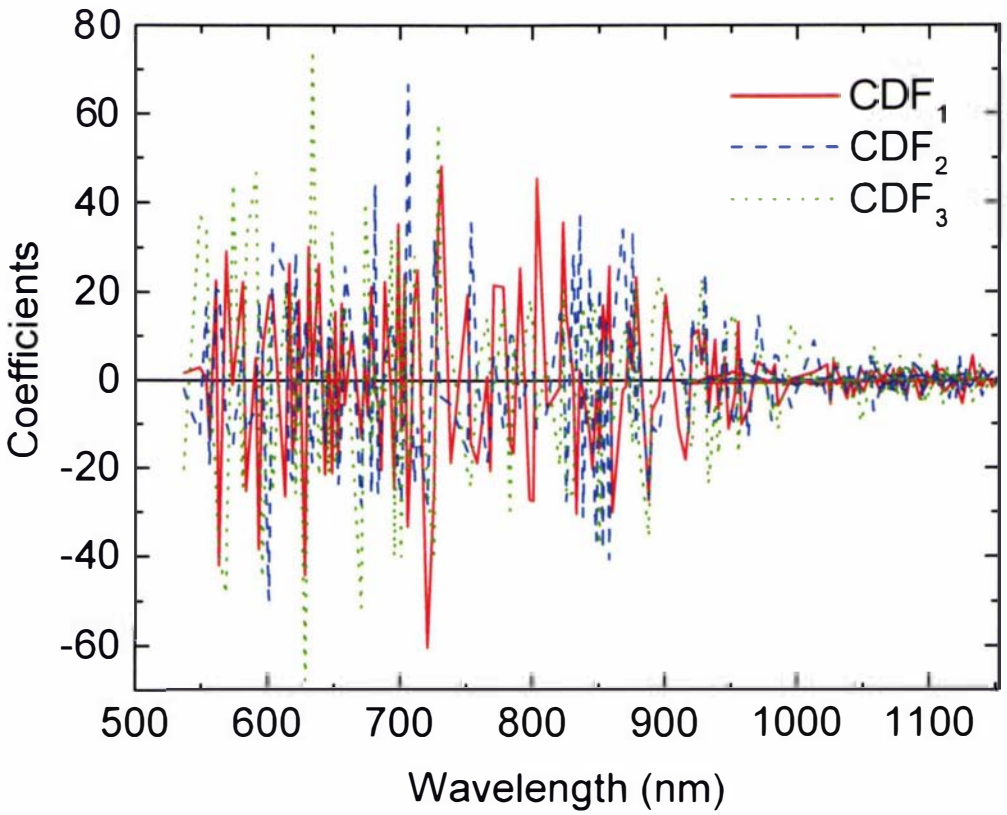

Figure 7.4 The standardized canonical coefficients (SCC) in three canonical functions (CDF_1, CDF_2 and CDF_3) used to discriminate groups of disordered fruit in experiment 3.

Fruit in the validation data set had similar distribution patterns along the CDF_1, CDF_2 and CDF_3 axes as those in the model-building data set (Fig 7.3B).

Discrimination based on the three canonical functions yielded 98.7% healthy fruit in the predicted healthy group compared with 94.6% by chance. The percentage of SP, SEB and SR fruit in the predicted SP, SEB and SR groups were respectively 15.5%, 8.0% and 6.7% compared with 2.1%, 2.2% and 1.1% respectively by chance (Table 7.9). The cumulative percentage of disordered fruit (fruit with SP, SEB or SR) in the predicted healthy fruit group was 1.3% while the cumulative percentages of disordered fruit in the predicted disordered groups ranged from 13.9% to 22.2%.

Table 7.9 Effect of a four-group segregation based on three canonical functions for 9,612 fruit in the validation data set of experiment 3.

Actual group	Fruit number (percentage) in each predicted group				Total fruit number
	Healthy	Soft patches	Stem end botrytis	Side rots	(percentage)
Healthy	6985	488	951	666	9090
	(98.7%)	(77.8%)	(86.1%)	(82.8%)	(94.6%)
Soft Patches	23	97	51	35	206
	(0.3%)	(15.5%)	(4.6%)	(4.4%)	(2.1%)
Stem end botrytis	54	23	88	49	214
	(0.8%)	(3.7%)	(8.0%)	(6.1%)	(2.2%)
Side rots	15	19	14	54	102
	(0.2%)	(3.0%)	(1.3%)	(6.7%)	(1.1%)
Total fruit number	7077	627	1104	804	9612

7.3.2.4 Discrimination of disordered fruit in experiment 3 with respect to time

The 29,990 fruit assessed for disorders were assigned to high-risk, medium-risk, low-risk and healthy fruit groups (Table 7.10) for a four-group multivariate discriminant analysis. Two-thirds of the fruit in each group were randomly assigned to the model-building data set with the remainder being allocated to the validation data set.

Table 7.10 Four fruit groups formed for canonical discriminant analysis in experiment 3 with respect to time

Fruit group	Time when disorders were observed	Fruit number
High-risk	During storage and within 2 days after removal from storage	444
Medium-risk	Within 2-14 days after removal from storage	2001
Low-risk	Within 15-28 days after removal from storage	26761
Healthy	More than 28 days after removal from storage	784

The best discriminant results were produced from absorbance (ABS) spectra compared with the results produced from the spectra of other pre-calibration transformations. Stepwise CDA indicated that 171 out of the 249 wavelength data points had a significant contribution to discrimination of the fruit groups. CDA on the model-building data set revealed that the first, second and the third canonical functions (CDF_1,

CDF_2, and CDF_3) accounted for respectively 64.8%, 30.1% and 5.1% variation of the four fruit groups all have significant role in assigning fruit into different groups.

High-risk fruit had the highest CDF_3 score, the lowest CDF_2 score and moderate CDF_1 score compared with fruit of other groups. Medium-risk fruit had the highest CDF_1 and moderate CDF_2 and CDF_3 scores. Low-risk fruit had moderate scores in all the three canonical functions. Healthy fruit had the highest CDF_2 score, moderate CDF_1 score and moderate CDF_3 score (Fig 7.5A).

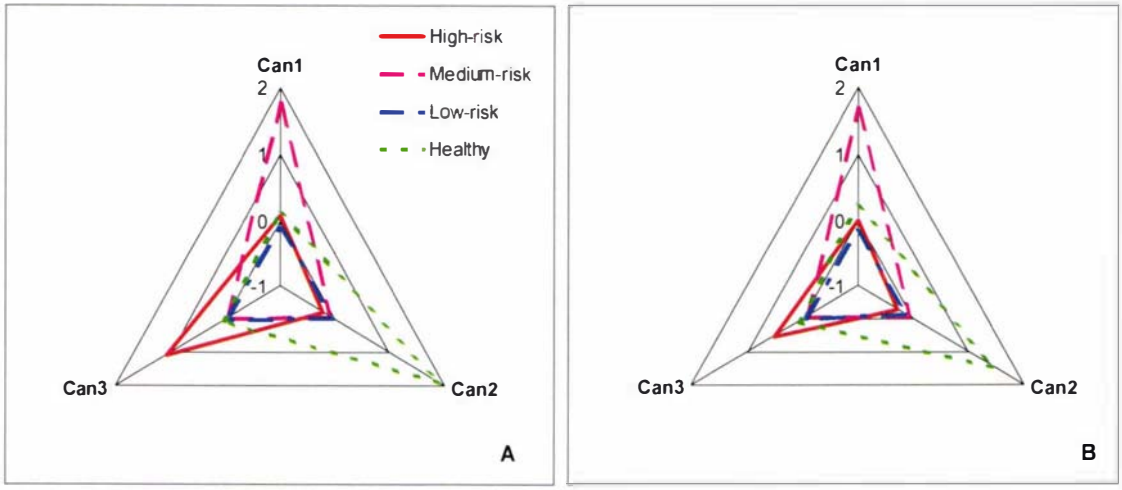

Figure 7.5 Means of the three canonical functions (CDF_1, CDF_2 and CDF_3) of high-risk, medium-risk, low-risk and healthy fruit groups in the model-building data set (A) and the validation data set (B) of experiment 3.

The standardized canonical coefficients (SCC) in CDF_1, CDF_2 and CDF_3 displayed many sharp peaks and troughs at wavelengths ranged between 550 and 1000 nm while the SCC values for spectra data beyond this range were small (Fig 7.6).

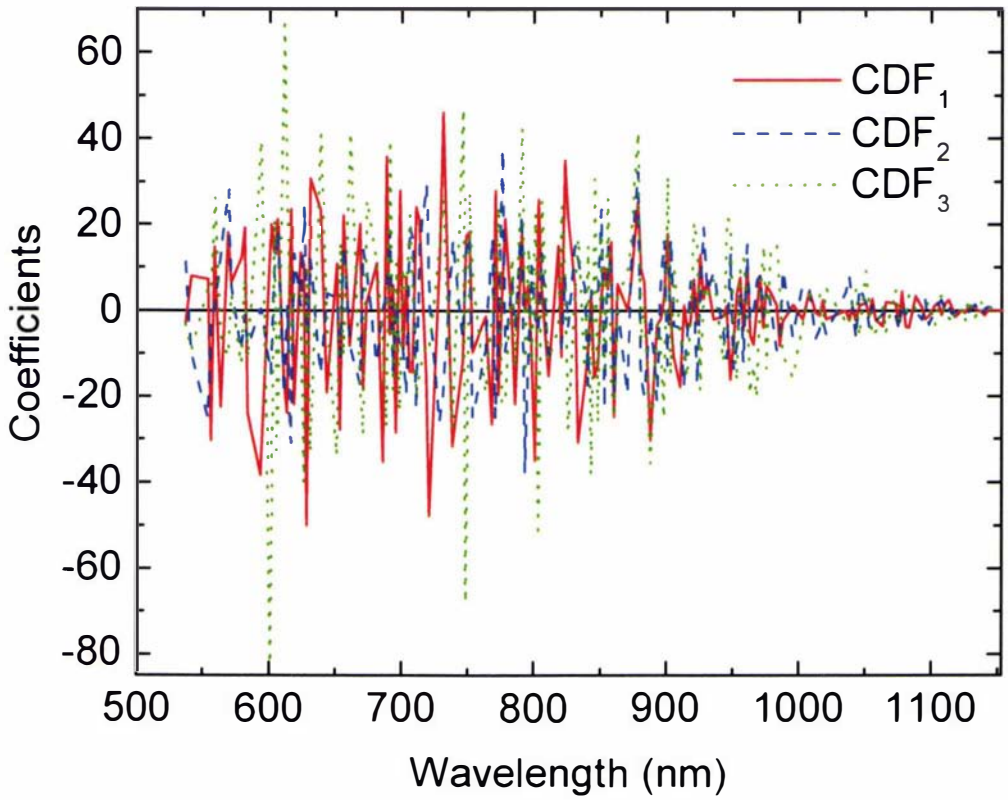

Figure 7.6 The standardized canonical coefficients (SCC) in three canonical functions (CDF_1, CDF_2 and CDF_3) used to discriminate fruit of high-risk, medium-risk, low-risk and healthy fruit groups in experiment 3.

Fruit in the validation data set had similar distribution patterns along the CDF_1, CDF_2 and CDF_3 axes as those in the model-building data set (Fig 7.5B).

Discrimination based on the three canonical functions yielded 15.0% healthy fruit in the predicted healthy group compared with 2.4% by chance. The percentage of high-risk, medium-risk, low-risk and healthy fruit in the predicted high-risk, medium-risk, low-risk and healthy groups were respectively 2.75%, 30.9%, 96.9% and 15.0% compared with 1.5%, 7.0%, 89.1% and 2.4% respectively by chance (Table 7.11). The percentage of high-risk fruit in the predicted high-risk group (2.7%) was more than twice that in the predicted medium-risk (1.3%), low-risk (1.1%) and healthy (0.8%)groups.

Table 7.11 Effect of a four-group segregation with respect to time based on three canonical functions for 9444 fruit in the validation data set of experiment 3.

Actual group	Fruit number (percentage) in each predicted group				Total fruit number (percentage)
	High-risk	Medium-risk	Low-risk	Healthy	
High-risk	53 (2.7%)	20 (1.3%)	58 (1.1%)	6 (0.8%)	137 (1.5%)
Medium-risk	82 (4.2%)	473 (30.9%)	54 (1.0%)	52 (6.6%)	661 (7.0%)
Low-risk	1781 (92.3%)	989 (64.6%)	5036 (96.9%)	611 (77.6%)	8417 (89.1%)
Healthy	14 (0.7%)	50 (3.3%)	47 (0.9%)	118 (15.0%)	229 (2.4%)
Total fruit number	1930	1532	5195	787	9444

If the high-risk fruit and medium–risk fruit are combined into a poor fruit group (i.e. fruit that developed detectable storage disorders during the 6-month storage and 2 weeks subsequent shelf life) and combine low-risk fruit and healthy fruit into a good fruit group (i.e. fruit that did not shown any disorders until 2 weeks at the shelf life), the number of poor fruit accounted for 18.1% in the predicted poor fruit group compared with 2.8% in the predicted good fruit group (Table 7.12).

Table 7.12 Effect of a two-group segregation with respect to time based on three canonical functions for 9444 fruit in the validation data set of experiment 3.

Actual group	Fruit number (percentage) in each predicted group		Total fruit number (percentage)
	Poor	Good	
Poor	628 (18.1%)	170 (2.8%)	798 (8.4%)
Good	2834 (81.9%)	5812 (97.2%)	8646 (91.6%)
Total fruit number	3462	5982	9444

7.3.3 Comparison of the predictive models between experiments

For the prediction of CFS, the PLS regression coefficient showed some significant values (either positive or negative) at wavelengths between 437-990 nm in experiment 1 while the significant coefficients of experiment 2 occurred at wavelengths between 586-979

nm (Fig 7.7A). The coefficients had no similarity between experiments except for two common peaks at wavelengths around 622 and 785 nm respectively.

For the prediction of FFS, the PLS regression coefficient showed some significant values at wavelengths between 500-1104 nm in experiment 1 while the significant coefficients of experiment 2 occurred at two separate wavelength regions, one at wavelengths between 622-630 nm and another one at wavelengths between 923-973 nm. The significant coefficients for the prediction of FFS in experiment 3 occurred at wavelengths between 546-1153 nm (Fig 7.7B). The coefficients had no similarity between experiments except for a common peak at wavelengths around 966 nm for experiment 2 and experiment 3.

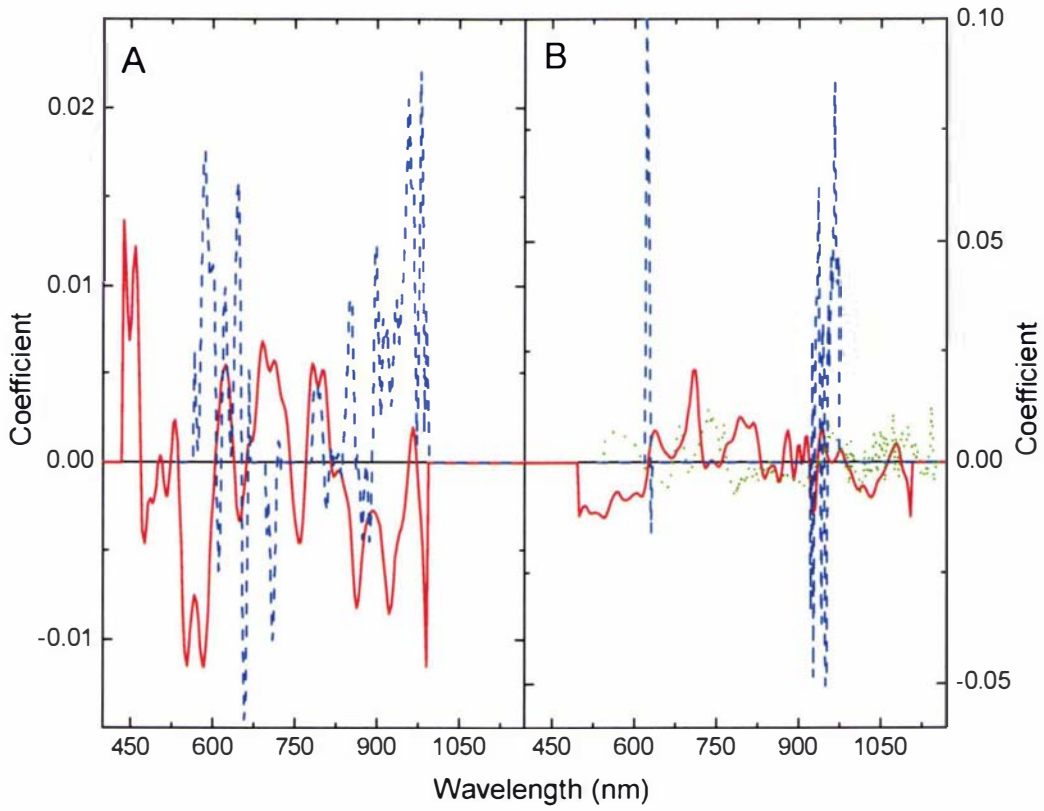

Figure 7.7 Partial least square regression coefficients for the prediction of compression force (A) and flesh firmness (B) at the end of storage obtained in experiment 1 (solid line), experiment 2 (dash line) and experiment 3 (dot line).

7.4 Discussion

Despite the limited prediction accuracies for fruit firmness measured at the end of storage (Table 7.1), fruit segregation based on predicted CFS or FFS yielded significantly higher firmness and lower percentages of soft fruit in the predicted firm fruit groups compared with those of the predicted soft and medium firmness groups (Tables 7.2). The PLS regression based on reflectance spectra measured using the commercial VNIR instrument in experiment 1 produced the best prediction results where the model explained about one-third of the firmness variation in the validation data set (Table 7.1). When fruit in the validation data set were segregated into three groups according to predicted CFS or FFS, the measured CFS or FFS of the hard fruit groups were 2.4 N (CFS) and 3.8 N (FFS), respectively, higher than that of the soft groups. The numbers of actual soft fruit in the predicted hard fruit groups were negligible while those in the predicted soft fruit groups exceeded 20% (Table 7.2). This indicates that the commercial VNIR instrument can be adapted to segregate fruit susceptible to premature softening from those capable of maintaining higher firmness during storage.

The PLS regression models based on the interactance spectra measured using the USB2000 fibre optic spectrometer in experiment 2 and experiment 3 predicted CFS and FFS poorly (the models explained less than a quarter of the firmness variation in the validation data sets) compared with that based on the reflectance spectra measured using the commercial VNIR instrument in experiment 1 (the models explained about one third of the firmness variation in the validation data sets; Table 7.1). However, segregation of fruit in the validation data set of experiment 2 and 3 according to predicted CFS or FFS also resulted in significantly higher firmness (CFS or FFS) of the predicted hard fruit groups compared with that of the medium or the soft fruit groups. In experiment 2, the percentage of soft fruit in the predicted firm group (20.3% based on CFS and 20.4% based on FFS) was about half the percentage in the predicted medium group (45.3% based on CFS and 39.6% based on FFS) and less than one-third of the percentage in the predicted soft group (66.4% based on CFS and 65.3% based on FFS). The overall high percentage of soft fruit in experiment 2 can be attributed to the extended storage time (9 months compared with 6 months in experiment 1 and 3). It is surprising that no fruit in experiment 3 had softened to

less than export criterion after 6 months of storage (Table 7.2) while the fruit in experiment 2 sampled in the same year had 4.5% soft fruit when checked after 6 months storage. The difference could be attributed to the differences between experiments in the instruments used for firmness measurement and storage conditions. The Quick Measure Penetrometer System (HortPlus Ltd, New Zealand) used in experiment 3 tends to produce higher readings than the Texture Analyser (TA.TX2, Stable Micro Systems, England) used in experiment 2 (Jeffery, 2003, personal comm.). Ethylene concentration of the cool stores may differ due to the differences in fruit load, ethylene scrubbers and environmental contamination although the ethylene concentrations in all the cool stores were under detectable level. Actual fruit temperature may fluctuate between $-1^{o}C$ and $1.5^{o}C$ although the storage temperatures were set to $0.5^{o}C$.

The predictions of CFS and FFS using the reflectance spectra measured with the commercial NIR instrument in experiment 1 were more accurate than those using the interactance spectra measured with the USB2000 fibre optic spectrometers in experiment 2 and experiment 3 (Table 7.1). Similarly, the predictions of at-harvest attributes were more accurate using the reflectance spectra measured with the commercial NIR instrument than those using the interactance spectra measured with the USB2000 fibre optic spectrometers (chapter 6). The agreement could be attributed to the relationships between at-harvest attributes and the rate of fruit softening (chapter 5).

The discriminant results in all the three experiment showed a common problem of incorrect classification between groups (Tables 7.5, 7.8, 7.9 and 7.11). The incorrect classification might be partially attributed to the fact that the cause of some disorders, particularly fungal rots and soft patches are more closely related to initial infection (Michailides and Morgan, 1997) and mechanical damage (Davie, 1997) rather than physiological factors. Fruit with physiological disorders (i.e. fruit with LTB) were discriminated at higher accuracies than fruit with soft patches were. For example, 72.7% LTB fruit in experiment 1 and 57.5% LTB fruit in experiment 2 were correctly classified compared with 45.8% and 39.6% correct classification of soft patch fruit in experiment 1 and experiment 2, respectively. Fruit with purple patches were discriminated with high accuracy (75% in experiment 1) as expected because purple patches are associated with lower fruit chroma (C) and hue (H) as measured externally at harvest (Feng et al., 2002)

and C and H can be predicted using the reflectance spectra measured with the commercial NIR instrument with R^2 of 0.64 and 0.69, respectively (Chapter 6). Fruit with stem end *Botrytis* rot or side rots were discriminated at accuracies (41.1% and 52.9% respectively) comparable to that of fruit with soft patches (47.1%) in experiment 3 (Table 7.9).

The incidence of storage disorders differed considerably between experiments. As checked after 5 days simulated shelf life, fruit with soft patches, LTB, purple patches and fungal rots accounted for 7.7%, 3.6%, 4.9% and 0.9% respectively in experiment 1 with storage durations ranged from 6 to 11 months depending on grower line (2000 season) while the disordered fruit accounted for 23.5%, 26.5%, 2.0% and 0.3% respectively in experiment 2 with a storage duration of 9 months (2001 season). Fruit developed soft patches and fungal rots within 5 days shelf life following a 6-month storage accounted for 0.1% and 0.9% respectively in experiment 3 (2001 season) while LTB and purple patches were not observed in this experiment.

In experiment 1, most of the purple-patched fruit were observed in grower lines which had been stored for 6-9 months. This means that the higher percentage of purple-patched fruit in experiment 1 than that of experiment 2 was not likely to be caused by the difference in storage duration between the two experiments. Instead, it is likely to be caused by the hot weather of 2000 season compared with that of 2001 season (the average maximum temperatures of February to May in 2000 were 0.7°C higher than that of the same time in 2001 according to the record at Tauranga, the centre of the fruit sample region). This result supports the postulated cause of purple patches being the latent chemical burn resulted from preharvest spray during hot days (Feng et al., 2002).

