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EFFECTS OF POSTHARVEST TREATMENTS ON STORAGE QUALITY OF LIME (*CITRUS LATIFOLIA* TANAKA) FRUIT

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Abstract

Limes (*Citrus latifolia* Tanaka) are an attractive fruit crop but generally suffer a loss in value as their colour changes from green to yellow. Various approaches were taken to slow degreening including low temperature storage, use of controlled atmosphere (CA) environments, and treatment of fruit with physiologically active agents such as gibberellic acid (GA_3). However, the cold storage life of lime fruit can also be restricted by a number of factors including chilling injury (CI) and rots. Various pretreatments such as the use of fungicide (thiabendazole, TBZ) and hot water dipping (HWD) and several postharvest regimes based on temperature conditioning (step down technique) and intermittent warming (IW) regimes were further investigated to protect the fruit against rots and CI during cold storage. The objective of this study was to determine what storage conditions and pretreatments would permit long term storage of NZ limes with minimal loss of quality.

CA storage (10% O_2 with 0 or 3% CO_2) was compared to regular air storage (RA) and IW (varying durations) treatments across a range of temperatures. Although some CA storage regimes could assist in delaying degreening, none of the treatments provided protection against CI. CA storage at 3% CO_2 delayed yellowing and gave better fruit quality than the low CO_2 treatment. High CO_2 CA treatments at 5 or 7°C decreased the rate of colour change compared to other constant temperature treatments but did not protect against CI. CI limited storage of fruit under all conditions at constant low temperatures.

Including fungicide (TBZ) in the dip water reduced the incidence of rots and had a secondary effect on protection against CI of lime fruit. However, fungicide use may sometimes exacerbate stresses such as heat injury on lime peel. Hot water dipping has been shown previously to hold potential as a storage pretreatment, but this technique may give risk of damage on produce if it is dipped at too high a temperature. Some HWD treatments did delay degreening, but there was no major effect on CI. HWD at > 47°C for ≥ 4 min caused heat injury to NZ limes. All HWD treatments showed severe CI (>15%) after 10 weeks of cold storage; and HWD fruit stored under RA at 13°C did not

show any CI but showed some pitting (≤ 10%) and degreened rapidly. Overall no suitable HWD treatment for limes was identified in this trial.

This project identified the critical periods and temperature conditions for successful IW of limes. The IW conditions successfully delayed losses in quality of lime fruit provided the first warming period was applied within the first 20 days of storage. At least 2-cycle IW was required to maintain lime quality during long term storage. Some benefits were found after just one cycle of IW treatment but there were not enough to extend storage life.

IW storage benefited fruit quality and provided the highest overall fruit quality of all postharvest treatments tested. The degreening of lime during cold storage at 5°C could be delayed by IW treatments in which the fruit were stored at 5°C for 12, 16 or 20 days then moved to 15°C for 2 days. Both 2- and 6-cycle IW treatments proved satisfactory for maintaining colour on the green and yellow side of lime for 12 weeks of storage. IW treatments in which fruit were warmed within 20 day of cold storage did not show significant CI symptoms after 12 weeks of storage, and the 2-cycle IW treatment showed only a low percentage of CI fruit at this time. A 2-cycle IW treatment was almost as effective as 6 cycles, and a step down treatment also showed some promising results, indicating that it may be possible to further optimize the time and duration of variable temperature storage regimes to meet both quality requirements and the constraints of temperature management in commercial coolstores. The application of these regimes to other citrus species may also be beneficial. There are a number of physiological explanations that may account for the effectiveness of IW including positive effects on heat shock protein (HSP) and cell membranes. Nutritional factors such as vitamin C and flavonoid compositions were also investigated and fruit that did not show visible CI were found to retain at-harvest levels of these factors. Practical ways of implementing IW are discussed.

In order to understand the effectiveness of IW on degreening, I used a logistic model to describe degreening of lime peel. This modelling approach demonstrated that IW did not change the mechanism of lime degreening based on the similarity between the hue values predicted by the model and the actual hue values measured during lime storage. The activation energy (E_a) for degreening based on either hue angle (H°) or colour score (CS) during air storage was estimated to be ~53 and ~86 $KJ.mol^{-1}$, respectively. Relationship

between colour (H° and CS) and chlorophyll content, relationship between reflectance spectra (%), chlorophyll content and H° of lime fruit stored under different conditions are presented and discussed. This data allowed deduction to be made about the changes in individual pigments that are driving colour change during "good" and "bad" storage.

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Abbreviations

a*	CIE Lab 'a' value measured by a colorimeter
ABA	abscisic acid
ACC	1-aminocyclopropane carboxylic acid
ANOVA	analysis of variance
APX	ascorbate peroxidase
b*	CIE Lab 'b' value measured by a colorimeter
°C	degrees Celsius
CAT	catalase
cm	centimetre
C_2H_4	ethylene
$C_{20}H_{39}$	phytol
C*	chroma
CA	controlled atmosphere
C_a	chlorophyll *a*
C_b	chlorophyll *b*
C_{x+c}	carotenoids
CF	compression firmness
d	day
PFR	Plant and Food Research
CI	chilling injury
CO_2	carbon dioxide
CS	colour score
DNA	deoxyribonucleic acid
DSM	diosmin
e	exponential
E_a	activation energy
EB	extraction buffer
Eq.	equation
ERC	eriocitrin
°F	degrees Fahrenheit

FC	fluorescent compound
FW	fresh weight
GA_3	gibberellic acid
GAs	gibberellins
GC	gas chromatography
GR	glutathione reductase
hr	hour
H°	hue angle
$H°_C$	$H°_{CR200}$ (hue angle measured by chromameter CR200)
$H°_S$	$H°_{Spectrophotometer}$ (hue angle measured by spectrophotometer CM-2600d)
H-∞	maximum hue
H+∞	minimum hue
H1	Harvest 1
H2	Harvest 2
H3	Harvest 3
H4	Harvest 4
H5	Harvest 5
HI	heat injury
HPLC	high performance liquid chromatography
HSP	hesperidin
HWD	hot water dipping
HWRB	hot water rinsing and brushing
IRF	isorhoifolin
IW	intermittent warming
J	Joule
K	degrees Kelvin
k	reaction rate constant
k_0	preexponential Arrhenius constant ($time^{-1}$)
kD	kiloDaltons
kg	kilogram
kJ	kiloJoule
KOAc	potassium acetate
L*	Lightness measured by a colorimeter

l	litre
LCMS	liquid chromatography mass spectrometry
LSD	least significant difference
MA	modified atmosphere
MAV	measurement area value
MeOH	methanol
MET	methionine
Mg^{2+}	magnesium ion
mm	millimetre
min	minute
mol	mole
µg	microgram
mg	milligram
µl	microlitre
ml	millilitre
n.d.	no data
nl	nanolitre
nm	nanometre
NPO	neoponcirin
NRG	naringin
NRT	narirutin
O_2	oxygen
PAs	polyamines
PCA	principal components analysis
PC1	principal component 1
PC2	principal component 2
PGRs	plant growth regulators
ppm	parts per million
Put	putrescine
R	the universal gas constant
R^2	R-squared value
R800	reflectance at 800 nm
R700	reflectance at 700 nm

R680	reflectance at 680 nm
R520	reflectance at 520 nm
R480	reflectance at 480 nm
RA	regular air
RH	relative humidity
RNA	ribonucleic acid
ROS	reactive oxygen species
RP	rusty pigment
RTN	rutin
rpm	revolutions per minute
s	second
SAM	S-adenosylmethionine
SAM dec.	S-adenosylmethionine decarboxylase
SCE	spectral component excluded
SCI	spectral component included
SE	standard error
SOD	superoxide dismutase
Spd	spermidine
Spm	spermine
SSC	soluble solids content or Brix°
t	time
t_0	reference time at day 0
t_{ref}	reference time (d)
T	temperature (Kelvin)
TA	titratable acidity
TBZ	thiabendazole
TC	temperature conditioning
UV	ultra violet light

CHAPTER 1

Introduction

Limes are an attractive fruit sought after by consumers in many countries for their unique flavour and acidity, and also serve as a source of industrial and added-value food products (Bosquez-Molina *et al.,* 2004). As with many horticultural products, the price of the fruit is strongly dependent on its availability and quality characteristics. In New Zealand, lime is only a minor citrus crop with the main season being April – June, although fruit is also available later in winter and spring. Due to the colder climate relative to more favourable growing regions, NZ limes (*Citrus latifolia* Tanaka) can suffer from early degreening which can limit grower returns. Limes in local NZ supermarkets are often of poor quality, frequently being quite yellow, shrivelled, soft and showing some disorders, so losses are anticipated to be high and supermarkets cannot expect to command premium prices. If an optimal postharvest regime is used to extend storage life while retaining lime quality, NZ growers will gain benefits from improved presentation in the domestic market. In Thailand, lime (*Citrus aurantifolia* Swingle) is a widely consumed and economically significant crop and the main production season is July to September. Outside of this season, the price rises gradually and reaches the highest price in the dry season (February to April). Due to this price increase there will be an economic benefit from extending their storage life.

Limes are susceptible to chilling injury therefore are traditionally stored at moderate temperatures. During storage at 9 to 10°C, limes typically develop undesirable yellow colour after only 3 or 4 weeks and may become fully yellow after 8 weeks (Murata, 1997; Ladaniya, 2004). This gradual reduction of green skin colour of limes during storage and marketing adversely influences customer acceptance (Kluge *et al.,* 2003a; Bosquez-Molina *et al.,* 2004). Therefore, maintenance of a green skin colour is desirable. Nutrient retention in limes also needs to be studied because citrus fruits can be a valuable source of nutrients including antioxidants, vitamins and minerals (Craig, 1997).

Prestorage techniques such as application of gibberellic acid (GA_3), fungicide and hot water dipping (HWD) and postharvest technologies including modified and controlled atmosphere (MA and CA) storage, intermittent warming (IW) and temperature conditioning (TC) treatments could all be helpful in prolonging the high quality storage life of limes when applied individually or in combination. The benefits of these techniques have been reported for many kinds of fruit including citrus, however all of these techniques must be applied in combination with refrigeration as temperature is the dominant factor influencing all plant processes (Pearce, 1999). Lowering the temperature of fresh produce decreases its rate of deterioration (e.g. change in texture, loss of vitamin C), metabolism and hence not only reduces ethylene production, but also the rate of response of tissue to ethylene. However, storing the produce at too low temperature can cause adverse effects in the produce such as chilling injury (CI) (Wills *et al.,* 1998a). In common with other citrus varieties, CI can manifest in various forms including sunken scald-like discolouration, watery breakdown and pitting (Porat *et al.,* 2004). Rots may also limit storage life and their incidence is likely to be exacerbated by chilling injury.

The overall objective of this research was therefore to determine the optimal storage regime that best delays all of the physical, physiological and biochemical changes of lime fruit after storage and also could effectively protect against rots and disorders such as pitting or chilling injury. The further requirements of this work were that (1) the best regimes should be possible to implement commercially (2) the maintenance of colour was a key quality requirement and should be characterised and modelled and (3) the influence of the regimes on the nutritional status of the fruit must be characterised to ensure the benefit of lime fruit consumption was maintained.

CHAPTER 2

Literature review

2.1 Lime cultivars and their characteristics

The centre of origin of citrus species was in Southeast Asia. From there citrus crops have been spread all over the world since prehistoric times and the inter-country movement of each variety along trade routes makes a fascinating story. The oldest known reference to citrus appears before 800BC in Sanskrit literature and citrus fruit are now cultivated in tropical, subtropical and temperate regions from 40°N latitude to 40°S latitude (Baldwin, 1993; Murata, 1997; Moore, 2001; Mukhopadhyay, 2004b). Citrus is one of the most important fruit crops in the world because of the scale of its production and the health benefits derived from consumption of either fresh fruit or juice. There are many commercial species of citrus available such as orange (*Citrus sinensis* (L.) Osb.), mandarin and tangerine (*Citrus unshiu* Marc. and *Citrus reticulata* Blanco), lemon and lime (*Citrus limon* Burm.f. and *Citrus aurantifolia* Swingle), grapefruit (*Citrus paradisi* Macf.), pomelo (pummelo) (*Citrus grandis* Osb.), and their hybrids (Murata, 1997).

The genus *Citrus* (Ramesh Yadav *et al.,* 2004) is in the family Rutaceae. The fruit is classified as a hesperidium or berry of special structure. Each fruit segment consists of a juicy pulp enclosed in vesicles (Baldwin, 1993). There are two groups of lime: (1) low acid (sweet) lime (*Citrus limettioides* Tan.) cultivars such as 'Indian sweet lime' (also known as Palestine lime), which are popular for fresh consumption (Samson, 1986; Baldwin, 1993; Berry, 2003) and used for medicinal purposes (Berry, 2003), and (2) acid (sour) cultivars including 'West Indian' or ('Mexican' or 'Key') lime (*C. aurantifolia* Swingle), which is small-fruited and the predominant type grown in the world, and the larger 'Tahiti' or 'Persian' lime (*C. latifolia* Tan.) (Samson, 1986; Baldwin, 1993; Berry, 2003). Both of these acid limes are consumed fresh and also used in food or flavour industries (Murata, 1997; Ziena, 2000; Lota *et al.,* 2002; Chisholm *et al.,* 2003; Ramesh Yadav *et al.,* 2004; Ubando-Rivera *et al.,* 2005).

The main species of this large-fruited, acid lime is *C. latifolia* Tanaka (Spiegel-Roy and Goldschmidt, 1996; Ladaniya, 2004). The fruit is seedless (Samson, 1986; Thompson, 2003). Berry, (2003) reported the size and shape of 'Tahiti' lime is similar to the Bearss lemon and this lime may be a hybrid of the citron and/or lemon, but the parentage and true source are not known. The juice of all acid limes provides a high quality characteristic lime flavour and contains typical 'lime-like' aroma compounds, which are similar throughout most of the world.

Lime has the most sour taste of all citrus fruits and is too acidic to be consumed as undiluted juice (Berry, 2003). However, lime juice is popular and is consumed in different styles such as in juice mixtures and carbonated or alcoholic beverages, or is used in salads or as a flavouring ingredient in dishes ranging from hot cooked foods to cold desserts. It is also used in pickling or with fresh fruits in syrup manufacture to protect against discolouration, and is used in medical applications (Ziena, 2000; Berry, 2003). Chaisawadi *et al*., (2005) reported that compounds from lime and its derivatives such as juice, skin and oil have a wide range of medicinal properties. They suggested that d-limonin, extracted from lime oil, has cancer-preventive and anticarcinogenic properties. In Thailand, lime (*C. aurantifolia* Swingle), locally known as 'Ma-nao', has been used for providing a sour taste and its unique fragrance to enhance overall flavour and aroma in various Thai foods. It is also used in Thai traditional medicine.

The distinctive natural lime aroma volatiles (Chisholm *et al.,* 2003) are also widely used in other industries. For example, the volatile fraction of lime oil, limonene, is used in cosmetics (e.g. perfumery ingredients) and household products such as soaps and detergents (Dugo *et al.,* 1997; Berry, 2003; Chisholm *et al.,* 2003).

2.2 Lime production

The Bay of Plenty, Northland and Gisborne are the major growing regions for NZ limes and lemons. The climatic conditions of these areas are characterised by low rainfall, high sunshine and being usually frost free. Lime is only a minor crop in NZ. The export value of limes is included in the same figures as lemons (both fresh or dried). Based on the HortResearch's *Fresh* Facts 2006 and 2007 publication (Anon., 2006, 2007), the estimated export value of lemon products was ~NZ$2.5 million and based on the UN

Comtrade's website (http://comtrade.un.org) the exported values of lemon and lime products in 2006 was ~US$1.8 million (or ~NZ$2.6 million). This suggests the export values of lime may be about NZ$100,000 (dependent on the reliability of the US$ and NZ$ conversion). Even though the production and export value of limes in NZ are low, the retail prices of good quality limes within the country are higher than for other citrus consumed in NZ.

In Thailand, the major growing regions for limes are in the central (e.g. Phetchaburi, Kanchanaburi, Samut Sakhon, Nakhon Pathom), the southern (e.g. Nakhon Si Thammarat, Surat Thani) and the northern regions (e.g. Nakhon Sawan, and Chiang Mai). The consumption volume of limes in Thailand is many thousands of tonnes a year. Although limes can be grown in every region in Thailand and produce fruit throughout the year, the quantity of limes available varies from season to season and in the dry season (February to April) the price of limes can therefore increase by as much as five to ten times. In contrast, the price of limes is very low in the wet season because there is a lot of fruit in the market (Chaisawadi *et al.,* 2005; Sritananan *et al.,* 2006). There is therefore an economic benefit in extending lime storage life and possibly an opportunity for NZ and Australia to export fresh limes to Thailand and other South East Asian countries during selected periods of higher prices.

2.3 Consumers' acceptance of lime

Lime fruit are commonly harvested when fully developed, but still green (Kluge *et al.,* 2003a; Thompson, 2003). The optimum harvest time is when the skin becomes smooth and the rind turns from dark to pale-green (Murata, 1997; Thompson, 2003). Kader, (2004) proposed a rating scale for lime based on 5 levels where: stage 1 = dark green, 2 = light green, 3 = yellowish-green, 4 = greenish-yellow and 5 = yellow. A minimum colour considered as 'good green' is about level 2. Thompson (2003) also reported that the European market prefers fruit that is at least >42 mm in diameter. The fruit are normally harvested by hand, using secateurs to cut at the "button" of the fruit. Various prestorage treatments may be applied in the packing house before cool storage or the fruit may be directly transferred to the market. Sale, (2001) reported that NZ orchardists typically pick the fruit regularly using selective picking as their thinning programme. By this technique

they can get a better size of fruit and almost continuous flowering, which in turn provides them harvestable fruit for much of the year.

Green limes are strongly preferred for consumption by customers in many countries (Murata, 1997; Kluge *et al.,* 2003a; Thompson, 2003; Ladaniya, 2004) including New Zealand (Sale, 2001). In Thailand, green limes are also preferred but yellow limes are also used in cooking. Thompson (2003) reported that there are no other quality differences between light green limes and fully yellow fruits but customers clearly prefer green limes. Given that degreening, the gradual reduction of green skin colour of limes during storage and marketing, adversely influences customer acceptance, storage treatments to maintain the green skin colour of the fruits are desirable (Kluge *et al.,* 2003a; Bosquez-Molina *et al.,* 2004) but fruit must also be in sound condition and free of other defects.

2.4 Major quality changes of citrus fruit after harvest

The following section contains some basic information about citrus fruit growth and development. Because different citrus tissues are so biochemically different, they will have very different susceptibilities to responses when fruits are stored at suboptimal temperatures. The origin of the different tissues and the differences in composition of these tissues need to be considered in order to focus my research on the most sensitive regions.

2.4.1 The development of citrus fruit including the origin of different tissues and the differences in composition of these tissues

Citrus fruits are botanically classified as a special type of berry termed a 'hesperidium' (Baldwin, 1993; Spiegel-Roy and Goldschmidt, 1996). Citrus is a true fruit that develops from a superior ovary with all of the tissues derived from the ovary (Albrigo and Carter, 1977; Soule and Grierson, 1986; Baldwin, 1993; Spiegel-Roy and Goldschmidt, 1996). The citrus ovary is composed of 6 to 20 united carpels which form locules (Albrigo and Carter, 1977; Baldwin, 1993). The growth and development of a citrus flower's ovary takes 6-18 months or more to become a fruit ready to harvest, depending upon the type and particular cultivar of fruit (Soule and Grierson, 1986). Citrus fruits are

morphologically composed of two major regions, the first is the pericarp which is known as the peel or rind and the second is the endocarp, the edible portion of fruit called the pulp. The citrus peel can be further separated distinctly as the external coloured portion (the epicarp or the flavedo) and the internal white layer of the peel (the mesocarp or albedo) (Spiegel-Roy and Goldschmidt, 1996).

The flavedo consists of epidermis, hypodermis, and outer mesocarp. The epidermis consists of an epicuticular wax layer in platelets, a mixture of cutin, wax and cell wall-material, the primary cell wall and the epidermal cell wall. The flavedo also contains pigments in chloroplasts or chromoplasts and oil glands derived from special cells that produce terpenes and oils (Albrigo and Carter, 1977; Soule and Grierson, 1986; Baldwin, 1993; Spiegel-Roy and Goldschmidt, 1996; Izquierdo and Sendra, 2003).

The white albedo portion consists of large, deeply lobed cells with numerous large intercellular spaces and scattered vascular elements. The tissues contain large air spaces, providing a spongy nature. The albedo may occupy 60-90% of fruit volume during the peel development at the early stage of fruit development. The albedo becomes thinner during the development of the pulp (Baldwin, 1993; Spiegel-Roy and Goldschmidt, 1996). The albedo of grapefruit, orange, lemon and tangerine contains higher flavanone content than juice vesicles, section membranes or flavedo. The albedo is also regarded as an important source of limonin. Both albedo and flavedo (peel) contain a higher concentration of bitter principles and pectin than other parts of the fruit. Information on the structure and composition of the fruits can be used to improve the design of equipment for citrus processing (Albrigo and Carter, 1977; Baldwin, 1993).

Approximately 10, 25 and 40-50 % of citrus fruit is made up of flavedo, albedo and juice, respectively. The main constituents (%) of citrus are 85-90 % water, 6-9 % sugar and less than 2 % for acids, pectins, minerals, essential oils, fiber, protein and fat (Izquierdo and Sendra, 2003). The endocarp portion of citrus fruit is the most complex tissue. This edible portion called the pulp consists of segments, the ovarian locules, enclosed in a locular membrane and filled with juice sacs (or juice vesicles) (Baldwin, 1993; Spiegel-Roy and Goldschmidt, 1996). Juice sacs appear at first as dome shaped protrusions from the locular membrane into the locules, these tissues are initiated at about full bloom. The domes develop into juice sacs through apical meristem activity. Juice sacs are elongated

in the mature fruit and are mostly spindle- (or elliptical-) shaped multicellular structures (Spiegel-Roy and Goldschmidt, 1996). Vascular bundles form a loose network around the locules (Figure 2.1A). Juice sac cells are highly vacuolated and the narrow cytoplasm contains lipid droplets in plastids, leucoplasts and chromoplasts. Juice within the vacuole of these cells is rich in organic acids and other soluble compounds such as amino acids and salts. Calcium oxalate, hesperidine and naringin crystals can also be found in the rind and juice sacs of citrus fruits (Shomer *et al*., 1975; Baldwin, 1993). A diagrammatic representation of an equatorial cross-section through a citrus fruit is shown in Fig. 2.1B.

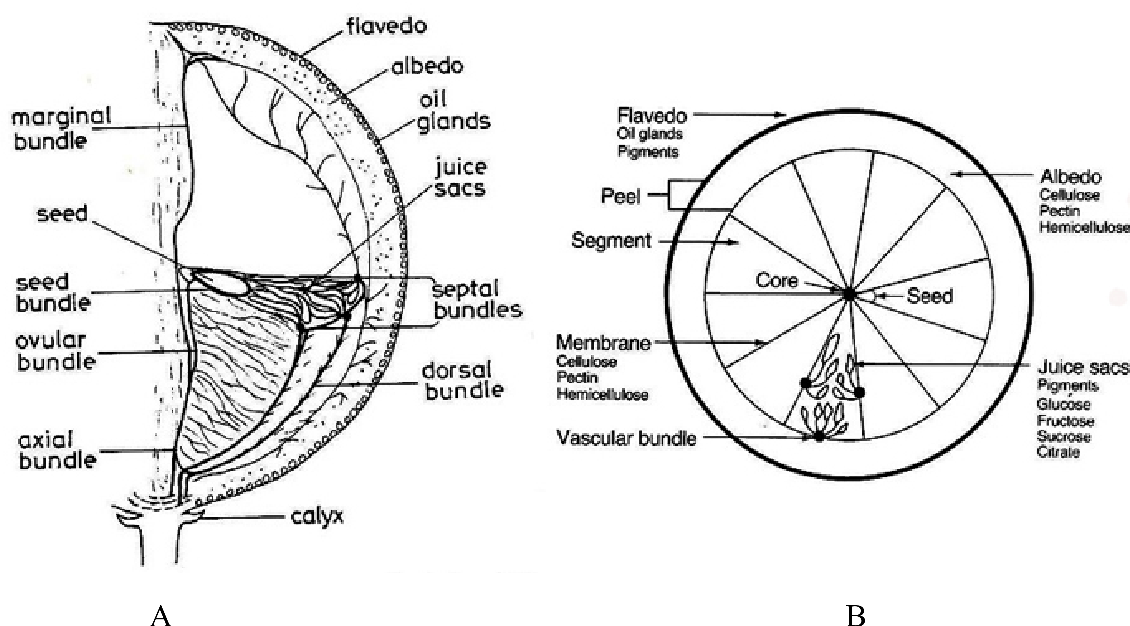


Figure 2.1 A: Schematic drawing of a mature citrus fruit emphasizing the vascular arrangement. (From Spiegel-Roy and Goldschmidt, 1996); B: Diagrammatic equatorial cross-section through a citrus fruit. (From McCready, 1977; Baldwin, 1993).