In experiment 2, all fruit were stored for 9 months and then brought to shelf life conditions for the measurement of firmness and disorders. The overall high percentage of fruit with soft patches and LTB in this experiment may be attributed to the over storing of some grower lines which do not have the innate storage potential for 9 months. For example, one-third of the 22 grower lines in experiment 2 had mean FFS of 6.1-8.1 N as measured at 20°C (equivalent to 7.9-11.2 N if measured immediately upon removal from coolstore, according to firmness-temperature coefficient of kiwifruit reported by Jeffery and Banks, 1994) and the percentage of soft fruit in these grower lines ranged from 55%-90%). Concomitantly, the overall low percentage of fruit with storage disorders in

experiment 3 may be attributed to the short storage duration (6 months) compared with that of experiment 1 (6-11 months depending on grower line) and experiment 2 (9 months). The overall low incidence of storage disorders of experiment 3 also agrees with the overall low softening rate of the grower lines (Chapter 5).

The results from experiment 3 indicated that disordered fruit accounted for 1.5% at the end of storage, and increased to 8.2% and 97.4% respectively after subsequent shelf life of 2 and 4 weeks (Table 7.10). A fruit showing a disorder after 14 days of shelf life was very similar to another one showing the same disorder in the following day, but the former was allocated to disordered group while the later was allocated to healthy group. In such a cases, small measurement error in both the VNIR spectra and the disorders could cause some incorrect classification between the disordered and healthy groups.

The misclassification between groups of different disorders may be attributed to the overlaps between disorders on the same fruit. For example, LTB, fungal rots and soft patches were usually associated with each other. This reflects the common physiological factors that affect fruit susceptibility to several storage disorders. For example, kiwifruit with low Ca are susceptible to soft patches (Banks et al., 1995; Davie, 1997; Benge, 1999; Benge et al., 2000), *Botrytis* rots (Banks et al., 1995; Davie, 1997) and physiological pitting (Ferguson et al., 2001). The relationship between Ca and LTB is not clear for kiwifruit, but the association of low Ca and high incidence of LTB were found in avocado (Chaplin and Scott, 1980), peach (Wade, 1981), and lime (Slutzky et al., 1981).

In the discriminant analyses for groups of fruit with different disorders, some fruit with minor disorders were excluded from analysis. Consequently, the discriminant models did not have the ability to separate fruit with minor disorders. Continuous monitoring for disordered fruit in experiment 3 facilitated the grouping of fruit with respect to the time when disorders were found regardless of what kind of disorder the fruit had developed. A four-group segregation of high-risk, medium-risk, low-risk and healthy fruit based on VNIR spectra taken at harvest yielded higher percentage of correctly classified fruit in all the four predicted fruit groups compared to the percentages by chance (Table 7.11). When high-risk fruit and medium–risk fruit were combined into a poor fruit group (i.e. fruit that developed detectable storage disorders during the 6-month storage and 2 weeks subsequent shelf life) and low-risk fruit and healthy fruit were combined into a good fruit group (i.e.

fruit that did not shown any disorders until 2 weeks at the shelf life), the number of poor fruit accounted for 18.1% in the predicted poor fruit group compared with 2.8% in the predicted good fruit group (Table 7.12). This indicated that VNIR technologies are capable of segregating fruit susceptible to storage disorders from less susceptible fruit.

However, large amount of healthy fruit have been incorrectly classified into groups of disordered fruit. In experiment 1, 66.7%-84.6% of the fruit in predicted disordered fruit groups remained healthy (Table 7.5) while the percentages of healthy fruit in the predicted disordered groups ranged from 32.9%-48.8% in experiment 2 (Table 7.8) and 77.8%-86.1% in experiment 3 (Table 7.9). This could result in great fruit loss if the low-grade fruit are rejected. Further work is needed to increase classification accuracy, to improve inventory management to ensure these fruit be sent to the market earlier, or to develop postharvest treatments to reduce the disorders.

Comparison of the predictive models for CFS or FFS obtained in different experiments indicated that the models were based on spectral data at different wavelength regions and the PLS regression coefficients rarely have common peaks and troughs within the same wavelength range (Fig 7.7). Consequently, it is hard to see the spectroscopic basis (e.g. absorption bands of known chemical and physical species) of the predictive models.

Comparison of the discriminant models between experiments is difficult because of the differences in fruit groups and instruments. Canonical functions of experiment 1 were calculated from reflectance spectra at 9 wavelength points with wavelengths ranged from 300-708 nm (Table 7.4) while that of experiment 2 were calculated from interactance spectra at 37 wavelength points with wavelengths ranged from 572-1149 nm (Table 7.7). Canonical functions of experiment 3 were calculated from interactance spectra at more than 150 wavelengths spread over the whole truncated wavelength range of 536-1159 nm (Fig 7.4 and 7.6). However, the most important wavelengths (i.e. wavelength with large standardised canonical coefficients in canonical functions) were located within a range of 550-1000 nm in both experiment 2 and 3. Large positive SCC (standardised canonical coefficient) values at one wavelength point or a group of wavelength points were usually associated with large negative SCC values at neighbouring wavelength points or *vice versa*. For example, the largest negative SCC value in CDF_1 of experiment 1 occurred at 605 nm (SCC=-80.6) which was associated with the largest positive SCC value at 595 nm (SCC=-

55.6) and a moderate positive SCC value at 609 nm (SCC=28.6). In experiment 2, the largest SCC value in CDF_1 occurred at 816 nm (SCC=156.6) which was associated with the largest negative SCC value at 819 nm (SCC=-121.9) and a moderate negative SCC value at 811 nm (SCC=-34.6). This indicated that the SCC values should be considered by groups of wavelength points, and each group of wavelength points may represent a spectral band which is associated with certain properties of the fruit. For example, the band of 605 nm showing the most significant importance for discriminating disordered fruit from healthy fruit in experiment 1 (Tables 7.3 and 7.4) is possibly a response to cytochrome *c* oxidase, a terminal respiratory enzyme (Vanneste, 1966). The high absorbance at 605 nm, but low absorbance at 609 and 595 nm of fruit with purple patches or soft patches may be an indication of high concentration of cytochrome *c* oxidase induced by chemical or mechanical damage, respectively. However, attempts to match other major groups of wavelengths with known spectral band were not successful. Furthermore, neither a spectral band between 595-609 nm was shown in the VNIR spectra of experiment 1 nor a spectral band between 811-819 nm was shown in the VNIR spectra of experiment 2 (data not shown). Therefore, the spectroscopic basis of the discriminant models is yet to be elucidated.

Among the 12 pre-calibration transformations applied to spectral data, absorbance spectra (ABS) in association with smoothing (SABS) and standard normal variate transformation (SNV) or normalisation with minimum and maximum values in each spectrum (NM) appeared sufficient for predicting firmness at the end of storage or for discriminating fruit of different storage disorders. Quadratic baseline compensation (QBC) and the second derivation (D_2) did not result in better prediction or discriminant results. This is similar to the estimation of at-harvest fruit attributes where QBC and D_2 have no advantage over ABS, SABS, SNV and NM (Chapter 6).

7.5 Conclusion

VNIR technology is useful to predict fruit firmness measured at the end of storage for fruit segregation purposes. Fruit with high risks of developing such storage disorders as soft patch, low temperature breakdown, purple patches, and fungal rots could also be

discriminated at harvest based on the VNIR spectra. Further work is needed to improve the prediction and discriminant accuracies and to elucidate the spectroscopic basis of the prediction and discriminant.

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Factors affecting VNIR measurements of kiwifruit attributes

8.1. Introduction

Visible-near infrared spectroscopy (VNIR) is a non-destructive technique capable of measuring various quality attributes of fruit and vegetables. For kiwifruit, VNIR has been used to estimate flesh firmness (FF), soluble solids content (SSC) and dry matter content (DM), flesh huge angle (FH) and density (McGlone and Kawano, 1998; Osborne and Kunnemeyer, 1999; Schaare and Fraser, 2000; McGlone et al., 2002a). However, the standard error of prediction (SEP) and coefficient of determination (R^2) achieved in different experiments differed considerably. For example, McGlone and Kawano (1998) achieved SEP=0.39 °Brix and R^2=0.90 for SSC and SEP=7.8 N and R^2=0.66 for FF using reflectance spectra from 800-1100 nm, while Costa et al. (1999) achieved R^2 of only 0.65 and 0.42 for SSC and FF respectively using reflectance spectra from 650-1200nm. The results from the current study also varied considerably from year to year even though spectral data were taken in the same mode and the same wavelength range were used for calibration (Feng et al., unpublished). The same problem has occurred when measuring other fruit such as apple (McGlone et al., 2002b).

Tracing the reasons for such variable results is not easy because the physical and chemical bases of the predictive models are not clear. McGlone and Kawano (1998) had related the regression coefficient vectors for DM and SSC to several absorbance peaks of starch, cellulose, sucrose and water within the wavelength range of 800-1100nm. But they found the regression coefficient vectors for FF related poorly to known absorbance bands of relevant chemicals such as pectin. In apple, McGlone et al. (2002b) found that the prediction models for background colour, starch pattern index, SSC, FF, quantitative starch and titratable acidity were primarily dependent on changes in the chlorophyll absorbance peak, which dramatically reduces in intensity during the progression of the fruit through the harvest period, rather than on the constituent or property of direct interest. Furthermore, current knowledge of NIR/VNIR absorbance of chemicals in solution were obtained from transmittance (William and Norris, 1987) rather than reflectance and interactance that are

actually used in fruit measurement, and data on the reflectance of chemicals were obtained from pure chemicals (William and Norris, 2001) rather than from solutions as they exist in fruit tissues.

VNIR spectra are affected not only by chemical components of the fruit, but also the physical properties of the fruit (McGlone and Kawano, 1998). Within-fruit gradients of both chemical and physical structure have been identified in previous studies (Ferguson, 1980; Clark and Smith, 1988; Hallett et al, 1992). Some variation in the spectra taken from the same fruit might be caused by within fruit gradients. If this is the case, techniques to reduce this variability would improve the accuracy of prediction using VNIR spectra.

Many pre-treatments of spectral data can be applied before calibration of the predictive model (Geladi et al., 1985; Barnes et al. 1989; Schaare and Fraser, 2000; Norris and Workman, 1997; Williams & Norris, 2001). Different pre-treatments tend to emphasise some aspects of the spectra by minimizing other aspects. For example, the differentiation of absorbance spectra retains absorbance band information, but baseline effects are minimised. Differential spectra should be used if only the absorbance bands are relevant to the fruit attribute to be predicted, otherwise the differentiation could be detrimental to the predictive results.

With respect to predicting storage potential of kiwifruit, it would be helpful to determine how fruit history affects VNIR spectra. Several important historical factors, such as prestorage delay (storage from harvest to packing), weight loss, compression and abrasion happening during the delay were tested. Kiwifruit is a berry fruit with hairy skin. Investigating how the hairs affect NIR spectra would provide information for making decisions of whether to measure fruit before or after hair brushing. This study was intended to provide information useful to improve understanding of the measurement and to identify possible aspects of improving the measurement procedure and data processing.

8.2 Materials and methods

8.2.1 VNIR transmittance of different chemical solutions and reflectance of kiwifruit subjected to different chemical dipping and storage treatments

Forty eight kiwifruit were randomly sampled on 13 April 2000 from bins at a packhouse in the Bay of Plenty, New Zealand's major kiwifruit production area. Fruit were sent to Massey University by overnight courier and hairs were brushed off using a piece of foam to simulate hair brushing on commercial packing lines. Fruit were randomly assigned to six groups with eight fruit in each group. Eight fruit in each group were dipped in one of the eight solutions for 35 minutes and then subjected to six storage treatments (Table 8.1). Fruit were washed with tap water to remove residual chemical solution on the fruit surface and dried with plain tissue before the start of the storage treatment.

Table 8.1 Chemical dipping and storage treatments

Storage treatments		Chemical treatments*****							
Days (d) and temperature (°C)	DD**** (°C d)	Starch (10%)	WPI** (3%)	AA*** (0.3%)	Na_2HPO_4 (2%)	KCl (2%)	$CaCl_2$ (2%)	$MgCl_2$ (2%)	Distilled water
3 d at 0.5	1.5	1-1*	1-2	1-3	1-4	1-5	1-6	1-7	1-8
15 d at 0.5	7.5	2-1	2-2	2-3	2-4	2-5	2-6	2-7	2-8
6 d at 0.5+9 d at 10	93.0	3-1	3-2	3-3	3-4	3-5	3-6	3-7	3-8
15 d at 10	150.0	4-1	4-2	4-3	4-4	4-5	4-6	4-7	4-8
6 d at 0.5+9 d at 20	183.0	5-1	5-2	5-3	5-4	5-5	5-6	5-7	5-8
15 d at 20	300.0	6-1	6-2	6-3	6-4	6-5	6-6	6-7	6-8

*The first number represents the storage treatment while the second indicates chemical treatment.

** Whey protein isolated. *** Ascorbic acid. **** Cumulative degree-days.

*** ** The pH of the solutions were adjusted to 5 (approximates the pH of fruit juice) using 0.1 % NaOH or 0.1 % HCl solutions. All the chemicals are of analytical purity.

Transmittance VNIR measurements were taken on the chemical solutions in a quartz cuvette and reflectance VNIR measurements were taken at two opposite points along the equator of each fruit after the factorial treatments. All fruit were brought to the lab (20°C and 50-60 % RH) 12 hours before VNIR measurement to allow temperature equilibration and the surface to dry.

The instrument was constructed around a TM300 monochromator (Bentham Instruments Limited, Reading, UK) fitted with a ruled grating (1200 lines mm^{-1}) and a 250 W quartz halogen lamp. The light from the exit slit of the monochromator was focussed onto one arm (6 mm in diameter) of a bifurcated fibre optic bundle (Oriel Instruments, Stratford, Connecticut, USA) and the light from this was focussed onto the quartz cuvette or a fruit at a right angle through a rectangular probe of 6 × 2 mm. Light from the sample passed back through the other arm of the fibre optic bundle and was focussed onto a silicon photodiode (1.75 mm^2 active area, RS Components, Auckland, NZ). The voltage output of the photodiode and associated circuitry was acquired using a DAS-1600 A/D board (Keithley, Taunton, Massachusetts, USA). All the operations of the instrument were controlled using in-house software. Spectra were collected at 2 nm intervals from 400 to 1200 nm and each point represents the average of 1000 acquisitions. In addition to carrying out the VNIR measurement in the dark, the sample and probe were covered by a piece of cloth during the measurement to avoid disturbance by extraneous sources of light. Reference spectra for chemical solutions were measured on the empty quartz cuvette in a transmittance mode. Reference spectra for fruit were measured on a Teflon block in an interactance mode.

Fruit weight (W), colour (L, C, H), and compression force (CF), flesh firmness (FF), flesh colour (FL, FC, FH) and soluble solid content (SSC) were measured within 4 hours before or after NIR measurement. Fruit weight was measured with a balance (0.001 g, Model PM1206, Mettler Toledo, Switzerland) and fruit colour with a Minolta chroma meter (CR-200). CF was measured by compressing fruit for 1.5 mm using a round-tip plunger (diameter = 11 mm) attached to a texture analyser (TA.TX2, Stable Micro Systems, England). The maximum force was recorded electronically. A force threshold (trigger force) was set to 0.2 N to allow fruit hairs to bend down and to make sure the probe had reached the fruit surface before the meter started counting the 1.5 mm compression distance. FF was measured using a 7.9 mm diameter plunger attached to the texture analyser. The plunger penetrated 9 mm into a fruit at a constant speed of 10 mm s^{-1} after a patch of skin about 15 mm in diameter was peeled off by a fruit peeler. Soluble solid content (SSC) was measured with a refractometer (Atago, 0-20%) using the juice extracted when measuring FF. Flesh colour was measured on the cut surface of two halves of each

fruit. Measurement of fruit colour, CF, FF, flesh colour and SSC were made at opposite positions along the equatorial line of a fruit, and the two readings were averaged to give L, C, H, CF, FF, FL, FC, FH, and SSC values of the fruit. A central piece of approximately 1 cm in thickness was cut from each fruit along the equator and freeze-dried for dry matter (DM) and mineral (N, P, K, Ca, and Mg) measurement (Feng et al., 2002).

8.2.2 VNIR interactance of different fruit tissues

Three 'Hayward' kiwifruit sampled in May 2000 from the Bay of Plenty, New Zealand's major kiwifruit production area were stored at 0.5°C for two weeks and equilibrated to room temperature for measurement. A central section of 8 mm in thickness was cut vertically from each fruit. This piece was further divided into outer pericarp (OP, flesh and skin) with or without skin, inner pericarp (IP, flesh with locule containing seeds) and core. Each section was placed vertically into a 10 mm quartz cuvette with its outside connecting the inner side of the cuvette wall. Interactance VNIR spectra were taken with light focussed onto the outside wall of the cuvette at a right angle. Reference spectra were measured on a Teflon block in an interactance mode.

8.2.3 Effect of post harvest delay and weight loss on interactance VNIR spectra

Two hundred freshly harvested fruit of similar size (90-105g) were sampled from field bins of a Bay of Plenty orchard at 10 am 9 June 2001 and brought to the laboratory in two polylined kiwifruit boxes (count 100) within 24 hours. The fruit were randomly assigned to 4 groups for the following treatments to generate differences in weight loss: (1) packed in a polylined single-layer kiwifruit trays located 150 cm away from fans; (2) packed in an open single-layer kiwifruit tray without polyliner located 120 cm away from fans; (3) packed in a moulded pulp tray located 90 cm away from fans; (4) packed in a moulded pulp tray located 30 cm away from fans.

Interactance VNIR spectra were taken on each fruit after two, three, four and eight days of weight loss treatment. The first two VNIR measurements were carried out under fluorescent light at Massey University, while the last two measurements were under

tungsten light in the Mt Maunganui. The VNIR measurement were taken at two opposite points along the equatorial line of each fruit using an USB2000 fibre optic spectrometer (Ocean Optics Inc.) fitted with a fibre optic interactance probe. The probe was a R400-7 reflection probe that consists of a tight bundle of 7 optical fibres of 400 μm in diameter (6 illumination fibres around 1 read fibre) in a stainless steel ferrule of 3.0" long and 0.25" diameter. The probe was pointed at the median equator of a fruit and a spectrum (519-1156 nm) was recorded at about 0.31 nm intervals (2048 points). Both white reference spectra (obtained when the probe was inserted into the RPH-1 reflection probe holder at 90° angle) and dark spectra (obtained by blocking the aperture to the optical fibre) were recorded for fruit of each treatment at each measurement time. Two separate spectra were taken on opposite sides of each fruit.

Fruit weight, colour and compression force were measured after each VNIR measurement. Flesh firmness was measured on 25 fruit from each weight loss treatment. Weight loss (WL) was calculated as the percentage of initial fruit weight using equation 8.1.

$$WL_t = \frac{W_0 - W_t}{W_0} \cdot 100\ \% \qquad (8.1)$$

where W_0 is initial fruit weight measured before storage and W_t is fruit weight measured after t hours of treatment).

8.2.4 Effect of compression and abrasion on VNIR spectra

One hundred freshly harvested fruit of similar size (90-105g) were sampled from field bins of a Bay of Plenty orchard on 9 June 2001 and brought to the laboratory in a polylined kiwifruit boxes (count 100) within 24 hours. The fruit were randomly assigned to 5 groups for compression and abrasion treatments. Fruit were placed over a wood (typical pine board used for kiwifruit bins) and compressed by 4.5, 3.5, 2.5, or 1.5 mm using a flat tip probe (20 mm diameter) attached to a texture analyser (TA.TX2, Stable Micro Systems, England). The probe was held at the maximum compression position for 3 seconds, and during this time the fruit was pulled out of the gap between the wood and the probe to generate abrasion damage. The treatment on fruit side facing the probe was considered as compression treatment (a simulation of fruit dropping into a harvest bin), and that on the

opposite side facing the wood was considered as abrasion treatment (a simulation of abrasion damage during harvest or transportation). Each fruit was treated on two positions at 90 degrees from each other along the equatorial line. Twenty untreated fruit were maintained as control.

Three separate interactance VNIR measurements were made 1, 3 and 7 days after the treatment using the USB2000 fibre optic spectrometer. The first VNIR measurement was carried out under fluorescent light, while the last two measurements were under tungsten light. Fruit colour and compression force were measured either before or after each VNIR measurement. Flesh firmness was measured after the final VNIR measurement. All measurements were taken on four sides of each fruit, two compressed sides and two grazed sides. Measured values from each treatment on a fruit were averaged.

8.2.5 Effect of hair brushing on interactance VNIR spectra

Twenty freshly harvested fruit of similar size (90-105g) were sampled from the orchard of Massey University on 5 June 2001 and kept at ambient condition for 24 hours to allow the surface to dry and temperature equilibration. Hairs on one side of each fruit were brushed off using a piece of foam and that on the opposite side were kept intact. Interactance VNIR spectra were taken on each side of the fruit using the USB2000 fibre optic spectrometer. Fruit colour, compression force and flesh firmness were measured after the VNIR measurement.

8.2.6 Data analysis

Spectral data at both ends of the wavelength range were deemed unreliable because of the low signal to noise ratio and detector constraints. VNIR spectra from the TM300 monochromator were truncated to a wavelength range of 470-1120 nm and that from the USB2000 fibre optic spectrometer were truncated to a wavelength range of 355.52-1154.44 nm. Boxcar averaging (Faber, 1999) of every 8 points (2.48 nm) was applied to spectral data from the USB2000 fibre optic spectrometer. This reduced the number of data points in each spectrum from 2048 to 251.

Spectral data from all experiment were baseline corrected to produce absorbance spectra (ABS) using equation 8.2.

$$ABS = -\log\left(\frac{I_s - I_d}{I_w - I_d}\right) \qquad (8.2)$$

where I_s is the raw spectral data taken on a sample (i.e. a chemical solution or a fruit), I_d is the raw spectral data of the dark reference, I_w is the raw spectral data of the white reference.

White reference spectra of the USB2000 fibre optic spectrometer were multiplied by two to ensure positive ABS values. Absorbance spectra were smoothed and second derivative spectra (D_2) were generated using a simplified least square procedure (Savitzky and Golay, 1964, Steinier et al., 1972) with a window size of 5 points (10 nm). The smoothed absorbance spectra (SABS), D_2 (in some cases) and fruit attributes were subjected to analysis of variance and regression analysis.

SPECTRUM (Brown, 2003) and SAS (SAS Institute Inc., 2003) were used for data processing and analysis.

8.3 Results

8.3.1 VNIR transmittance of different chemical solutions

VNIR transmittance differed considerably among chemical solutions tested. Compared with the absorbance of water, $MgCl_2$ (2 %) had the lowest absorbance, followed by Na_2HPO_4 (2 %), $CaCl_2$ (2 %) and KCl (2 %). The absorbance of ascorbic acid (AA, 0.3 %) was very close to that of water. Starch and whey protein had higher absorbance than water. The differences between chemical solutions and water decreased as wavelength increased to 960-990 nm where the differences reached the minimum and then increased as wavelength further increased to 1050-1100 nm (Fig 8.1).