The quality of citrus fruit gradually degrades after harvest in terms of both external and internal attributes so choosing appropriate storage conditions is extremely important to retain optimal quality. Physical and physiological changes during storage of the fruit can be used as quality indices and important citrus, including lime quality characteristics are reviewed in the following sections.

2.4.2 Colour, soluble solids content and acidity

Citrus fruits are classified as nonclimacteric (Baldwin, 1993; Murata, 1997; Porat *et al.,* 1999; Artes-Hernandez *et al.,* 2007; Barry and Giovannoni, 2007) therefore, the fruit can not be harvested immature as normal ripening off the tree does not occur. Peel colour of

citrus is not a good indicator of the fruit maturity, therefore the ratio of soluble solids content (SSC or Brix) and titratable acidity (TA) of the juice is used as a maturity index for many citrus varieties (Baldwin, 1993). Generally, a Brix/TA ratio ranging from 8-10 is taken as a minimum value and a range from 10-16 is considered as being of acceptable quality. If the fruit remain unharvested, the Brix increases while the acidity decreases continuously until the fruit become overripe. The taste is reported to be unpleasant when fruit reach a Brix/TA ratio of 20 or more (Samson, 1986). Lime and lemon are different from other citrus varieties in that their value is based primarily on the concentration of acid (citric) in the juice. The typical acid content (as citric acid) reported for lime juices in citrus processing quality-control and inspection laboratories ranges from about 6- 8.5% (w/v) (Berry, 2003).

Citrus is known as one of the most important sources of vitamin C (Lee and Kader, 2000). The recommended dietary intakes of vitamin C for women and men are 30 and 40 mg per day, respectively (Olson and Hodges, 1987). However, the vitamin C content in fresh products depends on the variety, climate, horticultural practice, maturity stage and storage conditions (Nagy, 1980). More information about vitamin C is further explained in section 2.4.5.1 and in chapter 7.

The relative concentration of chlorophyll and carotenoids in citrus fruit dictates the colour of the fruit. The pigments are located in the flavedo (peel) and juice vesicles (pulp) (Baldwin, 1993). Chlorophyll is found in chloroplasts and the carotenoids are both associated with chlorophyll in the chloroplasts and are also found in chromoplasts. Both groups of pigments are insoluble in water but soluble in acetone, ether and alcohols (Kays and Paull, 2004a). Degreening of lime peel is generally related to chlorophyll degradation and consequential unmasking of carotenoids. These changes occur continuously under room temperature conditions during marketing and also under low temperature storage, although the rate of degreening is then slower (Kluge *et al.,* 2003a). Chlorophyll breakdown causing degreening is a characteristic of many plant tissues during both ripening and senescence (Wang, 1977; Gong and Mattheis, 2003).

The major commercial species of citrus fruit and their composition including pigments are summarised in Table 2.1.

Table 2.1 Chemical composition in *Citrus* species

Citrus Species & cv. [1]	Vitamin C mg/100 ml juice	°Brix	TA % citric acid	Carotenoids		Essential oils e.g. d-Limonene, ppb of juice	Commer-cial uses	Ref
				Lycopene µg/g DW	ß-Carotene µg/g DW			
Grapefruit							juice and	
(*C. paradisi* Macf.).							fresh	
-Star Ruby				283.57±3.63	93.03±2.87		consumption	(1)
-albedo				46.77±0.42	n.d.			(1)
-juice	36.35	9.9	1.89					(2)
-NZ grapefruit								
juice	33.2-38.3	10.3-14.4	0.92-2.56					(3)
Mandarin							juice and	
(*C. reticulata*)							fresh	
-Fina -juice	42					34516±2657	consumption	(4)
-Nules - juice	33	12.65	0.66			10507 ± 810		(5)
-Malvasio -juice	33	10.4	0.93					(6)
Oranges							fresh	
(*C. sinensis* (L.) Osbeck)							consumption	
- Valencia -juice	60.26	11.40	1.82					(7)
- Cara Cara Navel				109.67±8.99	15.52±0.48			(1)
Lemon							fresh	
(*C. limon*) (juice)							consumption	
-Primofiori	45.9	9.1	6.7					(8)
-Maglino	56.66±3.04	9±0.19	6.74±0.24					(9)
Lime (juice)							juice and	
(*C. latifolia* Tan)	33.8-36.1	9.5-9.8	6.33-6.86				fresh	(10)
(*C. aurantifolia* Swingle)							consumption	
- Paan	23.65	7.06%	8.23					(11)
Pomelo							fresh	
(*Citrus grandis* (L.) Osbeck)							consumption	
-Fengdu red-fleshed				92.15±1.94	27.33±0.68			(1)
-albedo				13.41±2.36	4.85±0.22			(1)
- Khaonahmpeung				6.7±3.5 [2]	8.9±2.4 [2]			(12)
- Tongdee				288±87.4 [2]	25.6±8.1 [2]			(12)

Notes: [1] edible portion (flesh) unless otherwise specified
[2] µg / 100 gFW
n.d. = not detectable

References:
(1) Xu *et al*., (2006)
(2) Schirra, (1993)
(3) Robertson and Nisperos, (1983)
(4) Perez *et al*., (2005a)
(5) Perez *et al*., (2005b)
(6) D'Aquino *et al*., (2001)
(7) Erkan *et al*., (2005)
(8) Artes *et al*., (1993)
(9) Tsantili *et al*., (2002)
(10) Ziena, (2000)
(11) Win *et al*., (2006)
(12) Charoensiri *et al*., (2009)

For lime fruit, the retention of green colour is an important indicator of quality. Chlorophyll content of the peel is reduced when fruit reach maturity and it is possible to separate limes into different classes on the basis of estimated chlorophyll content. A photoelectric machine (ESM Model G) was developed for colour sorting citrus fruit, including limes, in 1972 (Thompson, 2003) although the extent of its current use is unclear.

2.4.3 Firmness

Degradation of insoluble protopectin to the more soluble pectic acid and pectins contributes to a decrease in firmness in many fruit. These changes occur relatively slowly and are less pronounced in citrus fruit as compared to climacteric fruits (Ladaniya, 2004). However, some softening of the fruit also occurs due to turgor pressure changes and/or respiratory loss of dry matter during growth, development and senescence. Furthermore, environmental conditions, irrigation practices, postharvest water loss, and aging of the fruit also influence the texture changes (Kefford and Chandler, 1970; Sasson and Monselise, 1977). For example, preharvest spraying of fruit with GA_3 retarded rind senescence of navel orange, carotenoid accumulation, and softening (Coggins, 1982). Some physiological disorders, such as chilling injury (CI) and watery breakdown, cause softening and 'spongy' fruit during low temperature storage (Murata, 1997). Paull and Jung Chen, (2000) similarly noted that postharvest heat treatments can affect fruit softening and contribute to loss of membrane integrity and flavour changes.

2.4.4 Respiration and ethylene production rates

Respiration and ethylene production are important indicators of physiological state and/or senescence in fresh products. A decrease in respiration rate during storage is usually beneficial to maintaining quality (Calegario *et al.,* 2001). However stresses such as pathogen infection, chilling injury, mechanical damage and treatment with external ethylene (Baldwin, 1993) and physiological disorders such as section drying (granulation of juice sacs) all increase the respiration rate of citrus fruit (Burns, 1990).

Citrus have a relatively low respiration rate and also produce only small amounts of ethylene (Porat *et al.,* 1999; Artes-Hernandez *et al.,* 2007). This is typical of

nonclimacteric fruit, in which endogenous ethylene production is consistently low and often almost insignificant. However, ripening-related changes associated with pigment changes and chlorophyll degradation in certain non-climacteric fruits such as citrus fruit are accelerated by external ethylene (Monselise, 1979; Vendrell *et al*., 2001).

Arpaia and Kader, (2000) reported that the ranges of respiration rate of lime at 10, 15 and 20°C were 3-5, 5-8 and 6-10 ml CO_2 kg^{-1} hr^{-1}, respectively, and the rate of ethylene production was lower than 0.1 µl kg^{-1} hr^{-1}. Win *et al*., (2006) reported that the respiration rate of untreated lime (*C. aurantifolia* Swingle) stored under ambient conditions (average about 27°C and 77% RH) was 11.2 ml CO_2 kg^{-1} hr^{-1} and the ethylene production rate was about 0.3 µl kg^{-1} hr^{-1} in the initial stages of storage.

2.4.5 Nutritional quality

Consuming a diet rich in fruits and vegetables provides natural antioxidants such as vitamins (A, C and E) and natural bioactive substances called phytochemicals (Craig, 1997). Citrus fruit contain a wide range of health promoting compounds including vitamin C, significant amounts of dietary fibre, various kinds of carotene (e.g. beta-carotene, lutein, zeaxanthin), and folic acid (Craig, 1997; Baghurst, 2003). These fruit also have a low ratio of sodium to potassium and are low in fat and dietary energy. Some major non-nutritive phytochemical compounds found in citrus are flavonoids, glucarates, coumarins, monoterpenes, triterpenes and phenolic acids (Baghurst, 2003). Humans require a diet containing relatively small amounts of vitamins for normal metabolism and growth, and plants are a major source of these essential vitamins with the exception of vitamin B_{12}, which is synthesized only by microorganisms, and vitamin D, which is obtained from sun exposure (Kays and Paull, 2004a). Some of most significant health-promoting compounds in lime fruit are vitamin C and the flavonoids, and these are reviewed in the following sections.

2.4.5.1 Vitamin C

Vitamin C is a water-soluble vitamin that is commonly found in relatively high concentrations in many fruits (Kays and Paull, 2004a) and particularly in citrus species. The term “Vitamin C” refers to both L-ascorbic acid and dehydroascorbic acid, the latter

being the first oxidation product of the former (Zumreoglu-Karan, 2006). There are many factors that influence the amount of vitamin C in fruit products, such as differences of genotype, preharvest climatic conditions, cultural practices, maturity, harvesting methods and postharvest handling procedures. For example, vitamin C in plant tissues can be increased when the intensity of light is high or less frequent irrigation is applied during the growing season. High rates of nitrogen fertilizers may decrease the vitamin C content in many fresh commodities and losses of vitamin C in fresh commodities are enhanced by extended storage, higher temperatures, low relative humidity, physical damage and chilling injury (Harris, 1975; Mozafar, 1993; Lee and Kader, 2000).

As noted, *Citrus* species are a rich source of vitamin C. For example, the Spanish Mandarin variety 'Hernandina', an offshoot of a Clementine variety (*C. reticulata* Blanco cv. Fina) reportedly contains about 42 mg.100 ml^{-1} vitamin C juice (Perez *et al.,* 2005a), mandarin (*C. reticulata* Blanco cv. Nules) contains 33 mg.100 ml^{-1} juice (Perez *et al.,* 2005b) and Eureka lemon about 48.4 mg.100 ml^{-1} juice (Shrikhande and Kaewubon, 1974). Ascorbic acid concentrations of 'Tahiti' lime have been reported to range from 39-62 mg.100 ml^{-1} lime juice (Ziena, 2000), while 'Tahiti' lime grown in Brazil contained 31 mg.100 ml^{-1} juice at the beginning of storage (Kluge *et al.,* 2003a).

2.4.5.2 Flavonoids

Phytochemicals are plant components which are currently being widely examined for their ability to provide health benefits (Dillard and German, 2000). The largest category of phytochemicals, and also the most widely distributed in the plant kingdom, are the phenolics. Flavonoids (the largest group of plant phenols), phenolic acids and polyphenols (commonly known as tannins) are the three most important groups of dietary phenolics (King and Young, 1999). Flavonoids (including flavones, isoflavones, flavonones, anthocyanins, catechin and, isocatechin) are found in fruit and vegetables and exhibit strong antioxidant activity; phenolic acids have also been implicated as active antioxidants (Lurie, 2003). Tannins are compounds of high molecular weight that are divided into two classes, hydrolysable and condensed tannins (King and Young, 1999). Apart from their antioxidant activity, plant phytochemicals are implicated as signalling compounds in the reduction of many diseases (Frankel, 1999).

Consumption of citrus fruit appears to contribute to a reduced risk of certain chronic diseases (Peterson *et al.,* 2006) and this activity is attributed to its phytochemical content. Most research on citrus species has concentrated on oranges as these are the most widely consumed member of this group. Citrus fruit typically contain more than 60 flavonoids and reports indicate a wide range of apparently beneficial properties, e.g. anti-inflammatory and antitumor activity, inhibition of blood clots and strong antioxidant activity (Craig, 1997).

Both the total amount of flavonoids and the particular types of flavonoids in a given citrus fruit are likely to contribute to its health-promoting properties. Flavonoid compounds have been studied in many citrus species such as oranges (Abeysinghe *et al.,* 2007, Mouly *et al.,* 1994, Nogata *et al.,* 2006, Kawaii *et al.,* 1999, Franke *et al.,* 2004 and Gattuso *et al.,* 2007), grapefruits (Mouly *et al.,* 1994, Nogata *et al.,* 2006, Kawaii *et al.,* 1999, Peterson *et al.,* 2006, Belajova and Suhaj, 2004, Franke *et al.,* 2004 and Gattuso *et al.,* 2007), lemons (Mouly *et al.,* 1994, Nogata *et al.,* 2006, Del Rio *et al.,* 2004, Kawaii *et al.,* 1999, Peterson *et al.,* 2006 and Gattuso *et al.,* 2007) and limes (Mouly *et al.,* 1994, Nogata *et al.,* 2006, Kawaii *et al.,* 1999, Peterson *et al.,* 2006 and Gattuso *et al.,* 2007).

Six flavonoids have been commonly reported in lime (*C. aurantifolia*) juice: eriocitrin (ERC), narirutin (NRT), hesperidin (HSP), diosmin (DSM), neoponcirin (NPO) and isorhoifolin (IRF). Two further flavonoids, rutin (RTN) and naringin (NRG), are also found in lime (*C. latifolia*) (Nogata *et al.,* 2006). Of these lime flavonoids, HSP is characteristically the most abundant.

Other *Citrus* species contain varying amounts of these compounds, plus other distinctive flavonoids, leading (Tripoli *et al*., 2007) to suggest that the relative content of these flavonoids can be used as a diagnostic test to confirm the origin of citrus juice. In a similar manner, Lim and Lim, (2006) reported that whilst orange is grouped in the same taxonomic class as pummelo and grapefruit, on the basis of its dominant flavonoids it is more similar to lime and lemon. The amounts of the various flavonoid compounds reported in different *Citrus* species are summarised in Table 2.2

Table 2.2 Flavonoid compounds in *Citrus* species

***Citrus* Species & cv.**	**Flavonoid compounds**								**Unit**	**Ref**
	ERC	**RTN**	**NRT**	**HSP**	**DSM**	**NRG**	**NPO**	**IRF**		
Lime (*C. latifolia*)										
juice vesicle tissue	47.1	13.4	2.2	56.0	2.2	4.0	1.1	5.5	FW	1
edible part	186.0	18.1	0.0	572.0	83.6	0.0	0.2	55.1	DW	2
Lime (*C. aurantifolia*)										
juice	0.29			1.77	0.08				vol	3
juice	1.38		0.23	15.64		0.0			aglyc	4
juice vesicle tissue	2.0	0.0	1.4	42.0	2.2	0.0	2.2	7.7	FW	1
(Brazil)	4.85		0.29	10.88					vol	5
(Mexico)	6.24		0.52	16.78					vol	5
Lemon (*C. limon*)										
juice	16.7			20.5	3.12				vol	3
juice	9.46		0.8	15.78		0.18			aglyc	4
-Eureka										
edible part	245.0	22.7	0.0	358.0	73.2	0.0	0.0	0.0	DW	2
mature fruit	630-670			720-760	250-310				DW	6
juice vesicle tissue	81.5	0.0	0.8	63.8	6.1	0.0	0.0	1.6	FW	1
-Lisbon	500-620			800-820	130-190				DW	6
-Fino 49	760-820			560-620	1000-1400				DW	6
(Spain) juice	8.77		0.68	15.44					vol	5
(France)	7.81		0.51	11.63					vol	5
Grapefruit (*C. paradisi*)										
juice	0.41	3.26	7.6	0.93		23.0			vol	3
juice				1.54		21.1			vol	7
juice	0.45		4.90	2.78		16.60			agylc	4
-Marsh										
juice vesicle tissue	27.5	5.4	208.0	0.0	0.0	1270	4.1	0.0	FW	1
edible part	1.2	0.0	500	5.0	0.0	1459	12.2	0.0	DW	2
-White										
juice	0.31		10.57	0.53		33.13			vol	5
(fresh & raw)			5.58	0.01		15.70			FW	8
juice	0.16		5.36	3.95		16.90			aglyc	4
-Red (juice)	traces		7.60	0.52		27.54			vol	5
-Red blush (edible part)	1.8	0.0	285.0	19.0	0.0	1143	15.1	0.0	DW	2
-Ruby red (fresh & raw)			11.30	0.32		33.61			FW	8
-Red & Pink	0.0		3.34	0.27		13.87			aglyc	4
-Pink	traces		5.61	0.46		15.91			vol	5
-Green	0.62		17.86	0.14		25.16			vol	5

***Citrus* Species & cv.**	**Flavonoid compounds**								**Unit**	**Ref**
	ERC	**RTN**	**NRT**	**HSP**	**DSM**	**NRG**	**NPO**	**IRF**		
Orange (*C. sinensis*)										
edible tissues				63-246		n.d.			FW	9
juice	0.31		5.2	28.6	0.09			0.07	vol	3
-Valencia										
juice	0.31		3.69	23.00					vol	5
juice vesicle tissue	19.7	21.6	54.3	93.2	0.0	0.0	2.9	0.0	FW	1
edible part	6.9	3.5	75.7	698.0	0.0	0.0	9.8	0.0	DW	2
-Navel										
juice	0.36		8.51	37.93					vol	5
fresh & raw			7.32	30.59		0.2			FW	8
-Morita navel										
edible part	11.9	13.7	444.0	1080	9.0	0.0	39.9	0.0	DW	2
-Blood	n.d.		4.33	36.30					vol	5
-Thomson	0.50		8.03	30.96					vol	5
-Malta	0.31		3.97	30.43					vol	5

Notes: FW = mg $100g^{-1}$FW, DW = mg $100g^{-1}$DW, vol = mg $100mL^{-1}$ and
aglyc = mg $100g^{-1}$FW (aglycone)
n.d. = not detectable

References:
(1) Nogata *et al.,* (2006)
(2) Kawaii *et al.,* (1999b)
(3) Gattuso *et al.,* (2007)
(4) Peterson *et al.,* (2006)
(5) Mouly *et al.,* (1994)
(6) Del Rio *et al.*, (2004)
(7) Belajova and Suhaj, (2004)
(8) Franke *et al.,* (2004)
(9) Abeysinghe *et al.,* (2007)

There appear to be only a few studies specifically investigating the health promoting properties of limes. For example 'Tahiti' lime was included in a Japanese study of anticancer substances in the readily extractable fractions of Citrus species and cultivars (Kawaii *et al.,* 1999a). Furthermore, lime (*C. aurantifolia*) was also studied in Iran for its efficacy on inhibiting proliferation of a human breast carcinoma cell line and a human lymphoblastoid B cell line (Gharagozloo *et al.,* 2002).

There are still many unanswered questions about how important the antioxidant properties of polyphenolics are to health. Many experts have argued for a more direct effect of the compounds than their antioxidant activity *per se*. For example, it has been suggested that the health benefit of consuming a single antioxidant is not equivalent to consuming a diet containing a range of antioxidants from fruit and vegetables (Frankel and German, 2006; Halliwell, 2006; Waterhouse, 2006). Frankel, (1999) reported that even though consumption of diets rich in flavonoids derived from fruit and vegetables is

associated with lower risks of diseases such as coronary heart disease and cancer, their nutritional importance is still being debated because the systematic study of their action is complicated by the vast multiplicity of flavonoid compounds. There is also controversy about these compounds because it is still not clear which flavonoids are absorbed, which tissues are affected, and whether or not possibly active metabolites are converted into more or less active products. Further research on the flavonoids is therefore required to clarify our knowledge of their mechanism of action.

2.5 Disorders of citrus during storage

2.5.1 Introduction

Physiological disorders significantly influence the quality of citrus fruits during storage and marketing periods. Both preharvest and postharvest factors affect the incidence of these fruit disorders (Grierson, 1986; Murata, 1997). Significant preharvest factors include: boron and copper deficiency, sunburn, wind scar and freezing. Postharvest factors such as temperature, humidity, atmospheric gas composition, mechanical stress and aging induce or contribute to physiology disorders such as rind staining, puffiness, granulation, oleocellosis, stem-end rind breakdown, stylar-end breakdown, watery breakdown, chilling injury and freezing injury (Murata, 1997).

Mechanical damage also frequently occurs during the postharvest handling of fruit and is considered as a type of stress. This stress results in physiological and morphological changes such as increased rates of respiration and ethylene production, bruising, cell rupture and ion leakage (Valero *et al.,* 2002). Mechanical damage can cause a peel injury of citrus fruit called oil spotting or oleocellosis (Wardowsky *et al.,* 1998). Oleocellosis is described as a physiological rind disorder that is caused by the action of phytotoxic oils on the rind tissue. These oils are released from glands located in the citrus rind as a result of mechanical damage (Knight *et al.,* 2001, 2002) and destroy nearby parenchyma, epidermal and subepidermal cells of the flavedo (Wardowsky *et al.,* 1998). Oleocellosis in citrus fruit is characterised by a sunken, discoloured appearance as observable greenish-brown areas on an orange or yellow background of the flavedo (Shomer and Erner, 1989; Knight *et al.,* 2002).

2.5.2 Pitting and chilling injury (CI)

In general, citrus fruit, and particularly limes, lemons and grapefruits stored at temperatures lower than 10°C, develop CI symptoms including rind pitting and sunken, water-soaked lesions on the fruit surface (Wills *et al.,* 1998b; Ladaniya, 2004). CI can manifest in various forms including superficial brown staining of the rind, browning of the albedo, and watery breakdown (Murata, 1997; Porat *et al.,* 2004). Lemons are sensitive to both heat and cold, and limes, which are well adapted to a tropical climate, are noted for their strong sensitivity to cold (Samson, 1986; Baldwin, 1993). Each cultivar has a critical temperature for the occurrence of CI and therefore each cultivar has an optimum temperature for keeping quality depending on the duration of storage (Murata, 1997).

Pitting is one form of CI of lime and is initially seen in the rind as small defects, while more severe symptoms show as brown discolouration or sunken areas of various sizes that develop from these small injury areas; the pits may subsequently coalesce and form leathery, brown, sunken areas on the rind. Severity increases with temperatures below 10°C (50°F) and longer durations of exposure to these temperatures (Spalding and Reeder, 1983; Arpaia and Kader, 2000). Murata (1997) noted the green rind colour of lime is retained better at 4°C, but the fruits are then subject to pitting and CI, which markedly shortens their storage life.

2.6 The current understanding of the physical, physiological and biochemical basis of chilling injury in plants

2.6.1 Definition and causes of CI

Chilling injury (CI) is a physiological disorder that can reduce the quality and value of plants and their products, particularly tropical and subtropical plant species, as a consequence of their exposure to low but non freezing temperatures (Lyons, 1973; Jackman *et al*., 1988; Parkin *et a*l., 1989; Marangoni *et al*., 1996). This physiological defect is exhibited at temperatures above 0 °C, around 8°C for subtropical plant species such as citrus, avocado and pineapple and around 12°C for tropical fruits such as banana (Lyons, 1973; Sevillano *et al*., 2009). A better understanding of the physiological and

biochemical causes of injury and mechanisms of resistance is necessary to design more effective control strategies and maximize shelf-life of the plant commodities and to develop more resistant cultivars through plant breeding (Markhart, 1989; Parkin *et al*., 1989).

2.6.2 Symptoms of CI

The exposure of chilling-sensitive plant species to low but non-freezing temperatures causes the alteration of multiple metabolic processes and leads to visual symptoms and subsequently cell death if the tissue is exposed to the damaging temperature for too long (Jackman *et al*., 1988; Raison and Orr, 1990; Saltveit and Morris, 1990; Serrano *et al*., 1996; Sevillano *et al*., 2009). Exposing sensitive plant species to this damaging temperature causes a variety of symptoms including irregular and abnormal ripening, increased water loss, surface pitting and discolouration, internal browning, breakdown of tissue, off-flavours, an increase in CO_2 and ethylene production. These symptoms may occur during low temperature storage or more commonly after transfer to a warmer temperature (Lyons, 1973; Cabrera and Saltveit, 1990; Saltveit and Morris, 1990; Lelièvre *et al*., 1995; Serrano *et al*., 1996). However, if the tissue is not stored at low temperatures for a long period, then many of these symptoms can be reversed by returning the tissue to non–chilling temperatures before damage occurs (Raison and Orr, 1990; Saltveit and Morris, 1990).

2.6.3 Different factors influencing CI

The problem of CI is not simple because the mechanisms involved in this disorder differ in different tissues. There are several intrinsic qualities of the tissue (e.g. species, cultivar, type of plant organ, developmental stage, growing conditions) and extrinsic qualities of the environment such as time and temperature interaction, relative humidity, composition of the atmosphere and postharvest treatments that affect the significance of CI (Lyons, 1973; Saltveit and Morris, 1990; Sevillano *et al.,* 2009).