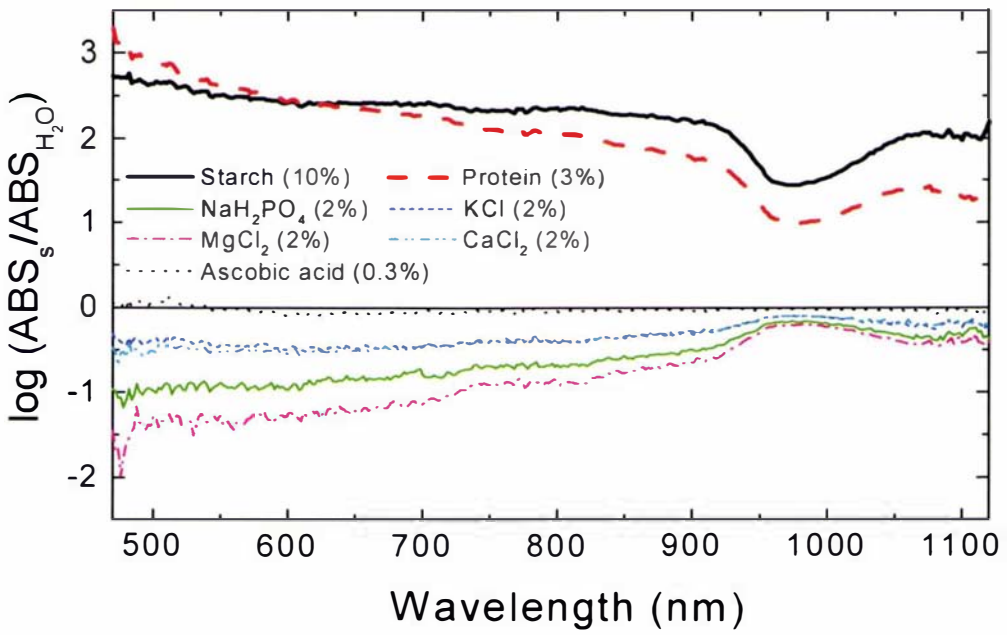

Figure 8.1 VNIR absorbance of chemical solutions (ABS_s) relative to that of water (ABS_{H_2O}) as measured in a transmittance mode

8.3.2 VNIR reflectance of fruit from chemical dipping and storage treatments

Analysis of variance for VNIR absorbance and fruit attributes indicated that there was no interaction between storage and chemical dipping treatments. Chemical dipping had no effect on VNIR absorbance and the fruit attributes measured. Storage treatments affected VNIR absorbance and most fruit attributes.

At all wavelength regions, VNIR absorbance of the fruit differed significantly among the storage treatments. Fruit stored at high temperatures for long periods showed higher absorbance compared with that of fruit stored at lower temperature for shorter periods (Fig 8.2). This trend was the most pronounced at 766 nm where the absorbance correlated with cumulative degree-days (DD) with a correlation coefficient of 0.63. The relationship between treatment mean absorbance and DD was log linear. A logarithmic model fitted to the data explained 47.9 % of the absorbance variation on individual fruit basis (Fig 8.3).

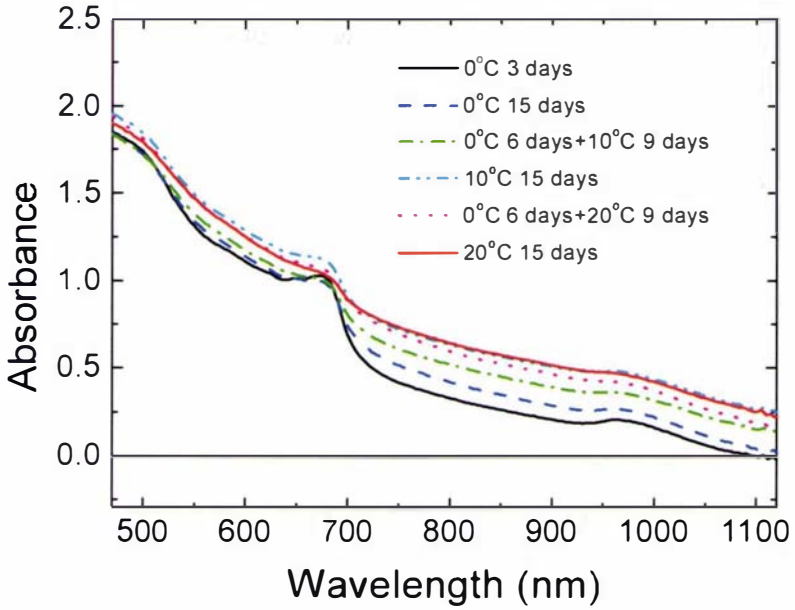

Figure 8.2 Absorbance spectra of fruit from different storage treatments. Each line is the mean of eight fruit.

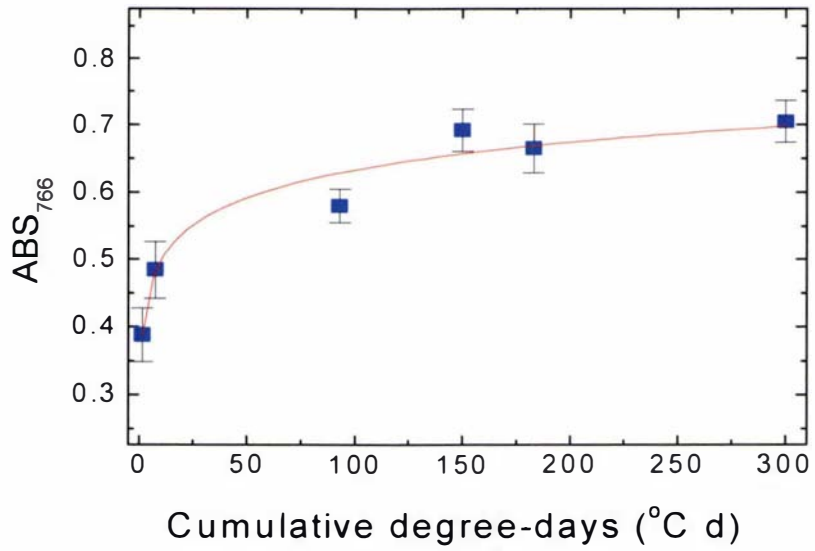

Figure 8.3 Relationship between cumulative degree-days (DD) of storage treatment and VNIR absorbance at 766 nm (ABS_{766}). Each point is the mean of eight fruit. Vertical bars represent standard error of the means. The solid line is a curvilinear relationship between ABS_{766} and DD:

$ABS_{766} = 0.36 \pm 0.03 + 0.059 \pm 0.006 \cdot \log_e(DD)$, n=48.

It was also noticed that the chlorophyll absorption band around 672 nm was narrower but more intense for fruit subjected to a short time at low temperatures compared with that of fruit stored for a longer time at higher temperatures (Fig 8.2). This was reflected more clearly in the second derivative spectra by a trough at 684 nm and a peak at 700 nm. The depth of the trough and the magnitude of the peak were more pronounced for fruit subjected to a short time at low temperatures compared with that of fruit stored for a longer storage time at higher temperatures (Figure 8.4 A). The log transformed difference between the peak and the trough ($LN(D_2)=\log_e(D_{2(700)}-D_{2(684)})$) related to DD with a R^2 of 0.64 on an individual fruit basis or a R^2 of 0.91 on a treatment basis (Fig 8.4B).

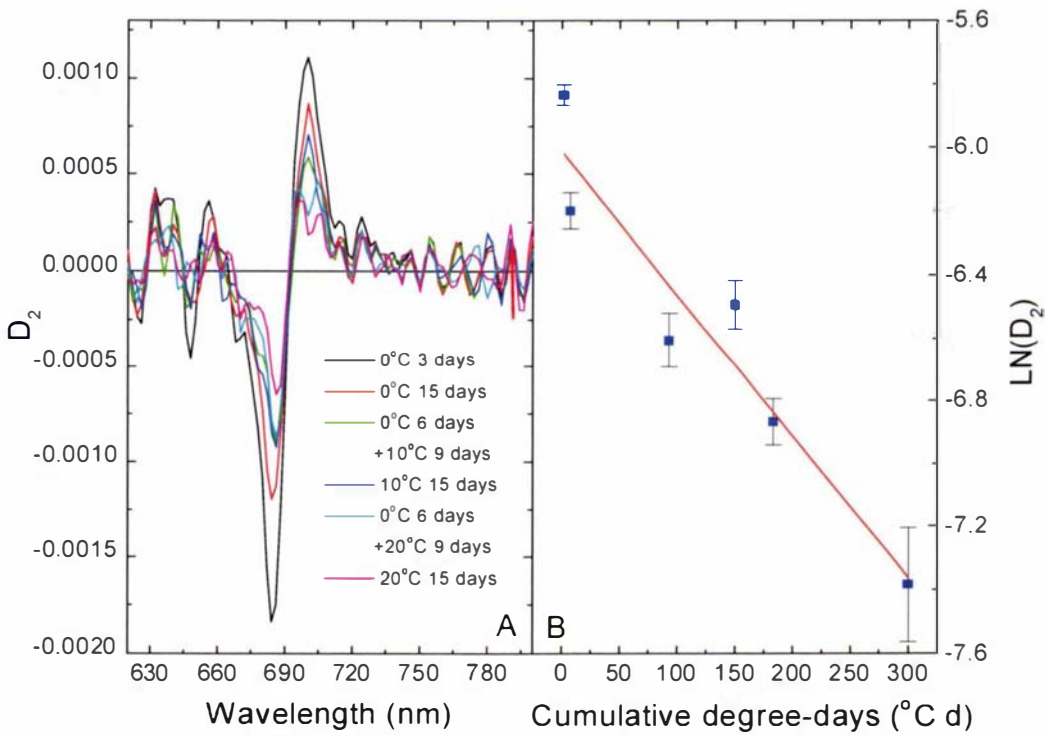

Figure 8.4 Second derivative spectra (D_2) of fruit from different storage treatments as a function of cumulative degree-days (DD). Each line in A is the mean of eight fruit. Each point in B is the mean of the log transformed difference between D_2 values at the peak (700 nm) and the trough (684 nm) of the eight fruit in each treatment ($LN(D_2)= \log_e (D_{2(700)}-D_{2(684)})$). Vertical bars represent standard errors of the means. The solid line in B is a linear relationship between $LN(D_2)$ and DD: $LN(D_2) = -6.01 \pm 0.06 - 0.0045 \pm 0.0004 \cdot DD$, n=48.

CF, FF, SSC, fruit colour and flesh colour (except FH) were significantly different for fruit subjected to different storage treatments (Table 8.2). CF, FF, SSC, FL and FC were exponentially related to cumulative degree-days (DD) while L, C and H were linearly related to DD (Fig 8.5).

Table 8.2 Compression force (CF), flesh firmness (FF), soluble solids content (SSC), fruit colour (L, C, H) and flesh colour (FL, FC, FH) of fruit in different storage treatments

Days (d) and Temperature (°C)	CF (N)	FF (N)	SSC (°Brix)	L	C	H	FL	FC	FH
3d at 0.5	25.3 a	55.8 a	9.4 a	50.3 a	25.9 a	83.9 ab	59.9 a	35.7 a	114.3 a
15d at 0.5	14.1 b	26.3 b	12.8 b	49.9 a	25.1 a	83.7 ab	53.0 b	30.0 b	115.3 a
6d at 0.5+9d at 10	6.3 c	6.2 c	14.0 c	47.2 bc	22.7 b	80.3 bc	45.9 c	24.6 c	115.9 a
15d at 10	4.9 cd	1.7 d	13.9 c	47.9 b	22.6 b	82.5 ab	44.2 cd	22.7 cd	115.0 a
6d at 0.5+9d at 20	4.4 cd	0.5 d	14.0 c	45.3 cd	21.4 bc	81.3 b	42.5 de	23.3 cd	115.4 a
15d at 20	3.7 d	0.0 d	14.5 c	43.5 d	20.1 c	77.0 c	41.5 e	21.9 d	114.7 a

*Values with different labels in each column are significantly different ($LSD_{0.05}$).

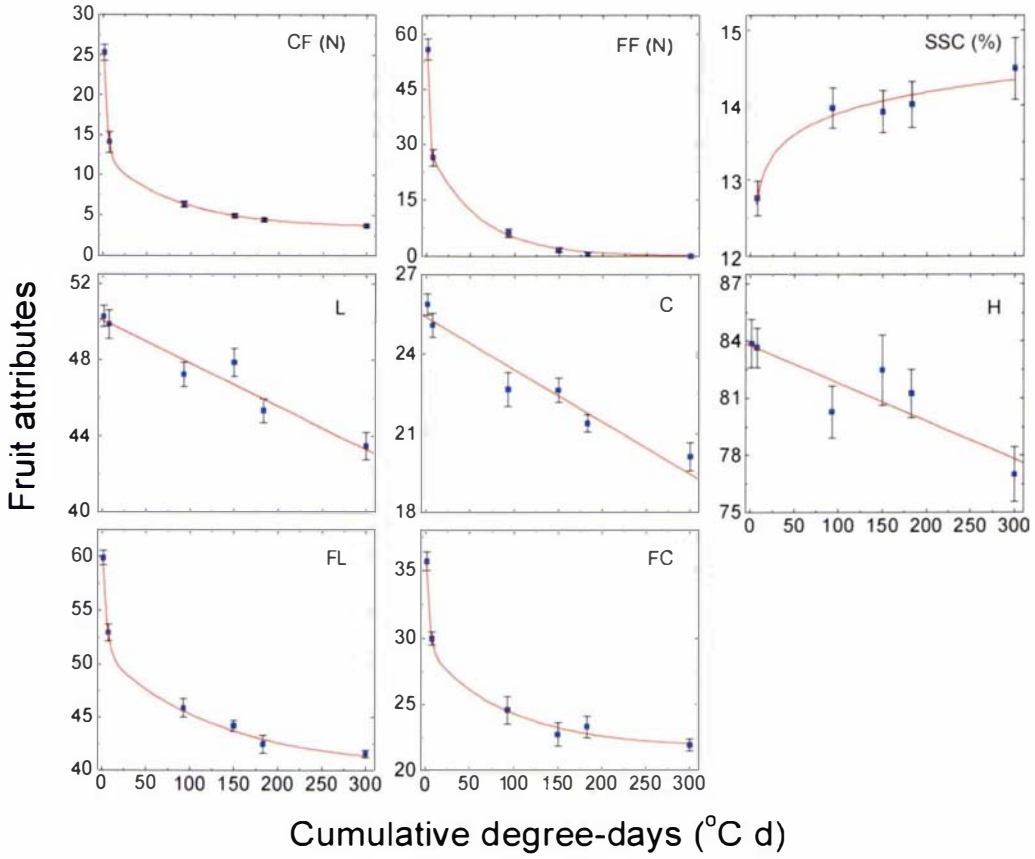

Figure 8.5 Changes in compression force (CF), flesh firmness (FF), soluble solids content (SSC), fruit colour (L, C, H) and flesh colour (FL, FC) in respond to cumulative degree-days of storage treatments. Each point is the mean of eight fruit. Vertical bars represent standard errors of the means. Solid lines are fitted non-linear, linear or curvilinear relationships between fruit attributes and DD (n=48):

$CF = 7.4 \pm 0.8 \cdot e^{(-0.0024 \pm 0.0005 \cdot DD + 1.4 \pm 0.1 \cdot e^{(-0.10 \pm 0.02 \cdot DD)})}$ R^2=0.92;

$FF = 30.3 \pm 2.0 \cdot e^{(-0.019 \pm 0.002 \cdot DD + 2.9 \pm 0.3 \cdot e^{(-DD)})}$ R^2=0.94;

$SSC = 12.0 \pm 0.4 + 0.40 \pm 0.09 \cdot log_e{}^{(DD - 1.499 \pm 0.003)}$ R^2=0.74;

$L = 50.1 \pm 0.4 - 0.022 \pm 0.003 \cdot DD$ R^2=0.50;

$C = 25.2 \pm 0.3 - 0.020 \pm 0.002 \cdot DD$ R^2=0.57;

$H = 83.9 \pm 0.9 - 0.020 \pm 0.005 \cdot DD$ R^2=0.16;

$FL = 39.9 \pm 4.2 + 8.6 \pm 1.4 \cdot e^{(-0.005 \pm 0.007 \cdot DD + e^{(-0.11 \pm 0.05 \cdot DD)})}$ R^2=0.88;

$FC = 21.5 \pm 1.4 + 6.4 \pm 0.8 \cdot e^{(-0.008 \pm 0.006 \cdot DD + e^{(-0.14 \pm 0.07 \cdot DD)})}$ R^2=0.79.

8.3.3 VNIR interactance of different fruit tissues

Inner pericarp showed the highest overall absorbance compared with other tissues. The absorbance of outer pericarp with skin was similar to that of peeled outer pericarp at wavelengths below 570 nm, but became lower at longer wavelengths. The absorbance of core was the lowest at wavelengths below 700 nm, but became higher than the intact outer pericarp above 700 nm. The absorbance spectra of all the tissues showed some common features, such as the chlorophyll absorption band around 672 nm and water absorbance band at 970-980 nm. However, the shape and the intensities of the bands differed considerably between fruit tissues. Peeled outer pericarp had the most intense chlorophyll absorption band while the inner pericarp had the most intensive water absorption bands. Intact outer pericarp displayed the flattest absorbance bands of both chlorophyll and water (Fig 8.6).

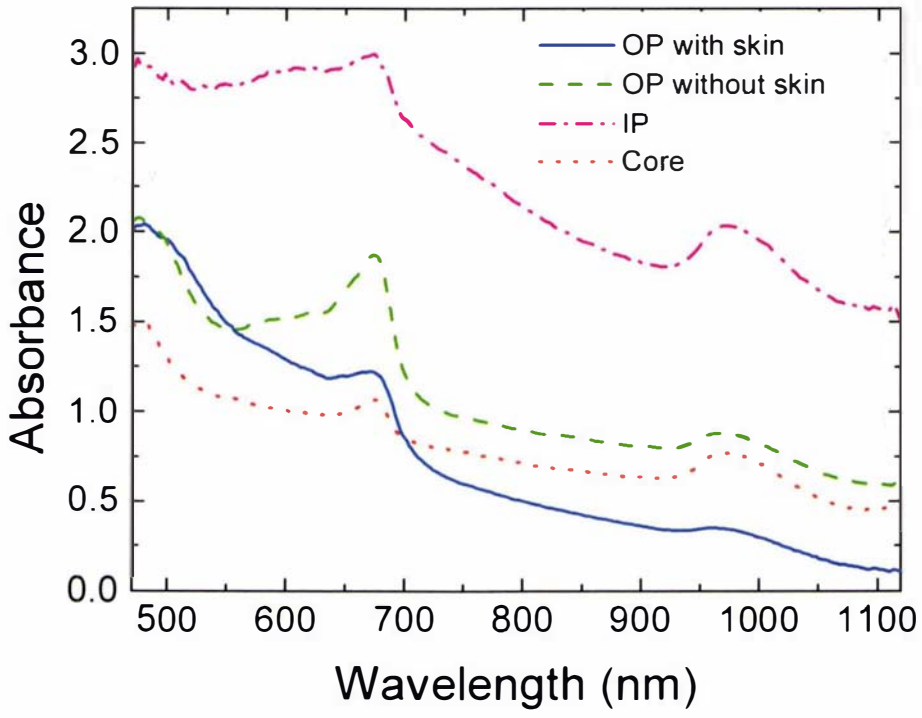

Figure 8.6 Absorbance spectra of outer pericarp (OP) with skin, OP without skin, inner pericarp (IP) and core measured in an interactance mode. Each line is the mean of three repeated measurements.

8.3.4 VNIR reflectance of fruit from different weight loss treatment

VNIR absorbance was affected by both weight loss treatment and treatment time. Fruit in polylined kiwifruit trays had lower absorbance than exposed fruit within the first four days (Fig 8.7A, B, C), but the trend was reversed after eight days (Fig 8.7D).

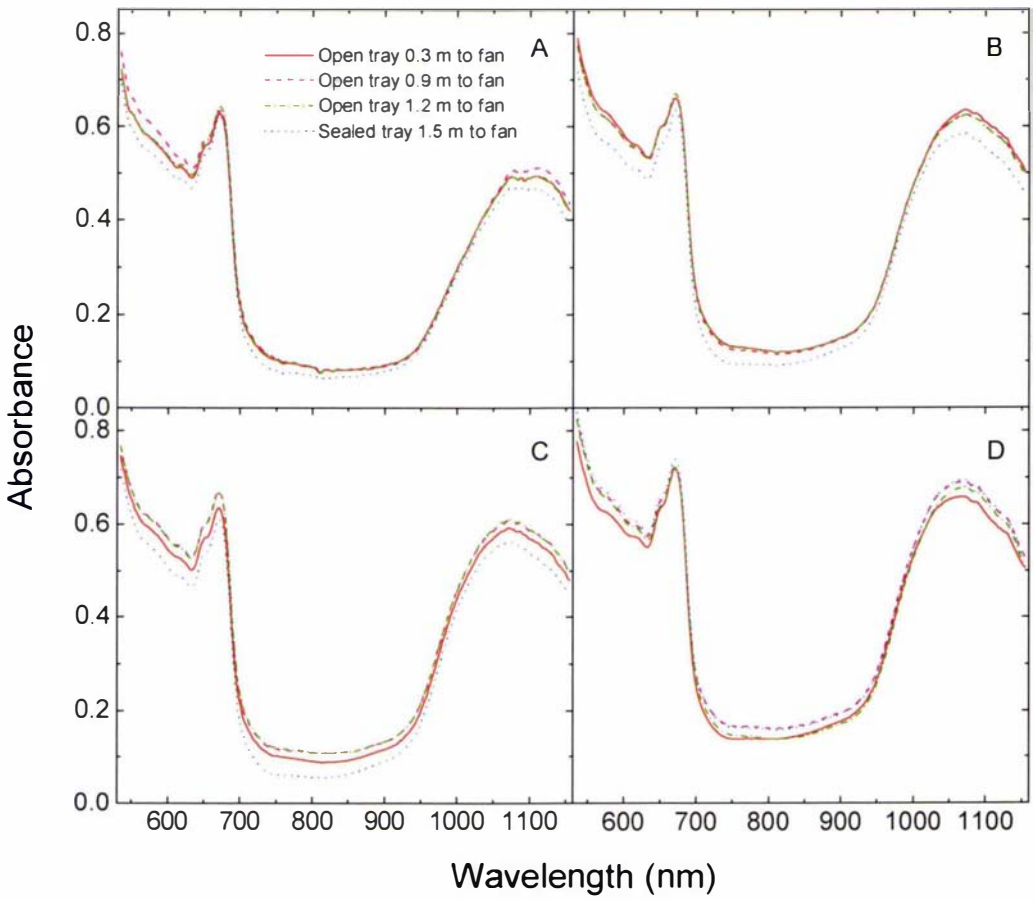

Figure 8.7 VNIR reflectance of fruit subjected to different weight loss treatments for two (A), three (B), four (C) and eight (D) days. Each line is the means of 50 fruit. The first two measurements were made under fluorescent light while the last two measurements were made under tungsten light.