2.6.4 Original hypothesis

Several hypotheses have been proposed to clarify the mechanisms of CI (Saltveit and Morris, 1990; Serrano *et al*., 1996). However, the exact mechanisms of this disorder and

its effects are not completely understood (Sevillano *et al*., 2009). The original hypothesis was proposed by Lyons, (1973) and cited phase transition changes of cell membranes as the primary cause of chilling-sensitivity. For several years CI was thought to be a direct consequence of the transition of lipids in cell membranes from a liquid to a gel state, occurring at a critical temperature, that led to a complete loss of permeability control (Lyons, 1973; Marangoni *et al*., 1996). The phase transition temperature of pure lipids or lipid mixtures is determined to a large extent by the fatty acid composition on the glycerol backbone of phospholipids. The greater the unsaturation of the fatty acids, the lower the phase transition temperature. Therefore, membranes that contain highly unsaturated fatty acids tolerate lower storage temperatures than membranes with more saturated fatty acids (Markhart, 1989). However, this suggestion is now regarded as an oversimplification (Parkin *et al*., 1989; Shewfelt and Erickson, 1991; Marangoni *et al*., 1996).

2.6.5 Ir- and reversible concepts

The membrane phase change theory has been refined in more recent years. It is still believed that a phase change in the cell membranes is perceived as a sign of chilling. However, the tissue can adaptively alter its membrane composition and lower the sensitivity threshold by increasing the proportion of unsaturated lipids. Once the threshold of sensitivity is passed, the tissue may respond with adaptive changes (e.g. increased antioxidant activity); but if the chilling stress is sufficiently prolonged, then irreversible deterioration will result. Exposure of the tissues to a short chilling temperature followed by a warmer temperature may allow the tissues to repair any damage and increase its protective systems, enabling the fruit to ripen normally. An increased respiration rate may be a sign of the tissue is elevated metabolic rate as it recovers from a disrupted metabolism (Sevillano *et al*., 2009). Fruits can recover after chilling if the threshold of irreversibility has not been reached. This reversibility is apparently lost at some stage once some secondary events have occurred (Platt-Aloia and Thomson, 1987; Marangoni *et al*., 1996). The irreversible phase of the reaction may cause permanent and extensive CI symptoms (Marangoni *et al*., 1996). These effects are the basis for the “intermittent warming” technique, fresh commodities are cycled periodically between cold and warm temperatures. IW can ameliorate chilling effects in some fruits (Lyons, 1973).

2.6.6 Revised hypothesis – the primary and secondary responses

A revised form of the Lyons hypothesis suggested that the physical properties of membranes can be affected by lipid composition which, in turn, can modulate the properties of bound proteins (Shewfelt and Erickson, 1991). This hypothesis has further evolved and there is general agreement that CI consists of primary and secondary events. The primary response occurs at the critical chilling temperature, it causes a dysfunction of the tissue, and may lead to damage because of secondary responses (Raison and Orr, 1990; Shewfelt and Erickson, 1991). The primary response is generally attributed to lipid phase changes in cellular membranes of sensitive species when exposed to cold conditions. This primary event would dispose susceptible tissue to the development of secondary events, a cascade of deteriorative reactions, resulting in loss of turgor, electrolyte leakage, imbalance of metabolism, disintegration of the photosynthetic systems including the generation of reactive oxygen species (ROS), cell autolysis and even cell death (Lyons, 1973; Parkin *et al*., 1989; Sevillano *et al*., 2009).

2.6.7 Differences between the chilling sensitive and chilling tolerant plants and specific examples

The difference between chilling sensitive and chilling tolerant plants is found in the capacity of cell membranes to resist or adapt to the phase transition, from a liquid crystalline state to a solid gel phase, that occurs when plants are placed in chilling temperatures. The transition temperature at which this lipid phase transition occurs depends mainly on the level of desaturation of fatty acids in membrane lipids (Sevillano *et al*., 2009). Lyons *et al*., (1964) speculated that there is a relationship between the physical nature of cellular membrances and chilling sensitivity in plants. They found that the chill-resistant plants had a greater degree of lipid unsaturation in mitochondrial membranes than the chilling-sensitive plants. Many researchers have noted that plants that acclimate to chilling temperatures show an increased degree of fatty acid unsaturation in their membranes (Markhart, 1989; Marangoni *et al*., 1996).

Lower phase transition temperatures were observed in microsomal membranes of chilling-resistant tomato fruit and leaf membranes when compared to the chilling-sensitive ones. This suggests that the chilling-tolerant species had a lower phase transition temperature, leading to a higher resistance towards chilling than the sensitive species

(Marangoni and Stanley, 1989). In other words, this may suggest that chilling resistant membranes maintain their liquid-crystalline phase at a lower temperature than the chilling-sensitive species (Marangoni *et al*., 1996). Similarly, it was noted that peach fruit (*Prunus persica* L.) stored at 0 °C manifested higher membrane lipid fluidity and higher membrane lipid unsaturation than fruit stored at 5 °C. The higher membrane lipid unsaturation in peach fruit stored at 0 °C was beneficial in enhancing chilling-tolerance (Zhang and Tian, 2009).

However, the degree of unsaturation does not always correlate with chilling resistance. (Markhart, 1989) demonstrated that the chilling sensitive soybean had more unsaturation than the chilling-resistant broccoli. They suggested that unsaturation is an important step to acclimate the plant species to growth at chilling temperatures but unsaturation is not the only criterion for determining chilling resistance. Furthermore, Wilson, (1983) reported that there was no difference in the degree of fatty acid unsaturation between water-stressed *Phaseolus vulgaris* L. and the non-water stressed plants. However, the water stressed plants had a greater chilling tolerance than the non-water stressed plants (Markhart, 1989). This leads to a suggestion that the fatty acid unsaturation is associated to chilling tolerance but other factors e.g. reactive oxygen species, plant hormones, are also necessary to be considered.

2.6.8 Reactive oxygen species (ROS)

Changes in lipid composition of the membrane during chilling temperatures are being among the other most significant forms cold damage such as an increase in electrolyte leakage, lipid phase transitions at critical temperatures, shown in Arrhenius diagrams. The changes in the lipid composition of the membrane during cold storage are similar to the lipid composition changes during senescence (Marangoni *et al*., 1996; Sevillano *et al*., 2009). CI may be a form of senescence breakdown related to the degradative processes of uncontrolled phospholipid hydrolysis and lipid peroxidation which are associated with senescence of plant tissues (Parkin *et al*., 1989). Chloroplasts and mitochondria are considered as major sources of active oxygen species production (Lim *et al*., 2009).

Plant chloroplasts are the photosynthetic apparatus of the cell. They contain chlorophyll and the photochemical apparatus for converting light into chemical energy (Wills *et al*.,

1998d). These organelles are particularly abundant sources of ROS because of the highly energetic reactions of photosynthesis and the abundant supply of oxygen that results (Mittler, 2002; Sevillano *et al*., 2009). Damage to chloroplasts by chilling generally occurs before damage to mitochondria (e.g. in tomato, Moline, 1976; Cheng and Shewfelt, 1988). If the primary response to chilling is the phase transition in chloroplast and mitochondrial membranes, this directly leads to the secondary response, an increase in reactive oxygen species (ROS) and consequential oxidative stress (Lyons and Raison, 1970; Scandalios, 1993; Sevillano *et al*., 2009).

If the plants cannot counteract this abnormal upsurge of ROS, a range of oxidative processes follows: lipid peroxidation, protein oxidation, enzymatic activity inhibition and damage occurring to DNA and RNA; leading to membrane deterioration and even cell death (Scandalios, 1993; Mittler, 2002; Sevillano *et al*., 2009). The harmful effects of ROS in plants can counteracted by a complex antioxidant system including metabolites such as ascorbic acid, glutathione, α tocopherol and β-carotene and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Vierling and Kimpel, 1992; Mittler, 2002; Rivera *et al*., 2004; Rivera *et al*., 2007; Sevillano *et al*., 2009). In chilling tolerant tissues, antioxidant systems have the ability to remove toxic oxygen species before lipid peroxidation occurs (Wise and Naylor, 1987).

An example of a result consistent with the concept of antioxidant enzymes being protective to CI has been reported in Mexican limes. Rivera *et al*., (2007) studied the membrane damage of Mexican limes by determining the level of lipid peroxidation in the frozen flavedo tissues of non-conditioned and cold conditioned (13°C, 48 hr and 85% RH) fruits after storage at 4, 10 and 25°C (~90% RH). The results showed that the non-conditioned fruits showed higher levels of lipid peroxidation compared with conditioned fruits stored at the same storage temperatures. This cold-conditioning treatment has the ability to reduce CI symptoms by inducing peroxidase activity and maintaining the activity of SOD. The authors suggested that the induction and maintenance of antioxidant enzymatic systems throughout the storage period influences the effectiveness of this cold-conditioning regime in enhancing chilling resistance in Mexican limes.

Earlier work by the same authors showed that HWD (53°C for 3 min) caused a significant increase in activities of peroxidase and SOD in the flavedo of the fruits immediately after treatment. However, the enzyme activities diminished rapidly during storage and HWD was not effective in reducing CI when fruit was stored at 4 or 8°C (Rivera *et al*., 2004).

2.6.9 Effects of hormones to CI:

2.6.9.1 Polyamines

The effects of plant hormones and growth regulators on the development of CI can be beneficial or detrimental depending on the plant organ, the developmental stage, growth and the temperature. Polyamines (PAs), ethylene and abscisic acid (ABA) are the compounds that seem to play a more important role in the development of CI. PAs such as the diamine putrescine (Put), the triamine spermidine (Spd) and the tetramine spermine (Spm) are ubiquitous in plant organisms. Both endogenous and exogenous PAs have been shown to have an antisenescent function (Galston and Sawhney, 1990; Valero *et al*., 2002; Sevillano *et al*., 2009). One of the main effects of PAs in fruit is to reduce chilling symptoms (Valero *et al*., 2002). PAs are also reported as effective scavengers for removal of free radicals (Drolet *et al*., 1986; Sevillano *et al*., 2009). However, the protective mechanism by which they reduce CI has not been completely determined. It seems that their capacity to associate with membrane phospholipids could play a significant role against CI (Sevillano *et al*., 2009). Furthermore, a relationship between ethylene and PAs has been reported during fruit ripening and senescence. PA biosynthesis appears to compete with ethylene biosynthesis because they share steps in their biosynthetic pathways (Smith, 1985; Galston and Sawhney, 1990; Valero *et al*., 2002; Sevillano *et al*., 2009). In higher plants, the amino acid methionine (MET) is the initial step for ethylene synthesis. This compound is converted to S-adenosylmethionine (SAM) by the addition of adenine and then SAM is changed to 1-aminocyclopropane carboxylic acid (ACC) by the enzyme ACC synthase (Saltveit, 1999). On the other hand, Spd and Spm are synthesized in plants by the addition of an aminopropyl moiety onto Put and Spd, respectively. This aminopropyl moiety results from the decarboxylation of SAM by the enzyme S-adenosylmethionine decarboxylase (SAM dec.). The PA and ethylene biosynthesis are associated to each other as they share this common precursor (SAM) therefore the biosynthesis of PAs and ethylene is competitive (Serrano *et al*., 1996; Bouchereau *et al*., 1999).

2.6.9.2 Ethylene

Ethylene is a natural plant hormone that has numerous effects on the growth, development and storage life of many fresh commodities at micromolar concentrations (Saltveit, 1999). The role of ethylene in CI development is complex because it may alleviate or potentiate CI depending on the crop (Lafuente *et al*., 2005). Ethylene production may be enhanced at sub-optimal temperatures (Field, 1990). Low temperatures are associated to the interruption of normal fruit ripening (Jackman *et al*., 1988). It is wildly recognized that low temperature stimulates subsequent autocatalytic ethylene production at warm temperatures. The increase in ethylene production is a result of the accumulation of ACC at low temperature (Wang and Adams, 1980; Yang and Hoffman, 1984; Jackman *et al*., 1988; Lelièvre *et al*., 1995).

CI development is accompanied by increased ethylene production in many chilling sensitive plants (Wang and Adams, 1980; Jackman *et al*., 1988; Sevillano *et al*., 2009). Granny Smith apples (*Malus domestica*, Borkh) showed a sharp increase in ethylene production during storage at 4°C after 5 days whereas the fruits stored at non-chilling temperature (17°C) showed the increase in ethylene production after 35 days. The authors reported that ACC oxidase is induced during chilling of pre-climacteric Granny Smith apples (Lelièvre *et al*., 1995). Exposure of some pear fruit cultivars, such as Bosc, Passe Crassane, D’Anjou to low temperature is absolutely required for normal ripening of the fruit on the tree. These fruits required the onset of ethylene production after cold storage to ripen autonomously after subsequent rewarming (Wang *et al*., 1971; Lelièvre *et al*., 1997). Lelièvre *et al*., (1997) reported that a burst of ethylene production in Passe Crassane pears is associated with high activity of ACC oxidase and ACC synthase upon rewarming. From these reviews, this suggested that rewarming the fruits induce the burst of ethylene production of the stored produce at low temperature and led to the acceleration of ripening. Therefore, the IW also accelerates ripening during higher temperature periods. IW may allow repair of organelles, membranes and/or metabolic pathways before irreversible damages occur, metabolizing excess toxic compounds that have accumulated during cold periods and restoring any essential substances that were not able to be synthesised during chilling (Wade, 1979; Jackman *et al*., 1988; Wang, 1990) and this may lead to a greater resistance to CI if the optimum IW is implemented. Exposing sensitive fruits periodically between warm and cold temperatures before the

fruits suffer from chilling has been found to delay the onset of CI in many commodities (Lyons, 1973; Jackman *et al.*, 1988; Wang, 1990).

Many researchers have reported that chilling stress stimulates ethylene production in non-climacteric fruits. In cucumbers, the storage temperatures at 2.5°C accelerated the ACC and ethylene production (Wang and Adams, 1980). In eggplants, the membrane damage was found in the fruits stored at chilling temperature (0°C). This cold stress stimulated ACC and ethylene accumulation and their high levels were remained until CI symptoms became severe (Concellón *et al.*, 2005). Low temperatures appeared to impair the conversion step between ACC, the metabolic precursor of ethylene, and ethylene. The abnormal ripening of many fruits partly after being placed in a cold store for long periods of time can be explained by this process (Jackman *et al*., 1988).

2.6.9.3 Abscisic acid

Regarding another plant hormone, abscisic acid (ABA) has been involved in the responses of plants to chilling stress and the increase in chilling tolerance in several plant species (Markhart, 1989; Sevillano *et al*., 2009). The endogenous ABA level in chilling sensitive plants increases when the plants are exposed to chilling temperatures (Daie and Campbell, 1981; Markhart, 1989; Pardossi *et al*., 1992; Anderson *et al*., 1994; Janowiak *et al*., 2002). Application of ABA to chilling sensitive species before, during or even shortly after a cold storage treatment has been revealed to protect against CI (Markhart, 1984; Pardossi *et al*., 1992; Anderson *et al*., 1994; Prasad *et al*., 1994; Janowiak *et al*., 2002). An accumulation of ABA during low temperature storage has been observed in tolerant plant species. ABA appears to be associated with the induction of CI resistance by regulating stomatal closure, thus minimising dehydration, a typical symptom of chilling stress e.g. in maize seedlings (Markhart, 1984; Janowiak *et al*., 2002; Sevillano *et al*., 2009). However, the physiological mechanism by which ABA reduces CI is not completely known. The regulation of stomatal closure prior to low temperature storage in order to prevent dehydration which is a main symptom of CI seems to be a key factor (Sevillano *et al*., 2009).

2.6.10 Protective mechanism

2.6.10.1 Transgenic

Transgenic research it was possible to increase chilling tolerance in transgenic tomatoes using cold–responsive transcription factors like DRE binding factor from tobacco. The transcription factor overexpression led to an increase in expression of a range of genes including catalase, which is a powerful antioxidant that increases chilling tolerance. This suggests that in tolerant tissue, there may be natural production of these transcription factors (Sevillano *et al*., 2009).

2.6.10.2 Heat shock proteins (HSPs)

Living organisms respond to high temperatures and other abiotic stresses (e.g. cold shock, ethanol, heat shock, osmotic shock, or salinity) by producing heat shock proteins (HSPs) (Vierling, 1991; Vierling and Kimpel, 1992; Sabehat *et al*., 1996; Sevillano *et al*., 2009). These HSPs appear to play a protective role as molecular chaperones (Vierling, 1991; Saltveit, 2005). HSPs assist to protect against stresses by controlling the proper folding and conformation of both structural (i.e. cell membrane) and enzymatic proteins (Vinocur and Altman, 2005; Sevillano *et al*., 2009). Exposure of plant tissues to a number of abiotic stresses before chilling increases chilling tolerance because of the accumulation of HSPs (Saltveit, 1991; Sevillano *et al*., 2009). Since low temperatures can alter the solubility and folding properties of many proteins, this chaperone activity play an important role for protection against chilling (Vierling, 1991; Saltveit, 2005; Sevillano *et al*., 2009). Intermittent warming may also act as an abiotic stress and induce HSPs. Further details about heat shock proteins and intermittent warming are described in section 5.6 (overall discussion of chapter 5 in this thesis).

2.7 Rots

Citrus species are susceptible to many diseases caused by different types of pathogens such as fungi, bacteria, viruses, viroids, phytoplasmas, spiroplasmas, and nematodes (Mukhopadhyay, 2004a). Postharvest decay is often the major factor limiting prolonged storage (Schirra *et al.,* 2000) and in particular, fungal diseases are a major cause of damage and losses in harvested fruit (Davies and Albrigo, 1994). Giudice, (2002) reported the major postharvest fungal diseases of citrus are: green mold (*P. digitatum*

Sacc.), blue mold (*P. italicum* Wehmer), sour rot (*Geotrichum candidum* Link ex Pers), gray mold (*Botrytis cinerea*), Alternaria rot (*Alternaria citri*) and brown rot (*Phytophthora citrophthora and Ph. parasitica*). Giudice (2002) reported typical symptoms of each of these fungal diseases. For example, in green and blue mold infections the infected surfaces are first covered with a white mold which then turns green or blue in the advanced stage of infection. In contrast, gray mold begins with some areas of the infected fruits showing yellowish brown to dark brown, after that a white mold occurs which later changes to a characteristic gray colour. In sour rot the symptom starts with a yellow cream spot, then the underlying tissue becomes soft and an acid odour is given off from the rotting fruit. Barkai-Golan, (2001) reported that the green (*P. digitatum*) and blue mold fungi (*P. italicum*) are the main wound pathogens causing the most common and devastating diseases after harvest in citrus fruit. They suggested the sour rot fungus (*G. candidum*) is usually less important although it is particularly important after extended storage in wet seasons. Giudice (2002) also noted that fruit infection by *Botrytis* is characterized by some areas of the rind turning from yellowish brown to dark brown at first, a white mould then appears which changes to a gray colour later. Tournas and Katsoudas (2005) reported that *Penicillium* caused a soft rot which firstly showed as a light-coloured patch with obvious sparse mycelium; after several days the lesions were covered with green or bluish conidia and had a strong off odour.

The incidence of rots is likely to be exacerbated by CI. Wills *et al.,* (1998b) reported that the release of metabolites, for example amino acids, sugars and mineral salts from cells suffering from CI, together with the degradation of cell structures, provides an excellent source of substrates for the growth of pathogenic organisms, especially fungi.

2.8 Heat injury (HI)

Fruit ripening and senescence can sometimes be delayed by heat treatment (see below) but fruit can also be damaged by these postharvest heat treatments. Heat treatments influence both major physiological processes (respiration rate and ethylene production rate) and gene expression more broadly. The extent of change in fruit ripening depends on the time and temperature of exposure and how fast the product is cooled following the heat treatment (Lurie, 1998; Paull and Jung Chen, 2000; Vicente *et al.,* 2006). Sensitivity of fruit to heat treatments is also modified by preharvest weather conditions, cultivar, rate

of heating and subsequent storage conditions. The tolerance to heat stress is related to the level of heat protective proteins at harvest and the production of heat shock proteins after harvest (Paull and Jung Chen, 2000). However, heat damage in plant tissue can occur and may be both external and internal (Lurie, 1998). For example, external symptoms of heat damage evident as scald damage (surface browning) in 'Eureka' lemons (*C. limon*) occurred after HWD at 53°C for 3 min (McLauchlan *et al.,* 1997) or brown sunken lesions on the flavedo developed within 24 h at room temperature (22°C) after lemon (*C. limon* (L.) Burm.) were dipped in 55°C water for 5 min; the induced rind injury could be detected with chlorophyll fluorescence imaging technique (Obenland and Neipp, 2005). In other crops, HI may appear as peel browning in apples (Klein and Lurie, 1992b), 'Fantasia' nectarines (Lay-Yee and Rose, 1994) and avocado (Woolf and Laing, 1996), and pitting in grapefruit (Miller *et al.,* 1988) or degreening of the peel of zucchini (Jacobi *et al.,* 1996) and cucumber (Chan and Linse, 1989). Similarly internal heat damage can manifest as poor colour development and abnormal softening in papaya (An and Paull, 1990; Paull, 1995).

The symptoms of heat damage and CI are similar and can be confused if the heat treatment is applied to produce which is then stored at low temperature (Lurie, 1998). Ghasemnezhad *et al*., (2008) reported that some citrus fruit appear quite sensitive to heat. For example 'Satsuma' mandarins treated with HWD at 45, 47.5, 50, 52.5 and 55°C for 2 and 5 min showed heat damage in the form of rind browning. The authors also observed that temperatures higher than 50°C increased fruit peel damage of this citrus fruit. Schirra *et al*., (1997) reported that HWD at 53°C for 3 min before storage of 'Tarocco' oranges at 3°C for 10 weeks plus 1 additional week of shelf life at 20°C caused severe heat damage to the peel in fruit picked both earlier and later in the season. Schirra and D'hallewin, (1997) also noted that HWD temperatures higher than 54°C induced heat damage in the form of rind browning to 'Fortune' mandarins after 30 days of storage at 6°C plus 3 days at 20°C.

2.9 Techniques for extending storage life of citrus fruit

2.9.1 Introduction

As noted, the storage life of limes can be limited by many factors such as degreening, disorders such as pitting and CI, or rots. Temperature and storage duration affect colour development in citrus. The rate of degreening of lime is slower when the fruit are stored at low temperatures (Kluge *et al.,* 2003a; Kluge *et al.,* 2003b) and low temperature storage can also minimize decay (Ladaniya, 2004).

The possible strategies to extend citrus (and specifically lime) storage life are common to other fruit crops and include:

1. Application of fungicide (e.g. thiabendazole (TBZ))

   TBZ is used to control fungal diseases of many oranges such as *C. sinensis* Linn. Obsek cv. Salustiana and grapefruits (*C. paradisi* Macf.) cv. Star Ruby and Marsh seedless (Schirra *et al.,* 1998; Cabras *et al.,* 1999).
2. Application of gibberellic acid (GA_3)

   Pre and postharvest application of GA_3 can delay the maturation and senescence of orange fruit (Coggins *et al.,* 1969).
3. Prestorage heat treatments

   Heat treatment may improve resistance to CI and also reduce pathogen development in citrus (Ben-Yehoshua *et al.,* 1995; Rodov *et al.,* 1995; Porat *et al.,* 2000; Lurie *et al.,* 2004).
4. Continuous low temperature storage
5. Modified and controlled atmosphere

   Optimal CA conditions have been shown to be very effective in inhibiting the ripening of some fruits such as apples and pears (Brecht *et al.,* 2003), and generally inhibiting storage disorders and decreasing susceptibility to decay in a wide range of fresh produce (Kader, 2003a).
6. Variable temperature storage

   CI in citrus fruit can be alleviated by temperature conditioning (TC) and intermittent warming (IW), linked with treatment with thiabendazole and benomyl (benzimidazole fungicides), imazalil and sucrose fatty acid ester, and by film-packaging (Murata, 1997).

Each of these techniques is described more fully below.

2.9.2 Prestorage treatment

2.9.2.1 Application of fungicide

The fungicide thiabendazole (TBZ) is well known to control a wide range of fungal diseases of citrus fruit, and may also reduce CI during cold storage and shelf life (Schirra and Mulas, 1995b). When tested on limes, fungicides such as thiabendazole (0.5%), benlate (0.05%) and bavistin (0.05%) were equally effective against rots caused by *P. digitatum* and *P. italicum* (Verma and Tikoo, 2003).

The efficacy of TBZ on fungal diseases and CI was also increased when it was used in combination with hot water (Schirra and Mulas, 1995b). Similarly Schirra *et al*., (2000) reported that heat treatment in combination with agrochemicals has been researched in recent years to control postharvest decay of horticultural products. For example, Schirra *et al*., (1998) dipped 'Tarocco' oranges for 3 min with and without TBZ at 200 ppm in water at 50°C, or with and without TBZ at 1,200 ppm in 19°C water (room temperature). The fruit were stored at 3°C for 6 weeks, followed by 1 additional week at 20°C. They concluded that hot water dipping at 50°C reduced CI. The application of the fungicide at 50°C proved to be more effective in reducing CI symptoms than its application at 19°C.

However Rodov *et al*., (1995) reported that the use of fungicides (imazalil or thiabendazole added alone at 1,000 ppm) in HWD (53°C, 2-3 min) did not increase the chilling protective effect for four citrus fruits, namely grapefruit (*C. paradisi* Macf., cv. March), lemon (*C. limon* Burm., cv. Eureka), oroblanco (*C. grandis* Osb. x *C. paradisi* cv. Oroblanco, syn. Sweety) and kumquat (*Fortunella margarita* Swingle cv. Nagami), compared with HWD for the same conditions without fungicide. However the fungicide did protect fruit against decay. Furthermore, the authors also observed that the risk from heat damage of fruit during the hot water dip may be increased when fungicides are added.

2.9.2.2 Application of gibberellic acid (GA_3)

There are five major categories of "classical" phytohormones, namely auxins, gibberellins (GAs), cytokinins, ethylene and abscisic acid (ABA). Generally these phytohormones affect plant growth and development by affecting the division, elongation and differentiation of the plant cells. GAs have been known as one of the "classical five" plant growth regulators (PGRs) since 1937 and more than 112 GAs had been identified by 1998 (Vivanco and Flores, 2000).

GA_3 has been used for both pre- and postharvest application in many crops. Preharvest GA_3 application reduced postharvest decay in nectarine fruits (Zilkah *et al.,* 1997) and also decreased cherry pitting (Looney and Lidster, 1980). Postharvest application of GA_3 is also reported. For example peaches treated with putrescine and GA_3 maintained higher fruit firmness during storage and the respiration rate and ethylene emission were reduced compared to the control (Martinez-Romero *et al.,* 2000). Postharvest GA_3 at 200 mg l^{-1} delayed 'Mallika' mango ripening, retarding chlorophyll degradation in the peel (Khader, 1992). As examples of its use in other crops, GA_3 or CO_2 treatment also retarded chlorophyll and protein degradation, amino acid accumulation and respiration rate in parsley (Lers *et al.,* 1998) and GA_3 use resulted in additive beneficial effects on ripening inhibition of Chinese jujube (Jiang *et al.,* 2004).

The effects of GA on the postharvest physiology of citrus fruits have not been extensively studied and overall relatively little is known about their mode of action on the postharvest physiology of fruits (Valero *et al.,* 1998). Postharvest dips in GA_3 (50 and 100 ppm) inhibited the decay development of stored lemon fruit and slowed the decline of citral (an antifungal compound formed in lemon fruit and exerting inhibitory activity against common postharvest pathogens) content, and of the overall antifungal activity in the flavedo tissue, for 90 days (Ben-Yehoshua *et al.,* 1995). Vacuum infiltration of 'Verna' lemons at two ripening stages with 100 ppm of GA_3 delayed senescence and retarded colour changes, especially in stage 1 (colour break) fruit. These fruit maintained lower levels of ABA during storage at 15°C for 3 weeks and exhibited increased fruit firmness, either in stage 1 or in stage 2 (uniform yellow colour) (Valero *et al.,* 1998). When applied as postharvest dip treatments at 100 mg l^{-1} for 20s GA_3 effectively retained green

'Oroblanco' citrus fruit colour, followed by storage at 2°C for 4-5 weeks (Porat *et al.,* 2001).

2.9.2.3 Heat treatment

Consumer demand, government regulations and environmental concerns have all promoted research into improving non-chemical procedures for improving storage life of horticultural produce (Schirra and D'hallewin, 1997). Prestorage heat treatments offer one such opportunity to reduce postharvest losses as an alternative or complement to appropriate storage regimes (Couey, 1989; Klein and Lurie, 1992a; Schirra *et al.,* 1996). There are two types of responses of fresh commodities to heat. The first response is associated with production of heat shock proteins (HSPs) and can lead to reduced chilling sensitivity, and delayed or slowed modification of quality and ripening; this is a normal cellular response induced by lower heat treatments, usually below 42°C. However, higher temperatures may provide a similar response providing the exposure time is short. Factors including preharvest conditions, species, cultivars, ripening stage, times and temperatures of exposure and variations of diurnal temperature modify this response. The second response is proposed to arise above the threshold for damage (typically >45°) and happens when a stress threshold is exceeded and the ability of cell to restore its normal function can be lost. Heat shock mRNAs may be poorly induced and there can be increased damage to proteins and membranes. If this response exceeds the injury threshold this will slow or prevent adequate repair processes in the cells and lead to necrosis (Paull and Jung Chen, 2000).

Heat may be applied to horticultural produce as hot water dips, vapour heat, hot dry air or by hot water rinsing and brushing (Schirra *et al.,* 2000; Fallik, 2004). Heat treatments may improve the resistance of citrus fruit to CI (Rodov *et al.,* 1995; Porat *et al.,* 2000) and inhibit or reduce pathogen development in citrus fruit (Ben-Yehoshua *et al.,* 1995; Lurie *et al.,* 2004). Williams *et al*., (1994) used hot water immersion at 45°C (core temperature) for 42 min for Valencia oranges and they claimed that the fruit were firmer than the control treatment after storage at 0°C and showed increased colour development. However, this seems a rather extreme and risky treatment. Hot water treatment by immersion of the citrus fruit at 52-53°C water for 2 min is recommended to control *P. digitatum* rots in lemons (Nafussi *et al.,* 2001) and hot water treatment at 53°C for 3 min

was deemed best for mid-season (January-February) 'Tarocco' oranges (Schirra *et al.,* 1997). A positive effect of HWD on reducing CI in 'Fortune' mandarins stored at 2°C for 45 days was found when fruit were dipped at 47°C for 6 min or 53°C for 3 min. These HWD treatments also reduced rots during storage of mandarins (Gonzalez-Aguilar *et al.,* 1997). HWD at 50°C for 2 min reduced chilling injury up to 60% in Marsh grapefruit during storage at 1°C (Wild, 1993).

2.9.3 Post-harvest techniques for improving storage life

2.9.3.1 Low temperature storage

Temperature is the most important factor for maintaining the quality of fresh products such as fruit, vegetables and ornamentals. The quality of fresh commodities declines after harvest because they are living tissues which continue to respire (Kitinoja and Kader, 1995; Wills *et al.,* 1998a), but product quality can be maintained by optimal temperature control. All plant processes are expected to be influenced by temperature (Pearce, 1999). The relationship between change in colour and change in temperature is not simple. For example, carotenoids synthesis occurs readily at 15-20°C (Ladaniya, 2004) but is impaired above 35°C or below 15°C. Similarly, chlorophyll breakdown is slowed by low temperature storage, but when citrus fruit are grown in the tropics, they often stay green because chlorophyll breakdown is not triggered in fruit which are constantly warm (Davies and Albrigo, 1994). In the case of chilling-sensitive products as long as the fruit is stored at low temperatures above the chilling threshold temperature, the processes associated with deterioration, for example respiration, firmness change and vitamin C loss, are all reduced compared to ambient storage (Wills *et al.,* 1998a).

Optimal storage temperatures vary among species, varieties and plant parts. Quality changes are hastened at higher temperatures and lower temperatures can cause CI (Hatton, 1990). For example, storage of a tangor citrus fruit cultivar 'Ortanique' ('Topaz') at 13°C led to fast deterioration whereas the fruit suffered from CI when stored at 2°C (Cohen *et al.,* 1990b). For lime, the recommended low storage conditions for both 'Tahiti' and 'Mexican' limes range from 7.2 to 12.2°C and 85-90% RH (Thompson, 2003). The lowest safe storage temperature for lime is claimed to be 7°C (Wills *et al.,* 1998b).

2.9.3.2 Modified atmosphere (MA) and controlled atmosphere (CA) storage

Storage life of horticultural products can be significantly affected by the composition of the storage atmosphere. Changes in the concentrations of the respiratory gases (O_2 and CO_2) may prolong storage life (Wills *et al.,* 1998c). O_2 and CO_2 are biologically-active molecules of importance in primary and secondary metabolic processes in plants. Changes in the availability of these gases influence plant behaviour and their metabolism, resulting in extending storage duration and shelf-life under certain circumstances (Beaudry, 1999). The application of controlled atmosphere (CA) techniques has been very successful for apples (*Malus sylvestris* (L.) Mill.) and pears (*Pyrus communis* L.). However, the use of CA and MA storage for many other horticultural produce has not always been as successful, partly because their economic value may be too low to justify CA storage or the extension of their natural postharvest life from CA and MA technology may be relatively short (Brecht *et al.,* 2003).

MA and CA storage may be used to maintain the quality of fresh produce when refrigeration alone is insufficient to achieve the required storage time. The application of these techniques is different, but they are very similar in basic principles and their effects on the product. CA uses ‘active’ techniques to control and maintain gas composition during the storage period. The desired gas composition is initially created by either passive or active techniques during MA storage, but generally there is no active maintenance technique applied thereafter (Vigneault *et al.,* 2004).

Porat *et al*., (2004) found that the use of modified atmosphere packaging (MAP) “bag-in –box” Xtend films effectively reduced the development of CI and rind disorders that were not related to CI (such as rind breakdown, stem-end rind breakdown and shrivelling and aging) in citrus fruit. Active MAP is a technique that attempts to control the composition of the gas (O_2, CO_2 and C_2H_4) through scavenging or emitting mechanisms (Charles *et al.,* 2003). There are many forms of active MAP packaging that can be used for horticultural products. These have been reviewed by Utto *et al*., (2005) and possible applications and systems for active MAP of fruit and vegetables were reported by Charles *et al*., (2003) and Utto *et al*., (2008).

CA equipment is used to generate an atmospheric composition that is different from air (nominally 78 mol% N_2, 21 mol% O_2, and ~ 0 (0.03) mol% CO_2): generally, O_2 concentrations below 8% and CO_2 concentrations above 1% are used. CA storage can be a supplement to proper temperature and relative humidity management in preserving quality and safety of fresh commodities throughout postharvest handling (Kader, 2003b). In combination with lowered temperature, CA reduces the respiration rate by both restricting oxygen availability (the substrate) and elevating carbon dioxide concentration (the product) in the storage environment. CA has been shown to be effective in inhibiting the ripening of fruit, suppressing the production of ethylene, inhibiting certain storage disorders and slowing the growth of decay organisms.

There have been several studies of the influence of CA on citrus fruit storage and quality. Extended storage life without development of adverse flavour was obtained for lemons under 0% CO_2 and 5-6% O_2 at 10°C, Valencia oranges under 0% CO_2 and 15% O_2 at 2°C, and Oval oranges under 0% CO_2 and 10% O_2 at 10°C (Monzini and Gorini, 1973). Shrikhande and Kaewubon, (1974) found that high CO_2 (10 and 20% CO_2), when applied with 2.5 and 21% O_2 after irradiation of the fruit in air, suppressed the production of C_2H_4, reduced the loss of chlorophyll and ascorbic acid, and delayed the onset of physiological disorders in lemons during storage compared to air treatment. Wild *et al*., (1976) studied lemons (*C. limon* (L.) Burm. f.) stored at 10°C in air and CA of 3-5% O_2 and 0.1-0.2% CO_2 with and without C_2H_4 absorbent, during storage for 27 weeks. The lemons stored in CA showed high mold incidence where C_2H_4 accumulated but the development of mold was reduced when an C_2H_4 absorbent was used. CA storage also reduced the rate of green colour loss in lemons up to 27 weeks.

Artes *et al*., (1993) reported that the use of 12% O_2 and <2% CO_2 on 'Primofiori' lemons (*C. limon* Burn) at 13°C and >95% RH gave the best control of physiological disorders during 2 months of storage. They also treated the lemons with 10, 20 and 30% CO_2 for 24 hours at weekly intervals then stored the fruit at 13°C. The results showed that CO_2 treatments effectively prevented membranosis (a CI of the carpellary membranes), rind pitting and oleocellosis but did not prevent the development of Alternaria (*Alternaria citri* Ell. & Pierce) rot and red blotch disorder. Bertolini *et al*., (1991) stored 'Femminello comune' lemons fruit at a yellow-green colour stage at 0, 2, 5, 8, 10 or 12°C for 90 days and found that highest incidence of membranosis occurred at 5°C whereas the lowest

incidence of the disorders were seen at 0 or 2°C. Fruit stored at 8, 10 and 12°C had intermediate values of disorders however the fruit stored at 0 or 2°C developed rind pitting (another form of CI). This study also found that membranosis was reduced after the fruit were exposed to 40% CO_2 for 3, 6 and 9 days and then stored at 0 or 12°C. The beneficial effect of high CO_2 on membranosis increased with time. They also confirmed that CO_2 treatments reduced rind pitting of lemons.

There have been few studies of CA storage of lime fruit. Salama *et al*., (1965) found that 'Tahiti' limes stored under four CA conditions in a range of (<4-19%) O_2 and (0->30%) CO_2 between 10-21.1°C (50-70°F) increased in decay, especially from *Penicillium*. The best CA condition to control colour change was 4% O_2 and 30% CO_2 for fruit stored at 21.1°C (70°F), but decay (ranging from 20-80%) was the main problem under this storage regime. The authors also reported that the colour change of the fruit from green to yellow was slowed or arrested by increased levels of CO_2, but decreased O_2 without increased CO_2 was comparatively less effective.

Hatton *et al*., (1975) reported that 5% O_2 plus 7% CO_2 was best for 'Tahiti' limes, but under this CA treatment the fruit showed increased decay, rind scald and reduced juice content, therefore the use of CA storage on limes was not recommended. Spalding and Reeder, (1974) also reported that 'Tahiti' lime often developed injury and decay under CA storage. However, they reported that the limes stored in a CA condition at 7 or 10% CO_2 with 21% O_2 at 10°C (50°F) for 6 weeks were acceptably green and had normal rind thickness and acceptable juice content.

Sritananan *et al*., (2006) studied the effect of combinations of 3 and 5% of O_2 and CO_2 (3% O_2 + 3% CO_2, 5% O_2 + 3% CO_2, 3% O_2 + 5% CO_2 and 5% O_2 + 5% CO_2, respectively) and air on limes (*C. aurantifolia*) stored at 10°C. They found that CA storage reduced loss of chlorophyll and colour change of peel (measured as a* and b* values); furthermore, CA suppressed C_2H_4 production and respiration rate when compared to the fruit stored under air treatment. Similar to other reports, they found an increase in physiological disorders (pitting) in all treatments. Disorders were observed after 7 days in 5% O_2 and 5% CO_2 whereas the others treatments showed disorders at 14 days. The fruit showed skin injury after 1 week and this increased with time until it reached about 90% after 42 days of storage. The least disorder development was found in

the fruit stored under 3% O_2 + 3% CO_2 but these fruit still showed skin injury at 42 days of storage. They suggested that CA reduced severity of the disorder by ca. 50% compared to normal air storage.

2.9.3.3 Intermittent warming (IW)

Variable temperature treatments may offer benefits over continuous cold storage, especially with regard to chilling injury. IW is a technique that involves periodic interruption of cold storage by exposure of fruit to short periods of warming (Schirra and Cohen, 1999; Porat *et al.,* 2003; Porat, 2004). IW treatments have been applied commercially to selected crops and IW treatments could be helpful in prolonging the high quality storage life of limes (Kluge *et al.,* 2003a; Kluge *et al.,* 2003b).

Kluge *et al*., (2003a) stored limes under three different IW conditions (IW-1; 20°C for 2 days and then 5°C for 7 days, IW-2; 20°C, 2 days then 5°C for 14 days and IW-3; 38°C for 1 day then 5°C for 14 days) and compared fruit quality with a control treatment stored at 5°C. They found that their IW-1 and IW-2 treatments extended 'Tahiti' lime storage life and reduced CI to 12.5-20% after 60 days of storage whereas the control fruit stored at 5°C showed 40-58% CI. All fruit showed CI at 90 days of storage. IW-1 and IW-2 conditions did not delay colour loss when compared with the control after 60 days of storage and the IW-3 showed adverse effects, promoting increased degreening and rot development, high respiration rate and increased levels of ethanol and acetaldehyde after storage. The fruit stored under IW-1 and IW-2 were not significantly different compared with the control fruit in soluble solids content (SSC), titratable acidity (TA) and percentage of juice.

Although IW can protect against chilling-induced damage this technique has to be applied before the irreversible stage of CI occurs in the tissues (Wang, 1990; Schirra and Cohen, 1999). Alteration of temperatures from cold to warm and then from warm to cold may increase synthesis of unsaturated fatty acids during a rapid readjustment of metabolism and this may mean the tissues are more resistant to CI (Wang and Baker, 1979; Wang, 1982). The warming period may also allow the tissues to metabolize excess intermediates accumulated during the cold cycle or to replenish any substances that were not able to be synthesised. It is postulated that chilling-induced damage to tissues may also be repaired

during the warming cycle of IW (Wade, 1979; Wang, 1990). CI can be prevented in many tissues, such as fruits, seeds and seedlings, if they are returned to a warmer temperature before the occurrence of degenerative changes of the tissues (Hatton, 1990).

The benefits of IW have also been demonstrated for other citrus fruits. For example IW has been shown to reduce CI and incidence of decay development during storage in lemons (Cohen *et al.,* 1983; Cohen *et al.,* 1990a; Artes *et al.,* 1993), 'Ortanique' ('Topaz') tangor citrus fruit (Cohen *et al.,* 1990b), 'Oroblanco' citrus fruit (Porat *et al.,* 2003), 'Fortune' Mandarin (Schirra and Mulas, 1995a) and 'Olinda' oranges (Schirra and Cohen, 1999) but the optimal conditions can vary with cultivar, cultivation conditions and species (Kluge *et al.,* 2003b). This technique can be very effective. For example warming for 7 days at 13°C following 21 days at 2°C is now used commercially for 'Eureka' and 'Villa franca' lemons and the fruit can be kept in storage for 6 months or longer without CI during marketing periods (Cohen, 1988).

Another technique, called a dual temperature (shipping) regime, has been studied in South Africa for storage of four Japanese plum (*Prunus salicina*) cultivars ('Sapphire', 'Songold', 'Laetitia' and 'Angeleno') in combination with either RA (7.5°C) or CA (5% O_2 and 10% CO_2) storage. All the fruit were stored at -0.5°C for four days to simulate a commercial acclimation period. During simulated shipping, the fruit were randomly assigned and stored under RA or CA and either under dual temperature conditions (-0.5°C for 6 days and 7.5°C for 12 days) or a single mild temperature condition (7.5°C) for 18 days. Thereafter, the fruit temperature was again lowered to -0.5°C for 14 days for 'Sapphire' and 'Laetitia' or 21 days for 'Songold' and 'Angeleno' cultivars to simulate distribution and storage in the market. During the simulated shelf life, the fruit were stored at 15°C for 5 days. The results demonstrated that the storage life of all four cultivars could be extended for an additional two to three weeks under all regimes. The CA treatment with dual temperature regime retained fruit firmness the best. The lowest respiration rates, ethylene production rates and internal of ethylene content were observed in the CA fruit. Development of skin colour of the fruit was better under RA than CA shipping, and when stored under RA and also under the single mild temperature than the dual temperature condition (Mare *et al.,* 2005).

2.9.3.4 Temperature conditioning (TC) or step down technique

Prestorage temperature conditioning (TC) is another technique used in commercial practice. TC is performed by storing the fruit at progressively lower temperatures for short periods of time before continuous storage at the final (lowest) temperature (Hatton, 1990; Woolf *et al.,* 2003). Several high temperature conditioning (or curing) treatments have been studied to reduce the severity of CI on citrus. For example, curing 'Marsh' grapefruit and 'Eureka' lemons at 36°C for 3 days gave positive and similar effects compared to HWD at 53°C for 2-3 min. The authors suggested however that HWD was much easier to implement and could be combined with regular packhouse treatment procedures (Rodov *et al.,* 1995). Curing treatments at 21°C for 3 days, 16°C for 7 days and 36°C for 3 days of 'Star Ruby' grapefruit reduced CI when compared to the control fruit stored at 2°C plus an additional week at 20°C, but these treatments significantly increased fruit weight loss and enhanced peel colour changes. Curing treatments at 36°C for 3 days (under water-saturated conditions) increased decay development (Porat *et al.,* 2000). A heat-conditioning treatment at 37°C for 3 days was highly effective at increasing the CI tolerance for 'Fortune' mandarins during storage at a chilling (2°C) temperature (Holland *et al.,* 2002). Heat-conditioning (37°C, 3 days) also increased the CI tolerance of 'Navelate' oranges held at 2°C for 88 days; in contrast the control fruit stored at 2°C showed CI after 30 days of storage (Holland *et al.,* 2005).

'Oroblanco' citrus fruit were stored under RA at 2 and 11°C, IW (2°C, 3 weeks and 11°C, 1 week) and temperature conditioning (16°C, 1 week before continuous storage at 2°C) during long term storage (16 weeks). All the fruit retained their green colour up to 16 weeks, except the fruit stored at 11°C, which retained its green colour for only 8 weeks. IW reduced the amount of severe CI to only 5, 7 and 11% after 8, 12 and 16 weeks, whereas the fruit stored continuously at 2°C showed 40, 51 and 68% CI after the same periods of time, respectively. The temperature conditioning treatment effectively reduced the CI to only 5% after 8 weeks of storage, but was ineffective in reducing CI after that time. The IW and TC treatments also reduced the incidence of decay development during storage (Porat *et al.,* 2003). Temperature conditioning of California-Arizona desert lemons at 5 or 15°C for 1 week before quarantine treatment (0-2.2°C for 10-22 days) resulted in less CI than in non-conditioned fruits (Houck *et al.,* 1990).

Rivera *et al*., (2007) reported that when low temperature conditioning (13°C for 2 days) was applied to 'Mexican' lime (*C. aurantifolia* Swingle) before storage at 4°C, CI symptoms of limes were reduced. Furthermore, they observed a significant increase of activity of peroxidase and the maintenance of the activity of superoxide dismutase in limes during storage at 4°C. The authors suggested that the effectiveness of the low temperature conditioning in reducing CI in limes depended on the induction or maintenance of antioxidant enzymatic systems during the storage period.

2.10 Aims and project objectives

This review has identified several possible prestorage and postharvest methods that may prove successful for prolonging storage quality of lime fruit after harvest. Gibberellic acid application (GA_3), controlled atmosphere (CA), hot water dipping (HWD), intermittent warming (IW) or temperature conditioning (TC) have all been shown to offer some benefits for prolonging citrus storage life. However for some regimes (e.g. CA, HWD + fungicide) the literature is contradictory regarding the potential benefit for storage quality. To distinguish the relative benefits of these techniques and attempt to resolve these contradictions requires a comprehensive investigation of their effects on fruit of similar quality obtained from the same source.

The overall goals of this research were to (1) determine the optimal regimes that best retain the green colour of lime peel during storage and are also effective in protecting against fungal diseases and disorders such as CI or pitting, and (2) to characterise the influence of these regimes on nutritional quality. In order to achieve these goals the specific objectives to be investigated included:

1. To establish a baseline of the effect of storage temperature on lime quality during storage under regular air (RA).

2. To identify which other prestorage or postharvest treatments best delayed degreening and prevented CI when used in combination with low temperature storage.

3. To further investigate and optimise these selected storage regimes and to quantify their influence on fruit quality through measurement of physical and physiological quality attributes.
4. To evaluate mathematical models and other quantitative indices of colour change as possible decision support tools to assist in optimising storage regimes.
5. To determine what changes in possible health promoting compounds occur under differed storage regimes and also to identify possible early indicators of fruit injury.

CHAPTER 3

Methods and materials

3.1 Fruit supply and handling

All limes used in this research were 'Tahiti' or 'Persian' limes (*C. latifolia* Tanaka) as this is the major cultivar grown in New Zealand (Sale, 2001). The major growing regions for limes are the Bay of Plenty, Northland and Gisborne, and fruit were sourced from three orchards in the first two of these regions. The orchard location and size ranges of the fruit used in this research are shown in Table 3.1. Fruit were harvested as green as possible with a smooth skin.

Table 3.1 Orchard locations and fruit sizes for each harvest.

Year	Harvest #	Month	Season	Orchard location	Fruit size (g)
2004	1	May	Main	Bay of Plenty, orchard 1	50-85
	2	October	Late	Kerikeri, Northland	50-85
2005	3	May	Main	Bay of Plenty, orchard 2	50-85
2006	4	September	Late	Bay of Plenty, orchard 2	40-80
	5	November	Late	Bay of Plenty, orchard 2	50-85

In order to minimise variation in this research, the fruit were sourced from the same orchard in the Bay of Plenty for H3 to H5 (Table 3.1). However, the weather pattern in each year also affected the availability quality and (probably) behaviour of the fruit. The years 2004 and 2005 were judged 'normal' years by the industry as the winter temperature was not too cold, whereas in 2006 fruit experienced more severe and early chilling which promoted earlier and more pronounced yellowing than the previous years. This influenced orchardists' picking regimes and may have contributed the fruit available in September 2006 being smaller than other harvests.

Harvest 1 fruit were picked and supplied directly by the orchard whereas the fruit obtained for harvests 2-5 were picked by the researcher and other Massey University staff. These fruit were cut at the button using secateurs and transferred by car on the same day to the laboratory at The New Zealand Institute for Plant and Food Research Limited (PFR), Palmerston North. After arrival of the fruit at the laboratory, setting-up the experiments took one day. A summary of treatments employed and the preparation and handling of fruit in each harvest is shown in Table 3.2.