It was also noticed that VNIR absorbance measured under fluorescent light after three days of weight loss treatment was significantly higher than that measured under the same light after two days of weight loss treatment, and VNIR absorbance measured under

tungsten light after eight days of weight loss treatment was significantly higher than that measured used the same light after four days of weight loss treatment. However, the absorbance measured under tungsten light after four days of weight loss treatment was lower than that measured under fluorescent light after three days of weight loss treatment (Fig 8.8).

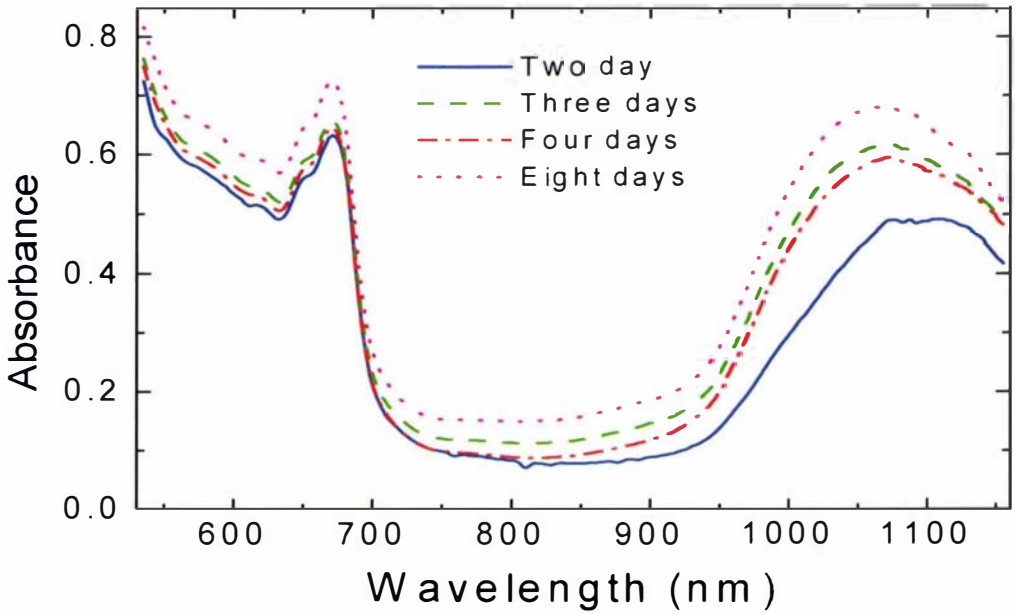

Figure 8.8 VNIR interactance of 'Hayward' kiwifruit measured after two, three, four and eight days of weight loss treatment. Each line is the mean of 200 fruit. The first two measurements were made under fluorescent light while the last two measurements were made under tungsten light

Fruit lost more weight in open trays compared with those in sealed trays. Weight loss of fruit in open trays was negatively related to the distance from the fan. The closer to the fan, the faster was the weight loss (Fig 8.9A). Lightness of fruit in sealed trays was lower than that of fruit in open trays except on the second day (Fig 8.9B). Fruit in sealed trays maintained higher C than fruit in open trays during the first four days. However, the difference diminished after 8 days (Fig 8.9C). Fruit H was not significantly affected by weight loss treatments (Fig 8.9D). Compression force was higher for fruit in sealed trays than that of fruit in open trays as measured after 5 days of weight loss treatment. However,

the difference was reduced after 7 days and eliminated after 9 days (Fig 8.9E). Flesh firmness measured after 10 days of treatment was lower in sealed fruit compared with exposed fruit. The highest FF occurred in exposed fruit located the closest to the fans (Fig 8.9F).

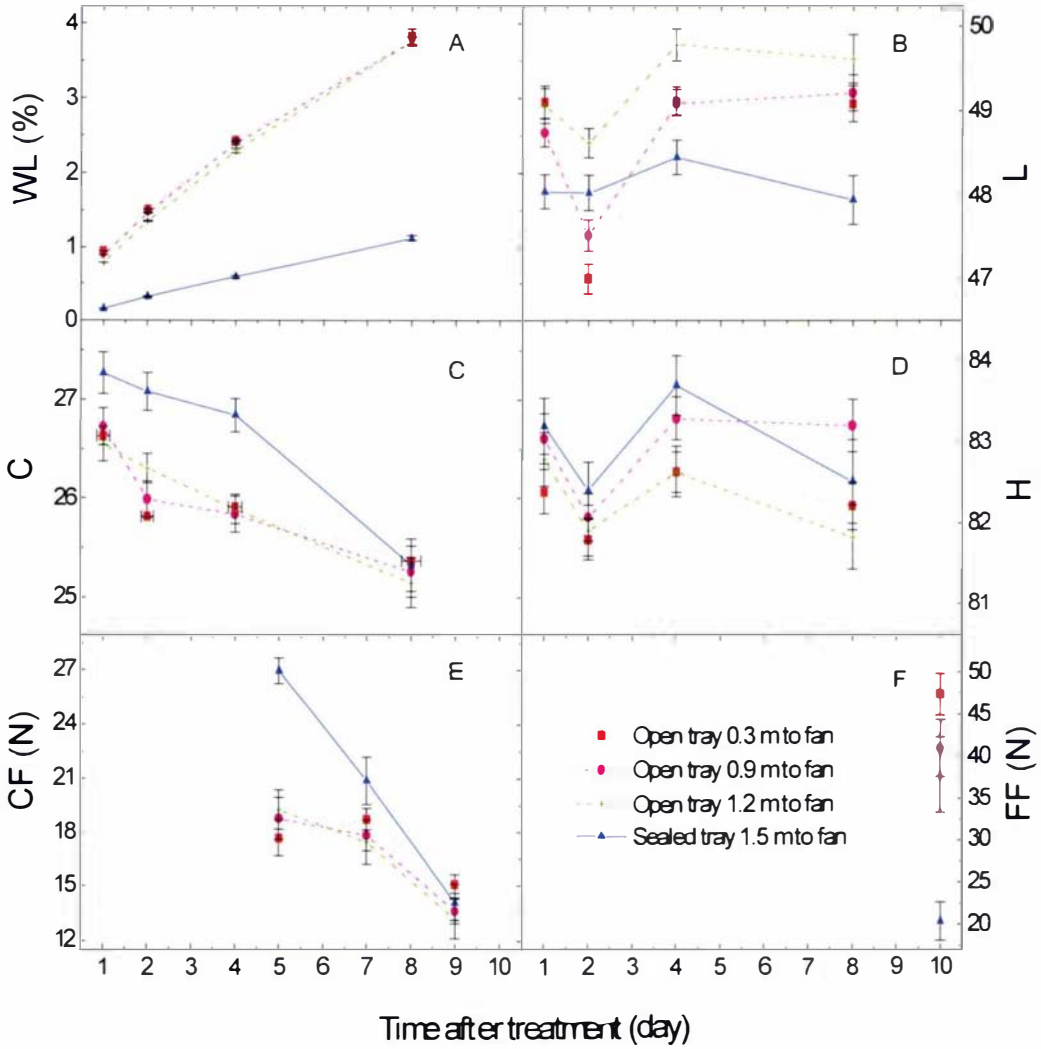

Figure 8.9 Effects of weight loss treatments on fruit weight loss (WL), colour (L, C, H), compression force (CF) and flesh firmness (FF). Each point is the mean of 50 fruit except for FF, which is the mean of 25 fruit. Vertical bars represent standard errors of the means.

8.3.5 Effect of compression and abrasion treatments on VNIR absorbance

Compression force applied to the fruit ranged from 45.7-153.7 N depending on the compression distance (Fig 8.10).

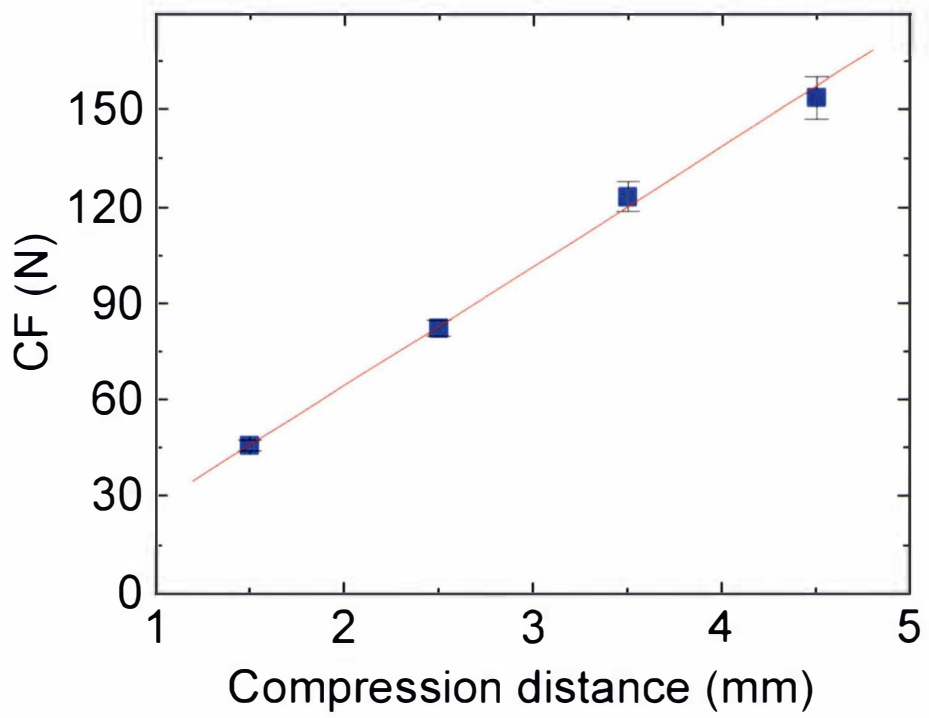

Figure 8.10 Compression distance (D) and compression force (CF) applied to fruit for compression and abrasion treatments. Each point is the mean of 20 fruit. Vertical bars represent standard errors of the means. Solid line represent fitted linear relationship between CF and D: $CF = 34.1 \pm 0.7 \cdot D$, n=80, R^2=0.82.

Both compression and abrasion treatments increased absorbance. The difference between treated fruit and the control was observed one day after the treatment, and became increasingly pronounced with time. Compression and abrasion treatments with the largest compression distance resulted in the highest VNIR absorbance. The effects of the compression treatments were less pronounced than that of the abrasion treatments. The effects of abrasion were most pronounced at the chlorophyll absorption peak around 671 nm while that of the compression were less dependent on wavelengths (Fig 8.11).

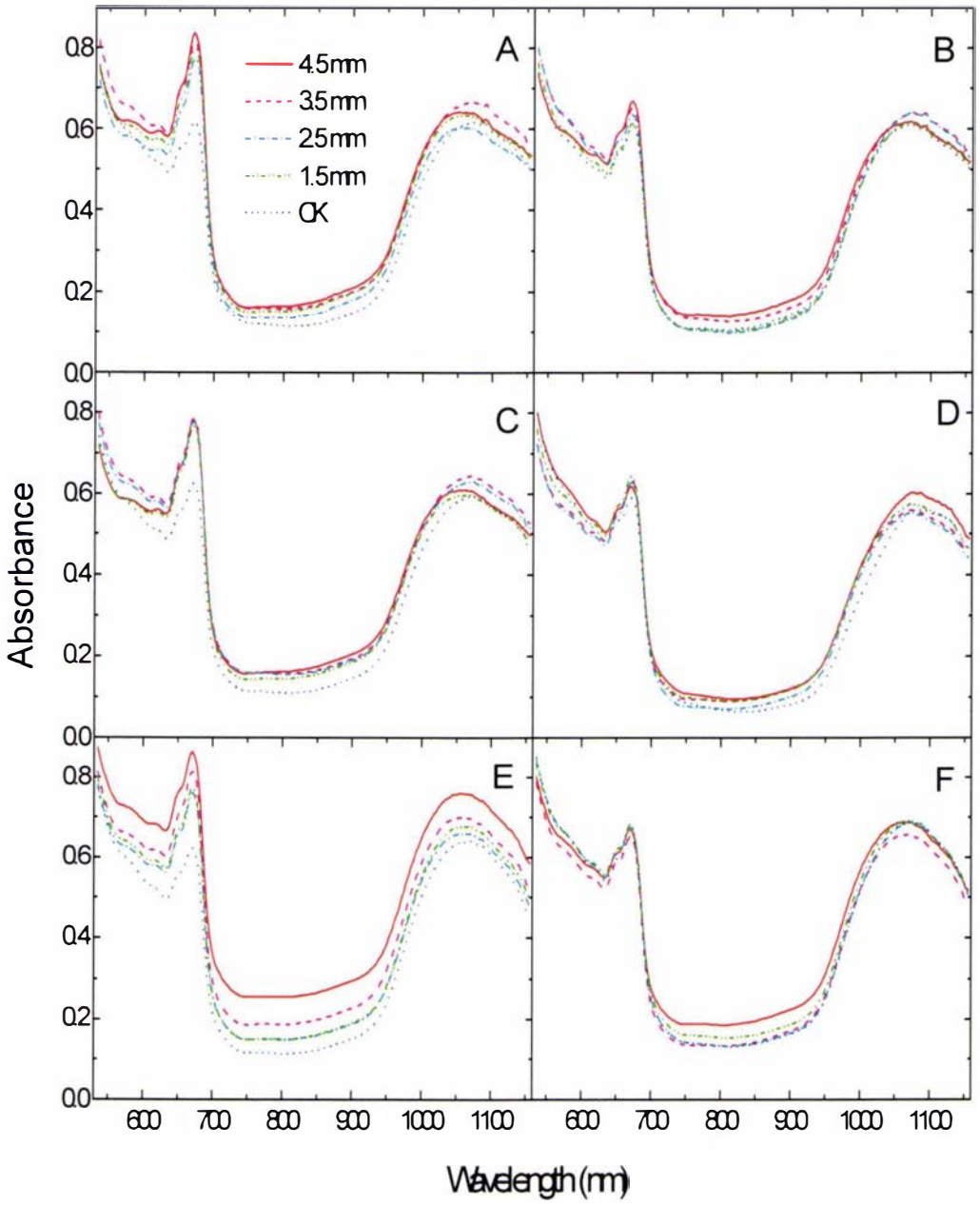

Figure 8.11 VNIR reflectance taken one (A and B), three (C and D) and seven (E and F) days after abrasion (A, C and E), and compression (B, D and F) treatments with compression distances of 4.5, 3.5, 2.5 and 0 (CK) mm. Each line is the mean of 20 fruit. The first measurements were made under fluorescent light while the last two were made under tungsten light

The effect of the abrasion treatments on the absorbance at 671 nm (ABS_{671}, chlorophyll absorption peak) increased as the compression distance increased (Fig 8.12A) while the effect of the compression treatments at this wavelength was not dependent on the compression distance (Fig 8.12B). The absorbance at 824 nm (ABS_{824}, a wavelength in the region of least absorbance) related positively to the compression distance for both the abrasion and the compression treatments although this trend was more pronounced in the abrasion treatments (Fig 8.12C and D). Both ABS_{671} and ABS_{824} measured seven days after the treatments were higher than those measured three days after the treatments. However, the absorbance measured one day after the treatments were higher than that measured three days after the treatments.

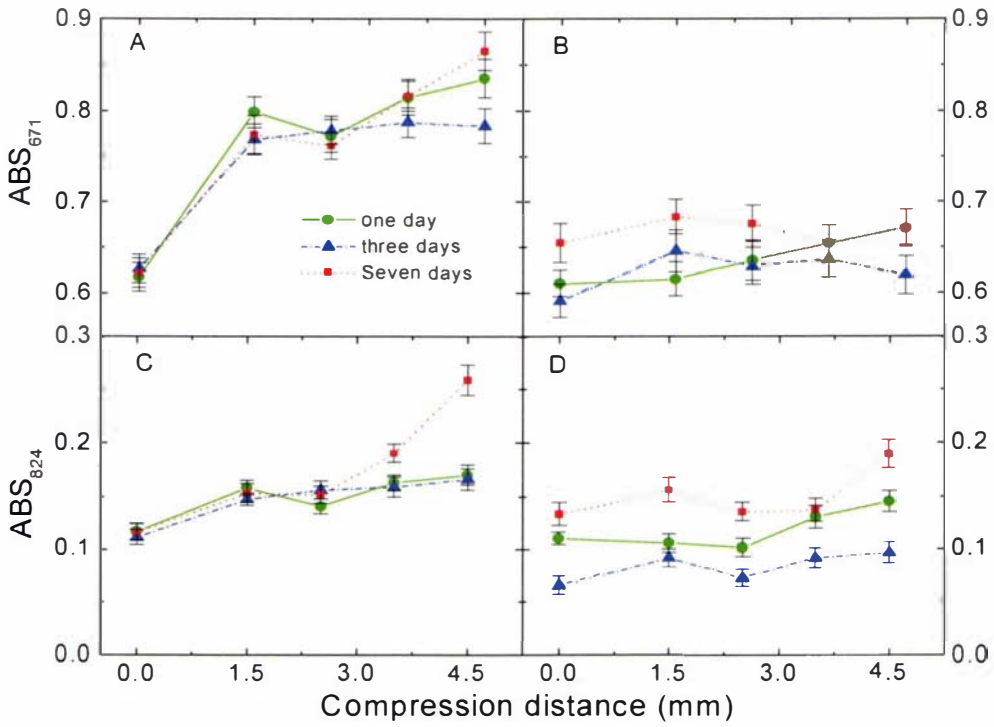

Figure 8.12 VNIR absorbance at 671 nm (ABS_{671}) and 824 nm (ABS_{824}) one, three and seven days after the abrasion (A and C) and compression (B and D) treatments with different compression distances. Each line is the mean of 20 fruit. Vertical bars are standard errors of the means. The first measurement was made under fluorescent light while the last two were made under tungsten light

Abrasion treatment at a compression distance above 2.5 mm accelerated fruit softening and resulted in high L, low C and high H values compared with the control (Fig 8.13 A, C, E, G and I). This effect was more pronounced at high compression distance than those at low compression distances. Abrasion treatment at 1.5 mm compression distance did not affect fruit firmness, but changed fruit colour. Compression treatment had similar effect as that of abrasion, but to a lesser extent (Fig 8.13 B, D, F, H and J).

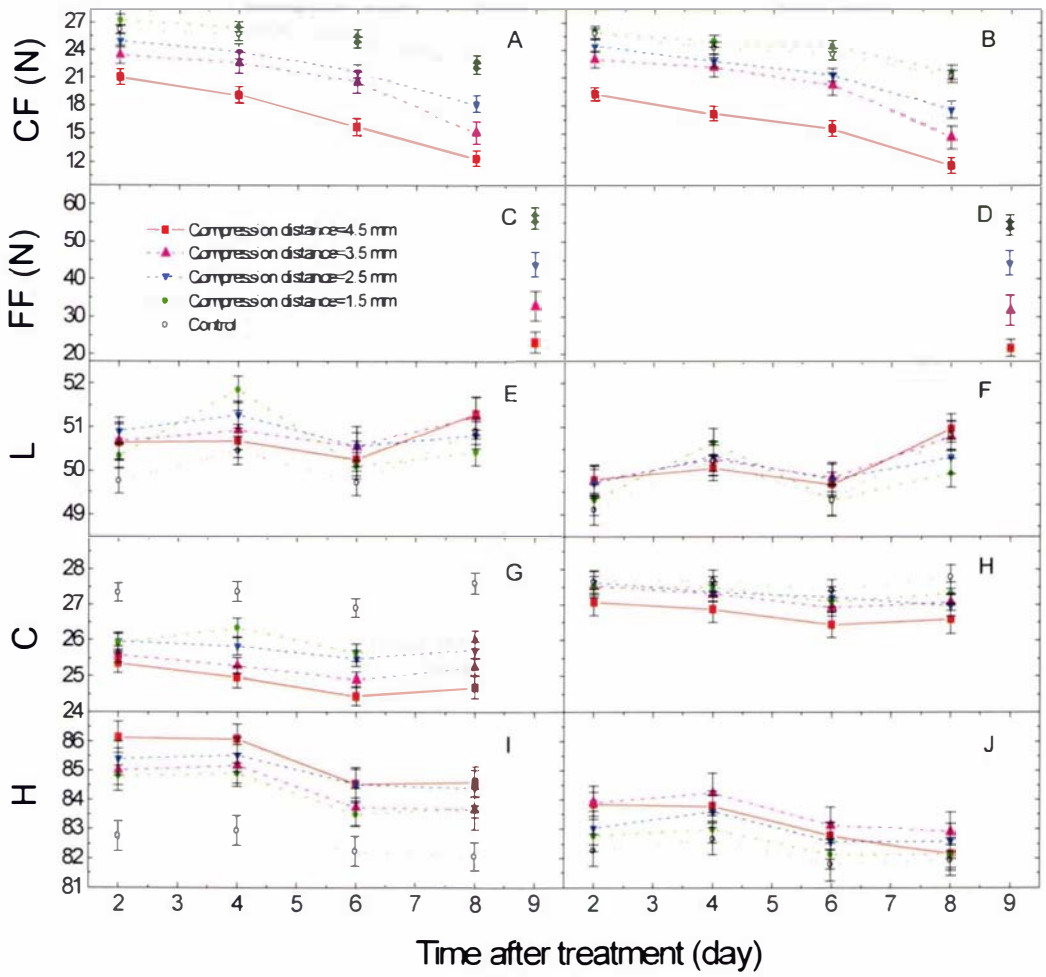

Figure 8.13 Effects of abrasion (A, C, E, G and I) and compression (B, D, F, H and J) treatments on compression force (CF), flesh firmness (FF) and fruit colour (L, C, H). Each point is the mean of 20 fruit. Vertical bars represent standard errors of the means.

8.3.6 Effect of hair brushing on VNIR absorbance

Hair-brushing of the fruit increased the absorbance within the wavelength region from 608-1051 nm. The difference was most pronounced at the chlorophyll absorption peak around 671 nm and the wavelengths around 984 nm (Fig 8.14).

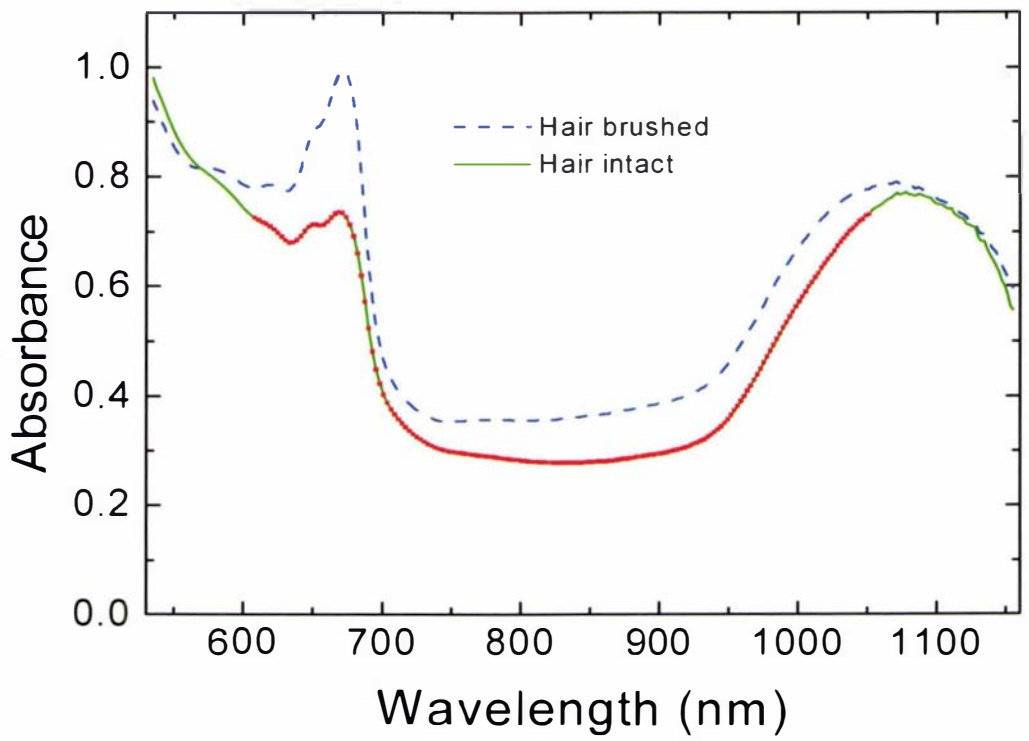

Figure 8.14 Effect of hair-brushing on VNIR absorbance. Each line is the mean of 20 fruit. Regions with significant difference (t test with $P<0.05$) are highlighted with dots.

Hair-brushed fruit had significantly higher CF, L and H, but lower C than un-brushed fruit while FF was the same for both treatments (Fig 8.15).

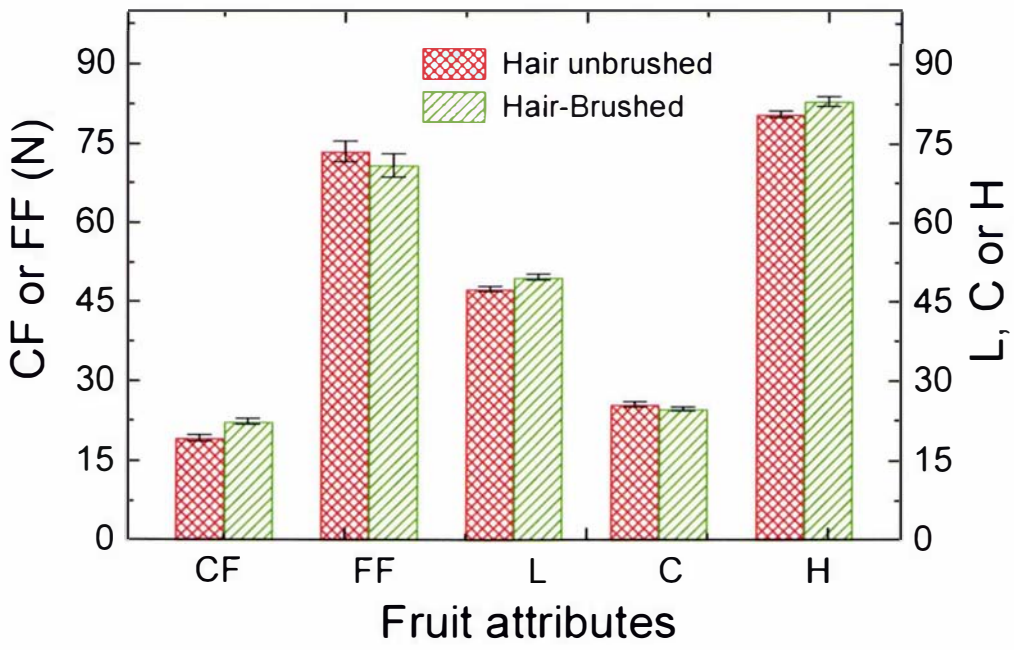

Figure 8.15 Effect of hair-brushing on compression force (CF), flesh firmness (FF) and fruit colour (L, C and H). Each column is the mean of 20 fruit. Vertical bars represent standard errors of the means.

8.4 Discussion

8.4.1 Physical and chemical basis of VNIR absorbance

Chemical solutions had different absorbance spectra relative to that of water (Fig 8.1). When light past through the chemical solutions, the amount of light lost depends on depends on wavelength, chemical species, concentration and optical path length of the solution. Spectroscopic measurement of the concentrations of the chemical species in the solution heavily depends on the attenuation of light at particular wavelengths relevant to the chemicals of interest. Light may also be scattered when particles exist in the solution. The scattering effect of particles depends on particle size and wavelength. Absorbed light could be re-emitted as fluorescence at a wavelength different to that of absorbed light. However, fluorescence is emitted in all directions and the proportion of re-emitted light reaching the

detector is usually negligible (William and Norris, 2001). Transmitted light detected in this experiment is the remaining light after absorption and scattering. Therefore, the absorbance spectra measured in this experiment in a transmittance mode represent the sum of light absorbed and scattered. The higher $\log(\frac{ABS_s}{ABS_{H_2O}})$ values of NaH_2PO_4, KCl, $CaCl_2$ and $MgCl_2$ solutions (Fig 8.1) indicated that the solutions transmitted more light than water did. Further work is required to clarify how these solutions had higher transparency to the source light. Solutions of starch and whey protein, on the other hand, showed very hight absorbance compared with water and other solutions. This could be explained by light scattering caused by the small particles of starch and whey protein suspended in the solutions.

Water has overlapping absorption bands at 950-1030 nm (William and Norris, 1987; McGlone and Kawano, 1998). The absorbance of KCl, $MgCl_2$, NaH_2PO_4 and $CaCl_2$ solutions at this wavelength region were the closest to that of water ($\frac{ABS_s}{ABS_{H_2O}}$ close to 1 and $\log(\frac{ABS_s}{ABS_{H_2O}})$ close to 0) compared with that at other wavelengths (Fig 8.1), indicating that the absorbance in this region was dominated by the absorbance of water. The effects of chemicals on absorbance spectra were better detected at either shorter or longer wavelengths outside the water absorption region.

Carbohydrates have absorption bands at 970-1030 nm that overlap with water absorption bands (William and Norris, 1987; McGlone and Kawano, 1998). However, this is not shown in Fig 8.1. Instead, $\log(\frac{ABS_s}{ABS_{H_2O}})$ values of both the starch and whey protein solutions showed a trough at about 978 nm. This implies that the light attenuation at this wavelength region was dominated with light absorption by water while that in other wavelength regions was dominated with light scattering. The whey protein solution had a lower $\log(\frac{ABS_s}{ABS_{H_2O}})$ values than the starch solution at long wavelengths (above 618 nm) but the trend was reversed at shorter wavelengths. This may reflect the difference in the distribution of particle size between whey protein and starch. The VNIR spectra of the 0.3 % ascorbic acid solution was very close to that of water due, possibly, to its low concentration.

These results imply that VNIR spectra of kiwifruit could be affected by water content and concentrations of starch and whey protein, and to a lesser extent, by the concentrations of Ca, P, Mg and K. Ascorbic acid is not likely to have significant effect on the absorbance of the fruit. Given that fruit dry matter content corresponds to water content and the increase in SSC and the decrease in fruit firmness during harvest season is associated with starch degradation (Arpaia et al., 1987; Redgwell et al., 1990; Redgwell and Percy, 1992; Bonghi et al., 1996), links between VNIR spectra and DM, SSC or fruit firmness (CF and FF) could be mediated by water content and starch concentration in addition to the direct effects of dry matter, SSC and firmness on the spectra. This is in agreement with the results of chapter 7 where DM, SSC, CF, FF and N concentration were predicted from VNIR spectra with higher R^2_p values than the concentrations of P, Ca and Mg, while K concentration was the least predictable variable (average of the three experiments).

Different tissues of a kiwifruit showed different interactance spectra in both magnitude and shape (Fig 8.6). The highest overall absorbance of the inner pericarp may be attributable to the dark brown to black coloured seeds. The flattening of the absorbance spectrum at short wavelengths (below 674 nm) was caused by the extremely low signal that approached the limit of the detector. The intense water absorption bands of the inner pericarp may be attributed to the high water content of the juicy locule cells surrounding the seeds (Hallett et al., 1992). Both chlorophyll and water absorption bands were less intense for outer pericarp with skin compared with that of the peeled outer pericarp. This implies that the skin could be a major barrier for detecting internal properties of kiwifruit. The barrier effect could be reduced by hair brushing because brushed fruit showed higher absorbance at wavelength above 570 nm, particularly at the chlorophyll and water-carbohydrate absorption regions compared with un-brushed fruit (Fig 8.14).

Due to the asymmetrical structure of a kiwifruit, the distance from locule cells, seeds or core to skin varies within a fruit depending on location along the equatorial line. This variability would alter the relative contribution of the inner structures to the detected VNIR signal. Therefore the variability of VNIR spectra could be reduced by keeping a consistent probe location (i.e. flat or curved sides), using a large probe and/or taking more

measurements on each fruit at different locations. This agrees with previous study (chapter 7) where experiments with more repeated scanning at each fruit gave better prediction of fruit attributes.

8.4.2 Effects of prestorage delay, storage and weight loss

Currently, kiwifruit are hand picked and put into picking aprons. When an apron is full, the fruit is tipped into fruit bins. Loaded bins are transported from orchard to pack houses where fruit may stay in a shed for up to 5 days before grading and packing. The grading and packing start from tipping fruit onto a conveyer belt where fruit pass through a brusher (brushing off hairs) and a grading table (taking out fruit with such visual defects as fan shape and scars). Then fruit are singulated by a singulator and transferred to a conveyer where fruit are weighed and sorted according to size). Finally sorted fruit are loaded into commercial packages, in which fruit are stored and sold. If fruit are to be segregated for storage potential based on VNIR measurement, the measurement is likely to be installed some where on the conveyer. Under certain circumstances, sorted fruit may be packed in fruit bins for storage. But the fruit has to be transferred to commercial packages before sale. In such situations, VNIR measurement could also be made after certain period of storage.

The overall magnitude of VNIR absorbance increased during prestorage delay and storage (Fig 8.2 and 8.8) in association with the decline in CF, L, C, H, FF, FL and FC, and increase in SSC (Fig 8.5). The results agree with previous study where ripe kiwifruit was found to be more transparent to VNIR light than unripe fruit (Schaare and Fraser, 2000). The increased light transition during fruit ripening could be attributed to the degradation of starch and cell wall polymers that scatter light. The increased transparency may also increase the contribution of inner part of the fruit tissue to the detected VNIR signal at an interactance mode because of the increase in effective distance the source light could have travelled inside the fruit. This may also contribute to the changes in the shape of the absorbance spectra (Fig 8.3 and 8.8) and to the increasingly accurate estimations of SSC of kiwifruit using VNIR spectra as fruit became ripe (McGlone et al., 2002a).

In VNIR absorbance spectra, absorbance at 766 nm showed the strongest correlation with DD (Fig. 8.2 and Fig 8.3) while in second derivative spectra, the wavelengths showing the strongest relationship with DD shifted to a peak at 700 nm and a trough at 684 nm (Fig 8.4). This implies that pre-treatment of VNIR spectra could result in prediction models based on spectral data from different wavelength regions. The trough in second derivative spectra at 684 nm (Fig 8.4 A) did not match the chlorophyll absorption peak around 672 nm in the absorbance spectra because of an overall declining trend of the absorbance spectra along the wavelength axis (Fig 8.2). The overall decline in absorbance spectra towards long wavelengths was caused by light scattering which is stronger at short wavelengths than at long wavelengths (William and Norris, 2001).

VNIR absorbance spectra of fruit experienced the same period of prestorage delay differed with weight loss treatments (Fig 8.7). The overall high absorbance of exposed fruit compared with fruit in sealed packages may be caused by a drier fruit surface that reflected less light. However, this trend was reversed after eight days of treatment. This might be attributed to a higher transparency of fruit tissue associated with lower FF of the sealed fruit compared with the exposed fruit (Fig 8.9 F).

The lowest FF of sealed fruit compared with exposed fruit as measured after 10 days of weight loss treatment (Fig 8.9 F) also raised an interesting issue about the mechanism of kiwifruit softening with respect to ethylene. Kiwifruit soften markedly when exposed to even minute (i.e. 0.01 l L^{-1}) concentrations of ethylene (Arpaia et al., 1987; Mitchell, 1990), but the fruit itself produces very little ethylene until it softens to a FF less than 14 N (Hyodo and Fukasawa, 1985; Bonghi et al., 1996; Kim, 1999; Ritenour et. al 1999; Feng et al., 2003). It has long been debated whether the fruit softening at the beginning of commercial storage, when ethylene concentration is controlled under the detectable level by an ethylene scrubber system, is an ethylene independent process or an ethylene dependent process powered by trace amount of ethylene produced by the fruit itself (Banks and Hewett, personal comm., 2000). Because the sealed trays in the weight loss treatment were opened at least once a day during the experiment, it was impossible for the ethylene levels inside the packages to reach a detectable level. However, the resistance of kiwifruit skin to ethylene (22.3 h cm^{-1} atm^{-1}) is four times that of Granny Smith apple

and 12 times that of a banana (Banks et al., 1991). It is believed that the internal fruit ethylene level could be higher in sealed fruit compared with that of exposed fruit due to the low air velocity and thick boundary layer for ethylene diffusion. Therefore, the fruit softening of the sealed fruit was likely to be enhanced by the higher internal ethylene concentration.

The lower CF of exposed fruit compared with sealed fruit as measured after 5 days of weight loss treatment could have been caused by a reduction in whole fruit stiffness due to the reduction of cell turgor associated with weight loss (Rojas et al., 2001). However, the difference was reduced after 7 days and eliminated after 9 days (Fig 8.9E). These changes were more likely to have resulted from the rapid fruit softening of sealed fruit rather than the weight loss of the sealed fruit because the difference in weight loss between sealed and exposed fruit was increased with time (Fig 8.9A).

During the prestorage delays at packhouses, fruit located on top of the bin are similar to the exposed fruit and those located at the inner part of the bin are similar to the sealed fruit. This difference could generate variations in VNIR absorbance, fruit attributes and subsequent storage life.

When the VNIR measurement of fruit in the weight loss experiment were shifted from the Massey laboratory to a laboratory at Mt Maunganui, ambient light changed from fluorescent light to tungsten light. VNIR absorbance measured after the shift was unexpectedly lower than that measured earlier on the same fruit (Fig 8.8). Similar change was also observed in the compression and abrasion experiment (Fig 8.11 and 8.12). This implies that the relationship between VNIR spectra and fruit attributes relating to prestorage delays could be distorted by changes in ambient light conditions. Therefore, the measurement should be carried out in darkness to reduce the disturbance.

8.4.3 Effects of mechanical damages and hair-brushing

Kiwifruit is sensitive to mechanical damage during harvest, transport, grading and packing (Davie, 1997). Compression, abrasion or even hair-brushing could stimulate

ethylene production, enhance fruit softening and induce soft patches later in storage (Hopkirk, 1990; Banks et al., 1992; Massantini et al., 1995; Mencarelli et al., 1996; Davie, 1997). Identifying and segregating fruit with such damage would be of great industrial interest. An increase in VNIR absorbance was observed one day after the compression and abrasion treatments, and became increasingly pronounced with time (Fig 8.11). This implies that VNIR measurement could be used to segregate kiwifruit according to mechanical damage. However, fruit with the abrasion treatment at a compression distance of 1.5 mm softened at the same rate as the untreated fruit did (Fig 8.13 A and C), but their VNIR absorbance increased to the same level as that of fruit subjected to harmful abrasion treatments at higher compression distances (Fig 8.11 A, C and E; Fog 8.12 A and C). Therefore it would be difficult to differentiate harmful abrasion from non-harmful abrasion. In addition, hair-brushing showed similar effects on the absorbance spectra as that of abrasion (Fig 8.14). It would be difficult to differentiate fruit with abrasion damage after hair brushing.

8.4.4 Impacts on predicting fruit attributes from VNIR absorbance

The accuracy of a predictive model for a fruit attribute depends on the causal relationship as well as the measurement errors of both the dependent and independent variables (Osborne et al., 1993). Prestorage delays, weight loss, compression, abrasion and hair-brushing affect both the VNIR spectra and the values of the fruit attributes measured by reference methods. Variability in these factors would introduce uncertainties to the predictive model. Therefore, it is advisable to control these factors at consistent levels so that to improve the accuracy of the predictions.

Some of the effects are common to different factors. For example, an intense chlorophyll absorption band could be caused by hair-brushing, abrasion or a combination of both. If it is caused by abrasion, the storage potential (STP) of the fruit would be reduced, if it is caused by hair-brushing, the STP would not be affected. In this case, VNIR measurement would be better taken before hair brushing. However, if the VNIR

measurement is to segregate fruit for SSC and DM, it would be better to take VNIR spectra after hair-brushing so that the spectral data would be less clouded by the hairs.

8.5 Conclusion

Starch, whey protein, $CaCl_2$, NaH_2PO_4, $MgCl_2$ and KCl altered the absorbance spectrum of water in both overall magnitude and shape. These effects would contribute to the predictions of such fruit attributes as DM, SSC, CF, FF and mineral concentrations. Different fruit tissues had very different features in their VNIR spectra. The relative contribution of the inner structures to the detected VNIR signal could be affected by the asymmetrical structure of the fruit with respect to the variable distance from core, seeds or locule cells to skin. Keeping a consistent probe location (i.e. flat or curved sides), using a large probe and/or taking more measurements on each fruit at different locations would increase the reliability of the measurement. Skin is a major barrier for detecting internal properties of kiwifruit. The barrier effect could be reduced by hair-brushing. Prestorage delays, weight loss, compression, abrasion and hair-brushing affect both the VNIR spectra and the values of the fruit attributes measured by reference methods. Variability in these factors would introduce uncertainties to the predictive models. It is desirable to control these factors at consistent levels for accurate predictions. Features of VNIR spectra indicating a fruit property at some wavelengths could be shifted to other wavelengths by such pre-treatment of spectral data as second differentiation. Careful choice of pre-treatment method could lead to superior predictive models. VNIR measurement should be carried out at darkness to reduce the disturbance by extraneous light.

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Non-destructive measurement of kiwifruit attributes using differential energy X-ray analysis

9.1 Introduction

X-ray radiography and X-ray computed tomography (CT) has long been used to measure split stones (Han et al., 1992), insect-damage (Bowers et al., 1988), bruise damage (Shahin and Tollner, 1998), disease (Yantarasri et al., 1998), disorders (Thomas et al., 1993, 1995); moisture (Tollner et al., 1992; Barcelon et al., 1999), maturity (Yantarasri et al., 1998; Barcelon et al., 1999), density, titratable acidity or pH, soluble solids content (Barcelon et al., 1999) of fruit. It was suggested that X-ray measurements on intact fruit could be used as an indicator of fruit quality, and has the potential to be adapted for on-line sorting and quality monitoring (Shahin and Tollner, 1998; Barcelon et al., 2000). For this purpose, differential energy X-ray analysis (DEXA) scanners used in modern airports are the ideal instrument for a preliminary trial because they allow bulk scanning of large amounts of fruit.

Airports use DEXA to differentiate material content in scanned baggage. The content can be broadly grouped as plastic or metal; organic or inorganic. The technique is primarily based on the measurement of effective atomic number of the material (Rapiscan Security Products Inc., 2002).

The quality and storage behaviour of kiwifruit are influenced by harvest maturity indicated by soluble solid content and dry matter content (Weet, 1979; Harman, 1981; Harman et al., 1982; Crisosto et al., 1984; Asami et al., 1988; Mitchell et al., 1992; Abdala et al., 1996; Pan et al., 1996; Costa et al., 1997; Benge, 1999), fruit size (Hall et al., 1996; Crisosto et al., 1999) and concentrations of minerals (Hopkirk et al., 1990; Prasad and Spiers, 1991; Banks et al., 1995; Lallu and Yearsley, 1995; Tagliavini et al., 1995; Dave, 1997; Lallu et al., 1999; Benge, 1999; Hertog and Jeffery 2000, 2002; Ferguson et al., 2001). Dry matter content (DM) of kiwifruit is closely related to fruit density (Jordan et al., 2000), and mineral elements have higher atomic number than elements in water or carbohydrates. Therefore, it is possible that a DEXA image which differentiates fruit on the

basis of variable atomic numbers could provide an indication of fruit quality and storage life (SL).

The premise for the investigation is to provide a preliminary evaluation of the DEXA technology for non-destructive measurement of kiwifruit attributes relating to fruit quality and SL.

9.2 Materials and methods

9.2.1 Fruit sample and general experiment procedures

Commercially packed count 36 'Hayward' kiwifruit from six grower lines were sampled on 30 May 2001 from the Bay of Plenty, New Zealand's major kiwifruit production area. Fruit were sent to Massey University by overnight courier and transported to the Institute of Geological and Nuclear Science (GNS) for DEXA measurement on 31 May. Forty fruit from each grower line were randomised and labelled with a number (1-40) before the DEXA measurement. Fruit were returned to Massey University on 1 June. Fruit weight (W), colour (L, C, H), and compression force (CF) were measured on all fruit. Then fruit 21-40 were used for destructive measurement of flesh firmness (FF), soluble solid content (SSC) and dry matter content (DM). Fruit 1-20 were packed in polylined single-layered trays (36 count) with 5 g $tray^{-1}$ of ethylene absorbent (Purafil, Papworth Engineering Ltd. New Zealand). Spare fruit from the same grower line were used to fill the trays. Loaded trays were placed in storage at 0.5°C.

The storage continued until 2 December 2001 (6 months storage). Fruit firmness was monitored three times during the last two months of storage using CF measurement. The CF measurement for a grower line was completed within 10 minutes after removal from storage; fruit temperature was maintained at 1-2°C by quickly closing the polylined single-layer-kiwifruit-tray after each individual fruit was taken out for measurement. After the final CF measurement, incidences of soft patches (SP), fungal rots (FR) and low temperature break down (LTB) were recorded as diameters (mm) of affected area on each fruit; FF, SSC, DM and seed number were measured after temperature equilibration for 16 h in open kiwifruit trays at 20°C. Mineral concentrations were measured on dry samples

after DM measurement. Measurements of W, L, C, H, CF, FF, SSC, DM, and minerals used the same methods described previously (Feng, et al., 2003).