Table 3.2 Summary of treatments used for each harvest.

Harvest #	Harvest and transport duration (day)	Application of 100 ppm GA_3	Application of the fungicide TBZ	C_2H_4 absorbent usage
1	2	√	×	×
2	1	×	×	×
3	1	×	√ Washing, 1500 ppm, 3 min in 20°C cold water √ Hot water dipping, 200 ppm, 2 min	√
4	1	×	√ Washing, 1200 ppm, 3 min in 15°C cold water	√
5	1	×	√ Cooling down after HWD into 15°C cool water, 1200 ppm, 3 min	×

Notes: √ = The chemical or process was applied in these selected treatments only
× = Chemical or process not applied

The fruit were washed and air-dried for one night except for harvests 1 and 2 (see details for each harvest below), then each fruit was marked by a pen on each of the (initially) most green and yellow sides of the fruit for skin colour measurement during the subsequent storage periods.

3.1.1 Harvest 1 (H1)

The fruit were harvested by the orchard staff and transferred to Palmerston North by truck. The supplying fruit company was interested in the effect of GA_3 on skin colour and as this was also appropriate for the research, one batch of fruit was supplied pre-dipped in GA_3. These fruit were dipped for 1 min in a 100 ppm GA_3 solution obtained by dissolving a tablet containing 1 g GA_3 in 10 l water; the fruit were then air-dried before being packed and transported to the laboratory. All the fruit were stored in a 13°C ± 1°C cold room at PFR for one night before being placed into each treatment.

3.1.2 Harvest 2 (H2)

The fruit were not treated with any chemicals after harvest. All the fruit were stored in a 13°C ± 1°C cold room at PFR for one night before storage in different regimes.

3.1.3 Harvest 3 (H3)

The fruit were stored in a 7°C ± 1°C cold room at PFR for one night. The fruit stored under regular air (RA) and intermittent warming (IW) conditions were washed for 3 min in 20°C cold water to which the fungicide thiabendazole (Alex McDonald Merchant Limited, Christchurch) (TBZ, 1,500 ppm) was added. One further IW treatment was not treated with the fungicide. For all the HWD treatments, the fruit were dipped in hot water (52-53°C) containing TBZ at 200 ppm for 2 min. A lower concentration was used in these experiments because the efficacy of TBZ against fungal diseases is claimed to be increased when it is used in combination with hot water (Schirra and Mulas, 1995b). Some HWD treatments also had a sachet of C_2H_4 absorbent (Purafil, IPSCO, Auckland, NZ) placed into their storage chambers.

3.1.4 Harvest 4 (H4)

The fruit were stored overnight in a 10°C ± 1°C cold room at PFR. All the fruit were then washed for 3 min in cold water (15°C) to which the fungicide TBZ (1,200 ppm) was added and air-dried as previously. C_2H_4 absorbent sachets were added to some RA treatments, the temperature step down treatment, and all the IW treatments in this trial.

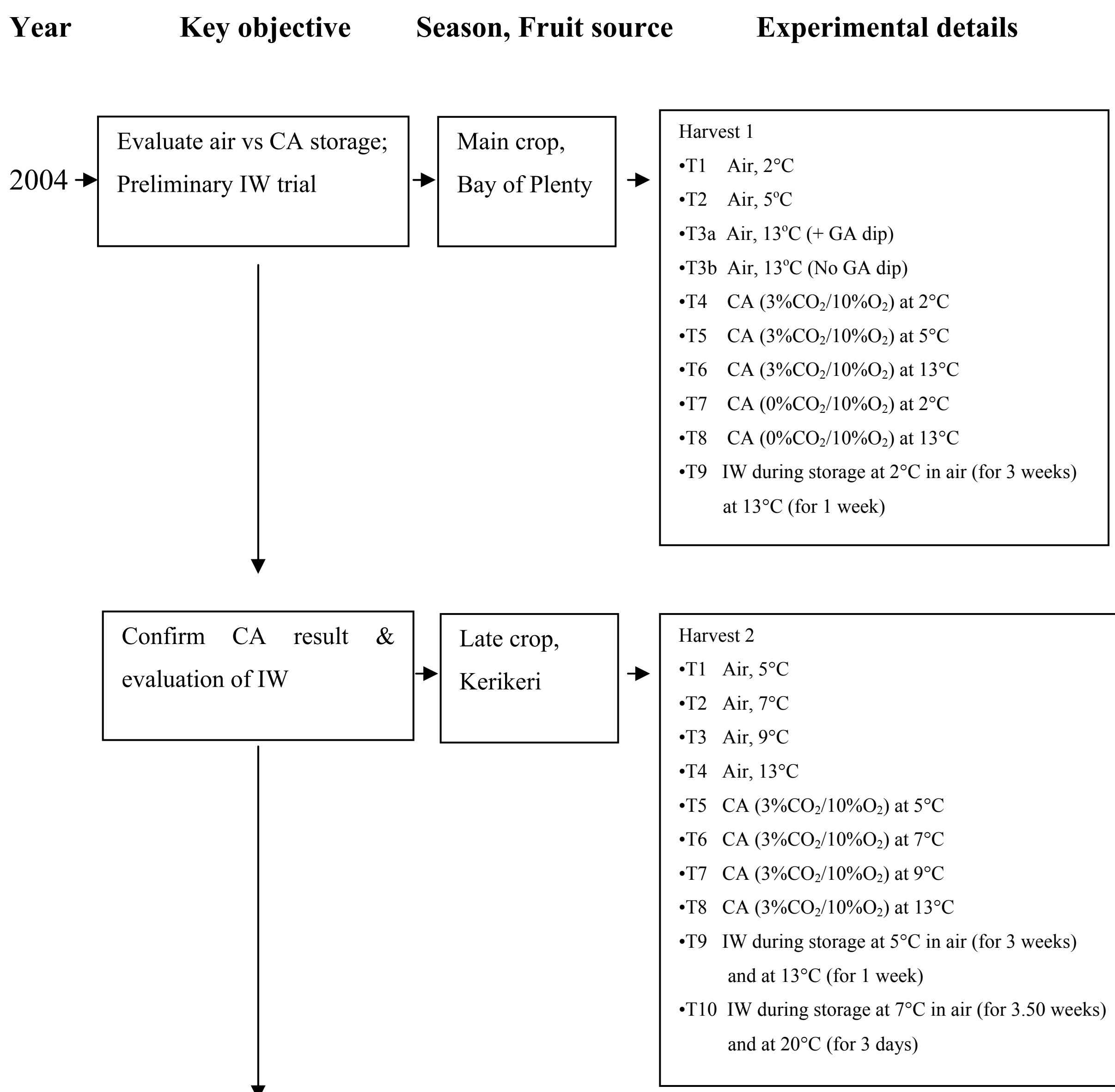
3.1.5 Harvest 5 (H5)

The fruit were stored in a 10°C ± 1°C cold room at PFR for one night. The fruit were dipped into hot water without fungicide in a water bath at a range of temperatures from 42°C to 57°C and dipping times from 2-6 min, then the fruit were cooled by dipping them for 3 min into 15°C cold water to which TBZ (1,200 ppm) was added. The fruit were again air-dried overnight. Those fruit being stored under RA at 5 and 13°C ± 1°C were dipped into 15°C cold water for 2 min before being dipped again for 3 min in cold water to which TBZ (1,200 ppm) was added; the fruit were then air-dried overnight.

3.2 Schematic overview of experimental programme

Fig. 3.1 provides an overview of the experimental programme, indicating the order in which the runs were made and the various treatments investigated.

Year **Key objective** **Season, Fruit source** **Experimental details**



2005 → Evaluate IW & HWD → Main crop, Bay of Plenty →

Harvest 3
- T1 Air, 5°C
- T2 Air, 7°C
- T3 Air, 15°C
- T4 Air, 20°C
- T5 In air at 5°C with IW at 15°C (12 days+2 days)
- T6 In air at 7°C with IW at 20°C (12 days+2 days)
- T7 In air at 7°C with IW at 20°C (3.5 weeks+3 days)
- T8 In air at 5°C with IW at 15°C (12 days+2 days, no fungicide (NF))
- T9a HWD (52-53°C, 2 min) then in air at 5°C
- T9b HWD (52-53°C, 2 min) + C_2H_4 absorbent, then stored in air at 5°C
- T10a HWD (52-53°C, 2 min) then stored in air at 5°C with IW at 15°C (12 days+2 days)
- T10b HWD (52-53°C, 2 min) + C_2H_4 absorbent, then stored in air at 5°C with IW at 15°C (12 days+2 days)

2006 → Refine IW treatments → Late crop, Bay of Plenty →

Harvest 4
- T1 Air, 5°C
- T2 Air, 5°C+ C_2H_4 absorbent
- T3 Air, 7°C
- T4 Air, 7°C+ C_2H_4 absorbent
- T5 Step down (10°C, 2 weeks), (5°C to…end) + C_2H_4 absorbent
- T6 IW 5/15°C (12 days / 2 days) + C_2H_4 absorbent (only 1 cycle)
- T7 IW 5/15°C (12 days / 2 days) + C_2H_4 absorbent (only 2 cycles)
- T8 IW 5/15°C (12 days / 2 days) + C_2H_4 absorbent (6 cycles)
- T9 IW 5/15°C (16 days / 2 days) + C_2H_4 absorbent
- T10 IW 5/15°C (20 days / 2 days) + C_2H_4 absorbent
- T11 IW 5/15°C (24 days / 2 days) + C_2H_4 absorbent

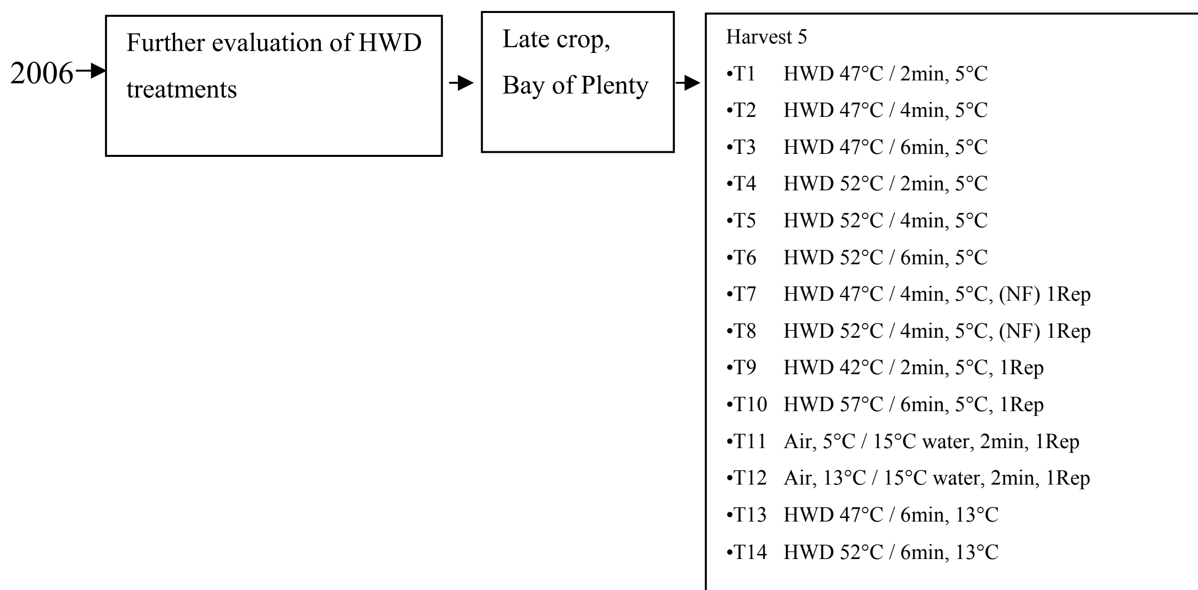


Figure 3.1 Schematic overview of experimental programme.

3.3 Storage regimes

All experiments were conducted at PFR. For each CA regime, each cold room was set up with three gas lines (polyethylene tube “LEDATHENE”, approximately 3 mm internal diameter). CA gas mixes were generated by mixing gas from cylinders of CO_2, N_2 and air (BOC Gases New Zealand Limited, Palmerston North) using a gas mixer (Fig. 3.2). Each gas concentration for each CA regime was measured by a Shimadzu model 9A TCD-GC (Shimadzu Corporation, Kyoto, Japan) and separately adjusted until the desired concentration was reached. Each mixed CA gas was then passed through a flask containing 500 ml of water to humidify the gas before continuing to each fruit storage container in a cold room. The temperature in each cold room was controlled and monitored for all storage periods for each harvest. Each container was sealed and flushed with air or the CA gas mixture at 30-40 ml min^{-1}. Flow rates were monitored with a mass flow controller (Matheson, Lyndhurst, USA) and subsequently checked throughout the experiment simply by counting the bubble rate when the tubes (closed with needles) were immersed in water. For harvests 1 - 4, the container lids were fitted with a rubber septum to enable regular sampling of the gas atmospheres.

Figure 3.2 The gas mixer used to prepare gas atmospheres for CA trials.

3.3.1 Glass jar storage system (H1)

CA systems were established in six cold rooms. This trial was conducted under three storage temperatures, each storage temperature was replicated using two cold rooms. Ten different temperature and atmosphere treatments were investigated (Fig. 3.1 and 3.3). Each treatment contained four replicates; two replicates from each treatment were placed separately into each of the two cold rooms at the desired temperature. Each treatment sample comprised four replicates of four or five fruit which were stored packed in glass jars (approximately 1 l total volume) (Fig. 3.3). Gas was introduced into the bottom of the jar via a hose fitting attached to the lid and the exit gas was removed from the top of the jar through a second fitting attached to the lid. For IW treatments, the fruit were physically moved between rooms maintained at the appropriate temperatures. Harvest 1 fruit were selected by weight and by colour (Table 3.1). Ideally I would have chosen only green fruit but I had to accept some fruit up to 75% yellow (Fig. 3.12). Fruit quality was measured after 1, 2, 4 (for selected treatments), 8, 10 and 12 weeks of storage and after 3 days of shelf life at 20°C ± 2°C at 8, 10 and 12 weeks of storage (Fig. 3.4).

Figure 3.3 Limes in CA storage using glass jars.

Figure 3.4 Limes during shelf life at 20 ± 2°C.

3.3.2 Polycarbonate box storage system (H2, H3 and H4)

For H2–4, four cold rooms were set up, one for each of the four storage temperatures. The fruit were packed as a single layer (20 fruit) in a polycarbonate box of approximately 7 l total volume. The fruit were selected by weight and by colour (Table 3.1). Trial 2 had 10 treatments; two replicates in each treatment were removed for fruit quality evaluation after 2, 4, 6 and 8 weeks of storage. The fruit were stored under RA, CA or IW conditions (Fig.3.5).

Figure 3.5 Limes in RA or CA and IW conditions during storage using polycarbonate boxes.

For H3, the fruit were selected by weight and colour to be similar to H2 (Table 3.1). Fruit stored under RA at 5, 7, 15 and 20 ± 1°C and IW conditions. Fruit were washed, dipped in fungicide (Table 3.2 and Fig. 3.6), and air-dried overnight before being placed into each polycarbonate box, as in H2 (Fig.3.5). One further IW treatment was set up (T8, Table 3.1) in which the fruit were stored without prior fungicide treatment. This trial had 12 treatments; two replicates in each treatment were taken for quality evaluation, either after 1 week for fruit stored at 15 and 20°C ± 1°C or every 2 weeks for all other treatments until 12 weeks. Each replicate (box) contained 40 fruit. All fruit for HWD treatments were dipped into the fungicide TBZ (200 ppm) in hot water in a water bath which was set up for the desired temperature and time as shown in Fig. 3.7. The fruit were held under the water with a gloved hand and agitated gently to ensure even distribution of heat to the fruit. HWD treatments with and without C_2H_4 absorbent were set up for this trial (Table 3.2 and Fig 3.1). In a preliminary experiment, internal temperatures of the limes were monitored with temperature probes at the fruit core and just under the skin (inside the albedo), and the rates of heating and cooling were monitored using Gemini data loggers, UK Ltd, Tinytag® (Energy Engineering Ltd) (see Fig. 5.20 and 5.21).

Figure 3.6 H3 fruit stored under RA at 5, 7, 15 and 20 ± 1°C and IW conditions were washed for 3 min in 20°C cold water to which the fungicide TBZ (1,500 ppm) was added.

Figure 3.7 Selected H3 fruit were treated hot water dipping (HWD) at 52-53°C; H5 fruit were dipped at 42-57°C, for 2-6 min.

For H4, the fruit were smaller than the previous treatments (Table 3.1) and the average colour score (see below) was >25% but < 50% yellow (Fig. 3.12). This trial had 11 treatments and two replicates in each treatment were taken for quality assessment every 2 weeks until 12 weeks of storage. Each replicate (box) contained 30 fruit and 10 fruit from each replicate were put at 20°C ± 2°C for shelf life evaluation at 8, 10 and 12 weeks for 3 days. C_2H_4 absorbent sachets were also added in selected treatments for this trial (Table 3.2 and Fig. 3.1).

3.3.3 Cylindrical storage container system (H5)

For H5, two cold rooms were set up. The sizes of H5 fruit were similar to H1-H3 (Table 3.1) and the initial average colour score was about 25% yellow (Fig. 3.12). Fruit were dipped into hot water in a 25 l water bath at controlled temperatures (similar to that shown in Fig. 3.7), and then were cooled by dipping them for 3 min into 15°C cold water to which TBZ (1,200 ppm) was added, before being air-dried (Table 3.2 and Fig. 3.8AB). The fruit stored under RA at 5 and 13°C ± 1°C were dipped into 15°C cold water for 2 min in a sink before dipping them into the 15°C cold water containing TBZ (1,200 ppm) for 3 min; the fruit were then air-dried overnight (Fig. 3.8AB).

Figure 3.8 H5 fruit were washed (or cool down after HWD) for 3 min in 15°C cold water to which the fungicide TBZ (1,200ppm) was added (A) then left to air-dry at room temperature for one night (B).

Once dry (after ~12 hours) 30 fruit were packed into each replicate cylindrical storage container before being placed in cool stores at 5 or 13°C ± 1°C (Fig. 3.9). This trial had 14 treatments and one or two replicates were applied in each treatment (Fig. 3.1). Each replicate was taken for quality assessment at 2, 4, 8, 10 and 12 weeks of storage; 10 fruit from each replicate were put at 20°C ± 2°C for shelf life only after 10 weeks of storage for 3 days. No C_2H_4 absorbent was applied for this trial.

Figure 3.9 Limes in RA storage using cylindrical storage containers.

3.4 Colour measurement

3.4.1 Introduction

Colour is one of the most important quality factors for fresh products. It is an aspect of appearance of the product which can affect a customer's perception of quality and therefore affect the overall acceptability of the product (Francis, 1995). Colour measurements are therefore often used as a quality indicator in postharvest research (McGuire, 1992). The colour of a sample can be described by many colour coordinate systems such as RGB (red, green, blue), Hunter Lab, CIE (Commission Internationale de l'Eclairage) L* a* b*, CIE XYZ, CIE L* u* v*, CIE Yxy and CIE LCH (Abbott, 1999). Most studies use either CIE L*a*b* or C* (Chroma) and H° (hue) as the measure of colour. McGuire (1992) noted that whereas the lightness (L*) is correctly reported, a* and b* values are difficult to interpret separately and these coordinates are not independent variables. Additionally some researchers may report their colour data using different systems, e.g. "L", "a", and "b" may be obtained from different instruments as either CIE L*a*b* or Hunter Lab values; these are related to each other, but are not identical. This can cause difficulty in interpreting colour data, therefore McGuire (1992) recommended that C* and H° are the most appropriate measures of colour and that the instrument's illuminant source (C or D65), calibration standard(s), and illuminant/viewing geometry (d/0 or 45/0) must be clearly stated.

For this work hue angle (H°) was used as the main parameter to describe the colour change of the green and yellow sides of the lime peel during the storage periods and was measured using a Minolta chromameter or spectrophotometer as described below. The hue of lime peel changed in the range from a maximum of 115° on the green side to a minimum 90° for those fruit measured with the chromameter or from a maximum of 110° on the green side to a minimum 80° for the spectrophotometer. Fig. 3.10 indicates approximate colour regions on a CIE chromaticity diagram.

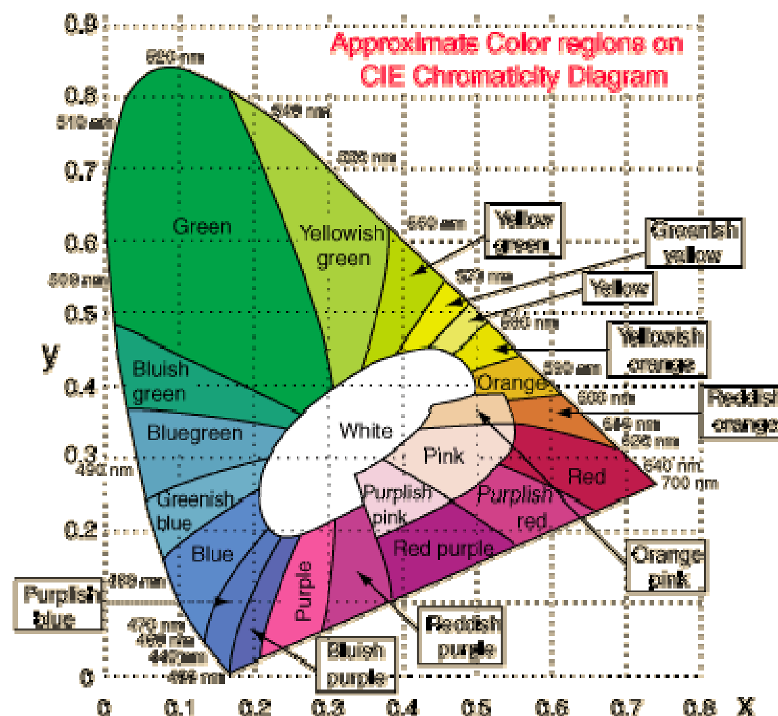


Figure 3.10 The CIE chromaticity diagram showing approximate colour regions from 400 nm to 700 nm wavelength (http://hyperphysics.phy-astr.gsu.edu/hbase/vision/cie.html; Georgia State University).

3.4.2 Chromameter

A Minolta chromameter (model CR-200, Minolta Camera Co., Ltd, Osaka, Japan) was used for measuring fruit skin colour during storage for H1 and H2 fruit. The machine was calibrated using a green colour standard (D/0 Diffused illumination, 0° viewing; light source C, Y 29.9 x 0.273 y 0.369). Each fruit was marked by a pen on the initially most green and yellow sides before measuring skin colour. Fruit were measured on subsequent occasions at the same spot (as far as was possible). The colour measurement was done under normal fluorescent light in the laboratory and the data were downloaded to a computer after finishing the measurements.

3.4.3 Spectrophotometer

A Konica Minolta spectrophotometer (model CM-2600d, Konica Minolta Sensing, INC., Osaka, Japan) was used for measuring skin colour of H3-H5 fruit. The spectrophotometer was selected for this work to acquire more spectral information to characterise changes in fruit colour. The machine was set up for the observer at 10° and illuminant C was selected. Instrument settings also were set up by choosing reflectance mode, specular component for both spectral component included (SCI) and spectral component excluded (SCE), and UV 100% full. The measurement area value (MAV) was 8 mm. The machine provided CIE L*a*b*, C* and H° values, and spectral reflectance at 10 nm intervals between 360 nm and 740 nm. The spectrophotometer was calibrated with a white colour standard (built-in) and all data were downloaded to a computer after finishing the colour measurements. The SCE data was selected as this accounts for differences in gloss as well as colour, but in practice there was very little difference (± 0.5 °hue) between the two systems (SCE and SCI).

Colour was measured using either the chromameter or spectrophotometer for H3 fruit whereas H4 and H5 fruit skin colour were measured only by the spectrophotometer under normal fluorescent light in the PFR laboratory.

3.4.4 Comparison of colour measurements between the chromameter and spectrophotometer

A comparison was made between the chromameter and spectrophotometer in order to be able to convert between measurements made with either machine. Several commercial paint colour cards in green and yellow shades were obtained from a local hardware store. Each card was measured 3 times using the chromameter and the spectrophotometer, using a consistent background. A sample of 30 green (25% yellow) New Zealand limes obtained from an orchard in the Bay of Plenty (orchard 2) and stored under RA at 20°C ± 2°C was also set up. The fruit skin colour was measured in the same position by both machines every 7 days for 6 weeks until the fruit skin became yellow; 3 sets of measurements from each fruit for each of the initially most green and yellow sides were collected with each machine. The data from the paint colour cards and lime skin colour from both machines were then compared. The correlation between these two samples is shown in Fig. 3.11 and summarised in Table 3.3.