9.2.2 DEXA measurements

A DEXA scanner (Rapiscan 500 series, Rapiscan Security Products Inc. USA, Fig 9.1) was used for DEXA measurements with the X-ray generator set to 110 kV. Fruit were placed on the conveyer in two parallel rows on the side of x-ray generator. A fan-beam of x-rays traversed through the fruit was detected by two sets of detector arrays located above the conveyer. The detector array at the top records the higher energy x-rays while the one at the bottom records the lower energy x-rays. Detectors provided two digital images that were saved to computer and retrieved to obtain DEXA variables (Table 9.1).

Table 9.1 DEXA variables retrieved from DEXA images of 'Hayward' kiwifruit

Variable	Definition
LEO	Low energy counts of out part (flesh and skin)
LEM	Low energy counts of middle part (seeds and core)
LEW	Low energy counts of the whole fruit
HEO	High energy counts of out part
HEM	High energy counts of middle part
HEW	High energy counts of the whole fruit
PixO	Area of outer part in pixels
PixM	Area of middle part in pixels
PixW	Area of the whole fruit in pixels
Lth	The length of the fruit in pixels
Wth	The width of the fruit in pixels
ECC	Eccentricity of the fruit (ECC of a round fruit is close to 0 and that of a long fruit is close to 1)

Figure 9.1 DEXA scanner used to measure kiwifruit (Rapiscan Security Products Inc., 2003). An x-ray generator is located under the right side of the conveyer and two sets of detector arrays are located on top of the measurement tunnel above the conveyer. Fruit were placed on the conveyer in two parallel rows on the same side of x-ray generator (i.e. right side) when passing through the measurement tunnel.

DEXA measurement was repeated on fruit 1-20 after three months storage. Fruit were warmed up to about 5°C for 1-2 hours during the repeated DEXA measurement. Fruit temperature during transportation to and from GNS was maintained at 0-1°C by packing fruit in insulated containers.

9.2.3 Data analysis

Storage life of DEXA fruit was estimated using an exponential model fitted to CF monitoring data (refer to chapter 4 for details). The relationship between CF and FF measured at the end of storage was used to determine the CF value equivalent to FF 8.5 N.

GLM and REG procedures of SAS (SAS Institute Inc., 1990) were used for data analysis. Squared and log transformed values for each fruit attribute were calculated for preliminary regression to take account of non-linear relationships. Ratios of DEXA variables (e.g. the ratio of X-ray counts to the image areas, ratio of high energy count to low energy count and ratio of DEXA variables measured in the middle or outer part to that of the whole fruit) were also calculated and used in analysis.

9.3 Results

9.3.1 Relationships between DEXA variables and fruit weight

Based on data from the first DEXA scanning, three DEXA variables, PixW, HEW and LEW together explained 96.3% of the W variation for fruit 21-40 (Fig 9.2A). Estimated model parameters are listed in table 9.2.

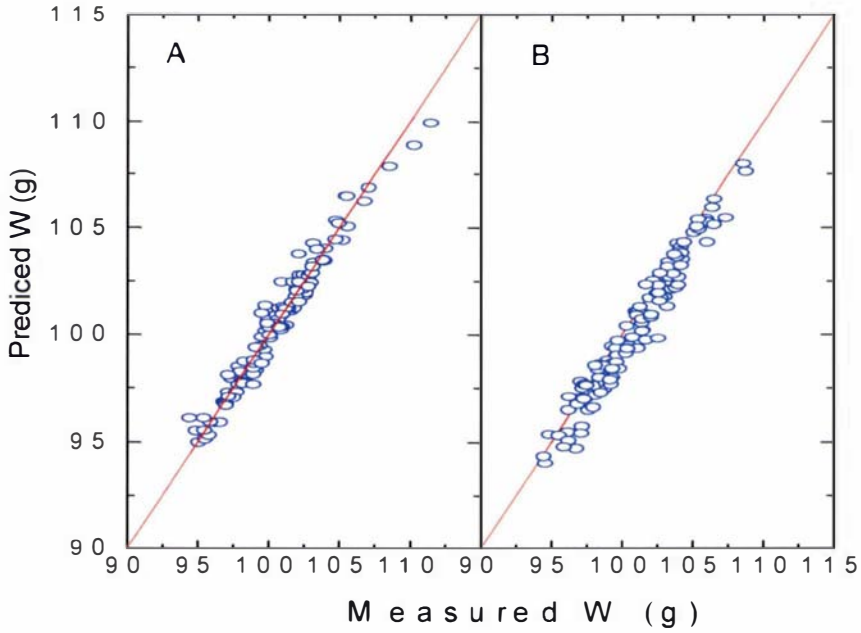

Figure 9.2 Measured and predicted fruit weight (W) using DEXA variables at the first (A) and second (B) DEXA measurement. Details of the predictive model are listed in table 9.1. Each point represents a fruit. The solid line represents a 1:1 line.

Table 9.2 Parameters estimated for a prediction model of fruit weight using DEXA variables at the first DEXA measurement

Variable	Coefficient	se	Model R^2	P>F
Intercept	194.92	3.21		<.0001
PixW	0.0495	0.0009	0.285	<.0001
HEW	-0.1013	0.0033	0.960	<.0001
LEW	0.0096	0.0032	0.963	0.0031

When the same model was used to calculate W of fruit 1-20 using DEXA data collected at the second DEXA measurement, the calculated W agreed with measured W with a R^2 of 95.7 % (Fig 9.2B).

9.3.2 Relationships between DEXA variables and dry matter content

Dry matter content showed a good relationship with DEXA variables for fruit located in the second row at the first DEXA measurement. Three DEXA variables (HEO, LEO and Lth) together explained 56.6% of the variation in measured DM of 60 fruit (Fig 9.3 A and Table 9.3). But the relationship was not confirmed with fruit in the first row at the same DEXA measurement or fruit in the same row at the second DEXA measurement (Fig 9.3B).

Table 9.3 Parameters of a predictive model for dry matter content using DEXA variables selected by stepwise regression. Data from 60 fruit located in the second row at the first DEXA measurement were used for the regression.

Variable	Coefficient	se	Model R^2	P>F
Intercept	106.7	11.1		<.0001
HEO	-0.192	0.025	0.138	0.035
LEO	0.174	0.024	0.507	<.0001
Lth	-0.286	0.104	0.566	0.0079

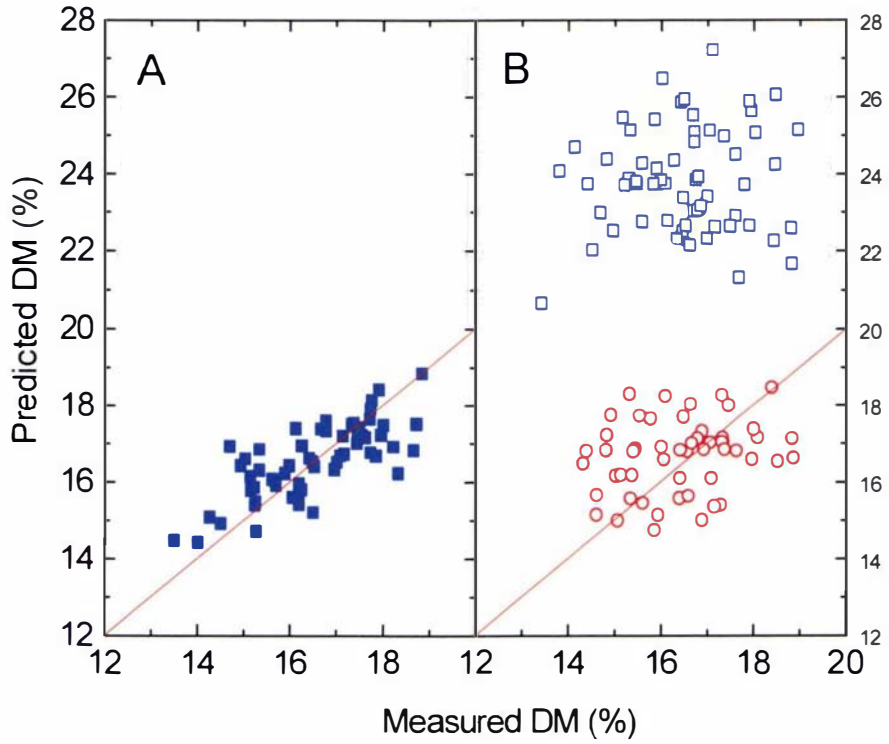

Figure 9.3 Predicted dry matter content (DM) and measured DM using DEXA variables for fruit located in the second row at the first DEXA measurement (■), the first row at the first DEXA measurement (□) or the second row at the second DEXA measurement (○). Each point represents a fruit. The solid line represents a 1:1 line.

9.3.3 Relationships between DEXA variables and nitrogen concentration

For fruit located in the second rows, nitrogen concentration (N) was found to be related to Lth, LEO, HEM, the ratio of HEM and HEO and the ratio of HEO and LEO at the first DEXA measurement (Table 9.4 and Fig 9.4A). But the relationship was not shown in the second DEXA measurement (Fig 9.4C).

Table 9.4 Relationship between nitrogen concentration and DEXA variables at the first DEXA measurement based on data from 30 fruit located in the second row

Variable*	Coefficient	se	Model R^2	P>F
Intercept	108129	48264		0.035
Lth	33.2	10.2	0.102	0.085
$\frac{HEM}{HEO}$	-114213	45294	0.299	0.010
HEM^2	0.018	0.007	0.390	0.059
LEO^2	-0.011	0.005	0.539	0.009
$(\frac{HEO}{LEO})^2$	-23598	12936	0.595	0.081

*Squared variables were used to take account for non-linear relationships.

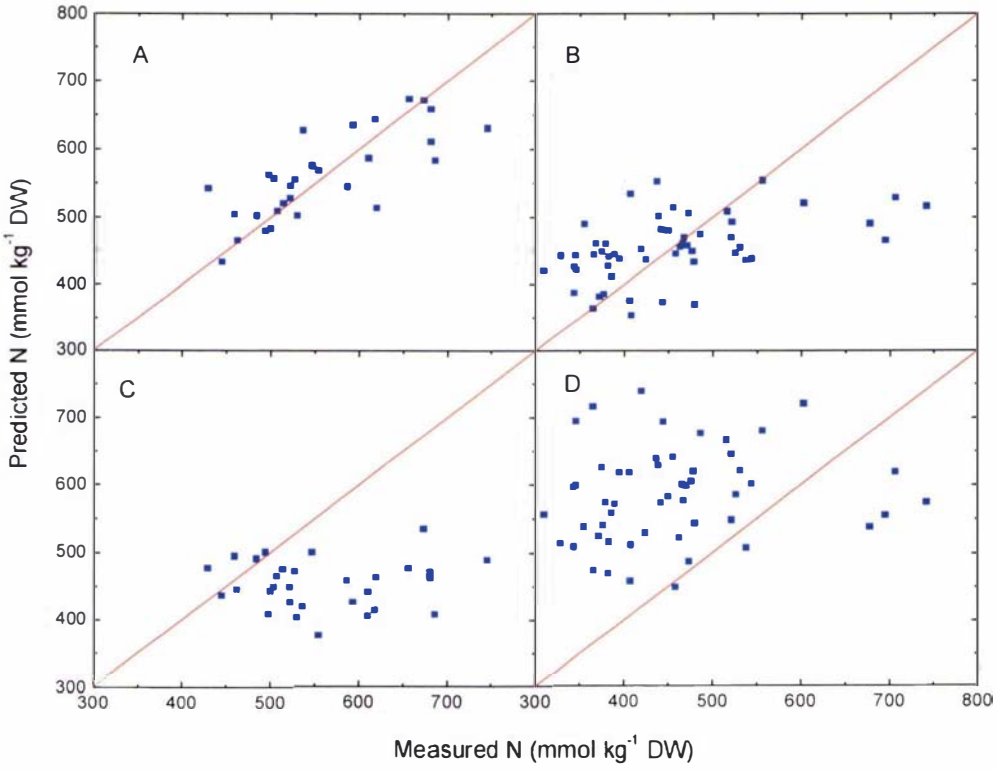

Figure 9.4 Predicted and measured nitrogen concentrations for fruit in the first (A and C) and second (B and D) DEXA measurement based on the relationships established with data from the first (A and B) or the second (C and D) DEXA measurements. Each point represents a fruit. The solid line represents a 1:1 line.

At the second DEXA measurement, N was related to HEM and the ratio of HEW and LEW (Table 9.5 and Fig 9.4B). But the relationship was not validated by data from the first DEXA measurement (Fig 9.4D).

Table 9.5 Relationship between nitrogen concentration and DEXA variables at the second DEXA measurement based on data from 52 fruit located in the second row

Variable	Coefficient	Se	Model R^2	P>F
Intercept	-20095	5332.5		0.0004
$\frac{HEW}{LEW}$	16515	4375.9	0.065	0.065
HEM	1.38	0.41	0.244	0.002

There was no other fruit attribute showing a consistent relationship with DEXA variables.

9.4 Discussion

DEXA image area (PixW) is an indication of fruit size in two dimensions (length and width). The energy counts of the image (LEW and HEW) offers the information at the third dimension (density and thickness). The relationship between W and PixW, LEW and HEW was consistent over repeated DEXA measurements of different fruit (Table 9.2 and Fig 9.2).

The relationship between DEXA variables and DM or N may be attributed to the larger atomic number of dry matter and N. Based on the ingredients of the dry matter of a normal kiwifruit, the atomic weight of dry matter is about 7.5 and that of water is 6. A change in DM from 14% to 19 % as measured in this study would increase the atomic number of the whole fruit by only 1.2 %. Minerals have atomic number much higher than water, but their concentrations were too low and the atomic number of the whole fruit changes very little within the possible variation range of minerals. For example, a change of N from 300 mmol kg^{-1} DW to 750 mmol kg^{-1} DW would increase the atomic number of the whole fruit by only 0.8 %. The detectors of the DEXA scanner may not be sensitive enough to pick up these small changes. Consequently, the predictions of DM and N from DEXA variables are deemed inaccurate.

The inconsistency in the relationship between DEXA variables and DM or N for fruit located in different rows may be attributed to variable sensitivities of the detectors across the conveyer that has been observed in a separate DEXA experiment (Tran et al., unpublished). The sensitivity variation of the detectors may also have affected the consistency of repeated measurement because row positions on the conveyer were not exactly the same in repeated DEXA experiments.

The lack of relationship between DEXA variables and other fruit attributes, such as fruit firmness, colour, SSC, P, K, Ca and Mg, may be attributed to the poor relationship between these fruit attributes and the overall atomic weight of the fruit. For example, fruit softening is associated with chemical changes at the molecular level (i.e. degradation of starch and cell wall materials to soluble solids), but not at atomic level (i.e. the elements remains the same).

Both the DEXA and X-ray CT measure the X-ray absorbance which is determined by the density of measured material. However, X-ray CT measures x-ray absorption of many small volumes known as voxels, which comprise the scanned region with defined length, width and thickness (Toller et al., 1992), while the DEXA scanner measures the X-ray absorption of the whole fruit. Because of the variable fruit thickness, it is impossible to differentiate the contributions of fruit density from that of fruit thickness to the absorbance. This might be another reason why DEXA failed to give consistent estimates of DM and mineral concentrations.

9.5 Conclusion

The DEXA scanner measures the X-ray absorbance that is determined by the thickness and density of measured material. Variables retrieved from the DEXA image give reliable estimation of fruit weight. The relationships between DEXA variables and dry matter content or mineral concentrations are weak and inconsistent between different positions on the conveyer and measurement times. Therefore, The DEXA is unlikely to be useful for assessment of kiwifruit quality.

9.6 Reference

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General Discussion

10.1. Introduction

Technologies for the segregation of 'Hayward' kiwifruit by storage potential (STP) at both grower line and individual fruit levels were investigated in this thesis. Fruit segregation at each level had two key components: a discriminant model for STP and rationalised methodologies to measure fruit attributes involved in the discriminant model. This chapter integrates the results and proposes segregation strategies for industry application.

10.2. Segregation on a grower line basis

10.2.1 Discriminant model on a grower line basis

On a grower line basis, STP can be measured as storage life (SL), the storage time for the fruit to soften to an average flesh firmness (FF) of 11.8 N, the minimum firmness required for exporting fruit from New Zealand (Hopkirk et al., 1996; Benge, 1999). SL can be estimated from an exponential model fitted to FF data measured at the last four firmness monitoring periods towards the end of storage if firmness monitoring is carried out monthly on 20 fruits per grower line each time (Feng et al., 2001). Of the two parameters of the exponential model (intercept and softening rate), softening rate was found to be the most important parameter determining SL (Section 5.3.1).

Canonical discriminant analysis on four groups of grower lines with different softening rates indicated that grower lines were separated by the two canonical functions (CDF_1 and CDF_2) incorporating fruit mineral concentrations (Ca/N and Mg), harvest date (HD), harvest maturity (indicated by proportion of solubilised dry matter measured at harvest, SSFDM), fruit colour (lightness, L) and prestorage delay (DL). Standardized canonical coefficients indicated that CDF_1 discriminated grower lines on Mg, Ca/N, harvest maturity and, to a lesser extent, HD and L. Grower lines characterised by high Mg, high Ca/N, advanced maturity, harvested late with low lightness and short cooling delays are

likely to soften at low rates and consequently have a long storage life. CDF_2 discriminated grower lines on prestorage delay. For grower lines harvested early at low maturity, extending prestorage delay improves storage potential. In contrast, extended prestorage delay would reduce the storage potential of grower lines harvested late at advanced maturity. This information is not only useful for fruit segregation, but also useful for growers and packhouses to determine the most appropriate harvest date and prestorage delay to achieve the best STP of each grower line (Sections 5.3 and 5.4).

The association of high Ca/N and Mg with low fruit softening rates found in this study (Section 5.3.2) agrees with previous reports where fruit with low Ca and Mg but high N tended to be susceptible to premature fruit softening and storage disorders (Wade, 1981; Poovaiah, et al., 1988; Prasad and Spiers, 1991; Wang, 1993; Banks et al., 1995a; Davie, 1997; Benge, 1999 and 2000). Principal component analysis on mineral composition of 'Hayward' kiwifruit indicated that individual elements could not be considered in isolation but rather in groups of elements; N, P, S, K, and Cu were classified into one group negatively related to FF after 12 weeks storage while Ca, Mg and Zn were in another group positively related to the FF. These groups were negatively associated with each other (Smith et al., 1994). Therefore the ratio of Ca and N (Ca/N) may represent the balance of the two groups of elements. Ca is a divalent element which is accumulated by kiwifruit largely during the very early stage of growth, possibly reflecting its low mobility in the phloem. By contrast, N, P, S, K, and Cu are relatively more mobile in the phloem, and have been found to accumulate at a steady rate throughout the growth of the fruit (Clark and Smith, 1988). Therefore, the balance of these two groups may reflect the pattern of fruit development under particular climate conditions and orchard management regimes. Climate conditions and orchard management regimes that increase sap flow to fruit through the xylem during early fruit development stage would benefit the accumulation of Ca to fruit. Because the sap flow to fruit is affected by transpiration of the fruit and the competition of leaves, N could affect Ca accumulation in fruit through promoting vegetative growth that reduces transpiration of fruit (by shading) and competes for sap flow with fruit (Buwalda, 1991; Johnson et al., 1997). The physiology of Mg in kiwifruit softening is not clear and merits further investigation.

The association of high SSFDM and late harvest with low softening rates during storage (Section 5.3.2) is consistent with previous studies in which early harvested fruit with low SSC were found to be firmer at harvest but softer after long-term storage (Weet, 1979; Harman, 1981; Harman et al., 1982; Crisosto et al., 1984; Mitchell et al., 1992; Abdala et al., 1996; Costa et al., 1997). The soft fruit problem of early harvested fruit may be attributed to the development of low temperature break down (LTB) that was observed in fruit harvested below 6.2% SSC and then stored at 0°C, whereas breakdown symptoms were absent in more mature fruit (Harman, 1981).

The effect of harvest date on fruit softening rate may reflect a chilling acclimation during late autumn (Sfakiotakis et al., 2002), or a large increase in cytokinin concentration in kiwifruit observed during the harvest season (Lewis et al., 1996), or both. However, it is not clear what the effective temperature for the chilling acclimation is, how diurnal temperature patterns influence the acclimation and how fruit cytokinin concentration relates to climate conditions, vine physiology and orchard management. The discriminant model developed in chapter 5 may have reflected a general trend where fruit harvested later experience longer chilling acclimation and accumulate more cytokinin than fruit harvested earlier. The actual chilling acclimation and cytokinin accumulation of grower lines harvested on the same date may vary considerably due to the differences in year, orchard site, vine physiology and orchard management. The variation in actual chilling acclimation and cytokinin accumulation would introduce some uncertainty to the discrimination. Replacing HD with more appropriate variables such as cumulative degree-hours of temperature below a certain temperature and measured cytokinin concentration etc. may increase the discriminant accuracy. With the rapid developments in temperature data logging, monitoring temperature on an orchard block basis is already a reality. Further development in biochemical assay techniques would be needed to measure cytokinin for industry applications if the relationship between cytokinin concentration and storage life can be established.

The effects of DL (prestorage delay) on fruit softening (Sections 5.3 and 5.4) may be a balance of two influences. First, prestorage delay may act as a postharvest curing to reduce the incidence of fruit rot caused by *Botrytis cinerea* (Poole and McLeod, 1994), consequently preventing fruit softening caused by ethylene production of affected fruit.

Second, fruit susceptibility to vibration damage during the grading and packing process increases as fruit becomes softer (Mitchell, 1990). The second influence may not be important for early harvested grower lines having a lag phase in fruit softening shortly after harvest (MacRae et al., 1990). However, the influence may become increasingly significant as the lag phase disappears in the late harvested grower lines (MacRae et al., 1990; Benge, 1999). These two influences of prestorage delay were reflected by its negative SCC (standardized canonical coefficient) in CDF_1 and its positive value in CDF_2 (Table 5.3). The actual effect of DL on each grower line may also depend on weather conditions during the delay, the level of latent pathogen infection and the level of mechanical damage during the grading and packing process. Replacing DL with more appropriate variables such as temperature, humidity, the levels of latent pathogen infections and vibration damage may increase the discriminant accuracy and enhance the ability to manipulate storage potential by choosing the right DL for each grower line.

Variation in fruit lightness may be attributed to the variation in micro-climate conditions and canopy management. Grower lines with low lightness are likely to be produced from a sunny orchard with a light canopy (Bible and Singha, 1993; Snelgar et al., 1998). The association of low fruit lightness with a slow softening rate may be attributed to enhanced Ca accumulation into fruit (Clark and Smith, 1988) or heat-shock protein (HSP) induced by high temperatures of exposed fruit (Ferguson et al., 1999) or both. The exposed side of apples was found to be firmer than the shaded side and avocado previously exposed to sunlight was found to have greater resistance to low temperature injury and a slower ripening rate (Ferguson et al., 1999). However, HSP of kiwifruit has not been reported previously and merits investigation.

Over 50% grower lines in the validation data set were correctly classified using the discriminant model compared with a percentage chance criterion of 25% (Section 5.3.2). The discrimination accuracy was higher for groups with softening rates at both ends of the softening-rate continuum (Tables 5.4 and 5.5). For example, 60% of the grower lines in the predicted slow-softening group (i.e. PKG_1) of the validation data set softened at the rates of the lowest quartile group (i.e. $k \leq 0.001$) while 80% of the grower lines in the predicted fast-softening group (i.e. PKG_4) softened at the rates of the highest quartile group (i.e. $k > 0.0034$). This suggests that the model could be used to select grower lines with excellent

storage potential for long-term storage and to identify grower lines with poor storage potential that need immediate attention.

The discriminant model of chapter 5 was developed based on pooled data from two experiments. The 37 grower lines of the first experiment (Section 5.2.1) were sampled in the 2000 (9 grower lines sampled weekly from 9 May to 23 May) and 2001 (28 grower lines sampled from 28 May to 9 June) seasons from the Bay of Plenty while the 72 grower lines of the second experiment (Section 5.2.2) were sampled in the 2001 season in an extended period (25 May to 27 June) from a wider production region (57 grower lines from the Bay of Plenty, 5 grower lines from Gisborne, 5 grower lines from Nelson and 5 grower lines from Northland). The two experiments also differ in instrument and measurement methodology. For example, FF in the first experiment was measured using a texture analyser (TA.TX2, Stable Micro Systems, Surrey, England) while that in the second experiment was measured using a HortPlus Quick Measure Penetrometer System (Hortplus Ltd, New Zealand); soluble solids content in the first experiment was measured using the juice extracted when measuring FF at the equatorial zone of each fruit while that in the second experiment was measured at two end caps of each fruit; mineral concentrations of each grower line in the first experiment were the average of 20–40 fruit measured separately while those in the second experiment were measured on a combined sample on grower line basis without internal replication (Sections 5.2.1 and 5.2.2). It was also noticed that two-thirds of the grower lines in the first experiment softened rapidly while two-thirds of the grower lines in the second experiment softened slowly. Concomitantly, grower lines in the second experiment had higher SSFDM and higher Ca/N than those in the first experiment (Sections 5.3 and 5.4). It is difficult to clarify whether the differences represent the true difference between grower lines of the two experiments or was caused by measurement bias. If measurement bias did exist between the experiments, the bias would have influenced the discriminant model developed based on pooled data from the two experiments. Therefore, further work is needed to validate the discriminant model using standardised measurement methodologies. It is also advisable to measure fruit cytokinin concentration and record climate conditions such as temperature, humidity and wind speed on grower line basis in future experiment so that to evaluate the possibility of replacing HD and DL with more appropriate variables.

Further work is needed to improve the discriminant model given that over 40% of the grower lines have been assigned to incorrect groups. Because some of the incorrect classifications could be attributed to the measurement errors of the variables used to calculate canonical functions (Section 5.4), reducing measurement errors of the fruit attributes using rationalised sample preparation and measurement methods (Chapter 3) is important for accurate discrimination of grower lines using the discriminant model.

10.2.2 Speculation on improving the discriminant model on a grower line basis

The physiological basis of the discriminant model can be considered with respect to internal ethylene concentration (IEC), because ethylene is a trigger force for the softening of kiwifruit (Hyodo and Fukasawa, 1985; Arpaia et al., 1987; Mitchell, 1990; Banks et al., 1991; Bonghi et al., 1996; Kim, 1999; Ritenour et. al 1999; Hertog and Jeffery, 2000).

The transfer of ethylene between fruit and the environment follows Fick's first law of diffusion (Maguire, 1998; equation 10.1),

$$r'_{C_2H_4} = \Delta p_{C_2H_4} \cdot A \cdot P'_{C_2H_4} \qquad (10.1)$$

where $r'_{C_2H_4}$ (mol · s^{-1}) is the rate of ethylene transfer from fruit to the environment (inside package); $\Delta p_{C_2H_4}$ (Pa) is the difference in partial pressures of ethylene between fruit and the environment; A (m^2) is the surface area of fruit; $P'_{C_2H_4}$ is the permeance of the fruit skin to ethylene (mol s^{-1} m^{-2} Pa^{-1}).

Assuming that ethylene production and diffusion is always at equilibrium, i.e. $r'_{C_2H_4} = r_{C_2H_4} \cdot M$, where M (kg) is the mass of the fruit and $r_{C_2H_4}$ (mol kg^{-1} s^{-1}) is the ethylene production rate of the fruit, then

$$\Delta p_{C_2H_4} = \frac{r_{C_2H_4} \cdot M}{A \cdot P'_{C_2H_4}} \qquad (10.2)$$

If the partial pressures of ethylene in the environment and inside the fruit are p_e (Pa) and p_i (Pa) respectively, then

$$p_i = \Delta p_{C_2H_4} + p_e = \frac{r_{C_2H_4} \cdot M}{\rho_{C_2H_4}} + p_e \qquad (10.3)$$

With a storage temperature of 0°C, equation 10.4 can be deduced by converting P_i (Pa) to *IEC* (mol L^{-1}) according to the Ideal Gas Law (Banks et al., 1995b).

$$IEC = \frac{4.4 \cdot 10^{-5} \cdot M \cdot r_{C_2H_4}}{A \cdot P_{C_2H_4}} + p_e \qquad (10.4)$$

Hertog and Jeffery (2000) assumed that fruit softening being a breakdown process of fruit firmness (F) catalysed by an enzyme (Enz; reaction 10.1).

$$Enz + F \xrightarrow{k_p} Enz \qquad \text{(Reaction 10.1)}$$

where k_p represents the rate constant of the reaction. A differential equation (equation 10.5) can be derived from reaction 10.1.

$$\frac{dF(t)}{dt} = -k_p \cdot Enz(t) \cdot F(t) \qquad (10.5)$$

where t represents the time in storage (day). The changes of F over time is the integration of equation 10.5 and the analytical solution for F(t) (remaining F after t days of storage) becomes equation 10.6 when Enz(t) is a constant.

$$F(t) = F_0 \cdot e^{-k_p \cdot Enz(t) \cdot t} \qquad (10.6)$$

where F_0 is the F value at the beginning of storage. Let $k = k_p \cdot Enz(t)$. FF(t)=F(t) or CF(t)=F(t), equation 10.6 becomes the exponential model (Equations 10.7 and 10.8) used in the current study for the calculation of storage life (Feng et al., 2001; Sections 3.3.3 and 4.3.3) and for characterizing grower lines for discriminant analysis (Sections 5.2.4 and 5.3).

$$FF(t) = FF_0 \cdot e^{-k \cdot t} \qquad (10.7)$$

$$CF(t) = CF_0 \cdot e^{-k \cdot t} \qquad (10.8)$$

where FF(t) and CF(t) represent flesh firmness (FF) and compression force (CF) after t days of storage. FF_0 and CF_0 represent theoretical values of FF and CF at the beginning of storage.

On a grower line basis, the exponential model fitted well to the firmness (FF or CF) monitoring data collected after 3-6 months storage. This indicated that Enz(t) of each grower line might indeed be stable during this period of storage. Because the rate constant

(k_p) of reaction 10.1 is common to all fruit regardless of grower lines (Hertog and Jeffery, 2000), the difference in k (fruit softening rate) between grower lines could be attributed to the difference in Enz(t) between grower lines. Because the activation of the enzyme from its precursor can be enhanced by ethylene (Bonghi et al., 1996; Hertog and Jeffery, 2000), the level of Enz(t) may be affected by internal ethylene concentration (IEC) as expressed in equation 10.9.

$$k = k_p \cdot \mathrm{Enz(t)} = k_p \cdot \alpha \cdot \mathrm{IEC} = k_p \cdot \alpha \cdot \left(\frac{4.410 \cdot M \cdot r_{C_2H_4}}{4 P'_{C_2H_4}} + p_e\right) \qquad (10.9)$$

where α is a coefficient between Enz(t) and IEC.

Given that the ratio of M and A is a constant (Maguire et al., unpublished), fruit softening rate is determined by $r_{C_2H_4}$ (ethylene production rate of the fruit), $P'_{C_2H_4}$ (permeance of fruit skin to ethylene), and p_e (partial pressures of ethylene in the environment). Ethylene production rate can be promoted by development of storage disorders such as fungal rot and low temperature break down (Wang, 1982; Feng et al., 2003) and the fruit's susceptibilities to storage disorders are affected by mineral concentrations (Wade, 1981; Poovaiah, et al., 1988; Hopkirk et al., 1990; Prasad and Spiers, 1991; Brook, 1992; Wang, 1993; Banks et al., 1995a; Benge 1999; Hewett et al., 1999; Ferguson et al., 2001; Mowat et al., 2002), temperature conditions before harvest (Sfakiotakis et al., 2002), harvest maturity (Weet, 1979; Harman, 1981; Harman et al., 1982; Crisosto et al., 1984; Mitchell et al., 1992; Abdala et al., 1996; Costa et al., 1997), postharvest curing (Poole and McLeod, 1994) and pre-cooling (Lallu, 1997; Lallu and Burdon, 2003). Therefore, the effects of minerals, harvest date, harvest maturity and prestorage delay on fruit softening rate could be attributed to their influence on ethylene production rate. In addition, a few disordered fruit could increase ethylene concentration inside the package (p_e) and promote fruit softening. The effect of fruit colour may be attributed to an association with skin permeance in addition to the association with mineral assimilation as discussed in section 5.4. Skin permeance of apple to water vapour was found to be affected by cultivars, growing conditions and harvest maturity (Maguire, 1998), however, there is no information available in the literature regarding the effects of cultivars, growing conditions, colour or harvest maturity on the permeance of kiwifruit skin.

In reality, many enzymes, for example amylase (Bonghi et al., 1996), β-galactosidase (Wegrzyn and MacRae, 1992; Bonghi et al., 1996), pectin methyl esterase (PME, Redgwell et al., 1990), endo-1,4-β-glucanase (EG, Bonghi et al., 1996), xyloglucan endotransglycosylase (XET, Redgwell and Fry, 1993) and polygalacturonase (PG, Redgwell et al., 1990; Bonghi et al., 1996) are involved in the softening of kiwifruit and the activity of each enzyme can change dramatically during storage. The exponential model (Equations 10.7 and 10.8) derived from a single-enzyme reaction (Reaction 10.1) with Enz(t) set as a constant is a simplified model. A constant Enz(t) can be approximated at storage duration between 3-6 months because the most significant changes in enzyme activity were observed shortly after harvest and when fruit softens to less than 10 N (Redgwell et al., 1990; Wegrzyn and MacRae, 1992; Redgwell and Fry, 1993; Bonghi et al., 1996). However, the approximation is not valid at the beginning of storage or during extended storage time when fruit softens to less than 10 N. Consequently, the exponential model cannot be extrapolated to describe the whole fruit softening process which includes a lag phase at the beginning of storage and an accelerated softening process during extended storage time (MacRae, et al., 1990).

Hertog and Jeffery (2000) assumed a reversible reaction of enzyme activation that could be accelerated by external ethylene and derived a complicated quasi-mechanistic model. This model is capable of describing the lag phase at the beginning of storage as a consequence of low enzyme activity, but is unable to describe the accelerated softening process at extended storage time. Further study on the relationship between enzyme concentration, IEC and other fruit attributes could improve physiochemical understanding of fruit softening. Once the physiochemical process is understood, it could be possible to develop a mechanistic fruit-softening model based on realistic physiochemical knowledge. Such a mechanistic model may facilitate a more robust discriminant model than what has been developed in the current study for fruit segregation purposes.

10.2.3 Rationalised methodologies for measuring fruit attributes involved in discriminant model on a grower line basis

Rationalised methodologies are essential for both model development and application. In developing and calibrating the discriminant model, the reference value of

fruit softening rate was calculated from firmness monitoring data. The reliability of measured firmness can be affected by instrument and fruit temperature (Feng et al., 2001; Chapter 3). Flesh firmness (FF) measured using a drill-mounted Effegi penetrometer is more variable than that measured using a texture analyser (TA.TX2, Stable Micro Systems, England; Section 3.3.1). The regression between FF values of duplicated measurements on the 180 fruit using a drill-mounted Effegi penetrometer had a coefficient of variance of 15.5% while that of the texture analyser had a coefficient of variance of 11.1% (Section 3.3.1). These results indicate that the texture analyser is more precise than the drill-mounted Effegi penetrometer, possibly due to the constant speed at which the plunger penetrated into the fruit and the objective data capture procedure.

The current industry practice of measuring FF immediately after fruit have been taken out of storage tends to result in higher FF readings and larger variation because of the rapid change in fruit temperature during the measurement (Jeffery and Banks, 1994; Feng et al., 2002; Section 3.3.2). It will be necessary for the industry to improve their current practice in order to gain more reliable measurements of FF.

Duplicate mineral analyses using dry tissue samples gave more consistent results than those using wet samples (regression between duplicated mineral analysis results on the same fruit using dry sample had R^2 of 0.86, 0.74, 0.59, 0.96 and 0.81 for N, P, K, Ca and Mg respectively, while the corresponding values were 0.47, 0.13, 0.26, 0.75 and 0.48 respectively when wet samples were used; Section 3.3.4). Therefore, samples should be dried and ground to fine powders for mineral analysis.

Colorimetric values can be altered by water condensation over the fruit surface (Section 3.3.2). Therefore, fruit should be allowed to dry before the measuring fruit colour.

Harvest maturity was calculated from soluble solids content (SSC) and dry matter content (DM). SSC was measured on juice expressed during FF measurement at the opposite sides along the equator of each fruit (Section 5.2.1) or measured on two end caps of each fruit (Section 5.2.2) while DM was measured on the equatorial region immediately next to the penetrometer holes (Section 5.2). It would be advisable to keep the sample region consistent because of within-fruit gradients in SSC (Hopkirk et al., 1986; MacRae et al., 1989; Schaare and Fraser, 2000), DM and mineral concentrations (Ferguson, 1980; Clark and Smith, 1988; Clark and Smith, 1991). It is also noticed that DM measured using

the freeze-drying method are slightly (0.6% FW on average) higher than that measured using oven-drying (Jordan et al., 2000) due possibly to the loss of volatile materials at high temperatures (Dulphy et al., 1975). A SSFDM of 35.2% calculated from a SSC of 7% and DM of 17.6% measured using the freeze-drying method would be about 36.7% if the DM were measured using the oven-drying method. Measurement of SSC can be made on juice expressed during FF measurement on the opposite sides around the equator of each fruit (Harker and Hallett, 1994) or at two end caps of each fruit (Hall and Mcpherson, 1997). The SSC measured on the opposite sides around the equator of each fruit is similar to the average SSC of the two end caps of the same fruit if the measurements are made at similar time (Hopkirk et al., 1986). However, in practice, the measurement of SSC on two end caps of each fruit may be delayed for a few hours after FF measurement. The changes in SSC during the delay could be enhanced by the wound made on FF measurement. Therefore, it is advisable to measure SSC immediately after FF measurement.

Compression force (CF) is a non-destructive measurement of fruit firmness. CF measurement has been successfully used to monitor fruit softening on a grower line basis for the development of a mechanistic fruit softening model (Hertog and Jeffery, 2000). Under similar storage conditions, storage life calculated from an exponential model fitted to CF data is similar to that calculated from FF data (Section 4.3.4). The advantage of using CF is that the measurement can be repeated on the same fruit, meaning that fewer fruit are required for monitoring and the measured data would be free from sampling error. However, CF as a measurement of whole fruit stiffness is vulnerable to the loss of cell turgor pressure associated with fruit weight loss (Section 8.3.4). Variation in fruit weight loss could be generated by fruit location in bins during the prestorage delays at packhouses. Whether or not this variation could be reduced after fruit has been packed in polylined kiwifruit trays is still to be investigated. To use CF within the kiwifruit industry would require development of a cheaper device for CF measurement at packhouses because, at a price of over $40,000, current instruments such as the texture analyser (TA.TX2, Stable Micro Systems, England) capable of measuring CF are cost prohibitive.

10.3. Segregation on an individual fruit basis

The current study did not repeatedly measure CF during storage. Consequently the softening rate cannot be estimated on an individual fruit basis. Given that low firmness at the end of storage results from premature softening and premature softening is usually associated with development of storage disorders such as soft patches, LTB, and fungal rots (Davie, 1997; Benge, 1999; Feng et al., 2003), fruit segregation on an individual fruit basis focused on predicting fruit firmness at the end of storage and discriminating disordered fruit from healthy fruit using non-destructive technologies such as visible-near-infrared spectroscopy (VNIR) and differential energy X-ray analysis (DEXA).

As grower lines with different softening rates can be discriminated based on fruit attributes measured at harvest (Chapter 5), it is logical that individual fruit with different softening rates could be discriminated by non-destructive technologies if at-harvest fruit attributes can be estimated using non-destructive technologies. Therefore, the abilities of VNIR (Chapter 6) and DEXA (Chapter 9) for estimating at-harvest attributes were also evaluated.

10.3.1 Measurement of at-harvest fruit attributes using VNIR

The results presented in chapter 6 indicated that soluble solids content (SSC), fruit colour (L, C, H), flesh colour (FL, FC, FH), and nitrogen concentration (N) were predicted with coefficient of determination (R^2_p) above 0.5 in at least three out of the four experiments while CF and FF could only be predicted using the interactance spectra; DM was predicted very well (R^2_p=0.85) using the reflectance spectra from the commercial VNIR equipment, but its estimates in the other three experiments were poor (R^2_p<0.37); mineral concentrations other than N were poorly predicted except for P, Ca and Mg which were predicted at $R^2_p \geq 0.5$ using the reflectance spectra (Sections 6.3).

The best predictive models for SSC and DM obtained in the current study (Table 6.4) had R^2_p and standard error of prediction (SEP) similar to those reported elsewhere (McGlone and Kawano, 1998; Costa et al., 1999; Osborne et al., 1999; Schaare and Fraser, 2000; McGlone et al., 2002a). The best predictive model obtained for FF had smaller R^2_p (0.59) and higher SEP (10.8 N; Table 6.3) than those reported by McGlone and Kawano

(R^2_p =0.66 and SEP=7.8 N; 1998). The superior estimation of FF in McGlone and Kawano's experiment might be attributed to more repeated VNIR scanning (50 scans of each fruit; McGlone and Kawano, 1998) compared with 2 scans per fruit in experiment 6.2.1. The predictive models obtained for FH (Table 6.3, 6.4 6.5 and 6.6) had smaller SEP values than the SEP of 1.6 obtained by Schaare and Fraser (2000). The difference may have resulted from the small variability of 'Hayward' kiwifruit used in the current study (FH ranged from 112-116) compared with that of the yellow-fleshed kiwifruit used by Schaare and Fraser (FH ranged from 98-115). Estimations of CF, L, C, H, FL, FC and mineral concentrations of kiwifruit using VNIR technology are not reported in the literature.

The reflectance spectra measured using a commercial VNIR instrument gave the best estimates for SSC, DM, H, FH, N, P, Ca, Mg, CFS and FFS but the worst estimates for CF and FF compared with the estimates of the interactance spectra in other experiments. The reason for these differences is not clear because the details of the commercial VNIR instrument are commercially sensitive and not available for inspection. The reflectance mode is not likely to be the reason for the superior estimates, but it could be responsible for the poor estimations of CF and FF because interactance is superior to reflectance in the estimation of SSC (closely related to both CF and FF), density (closely related to DM) and FL of yellow-fleshed kiwifruit (Schaare and Fraser, 2000). Wavelength range appears not a limiting factor because analysis of data from different experiments with spectral data truncated to the same range did not change the relative estimation accuracies (Section 6.3). It is possible that the more repeated scanning of the commercial VNIR instrument (20 scans per fruit) improved the signal to noise ratio and allowed for more relevant factors to enter into the PLS models, consequently resulted in more accurate estimation of fruit attributes. In fact, the average PLS factor numbers for the estimation of the 11 at-harvest fruit attributes (L, C, H, FL, FC, FH, CF, FF, SSC, DM and N) were 19.4 for the reflectance spectra measured using the commercial VNIR instrument compared with 9.3 and 14.6 for interactance spectra measured using a TM300 monochromator or a USB2000 spectrometer, respectively. This indicates that the estimation of fruit attributes could be improved by increasing the number of VNIR scans on each fruit. However, it is not clear whether the instrument background noise or the variability of fruit surface is the major source of the noise in VNIR spectra. If surface variability is the major source of the noise, it would be

possible to increase the probe size to cover a larger area of fruit surface for a better representation of the fruit by each VNIR spectrum. In other words, a few repeated scans with a large probe might produce an estimation accuracy comparable to that achieved by more repeated scanning with a smaller probe. Fewer repeated scans is preferable for inline measurement because it would allow more fruit to be measured per minute. Further work is needed to select the most suitable probe size.

The accuracy of estimating the same fruit attribute varied considerably between the experiments where VNIR spectra were measured in the same mode (i.e. the interactance mode) but using different instruments (Sections 6.3.1, 6.3.3 and 6.3.4). Earlier work by Walsh and co-workers has compared the performance characteristics of three commercially available 'miniature' spectrometers with respect to the application of non-invasive prediction of sugar content of fruit (Wash et al., 2000). The results from this work will help further selection of the best instrument.

The best predictive model for SSC was based on absorbance spectra at wavelengths ranging from 799-1136 nm (Table 6.4). This range is similar to that of 800-1100 nm selected by McGlone and Kawano (1998). PLS regression coefficient of SSC had significant values (either positive or negative) at wavelengths matching the spectral bands of carbohydrates at 870-890 nm, 900-930 nm and 970-990 nm (Williams and Norris, 1987). Similarly, the best predictive model for DM was based on absorbance spectra at wavelengths ranging from 500-1044 nm (Table 6.4), a range including shorter wavelengths than the 800-1100 nm range selected by McGlone and Kawano (1998). PLS regression coefficients of DM had significant values at wavelengths matching the spectral bands of carbohydrates at 900-930 nm and 970-990 nm (Williams and Norris, 1987) and the spectral bands of chlorophyll at 670-680 nm (Mowat and Poole, 1997; McGlone and Kawano, 1998). This indicates that the estimation model for SSC and DM was based on some chemical species. However, the chemical and spectroscopic analysis of the predictive models for fruit attributes other than SSC and DM did not show definite relationships to known spectral bands. Further more, PLS regression coefficients for the estimation of the same fruit attributes in different experiments showed different pattens within the same wavelength range (Fig 6.7). This implies that the PLS regressions in different experiments extracted information from the spectra in different ways. Matches between the coefficients

of the estimation models and the spectral bands of known chemical species are not straightforward.