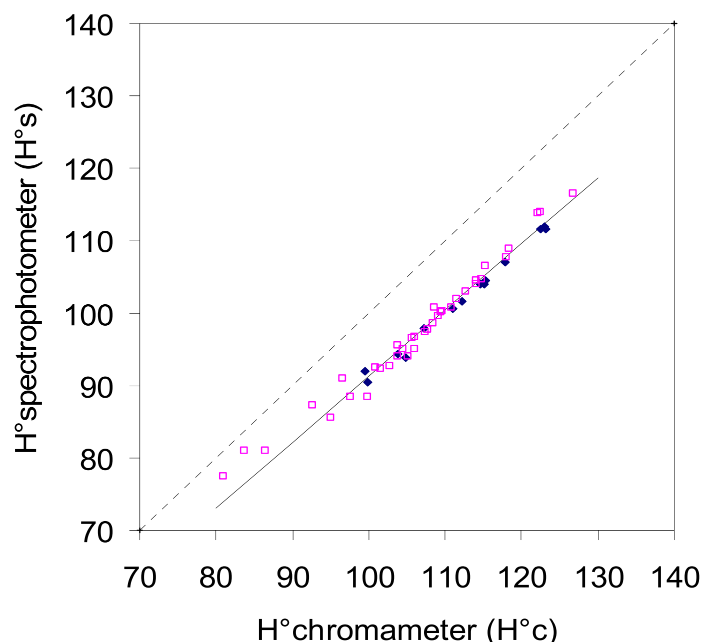


Figure 3.11 Correlation between hue (H°) of limes (♦) on the green (G) and yellow (Y) side and of paint colour cards (□) as measured by chromameter and spectrophotometer. The best fit lines shown is that given by Eq. 3.1. Each point represents the average of 30 replicate measurements.

Table 3.3 The summary of equations for correlating $H°_{Spectrophotometer}$ ($H°_S$) and $H°_{Chromameter}$ ($H°_C$).

$H°_{Spectrophotometer}$ (Y)	Slope	Intercept	R^2
Paint colour cards	0.8738	+4.516	0.9738
Lime	0.8955	+1.4038	0.9925
Both data sets	0.8695	+4.7969	0.9772
	0.9136	0	0.9747

Similar slopes and intercepts were obtained for fitting the paint card data, lime data and the combined data sets. Additionally the full data set was fitted for a forced intercept = 0 (Table 3.3). As the R^2 values were not greatly different in all cases the formula used to convert between the chromameter and spectrophotometer data sets was selected as:

$$H°_{Spectrophotometer} = 0.9136\ H°_{Chromameter} \qquad \textbf{Eq. 3.1}$$

This line is also shown in Fig. 3.11.

3.4.5 Colour score (CS, %)

A colour score was calculated by assigning a value of 0, 25, 50, 75 or 100% yellow to each fruit in a sample using a grading chart (Fig. 3.12) and calculating the mean colour value for the sample.

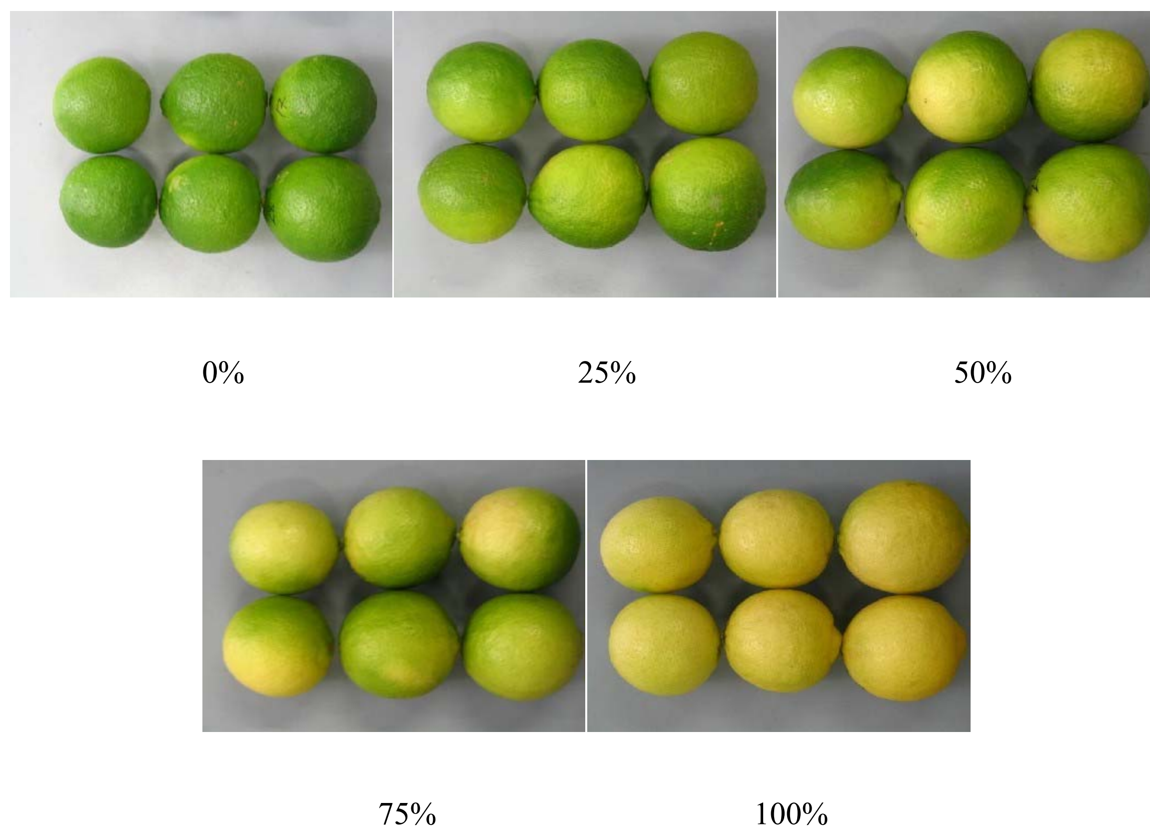


Figure 3.12 Colour score grading system for lime fruit ranging from green fruit = 0% yellow ($H^\circ \geq 115^\circ$) and yellow fruit = 100% yellow ($H^\circ \leq 95^\circ$).

3.5 Disorders

Limes are chilling sensitive and symptoms of chilling injury include pitting and brown discolouration. Pitting symptoms typically developed into CI during long term storage at low temperature. In this research, CI and pitting were classified using four-point scales as shown in Fig 3.13 and 3.14, respectively. When limes were dipped in hot water (either with or without fungicide) some heat injury resulted. A heat injury (HI) score was also applied (as shown in Fig. 3.15.) for fruit exposed to high temperature hot water dips (H4 and 5).

3.5.1 Chilling injury (CI)

CI was rated on a four point scale (Fig 3.13) based on the percentage of surface area showing CI symptoms following Kluge *et al.* (2003): 1 = <5%; 2 = 5 – 25%; 3 = 25 – 50%; 4 = >50%. The CI score for each treatment was determined based on individual assessment of each fruit in each treatment just before each sub-sample of fruit was removed for shelf life assessment. If a fruit was removed between scheduled samplings because of extensive CI or other disorders, it was counted as if it were a score 4 fruit at the next scheduled sampling. The number of fruit for each CI category in each sample was expressed as a percentage of the total fruit per treatment.

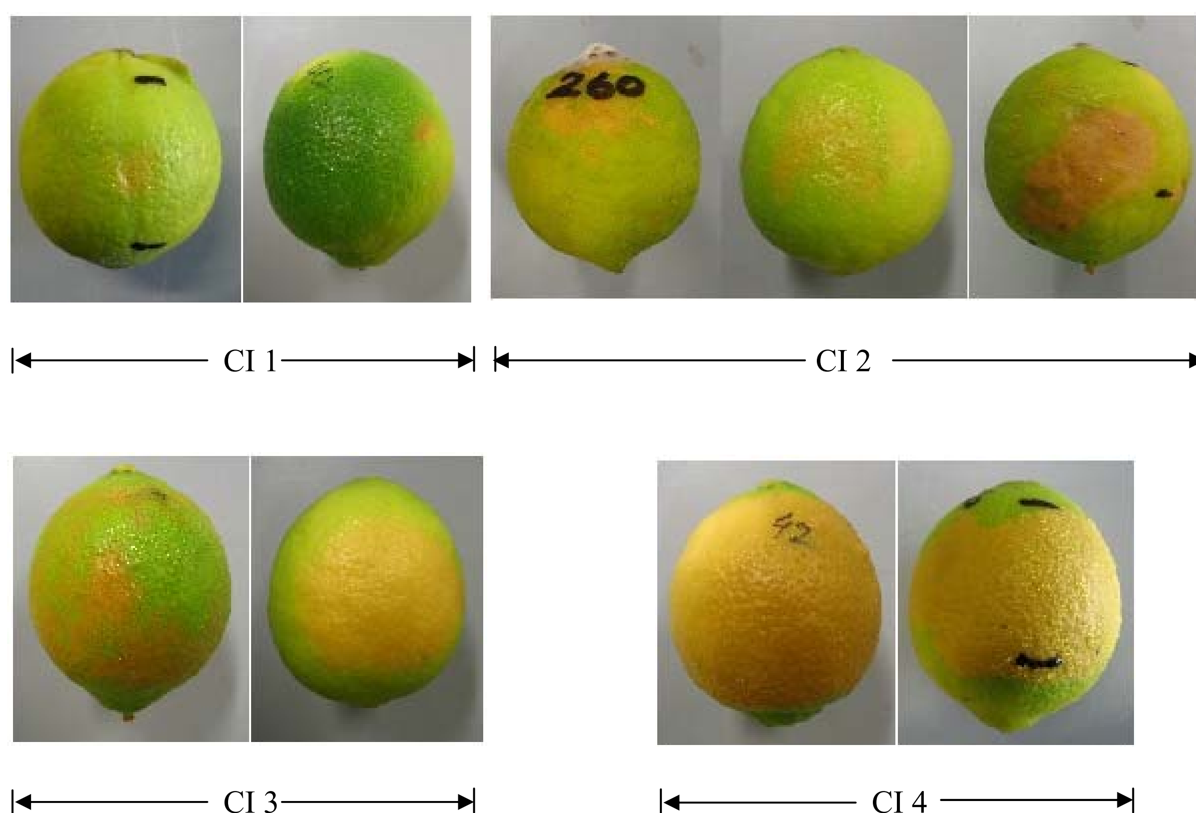


Figure 3.13 Grading scale for the percentage of surface area showing CI symptoms following Kluge *et al.* (2003): 1 = <5%; 2 = 5 – 25%; 3 = 25 – 50%; 4 = >50%.

3.5.2 Pitting

Prior to the detection of CI as described by Kluge *et al.* (2003), small skin indentations were frequently observed in stored limes (Fig 3.14) and described as pitting. Pitting was rated on a four-point scale: 1 = <5% surface area affected, 1-2 pits; 2 = 5-10% area

affected, > 2 pits; 3 = 10-15% area affected, >2 pits; 4 = >15% area affected, >2 pits. The number of fruit for each category in each sample was expressed as a percentage of the total fruit per treatment (as for CI).

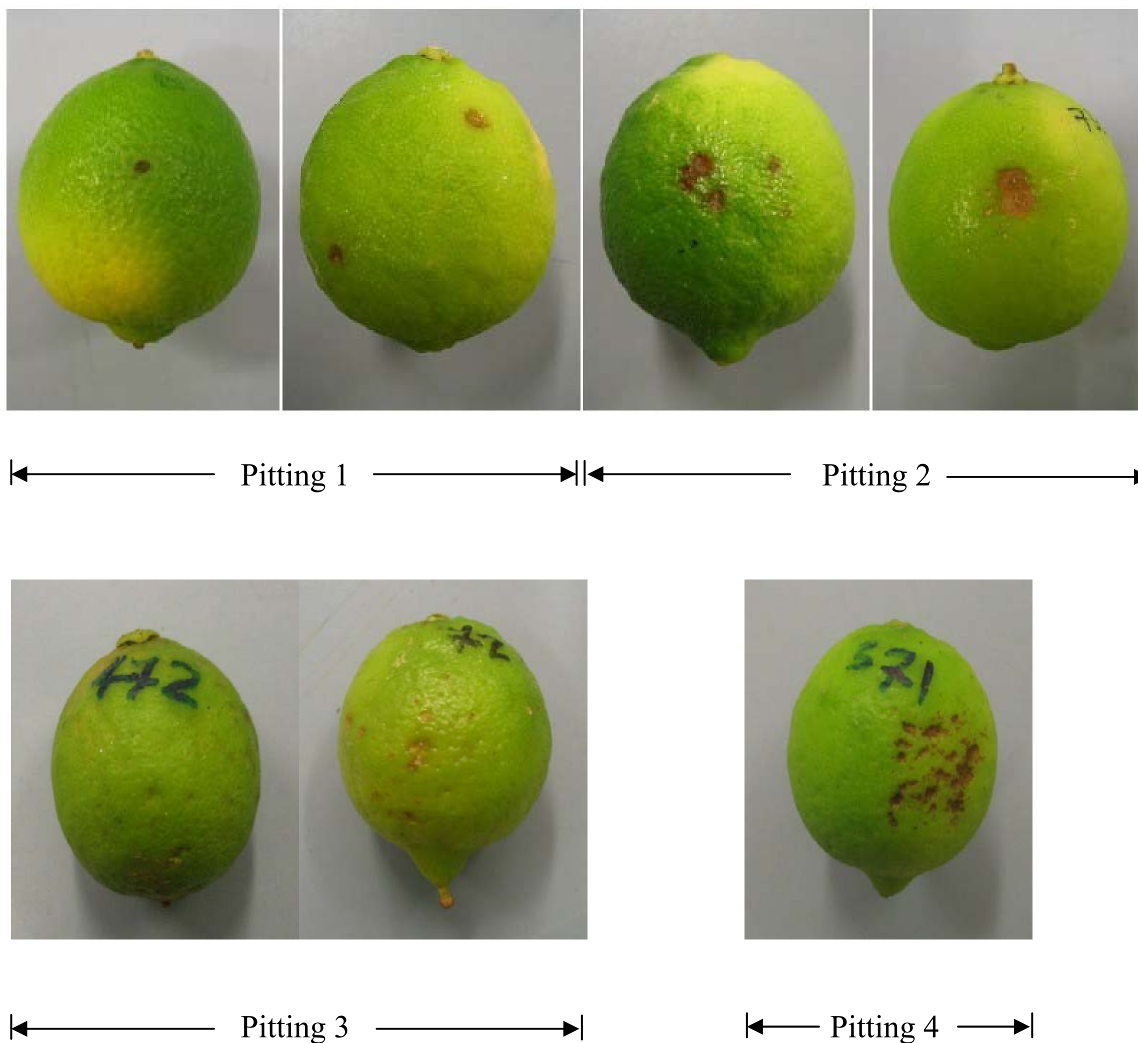
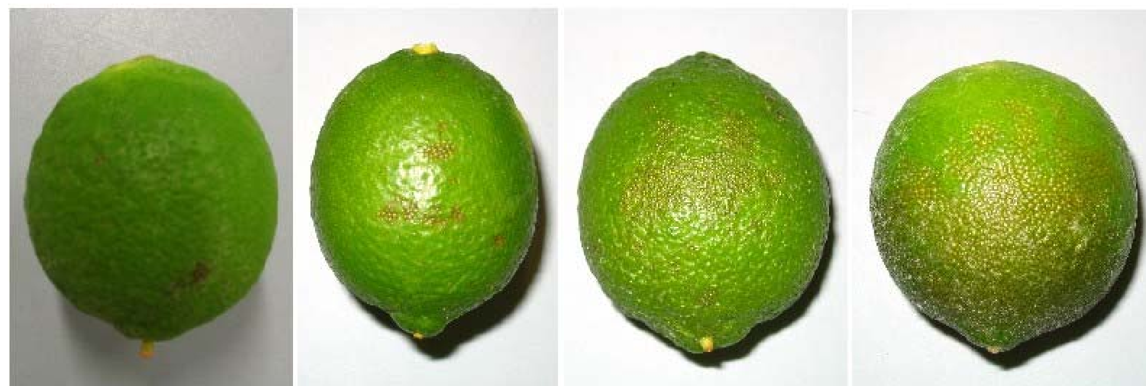


Figure 3.14 Grading scale for the extent of pitting symptoms: 1 = <5% surface area affected, 1-2 pits; 2 = 5-10% area affected, > 2 pits; 3 = 10-15% area affected, >2 pits; 4 = >15% area affected, >2 pits.

3.5.3 Heat injury (HI)

After dipping limes into high-temperature hot water, a distinctive pattern of skin damage was seen with localised patches of browning (Fig 3.15). HI was rated on a four point scale based on the percentage of surface area showing HI symptoms: 1 = <5%; 2 = 5 – 25%; 3 = 25 – 50%; 4 = >50%. Only fruit treated by HWD was assessed in this way. The percentage of HI fruit was calculated as above.



HI 1 HI 2 HI 3 HI 4

Figure 3.15 Grading scale for the percentage of surface area showing HI symptoms: 1 = <5%; 2 = 5 – 25%; 3 = 25 – 50%; 4 = >50%.

3.6 Rots

Postharvest rots can significantly detract from the quality of lime fruit during storage. Rots were tentatively identified as *Botrytis sp.*, *Penicillium italicum, Geotrichum candidum, Penicillium digitatum*. In rare cases, there was an unknown soft rot which may not have been caused by a pathogen (Barkley, 2004). The number of fruit affected by each type of rot was assessed for each treatment and recorded as a percentage of the total fruit. A typical example of each rot category is shown in Fig. 3.16.

3.7 Other quality parameter measurements

3.7.1 Compression firmness (CF)

Compression firmness (CF) was measured using a food texture analyser, either the TA-XT2, (H1 and H2) or TA-XT *plus* (Stable Micro System, Godalming, UK; H3 and H4), interfaced to a computer. H1 and H2 fruit were compressed by 2 mm with parallel plates at a crosshead speed of 1 mm s^{-1}, whereas CF of fruit for H3 and 4 was measured using a P/35 (35mm DIA cylinder aluminium) probe at the same conditions. The peak force in Newtons was recorded in both cases and used as the firmness index.

Botrytis sp. *Penicillium italicum* *Geotrichum candidum*

Penicillium digitatum Soft rot

Figure 3.16 Examples of the different types of rot observed on limes during storage.

3.7.2 Respiration and ethylene production rate

Respiration rate and ethylene production rate were measured for H4 fruit. A 550 ml glass jar was used and contained 3 fruit per treatment. Glass marbles (1 cm diameter, 40) were added to each jar in order to reduce the void volume of the jar, permitting measurements to be taken in a shorter time (3 hours). Gas samples were withdrawn using a 3 ml gas-tight syringe and injected into a Shimadzu TCD-GC model 9A (Shimadzu Corporation, Kyoto, Japan) fitted with an Alltech CTR 1 column. The temperatures of injector, oven, and detector were 150, 80, and 150ºC, respectively. The flow rate of carrier gas (helium; BOC Gases NZ Limited, PN) was 50 ml min^{-1}. Respiration rate was determined by

measuring the increase in CO_2 concentration in the sealed treatment chamber over a 3 hour period.

Ethylene production was determined in a similar manner using a Hewlett Packard FID-GC (model 5890A, Pennsylvania, USA) fitted with a Porapak N column. The temperatures of injector, oven, and detector were 140, 45, and 150ºC, respectively. The flow rate of carrier gas (nitrogen; BOC Gases NZ Limited, PN) was 50 ml min^{-1}.

3.8 Chemical assessments

Key nutrients of lime such as flavonoids and ascorbic acid also were investigated after storage in selected harvest. Flavonoids were analysed for H1 and H3 fruit. Additionally, ascorbic acid was measured at 0 day, 4, 8 and 12 weeks of storage for H3 samples. Correlations between chlorophyll content on the peel and skin colour changes of H5 fruit were investigated at harvest and after 2 and 4 weeks of storage.

3.8.1 Flavonoids (Polyphenolic compounds)

Juice was squeezed from H1 and H3 lime fruit samples and frozen at -20°C. The frozen juice samples were freeze-dried and used for flavonoid assessment. A 50 mg sample of freeze-dried material was weighed into an Eppendorf tube then extracted by adding 1.5 ml of extraction buffer (EB) comprising 85% methanol (MeOH) (LiChrosolv, Merck KGaA, Darmstadt, Germany) and 15% (10% acetic acid (HiPerSolv, BDH, VWR International, Ltd, England) in water), and vortexing thoroughly for 10-15 min. Each sample was centrifuged for 3 min at 14,000 rpm, 4°C. Each sample was syringe-filtered through a 0.45 µm filter and 0.5 ml was put into a HPLC vial and measured by HPLC, using a Waters 600E system controller, a Waters 996 photodiode array detector and a Waters 717 plus autosampler (Waters, Milford, Mass.). The column used was a Phenomenex Ltd, Milford, Auckland, NZ) C18 (2), Luna 5 µm, 150 mm x 4.6 mm, fitted with a guard column (8 x 3 mm, C-18; (ODS, Octadecyl)), Phenomenex, Auckland, NZ).

Chromatographic traces were recorded using the Millennium program, scanning wavelengths from 220 nm to 600 nm. Solvents used for elution were (A) 10% acetic acid in fresh MilliQ water, and (B) acetonitrile (HiPerSolv, Merck KGaA, Darmstadt,

Germany). A linear gradient was used to elute flavonoids varying as described in Table 3.4.

Table 3.4 Solvent-gradient table for flavonoids analysis.

Time (min)	Flow rate (ml.min^{-1})	%A	%B
0	1	95	5
15	1	80	20
30	1	50	50
32	1	50	50
32.5	1	95	5
41	1	95	5

Vials were loaded in the autosampler and 20 µl samples were injected on the column which was maintained at 25°C. Eluted components were monitored at 280 nm for proanthocyanidins and phenols, 313 nm for phenolic acids and 350 nm for flavonols. A standard flavonoid mixture, which consisted of analytical-grade chlorogenic acid, caffeic acid, rutin, quercetin-3-glc, quercetin, luteolin, apigenin and kaempferol, was also injected each day. Index peak identifications were obtained by liquid chromatography mass spectrometry (LCMS) performed by Nigel Joyce, PFR, Lincoln, New Zealand.

3.8.2 Ascorbic acid

Lime fruit from H3 were squeezed and the expressed juice stored at -20°C. The frozen juice samples were freeze-dried and used for ascorbic acid assessment. A 50 mg sample of freeze-dried material was weighed into an Eppendorf tube, then extracted by adding 1.5 ml of extraction buffer (EB) comprising 0.1 M potassium acetate (KOAc) (Merck, KGaA, Darmstadt, Germany) (pH 3 with formic acid (AnalaR, BDH laboratory supplies, England)) and vortexing thoroughly for 10 min. Each sample was centrifuged for 15 min at 14,000 rpm at 4°C. Each sample was syringe-filtered and 0.5 ml sample was added into a HPLC vial and measured by HPLC, using a Waters 600E system controller, a Waters 996 photodiode array detector and a Waters 717 plus autosampler. An Econosil (Alltech Associates, Inc. Illinois, USA) C18, 10 µm, 250 mm x 4.6 mm column was used for this measurement. Solvents used for elution were (A) 0.1 M KOAc (pH 5 with formic

acid), (B) 50% acetonitrile. The flow rate was 1.5 ml min^{-1}. The linear gradient employed for vitamin C is described in Table 3.5.

Table 3.5 Solvent-gradient table for ascorbic acid analysis.

Time (min)	Flow rate ($ml.min^{-1}$)	%A	%B
0	1.5	95	5
3.0	1.5	84	16
3.5	1.5	0	100
5.5	1.5	0	100
6.0	1.5	95	5
14.0	1.5	95	5

Vials were loaded in the autosampler and 20 µl samples were injected on to the column which was maintained at 25°C. Vitamin C was monitored at 254 nm. The individual vitamin C content of each replicate was identified and quantified by comparison with standard solutions of known concentration. HPLC data were converted to mg. 100 ml^{-1} by comparison with a 100 mg. 100 ml^{-1} vitamin C standard.

3.8.3 Chlorophyll content

Chlorophyll was extracted from 200 mg of lime peel ground to a powder in liquid N_2 and analysed following (Albert *et al*., 2009). The powder was put into an Eppendorf tube and 1 ml extraction buffer (acetone:methanol (7:3) + 200 mg $CaCO_3$) was added and vortexed. Each lime peel solution was centrifuged at 14,000 rpm for 2 min at 4°C (Eppendorf centrifuge 5417R, Global Science, Auckland, NZ). The supernatant was removed into a clean 15 ml foil-covered tube. One ml of acetone:methanol (7:3) was added to the pellet, vortexed and centrifuged, and the supernatant was collected into the same foil-covered tube. This process was repeated four times until the tissue was colourless. The supernatants were stored at 4°C in the dark for one night. Diethyl ether (AnalaR, BDH laboratory supplies, England) (4 ml) was added to the combined supernatants, mixed gently by inversion, then 4 ml of water were added and the solution was mixed gently again. The solutions were separated, the upper phase containing the pigments was removed and collected into a labelled foil-covered glass vial. A further 2

ml of diethyl ether was added to the remaining bottom phase and mixed. The upper phase was collected and combined with the supernatants in the previous glass vial. The ether phases were dried under N_2 using a heating block at 30-35°C. Resuspended pigments in 1 ml ethyl acetate (AnalaR, BDH laboratory supplies, England) were transferred into Eppendorf tubes. A 50 µl ethyl acetate sample was measured directly in a 950 µl chloroform (AnalaR, BDH laboratory supplies, England) mix. The solution was measured at three different wavelengths (480, 648 and 666 nm) by a Jasco V-530 UV/VIS spectrophotometer (Science & Technology Ltd, NZ). The data were recorded and the Wellburn equation (in chloroform) was used to obtain the total chlorophyll content (Wellburn, 1994). Chlorophyll *a* (C_a), chlorophyll *b* (C_b) and carotenoids (C_{x+c}) contents were calculated using the formulas described below (Eq. 3.2-3.4).

$$C_a = 10.91A_{666} - 1.2A_{648} \quad \textbf{Eq. 3.2}$$

$$C_b = 16.38A_{648} - 4.57A_{666} \quad \textbf{Eq. 3.3}$$

$$C_{x+c} = (1000A_{480} - 1.42C_a - 46.09C_b) / 202 \quad \textbf{Eq. 3.4}$$

3.9 Statistical analysis

All routine statistical analysis was carried out using SAS software package version 9.1 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA). This software was used to generate least significant difference values (LSD) by analysing raw data by analysis of variance (ANOVA). The LSD values generated by ANOVA were averages of values at a number of time points following harvest in many cases.