It was also noticed that the estimations of CF using VNIR spectra were always better than the estimations of FF in all three experiments. Part of this may be attributed to the difference between the whole fruit stiffness and the flesh rupture force measured by CF and FF respectively (McGlone et al., 1997). However, it is not clear why VNIR spectra were more closely related to whole fruit stiffness than they did to flesh rupture force. The variation in peeled skin area and thickness during FF measurement may have caused extra measurement error in FF data compared with the measurement error in CF that was measured on intact fruit. The measurement error would contribute to the error term of the predictive models (Osborne et al., 1993). Therefore the accuracy of estimating fruit attributes using VNIR spectra relies on the reliability of both the reference data and the spectral data.

10.3.2 Predicting fruit firmness at the end of storage based on VNIR spectra

Of the three experiments where VNIR spectra were measured from fruit at harvest and compression force (CFS) and flesh firmness (FFS) readings measured at the end of storage, reflectance spectra measured using a commercial VNIR instrument (experiment 7.2.1) produced the best predictive models (R^2p of 0.32 and 0.35, respectively, for CFS and FFS) followed by interactance spectra measured using the USB2000 spectrometers in experiment 7.2.2 (R^2p of 0.24 and 0.22, respectively, for CFS and FFS) and experiment 7.2.3 (R^2p of 0.14 for FFS). This trend is similar to that of the predictions of at-harvest attributes which were more accurate using the reflectance spectra measured with the commercial NIR instrument than using the interactance spectra measured with the USB2000 spectrometers (Chapter 6). This implies that the prediction of CFS and FFS using VNIR spectra was based on the relationships between VNIR spectra and at-harvest attributes (Chapter 6) and the relationships between at-harvest attributes and the rate of fruit softening (Chapter 5).

A three-group segregation of the fruit in the validation data sets yielded significantly higher firmness and lower percentages of soft fruit in the predicted firm fruit

groups compared with those of the predicted soft and medium firmness groups (Section 7.3.1). These results indicate that VNIR is useful to segregation of 'Hayward' kiwifruit for storage potential with respect to fruit firmness at the end of storage.

Comparison of the predictive models for CFS or FFS obtained in different experiments indicated that the models were based on spectral data at different wavelength regions and the PLS regression coefficients rarely have common peaks and troughs within the same wavelength range (Fig 7.7). Consequently, it is hard to see the spectroscopic basis (e.g. spectral bands of known chemical and physical species) of the predictive models. This is not surprising because the chemical and physical bases of CFS and FFS are complicated (McGlone et al., 1997) and many changes in chemical and physical properties occurred during storage (Redgwell and Fry, 1993; Harker and Hallett, 1994; Redgwell and Harker, 1995; Redgwell, 1996; Redgwell et al., 1997; Terasaki et al., 2001).

10.3.3 Discriminating fruit for storage disorders based on VNIR spectra

In all three experiments where VNIR spectra were taken at harvest and storage disorders assessed during the storage and subsequent shelf life, segregation of fruit in the validation data sets based on VNIR spectra using the discriminant models developed from the model-building data sets increased the percentages of fruit correctly classified in all predicted groups (Section 7.3). Consequently, the percentages of disordered fruit in the predicted healthy fruit groups were significantly lower than those in the predicted disordered groups. For example, a four-group discrimination of fruit sampled in the 2000 season (experiment 7.2.1) based on reflectance VNIR spectra measured using a commercial VNIR instrument resulted in 6.6% disordered fruit in the predicted healthy group, compared with 15.4% disordered fruit in the predicted soft patch (SP) group, 26.8% disordered fruit in the predicted low temperature break down (LTB) group and 33.3% disordered fruit in the predicted purple patch (PP) group (disorders assessed on the 5th day of shelf life after 6-11 months storage depending on when the average FF of a grower line declined to 11.8 N); a three-group discrimination of fruit sampled in the 2001 season (experiment 7.2.2) based on interactance VNIR spectra measured using a USB2000 spectrometer resulted in 33.1% disordered fruit in the predicted healthy group, compared

with 57.5% disordered fruit in the predicted SP group and 67.1% disordered fruit in the predicted LTB group (disorders assessed on the 5th day of shelf life after 9 months storage); a four-group discrimination of fruit sampled in the 2001 season (experiment 7.2.3) based on interactance VNIR spectra measured using another USB2000 spectrometer resulted in 1.3% disordered fruit in the predicted healthy group, compared with 22.2% disordered fruit in the predicted SP group, 13.9% disordered fruit in the predicted stem-end-*Botrytis* group and 17.2% disordered fruit in the predicted side rot group (disorders assessed during storage and within 2 days of shelf life after 6 months storage).

Repeated assessment of fruit disorder during storage and shelf life in experiment 7.2.3 enabled a fruit classification according to the time when disorders were found (Section 7.3.2.4). A four-group segregation of healthy (fruit remained healthy until the 28th day at shelf condition), low-risk (disorders found during 15-28 days in shelf condition), medium-risk (disorders found during the 3-14 days in shelf condition) and high-risk (disorders found during storage or within 2 days in shelf condition) fruit based on interactance VNIR spectra measured using the USB2000 spectrometer resulted in 0.8% high-risk fruit in the predicted healthy group compared with 1.1% high-risk fruit in the predicted low-risk group, 1.3% high-risk fruit in the predicted medium-risk group and 2.7% high-risk fruit in the predicted high-risk group. The small differences between groups resulted from inaccurate classification as well as the low percentage of high-risk fruit in the whole fruit population (about 1.5%). When high-risk fruit and medium–risk fruit were combined into a poor fruit group (i.e. fruit that developed detectable storage disorders during the 6-month storage and within 2 weeks shelf life) and low-risk fruit and healthy fruit were combined into a good fruit group (i.e. fruit that did not shown any disorders until 2 weeks of shelf life), the number of poor fruit accounted for 18.1% in the predicted poor fruit group compared with 2.8% in the predicted good fruit group (Table 7.12). Separation of poor fruit from good fruit will facilitate marketing of fruit according to their innate storage potential and maintaining premium perception of brand.

Because storage disorders are the major cause of ethylene production (Feng 2002a) and ethylene is the driving force for fruit softening (Hyodo and Fukasawa, 1985; Arpaia et al., 1987; Mitchell, 1990; Banks et al., 1991; Bonghi et al., 1996; Kim, 1999; Ritenour et. al 1999; Hertog and Jeffery, 2000), excluding fruit with a high risk of developing storage

disorders in fruit targeted for long term storage would slow down the softening of the fruit. This is of great industry value to reduce fruit loss and ensure high quality fruit for late season marketing.

The discriminant results in all the three experiments showed a common problem of incorrect classification between groups (Section 7.3.2). The incorrect classification might be partially attributed to the fact that the cause of some disorders, particularly fungal rots and soft patches are more closely related to initial infection (Michailides and Morgan, 1997) and mechanical damage (Davie, 1997) rather than physiological factors. The fungal infection and mechanical damage are usually limited to local areas on the fruit surface which may not have been measured by the VNIR instrument. Within the same experiment, fruit with physiological disorders (i.e. purple patches and LTB) were discriminated at higher accuracies than fruit with soft patches were. For example, 72.7 % LTB fruit in experiment 7.1 and 57.5% LTB fruit in experiment 7.2 were correctly classified compared with 45.8% and 39.6% correct classification of soft patch fruit in experiment 7.1 and experiment 7.2 respectively. Fruit with purple patches were discriminated with high accuracy (75% in experiment 7.1) as expected because purple patches are associated with lower fruit chroma (C) and hue (H) as measured externally at harvest (Feng et al., 2002) and C and H can be predicted using the reflectance spectra measured with the commercial VNIR instrument with R^2_p of 0.64 and 0.69 respectively (Section 6.3.1). Fruit with stem end *Botrytis* rot or side rots were discriminated at accuracies (41.1% and 52.9%, respectively) similar to that of fruit with soft patches (47.1%) in experiment 7.3 (Table 7.9).

Classifying firm fruit to soft fruit groups (Sections 7.3.1) or classifying healthy fruit to disordered groups (Sections 7.3.2) could result in great fruit loss if the low-grade fruit were rejected. To solve this problem, three strategies could be followed; (i) increase classification accuracy; (ii) improve inventory management to ensure these fruit be sent to the market earlier; (iii) develop postharvest treatments to reduce disorders. These are three aspects on which future research should focus.

10.3.4 Spectroscopic basis of the discriminant models

Canonical functions of experiment 7.1 were calculated from reflectance spectra at 9 wavelength points with wavelengths ranging from 300-708 nm (Table 7.4) while those of

experiment 7.2 were calculated from interactance spectra at 37 wavelength points with wavelengths ranging from 572-1149 nm (Table 7.7). Canonical functions of experiment 7.3 were calculated from interactance spectra at more than 150 wavelengths spread over the whole truncated wavelength range of 536-1159 nm (Fig 7.4 and 7.6). However, the most important wavelengths (i.e. wavelength with large standardised canonical coefficients) were located within a range of 550-1000 nm in both experiment 7.2 and 7.3. Large positive standardised canonical coefficient (SCC) values at one wavelength or a group of wavelengths were usually associated with large negative SCC values at neighbouring wavelengths or vice versa (Tables 7.4 and 7.7, Figures 7.4 and 7.6). This indicated that the SCC values should be considered by groups of wavelengths, and each group of wavelengths may represent a spectral band which is associated with certain properties of the fruit. However, attempts to match the major groups of wavelength points with known spectral bands were not successful. Therefore, the spectroscopic bases of the discriminant models are yet to be elucidated.

Results from Chapter 8 indicate that VNIR spectra could be affected by prestorage delay (Section 8.3.2), weight loss (Section 8.3.4) and mechanical damage (Section 8.3.5). Fruit weight loss during prestorage delay can be affected by temperature, air velocity and humidity (Maguire, 1998). Therefore, VNIR spectra contain information about prestorage delay, weather conditions during the delay and mechanical damage happened to the fruit. It is also possible that latent pathogen infection altered fruit surface characteristics that had been recorded by VNIR spectra. This information together with the information about initial fruit firmness, colour, SSC, DM and mineral concentrations forms the basis for fruit segregation using VNIR spectra.

10.3.5 Rationalised methodologies for VNIR measurement and data processing

To further improve the discriminant accuracy and to use the discriminant model for fruit segregation, it is very important to choose the right VNIR instrument and to eliminate measurement error.

The commercial VNIR instrument (Compac Sorting Equipment Ltd, New Zealand) takes 10 VNIR spectra in reflectance mode on each fruit when the turning fruit passes the

measurement chamber. VNIR spectra from this system (experiment 6.2.2 or experiment 7.2.1) gave the best overall estimation of at-harvest fruit attributes and the best prediction of fruit firmness (CFS and FFS) measured at the end of storage compared with the results from the USB2000 spectrometers (Sections 6.3 and 7.3). The reflectance measurement mode of the commercial VNIR instrument is also an advantage over the interactance measurement mode for inline fruit segregation purposes because reflectance can be measured without touching the fruit while interactance has to be measured with probe touching the fruit surface (Schaare and Fraser, 2000).

Measurement error may come from instrument drift, environmental disturbance and differences in sample preparation. Instrument drift is out of the scope of the current study, however, frequent calibration is recommended to reduce its effects (Williams & Norris, 2001). The frequency of instrument calibration using reference materials should be determined according to the stability of instrument and changes in environmental conditions.

Sources of environmental disturbance at a pack house include light, temperature, dust (including kiwifruit hair) and power supply to the light source. It is advisable to conduct VNIR measurement in an isolated chamber and to use a stabilised DC power supply to the light source. Because light measurement is temperature sensitive, particularly in the wavelength range of 800-1100 nm (Zude-Sasse et al., 2002), it is important to keep fruit temperature consistent during VNIR measurement. Current packhouse practice is to stack fruit bins outside until the time of packing. Outside temperature during the harvest and packing season may vary from -2 to 23 ^{o}C according to the minimum and maximum temperature recorded in 2000 and 2001. It is common to see condensed water over the fruit surface when fruit is packed in the morning. Fruit temperature is unlikely to be equilibrated and the wet surface is unlikely to dry out completely during the time when the fruit are passing through the current hair-brushing, sorting and grading procedures. Therefore, an equilibration procedure should be introduced if VNIR measurement is to be used inline for segregation purposes.

The asymmetrical structure of kiwifruit (Section 8.3.3) and within-fruit variation in localized mechanical damage (Section 8.3.5) and hair density (Section 8.3.6) may also affect VNIR spectra, good coverage of measurement area on each fruit by repeating VNIR

scans at different positions using large probes is recommended to obtain reliable VNIR spectra.

Raw spectra contain background noise and are subject to instrument drift and baseline changes. Many pre-calibration transformations have been developed to reduce background noise and to remove linear baseline changes between spectra (Geladi et al., 1985; Barnes et al. 1989; Schaare and Fraser, 2000; Mowat and Poole, 1997; Norris and Workman, 1997; Williams & Norris, 2001). Comparison of the twelve pre-calibration transformations applied to spectral data (Table 6.1) in estimating at-harvest fruit attributes (Chapter 6), in predicting fruit firmness (CFS and FFS) at the end of storage (Section 7.3.1) and in discriminating fruit for disorders (Section 7.3.2) indicated that calculation of absorbance spectra in association with smoothing and standard normal variate transformation or normalisation with the minimum and the maximum appeared to be the most useful data processing methods. Quadratic baseline compensation and the second derivation of absorbance spectra did not result in better results. This agrees with Schaare and Fraser (2000) who found that second derivation of absorbance spectra did not produce better estimations for SSC, density and FI. compared with normalization and smoothing.

Boxcar averaging of densely sampled spectral data using the USB2000 spectrometer over 2.5 nm reduced the number of data points in each spectrum to 1/8 of the original number without increasing the estimation error of the subsequent PLS regression. Instead, it reduced the size of the spectral data set and facilitated more efficient data storage and analysis (Sections 6.3). These results agree with Faber (1999) who found that compression of spectral data by boxcar averaging not only speeds up computerized data analysis, but also produces superior models through the enhancement of signal to noise. This suggests that boxcar averaging is also a useful method for processing densely sampled spectral data.

10.3.6 Evaluation of differential energy X-ray analysis for kiwifruit segregation

A preliminary experiment was conducted on a small scale to estimate at-harvest fruit attributes and to predict fruit firmness at the end of storage using differential energy X-ray analysis (DEXA, Chapter 9). DEXA variables retrieved from the X-ray images were related to each fruit attribute using stepwise regression. The results indicated that fruit

weight could be predicted from DEXA image area and the counts of low and high energy X-rays with an R^2_p of 0.96. The relationships between DEXA variables and dry matter content or mineral concentrations were weak and inconsistent between different positions on the conveyer belt and different measurement times. No relationship was found between DEXA variables and fruit firmness measured at the end of storage. Therefore, the DEXA is unlikely to be useful for kiwifruit segregation.

10.4 Strategic application of fruit segregation at an industry level

Despite the successes of the current study on developing segregation technologies for 'Hayward' kiwifruit, application of these technologies at an industry level faces several problems: (i) cost of measurement for segregating grower lines; (ii) cost of upgrading fruit sorting systems; (iii) disposal of the low grade fruit which could contain a considerable number of good fruit. Strategies for the application of fruit segregation (Fig 10.1) can balance the advantages and disadvantages of fruit segregation techniques and become cost-effective solutions.

10.4.1 Establishing a database

A database should be established to record preharvest factors (e.g. orchard site, soil condition, microclimate, irrigation, fertilization, vine structure, pruning, crop load, pest and disease control and harvest date), postharvest factors (e.g. prestorage delay, temperature and humidity during the delay, postharvest treatment, packing date, cooling speed, storage duration and condition check profile) and information fed back from the market place. The database will facilitate subsequent data analysis and making use of the analysis results.

10.4.2 Strategic segregation

Many packhouses currently rank grower lines for STP according to the storage behaviours observed during the previous seasons and find historical data are useful for determination of marketing sequence (pers. commu., Aongatete Cool Stores, Huka Pack

Ltd, 2001). Therefore, grower lines can be divided into poor STP, medium STP and good STP classes according to historical information in the database. Poor STP grower lines should be segregated on an individual fruit basis using a VNIR sorting facility with special focus on taking out high-risk fruit. The remaining fruit from this class should be sent to market as soon as possible. Grower lines in a medium STP class can be graded with the normal grading system without further segregation for STP and sent to market after the poor STP fruit. Grower lines in a good STP class should be evaluated with segregation models on a grower line basis. Batches of fruit with confirmed good STP can be targeted for late market while others should be assigned to marketing regimes according to evaluated STP.

10.4.3 STP test on library fruit

As comparison of storage performance of different grower lines may become invalid if storage duration and storage conditions are not identical (this is inevitable in practice because of the difference in marketing schedule, storage design and storage operation), it is necessary to randomly draw some fruit (e.g. 300 fruit per batch) from each grower line (fruit harvested at a date from an orchard or a block) as library fruit and monitor their storage behaviours under the same storage conditions. This will provide accurate comparison between grower lines every year. The information would be useful for fruit classification in the future.

10.4.4 Condition check

In the case of fruit deterioration caused by unexpected factors, a condition check is still necessary before sending fruit to market. However, condition check during storage may be postponed to later storage time (e.g. after 5 months) if high-risk fruit have been eliminated from poor STP fruit or if the good STP has been confirmed based on both fruit history and at-harvest fruit attributes (Section 10.4.2). This could result in considerable saving in fruit, facility and labour.

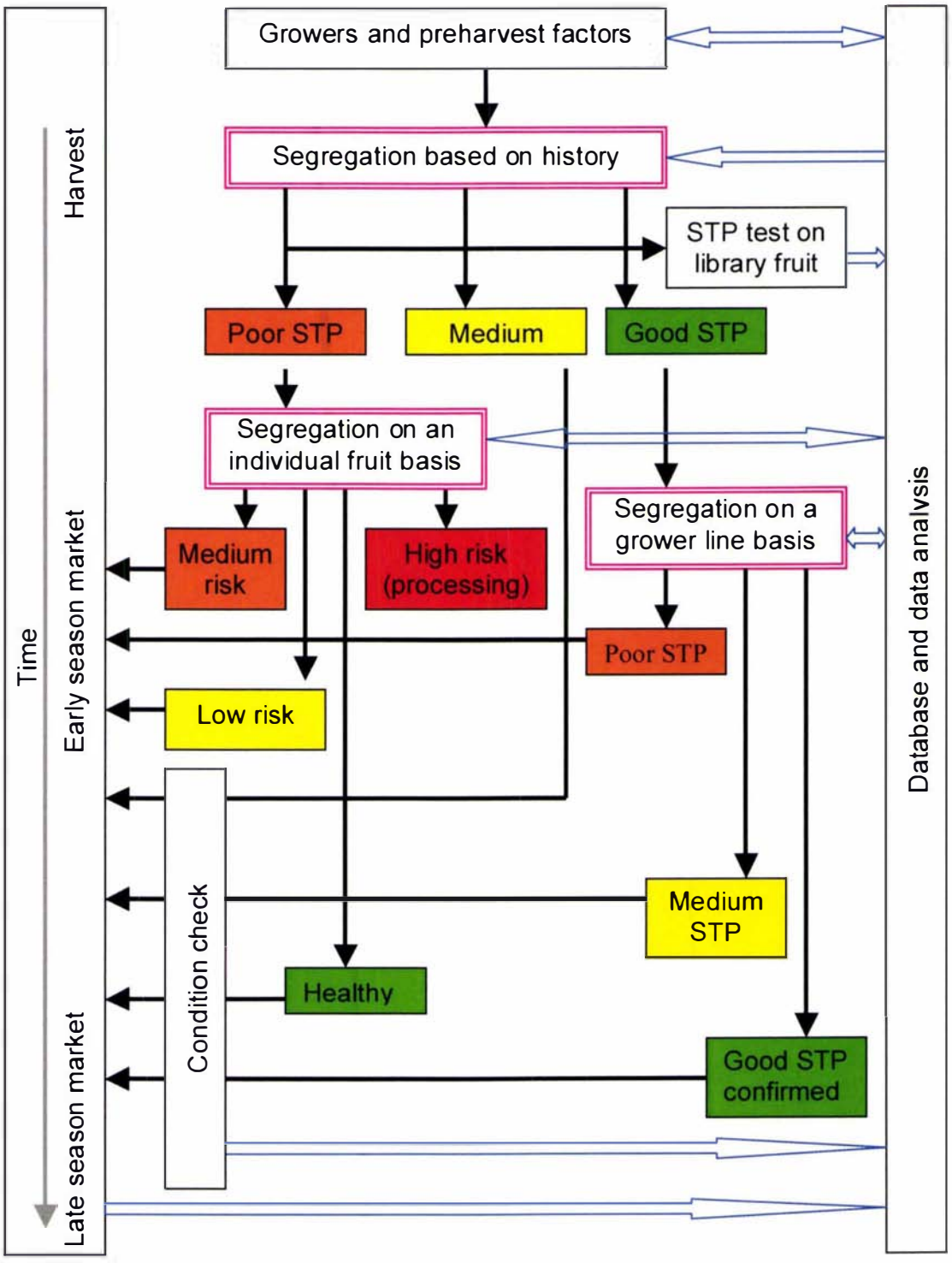

Figure 10.1 Strategies application of segregation technologies for storage potential (STP) at an industry level (Solid arrows indicate fruit flow and hollow arrows represent information flow; dark red indicates fruit unsuitable for sale, red indicates fruit to be sold first, yellow indicates fruit to be sold later and green indicates fruit to be sold last).

10.4.5 Information fed back from market places

Many disorders do not show clear symptoms until fruit are exposed to high temperatures in the market place. It would be useful to assess fruit quality at the market place and record the information in the database.

10.4.6 Database analysis

Database analysis should be updated every year. This will integrate information on preharvest factors, postharvest factors and fed back information from the market places to find relationships useful to improve orchard management, postharvest practice (including fruit segregation) and marketing regimes for the best benefit to the industry.

10.5 Conclusions

Storage potential of grower lines can be evaluated by fruit softening rate estimated by fitting FF monitoring data collected after three to six months storage to an exponential model. Discriminant model for fruit softening rate based on SSFDM, Mg, HD, Ca/N, DL, and L were able to classify over 50% grower lines to the right groups compared with percentage chance criterion of 25%. The discrimination accuracy was higher for groups with softening rates at both ends of the softening-rate continuum. The model can be used for fruit segregation on a batch basis and for improving orchard management and postharvest practice. Application of the discriminant model requires accurate measurement of at harvest fruit attributes using rationalised methodologies. Further work is needed to investigate the physiochemical basis of the predicting factors and select more reliable factors for the discrimination.

Storage potential of individual fruit can be evaluated by its susceptibility to fruit disorders. VNIR spectra taken at harvest are capable of estimating many at-harvest fruit attributes and to separate disordered fruit from healthy fruit. This technology can be used to segregate fruit on a per fruit basis because the measurement is non-destructive and quick. Further work is needed to select the instrumentation and incorporate the instrument with

grading machines in a way that external disturbance can be minimised and the measurement could cover sufficient fruit surface.

Due to the cost of instrumentation and measurement, segregation technologies should be used together with historical information of each grower line. Strategic application of segregation technologies could bring the industry a brighter future with the involvement of growers, packing houses, exporters and intellectual agencies.

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