Principal components analysis (PCA) was conducted on log-transformed values of peak area of 20 flavonoid peaks. Any missing peak height values (those values below the limit of detection) were replaced with a value of 1000 (half of the detection threshold of 2000). The peak heights were log-transformed using common logs (log to base 10) prior to performing a principal components analysis (PCA) using a variance-covariance matrix. The analysis was performed using Genstat software (9th Edition; Lawes Agricultural Trust, Rothamsted Experimental Station, VSN International Ltd, UK). Assistance with PCA analysis was provided by Dr. Duncan Hedderley and Dr. Andrew McLachlan, biometricians employed by PFR, New Zealand.

CHAPTER 4

Effects of gas atmosphere and GA_3 on lime (*Citrus latifolia* Tanaka) storage life

In this chapter the effects of regular air (RA), GA_3 and controlled atmosphere (CA) storage on quality of New Zealand limes are reported. The storage of limes under RA was used to provide a baseline of the effect of normal temperature conditions (ranging from 2–20°C) on lime storage. The storage of limes under different CA conditions was investigated because although this technique has been reported to delay colour change and reduce decay of fruit and vegetables after harvest (Salama *et al.,* 1965; Kader, 1986; Vigneault *et al.,* 2004; Sritananan *et al.,* 2006), disorders such as pitting, chilling injury and an increase in decay have also been reported for limes under CA storage (Salama *et al.,* 1965; Spalding and Reeder, 1974; Hatton *et al.,* 1975; Sritananan *et al.,* 2006). The physiological condition of fruits and vegetables may influence either the protection against CI or the increase of CI under a given concentration of CO_2 and so contribute to these variable results (Forney and Lipton, 1990). It might therefore be possible to identify a CA treatment that may both delay degreening and prevent CI and rots. The application of gibberellic acid (GA_3) to a batch of H1 fruit stored at 13°C was also tested and compared to other postharvest treatments.

4.1 Behaviour of lime fruit under regular air (RA) storage

4.1.1 Introduction

Regular air (RA) cold storage is widely used to extend the storage life of fruit and vegetables. Chilled RA storage contributes to quality maintenance and nutrient retention by reducing the rates of respiration and other metabolic processes of the product. Key benefits of RA storage are that it can be readily implemented in any cool store, is cheap and is easy to manage. Compared to controlled atmosphere (CA) storage, it requires less experienced staff and has fewer safety issues.

The objective of this initial study was to investigate the behaviour of NZ limes as a function of temperature under RA storage conditions. The materials and equipment used were as outlined in Chapter 3 and colour, texture, colour score, disorders and rots were monitored over 8 to 12 weeks of fruit storage. The influences of ethylene removal via inclusion of potassium permanganate (Purafil) adsorbent and GA_3 dipping were also investigated.

The key outcome of the work was the establishment of a baseline against which other treatments would be compared as part of the overall project goal of determining an optimum storage regime for long term storage of NZ limes.

4.1.2 Colour changes during RA storage

4.1.2.1 Introduction

Both hue (H°) and colour score (CS) were measured and recorded during five lime storage trials over the years 2004 to 2006. H° was measured on successive occasions at those points on the surface of individual fruit where the fruit was initially most green and initially most yellow, respectively. Colour score was assessed based on overall fruit colour.

Different temperature regimes were investigated ranging from ‘ambient’ (20°C) to very cool (2°C) temperatures. The different regimes employed in the later stages of the work were selected on the basis of the results of earlier experiments with the goal of optimising storage life with regard to both retention of green colour and minimisation of chilling and related disorders. A storage temperature of 5°C was included in every run as a reference condition.

The temperature ranges investigated are summarised in Table 4.1. All temperatures in all cold rooms were continuously monitored and remained within ± 1°C of the set point for all runs, except for ambient storage for which the range was 20°C ± 2°C. The relative humidity in each storage container was approximately 90-95% RH because humidified air was passed through each container during storage in all harvests.

Table 4.1 The air storage temperatures tested for five harvests of limes between 2004 -2006

Harvest #	Storage temperature (°C)
1	2, 5, 13
2	5, 7, 9, 13
3	5, 7, 15, 20
4	5, 7
5	5, 13

4.1.2.2 Trends in hue in RA storage

Typical changes in skin colour for the limes in RA storage are shown in Fig. 4.1AB for 2, 5 and 13°C for harvest 1 (2004) fruit for both the green (4.1A) and yellow sides of the fruit (4.1B). The fruit stored under RA at 2 and 5°C decreased only slowly in H° until 10 weeks of storage whereas the fruit stored at the higher temperature of 13°C decreased rapidly in H° after only 2 weeks of storage. After 8 weeks of storage the fruit stored at the low temperatures (2 or 5°C) clearly became more yellow and the colour (yellow-brown) was also influenced by some CI injuries on the skin. A hue value on the green side of 104° ($H°_C$), using the chromameter or 95° ($H°_S$), from the spectrophotometer, can be taken as the lowest limit of acceptable green colour (corresponding to a CS of about 75% yellow) (Fig. 4.1A). The dashed line on Fig. 4.1 represents this "yellow threshold' and it took about 4 weeks for fruit to yellow at 13°C but 8-10 weeks at 2-5°C.

In H2, intermediate temperatures were tested, and as expected yellowing was faster at higher temperatures (Fig. 4.2A, B). The H° of the H2 fruit stored under RA at 5°C and 7°C were not significantly different during storage until 8 weeks and gave H° values higher than the fruit stored at 9°C and 13°C, respectively, on both the green and yellow sides of fruit. Similarly, the fruit stored under RA at 9°C showed higher H° values (were more green) than the fruit stored under RA at 13°C after 6 weeks of storage on both sides of the fruit.

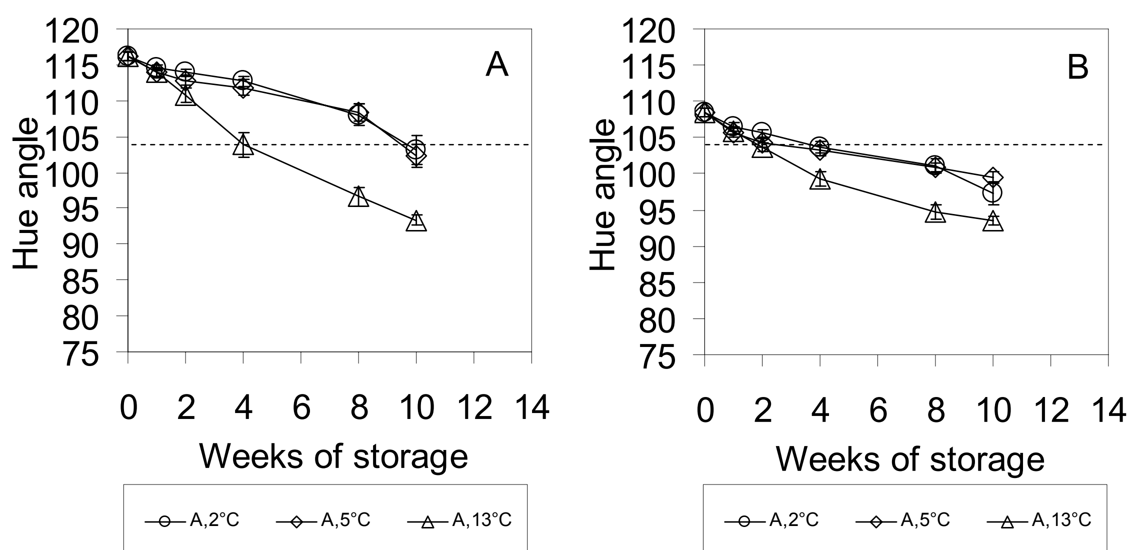


Figure 4.1 Hue angle in NZ limes stored under RA on the green side (A) or yellow side (B) at 2, 5 and 13°C, H1. Vertical bars indicate ± SE (n=18). H°$_C$ (chromameter data). (The dashed line represents "yellow threshold").

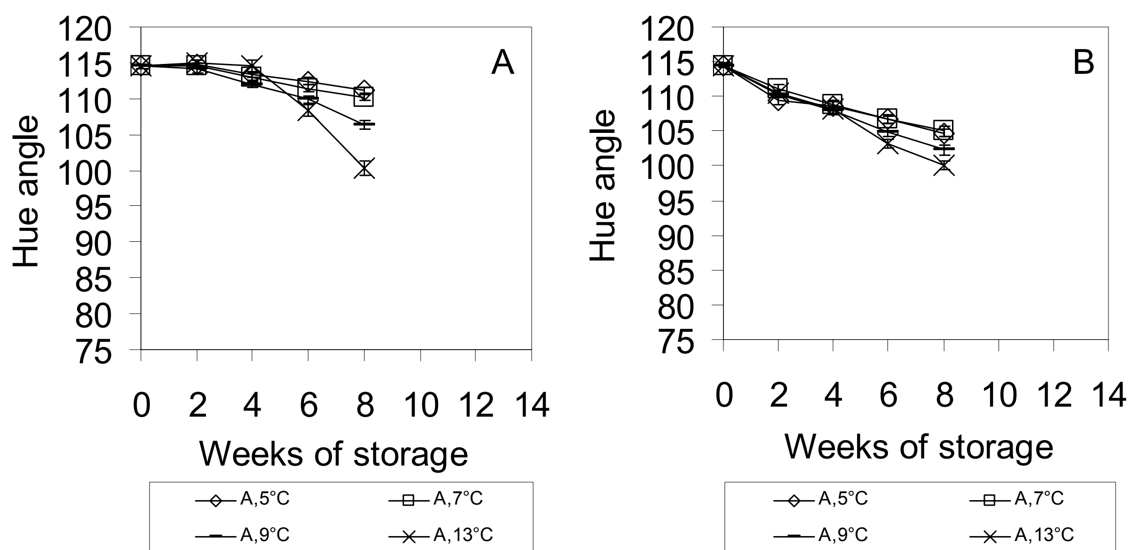


Figure 4.2 Hue angle in NZ limes stored under RA on the green side (A) or yellow side (B) at intermediate temperatures ranges 5, 7, 9 and 13°C, H2. Vertical bars indicate ± SE (n=40).

In H3 there was a clear distinction between the two higher and the two lower temperatures with respect to rate of H° change and end-point values with fruit becoming fully yellow after 8 weeks at 15 and 20°C (Fig. 4.3). Degreening was slightly slower at 20°C than 15°C, which is consistent with the observation that chlorophyll breakdown is somewhat inhibited at temperatures over 15°C (Davies and Albrigo, 1994).

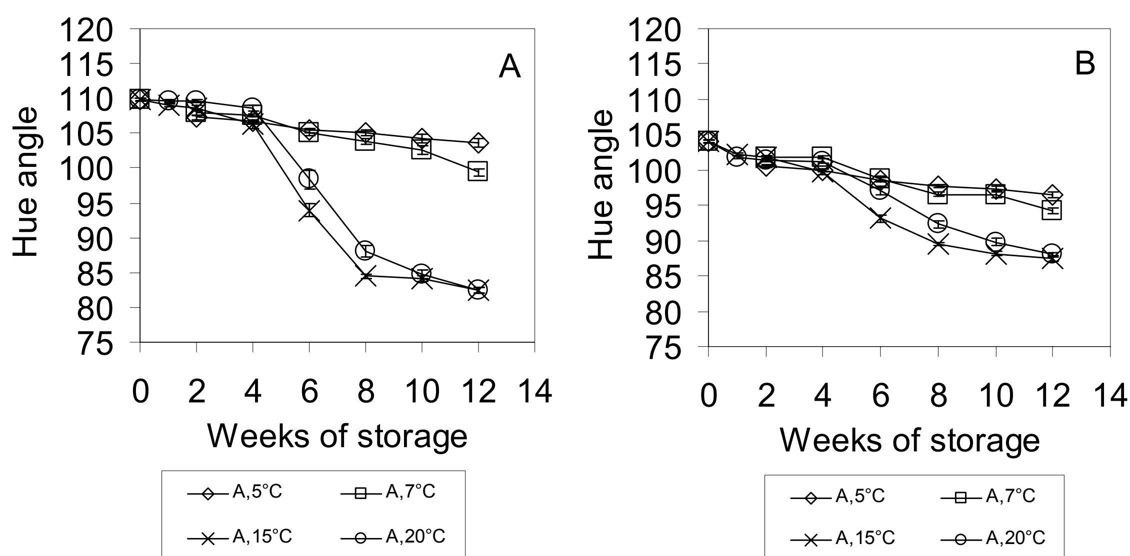


Figure 4.3 Colour change for NZ limes stored under RA on the green side (A) or yellow side (B) at 5, 7, 15, and 20°C, H3. Vertical bars indicate ± SE (n=80). $H°_S$ (spectrophotometer data).

Murata, (1997) and Ladaniya, (2004) reported that lime stored at 9-10°C developed an undesirable yellow colour after 3-4 weeks and may become fully yellow after 8 weeks. These data are consistent with the experimental data reported here and provide initial confirmation that NZ limes also exhibit a short high quality storage life with respect to colour when stored above 9°C.

When the H° of the fruit stored under RA at 5°C from H1-H5 are compared across all runs (Fig. 4.4) the data are reasonably consistent. It should be noted that fruit in H1 and H2 were measured with a chromameter (Minolta CR200) whereas the rest of the fruit were measured with a spectrophotometer (Minolta CM-2600D); (see section 3.4.4 on chapter 3 for details). Data in these graphs have been corrected (to $H°_S$) equivalent values using Eq. 3.1 to be comparable.

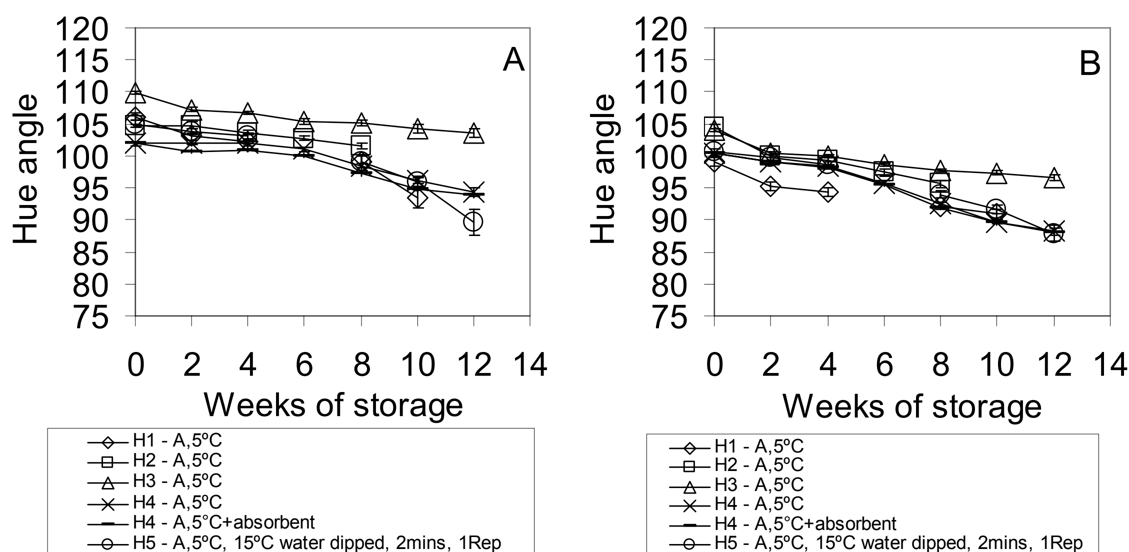


Figure 4.4 Colour change for NZ limes stored under RA on the green side (A) or yellow side (B) at 5°C across all runs. All H° of fruit from H1-H2 were adjusted for H° between chroma meter and spectrophotometer whereas the H° of H3 fruit were adjusted for only the beginning value (see chapter3, section 3.4.4).

The rate of colour change on the green and yellow sides was quite similar for H1, H2 and H5, and H2, H4 and H5, respectively. The rate of colour change of H3 fruit was slower whereas the rate of colour change of H1 fruit tended to be faster than other harvests, especially on the yellow side of the fruit (Fig. 4.4A, B). This reflected differences in initial fruit colour quality and possibly different climate conditions over the seasons. Overall, the quality of H3 fruit was very high with the fruit being of good size, more uniform and deeper green colour, and of generally a very healthy and appropriate appearance. It may be concluded that the H3 fruit were the best fruit of all harvests and the fruit was at an optimal fruit quality for harvest. Alternatively, the year 2005 may have been a particularly good growing season.

In a similar manner to the previous figure, $H°_S$ of the fruit stored under RA at 7°C from H2, H3 and H4 (Fig. 4.5A) and under RA at 13°C from H1, H2 and H5 are compared across all runs (Fig. 4.5B).

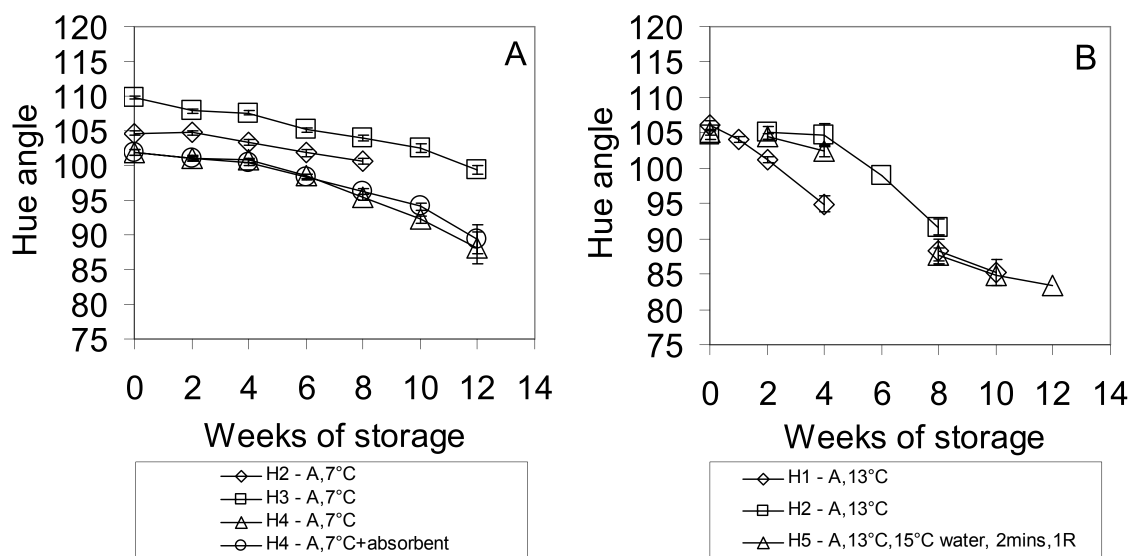


Figure 4.5 Colour change for NZ limes stored under RA on the green side at 7°C (A) or at 13°C (B) across three different harvest (H2, H3 & H4 at 7°C and H1, H2 & H5 at 13°C). All H° of fruit from H1-H2 were adjusted for H° between chroma meter and spectrophotometer whereas the H° of H3 fruit were adjusted for only the beginning value (see chapter 3, section 3.4.4).

The rate of colour change of the green side of H2–H4 fruit stored under RA at 7°C appeared reasonably consistent during storage for 12 weeks although the fruit differed in initial hue (Fig. 4.5A). The rate of change of hue of H2 and H5 fruit at 13°C appeared similar but H1 fruit yellowed more rapidly (Fig. 4.5B).

The H° of fruit stored under RA at 15 and 20°C decreased rapidly after 4 weeks of storage (Fig. 4.3A, B) until the green side colour reached a $H°_S$ about 95° and 85° at 6 and 8 weeks of storage, respectively (Fig. 4.3A). If the value of 95° ($H°_S$) is taken as the lowest limit of acceptable green colour, the average times taken for H1 and H3 fruit to reach this value under RA at different temperatures are shown in Table 4.2.

Table 4.2 Time to reach the limit of acceptable hue for fruit stored at 13°C, 15°C and 20°C (All H° data are expressed as $H°_S$ values, applying Eq. 3.1 as required).

Harvest # & initial CS	Colour side	Temperature (°C)	Weeks to 95°Hs
H1, 40% yellow	Green	13	4
H3, 25% yellow	Green	15	6
H3, 25% yellow	Green	20	6.5
H1, 40% yellow	Yellow	13	2
H3, 25% yellow	Yellow	15	6
H3, 25% yellow	Yellow	20	6.5

Unsurprisingly there was a marked harvest effect, with the initially more yellow fruit reaching the limit of acceptability sooner. For good quality fruit (H3), acceptable colour was retained for at least 6-6.5 weeks even at warm (ambient) temperatures.

Kluge *et al.* (2003b) reported initial lime H° values of approximately 120° (measured using a Minolta Chroma Meter CR-300) at-harvest and after continuous storage at 5°C for 60 days (~8 weeks) with 3 days shelf life at 20°C this had decreased to 113° (Table 4.3). Kluge *et al.* (2003b) did not state if they measured the green or yellow side, but presumably these high values indicate a measurement on the green side. Their H° data were compared with our H° data from H1 and H4 fruit after 8 weeks of storage plus 3 days shelf life at 20°C and H3 fruit after storage at 8 weeks (but with no shelf life). The respective final hue and change in hue values (as $H°_S$) and the comparison between these data is shown in Table 4.3.

Table 4.3 Comparisons of H° data from Kluge *et al.*, 2003b and H° data (in $H°_S$) from H1 and H4 at 8 weeks of storage plus 3 days shelf life at 20°C and H3* at 8 weeks of storage (no shelf life).

Sources of fruit	Temperature (°C)	Initial H° value on the green side	Hue angle (H°) (after 8 weeks)	ΔH°
Kluge *et al.,* 2003b	5	120 (average)	113	7
H1	5 ± 1	106 (G)	94	12
H3*	5 ± 1	110 (G)	105	5
H4	5 ± 1	102 (G)	99	3

While it is not possible to directly compare Kluge *et al.* (2003b)'s results with this study, (as different instruments were used) the change in hue (ΔH°) of ~7° for their fruit (after ~ 8 weeks) falls within the range of ΔH° values observed in this work of ~3° and 5° (H4 and H3) and 12° (H1).

4.1.2.3 Effect of inclusion of ethylene absorbent

4.1.2.3.1 Introduction

Ethylene is an endogenous hormone (Toivonen and Beveridge, 2005) which is synthesized by all plants (Wills, 2005). An ethylene concentration of 0.1 µl l^{-1} in air is often quoted as the threshold level for physiological activity (Kader, 1985) but concentrations as low as 0.005 µl l^{-1} can promote premature senescence in many fruit and vegetables (Toivonen and Beveridge, 2005). Wills and Warton, (2004) reported that any amount of ethylene can be detrimental consequently various strategies may be employed to reduce the level of ethylene in the atmosphere around fresh produce in order to delay the development of senescence of non-climacteric produce and the onset of ripening in climacteric produce. The ability of potassium permanganate to reduce the ethylene concentration was first reported by Forsyth *et al*., (1967) and it has been widely applied for apple and kiwifruit storage and to other fruits including banana, mango and avocado (Wills and Warton, 2004). There are fewer reports of its use for non-climacteric produce, but Wild *et al*., (1976) reported its use to extend the shelf life by retarding loss of green colour and reducing rots on lemon.

In this research, sachets containing 20 grams of potassium permanganate were prepared and placed into each selected treatment during storage. These would have reduced the free ethylene concentration but I did not attempt to measure the very low concentrations that may still have remained.

4.1.2.3.2 Colour changes of limes with C_2H_4 absorbent during RA storage

Effect of inclusion of C_2H_4 absorbent on the H4 fruit stored under RA at 5 and 7°C is shown in Fig. 4.6. There was very little effect of the presence of the C_2H_4 absorbent on the rate of degreening at 5 or 7°C on the green side of the fruit. At 7°C, the only slight

difference was found at 10 weeks, with a small decrease in yellowing caused by the absorbent ($p < 0.05$). At 5°C, there were small statistically significant differences in H° from weeks 2-10, with the presence of the absorbent increasing the rate of yellowing ($p < 0.05$), but this effect was tiny. Overall, the effect of C_2H_4 absorbent on the colour change of the green fruit was not commercially significant (e.g. with respect to time to reach 95° hue) so there is no apparent benefit of this approach to extend storage life of NZ limes.

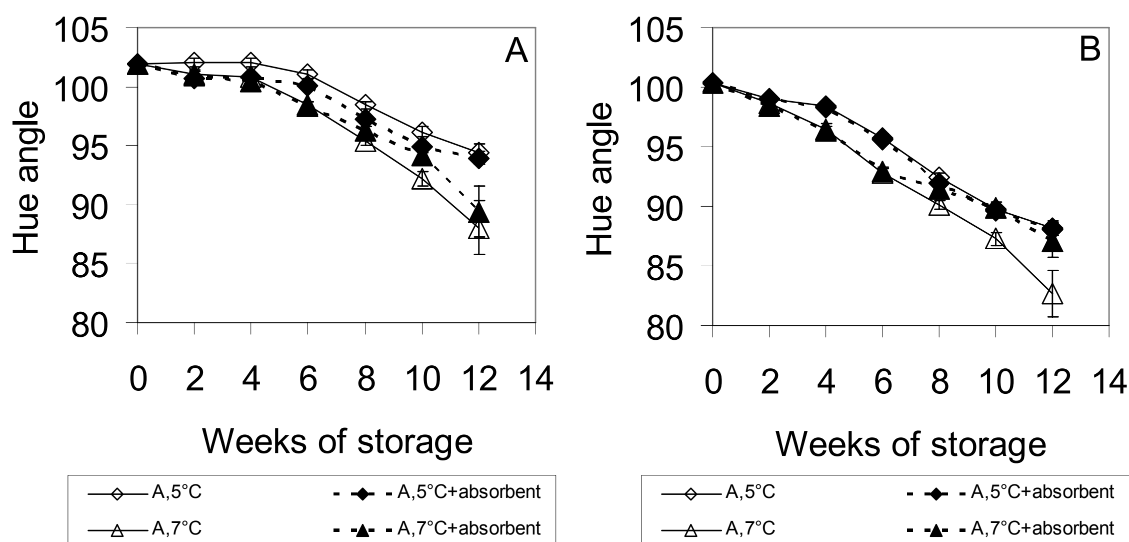


Figure 4.6 Colour changes for NZ limes stored under RA on the green side (A) or yellow side (B) at 5 and 7°C with and without C_2H_4, absorbent, H4. Vertical bars indicate ± SE (n=60 for 2-8 weeks, n=40 for 10 weeks and n=20 for 12 weeks).

4.1.2.4 The effect of GA_3 on lime stored in RA

The effect of GA_3 was tested in H1 fruit stored in RA. The results showed that degreening was delayed by approximately two weeks by treatment with GA_3 but after that time degreening increased faster than in the fruit stored under RA at 2 and 5°C (Fig. 4.7). I therefore did not use GA_3 for further experiments.

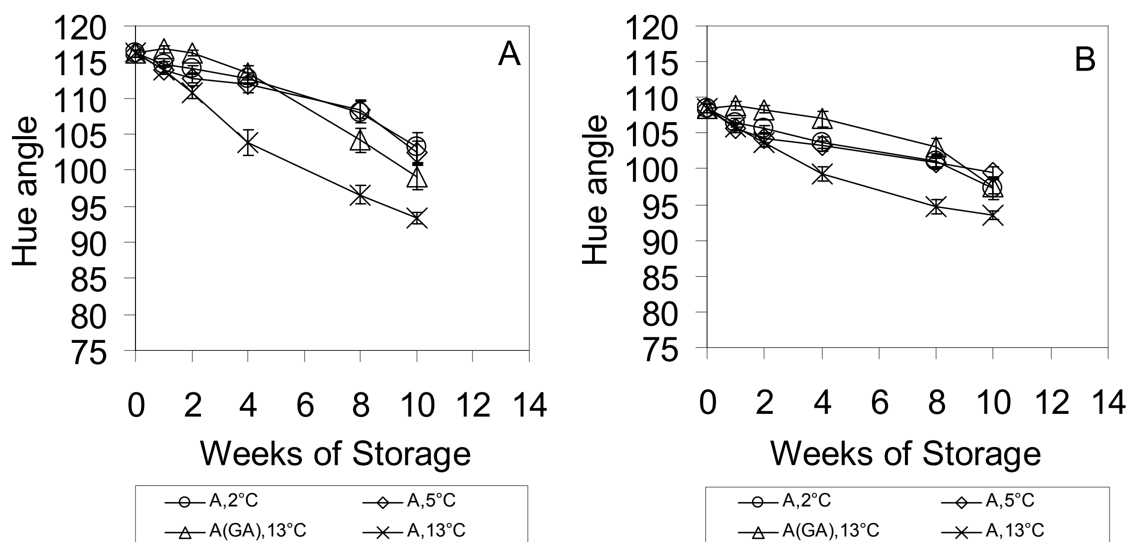


Figure 4.7 Colour change for NZ limes stored under RA on the green side (A) or yellow side (B) at 2, 5 and 13°C with and without GA_3, H1. Vertical bars indicate ± SE (n=18). $H°_C$ (chromameter data).

4.1.2.5 Colour score (CS) of lime under RA storage

The CS of H1 fruit were recorded at day 1 and then at various times until 10 weeks of storage. Trends in CS depended strongly on the storage temperatures and initial colour quality as I expected. The rates of change of CS of fruit stored at 2 and 5°C were markedly slower than the fruit stored under RA at 13°C (Fig. 4.8). A CS of 75% was estimated as the 'saleable limit', so applying this criterion the fruit stored at 2-5°C showed acceptable colour until 10 weeks.

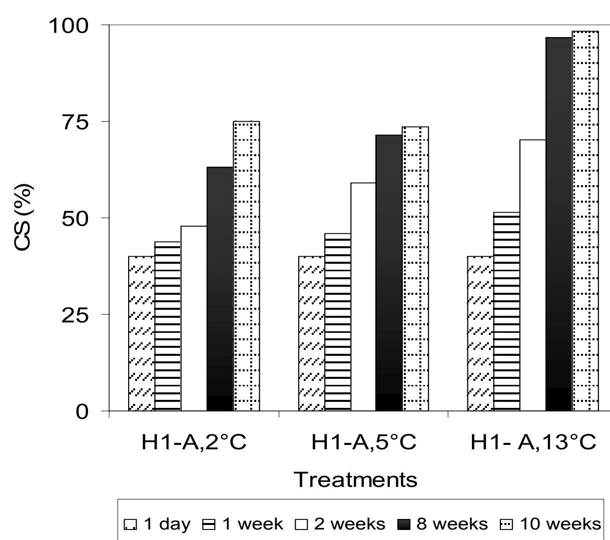


Figure 4.8 CS after harvest for NZ limes stored under RA at 2, 5 and 13°C until 10 weeks of storage, H1.

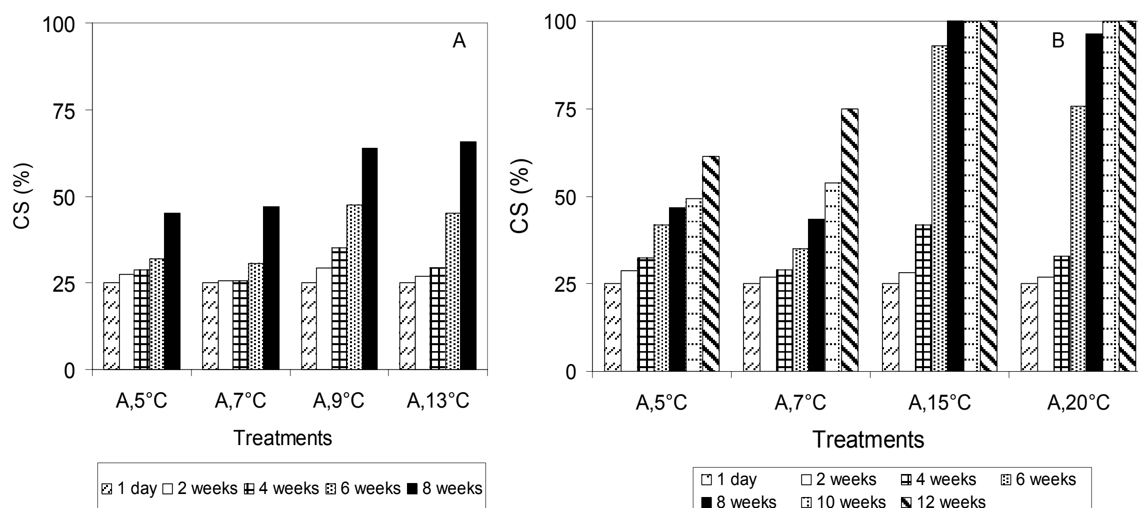


Figure 4.9 CS after harvest for NZ limes stored under RA at 5, 7, 9 and 13°C until 8 weeks of storage, H2 (A) or at 5, 7, 15 and 20°C until 12 weeks of storage, H3 (B).

There was rapid change for CS in storage temperatures above 10°C (Fig. 4.9A, B) which was similar to the change in H° (Fig. 4.2A, B and 4.3A, B). The rate of change of CS of the fruit stored under RA at 9 and 13°C was faster than the fruit stored under RA at 5 and 7°C (Fig. 4.9A), however it was quite difficult to distinguish between the CS of the fruit stored at 5 or 7°C, or the fruit stored at 9 or 13°C. The H2 and H3 fruit had the same starting CS (25% yellow) before storage and the difference in rate of change in CS was less marked than that observed with respect to change in H° at the lower temperatures (c.f. Fig. 4.4A and 4.5A with Fig. 4.9). Fruit from both harvests had similar CS of 40-45% after 8 weeks for both storage temperatures of 5 and 7°C. H3 fruit remained at an acceptable CS (≤75%) by 12 weeks at 5 or 7°C, whereas the fruit stored at 15 or 20°C reached 75% after only 6 weeks (Fig. 4.9B).

The colour change of lime is not uniform over the fruit surface as yellowing can occur over the whole surface or in certain patches. The use of CS to indicate overall colour change was both more simple, and possibly more correct than use of H°, as it provided an integrated assessment across the whole fruit surface. Despite the subjective nature of the use of a colour score chart, a strong and approximately linear correlation was observed between H° and CS for lime stored under RA as shown for fruit stored at 20°C in Fig. 4.10A. The trends in change of H° and CS under this condition are shown in Fig. 4.10B.

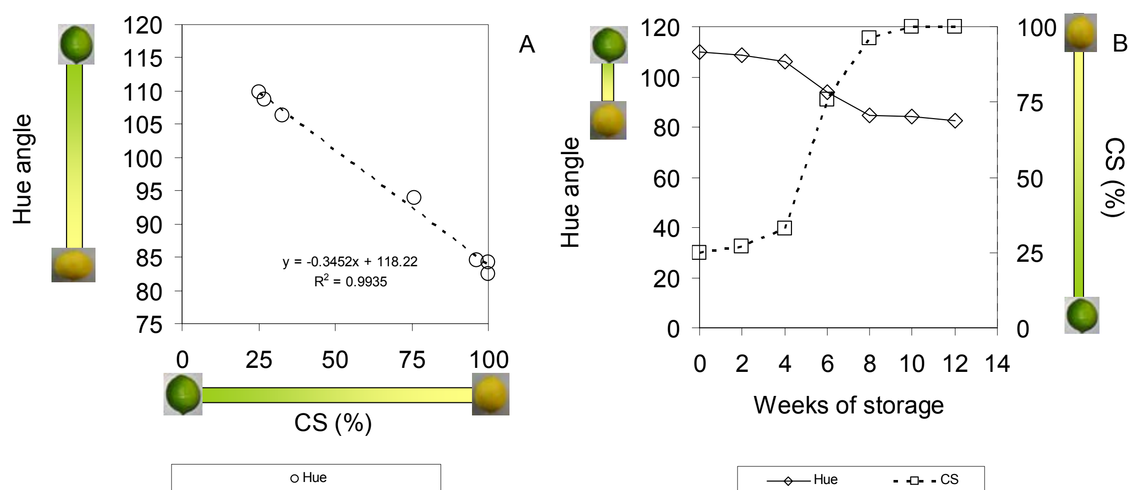


Figure 4.10 Correlation between H° and CS for NZ limes stored under RA on the green side (A) or association between H° (◊) on the green side and CS (□) (B), stored at 20°C, H3.

A grading chart is useful for determination of overall fruit quality and possibly better reflects the customer's perspective regarding the appearance of the produce. In contrast the colorimeter or spectrophotometer provides more objective information on colour change, but only at selected points on their surface. Spectrophotometers can also provide spectral data that may be influenced by changes in plant pigments. This aspect is further explored in chapter 6.

4.1.3 Disorders under RA

4.1.3.1 Introduction

Limes are chilling sensitive (Murata, 1997; Ladaniya, 2004) and in common with other citrus varieties, chilling injury can manifest in various forms including sunken scald-like discolouration, watery breakdown (pale brown areas characterised by cellular breakdown with fluid leakage) and pitting (Porat, 2004; Porat *et al.,* 2004). The severity of injury increases as the temperature is lowered below 10°C (50°F) and with longer durations of exposure to these temperatures (Arpaia and Kader, 2000). Murata (1997) reported that although the green rinds of lime are retained better at 4°C than at the recommended RA storage temperature (9-10°C), the fruits are subject to pitting which markedly shortens the storage life. The importance of these effects for NZ limes is considered in this section.

4.1.3.2 CI symptoms after storage of NZ lime under RA

There were some pitted or chilling injured fruit when the fruit were stored at 5°C or below for 8 or 10 weeks of storage but none in fruit batches stored under RA at 13°C for up to 10 weeks of storage (Fig. 4.11). The percentage of fruit stored under RA at 2°C that showed severe CI (i.e., CI-4) reached 20% and 45% at 8 and 10 weeks of storage, respectively. Less than 10% of the fruit stored under RA at 5°C showed less severe symptoms (CI-1 and CI-3) at 8 weeks of storage but more severe CI and a greater incidence of CI (CI-4; about 30%) was evident by 10 weeks of storage. Fruit could show either pitting or CI symptoms but there was a general tendency for pitting to appear first and then progress to CI. Thus, fruit that showed pitting subsequently showed more severe pitting and then CI symptoms. High-quality H3 fruit stored under RA at 5°C showed significant pitting (~15%) of fruit but no CI after 8 and 10 weeks and the fruit stored at 15 or 20°C did not show any pitting or CI after 12 weeks of storage (data not shown).

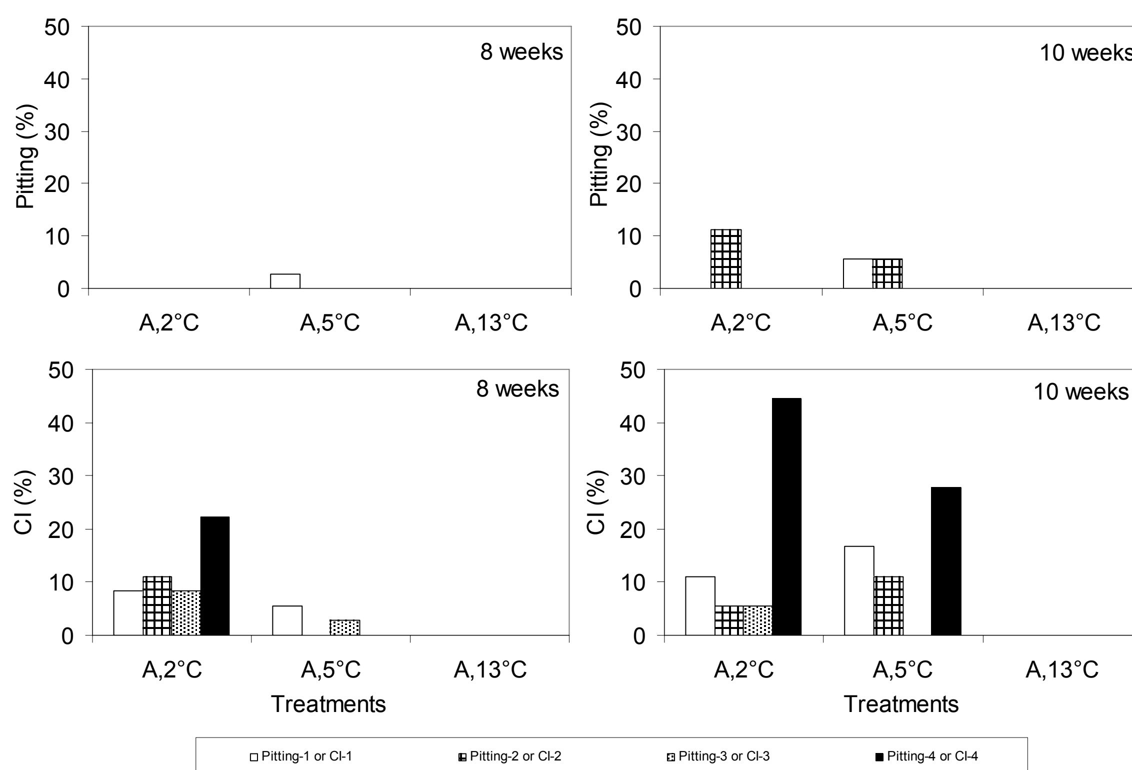


Figure 4.11 Incidence of pitting injury and CI after 8 (left) and 10 (right) weeks of storage for H1 fruit and RA storage at low and non-chilling temperatures (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

The H2 fruit suffered from CI when stored under RA at 5°C, but those fruit stored at 7°C only showed symptoms of CI after 8 weeks of storage (Fig. 4.12). The H2 fruit stored under RA at 9 or 13°C did not show any disorders at 6 and 8 weeks of storage. Overall, these data confirm that NZ lime fruit exhibit CI when stored at or below 7°C (for 8 weeks or more).

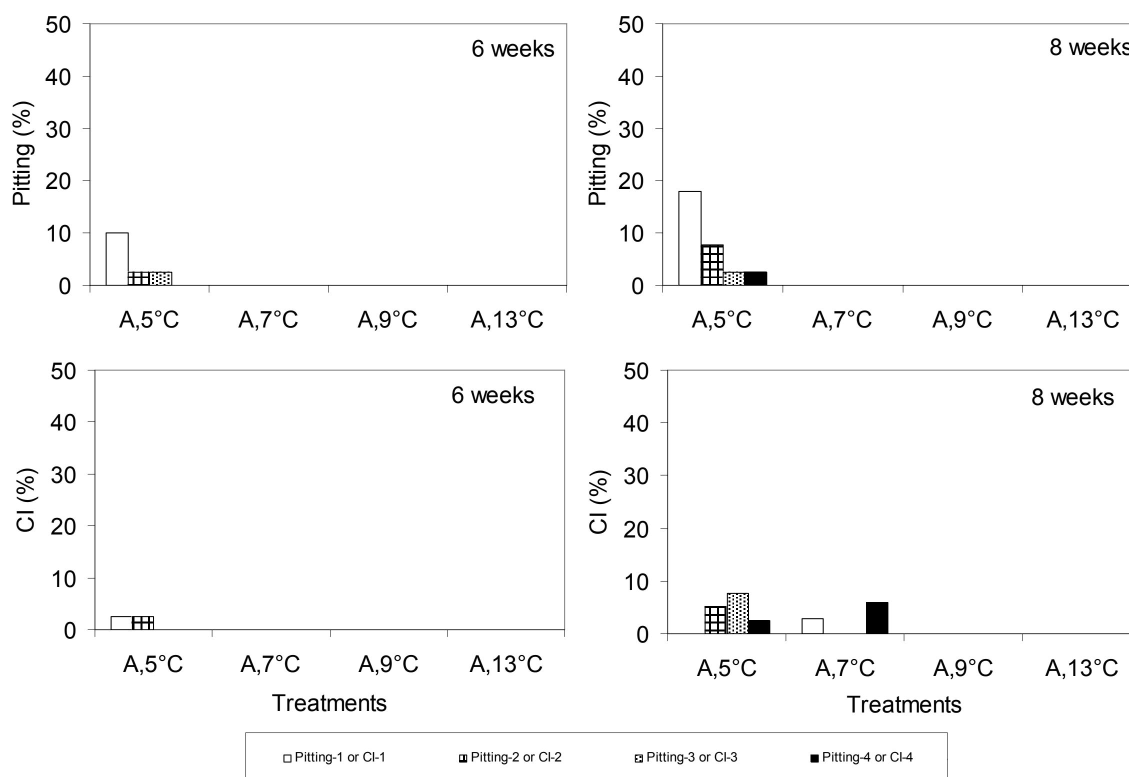


Figure 4.12 Incidence of pitting injury and CI after 6 (left) and 8 (right) weeks of storage for H2 fruit and RA storage (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

The H4 fruit stored under RA at 5 and 7°C with and without ethylene absorbent showed less than 20% pitting at 8 weeks and more severe pitting (up to 25%) was observed at 10 weeks of storage. The H4 fruit stored at 5°C (with no ethylene absorbent) or 7°C, with and without ethylene absorbent, did not show significant CI at 8 weeks. However, the incidence and severity of CI had increased by 10 weeks for all treatments (Fig. 4.13). The use of ethylene absorbent therefore appeared to provide no benefit in protecting against these disorders, but I do not know what actual ethylene concentration was present in the chamber.

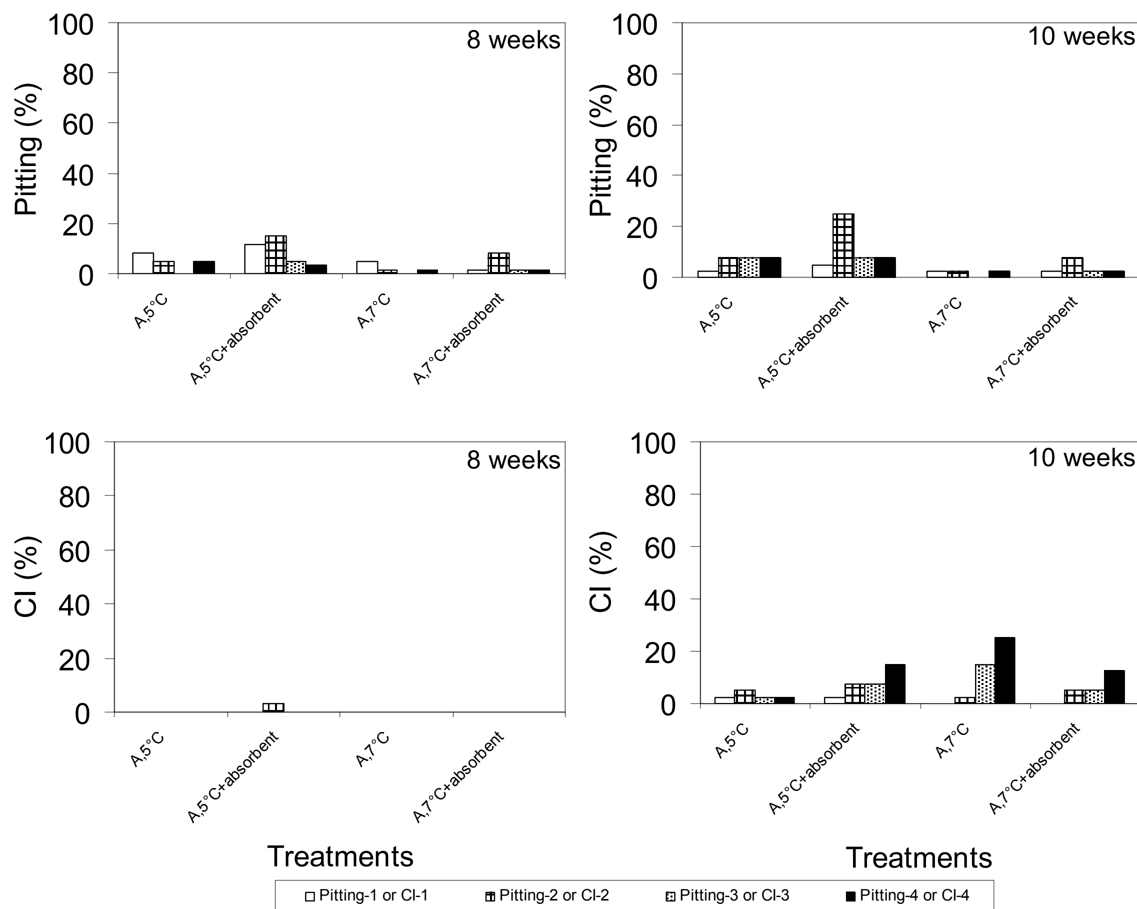


Figure 4.13 Incidence of pitting injury and CI after 8 (left) and 10 (right) weeks of storage for H4 fruit (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

4.1.4 Disorders under RA after storage and shelf life

4.1.4.1 Introduction

Twenty fruit were randomly selected from each treatment stored for 8 and 10 weeks at 5 and 7°C in H4, respectively. These fruit were placed in a 20°C room for 3 days shelf life as Saltveit and Morris, (1990) reported that while CI symptoms may appear during exposure to the chilling temperature, after removal of the produce to a warmer temperature the appearance of this disorder is usually rapid.

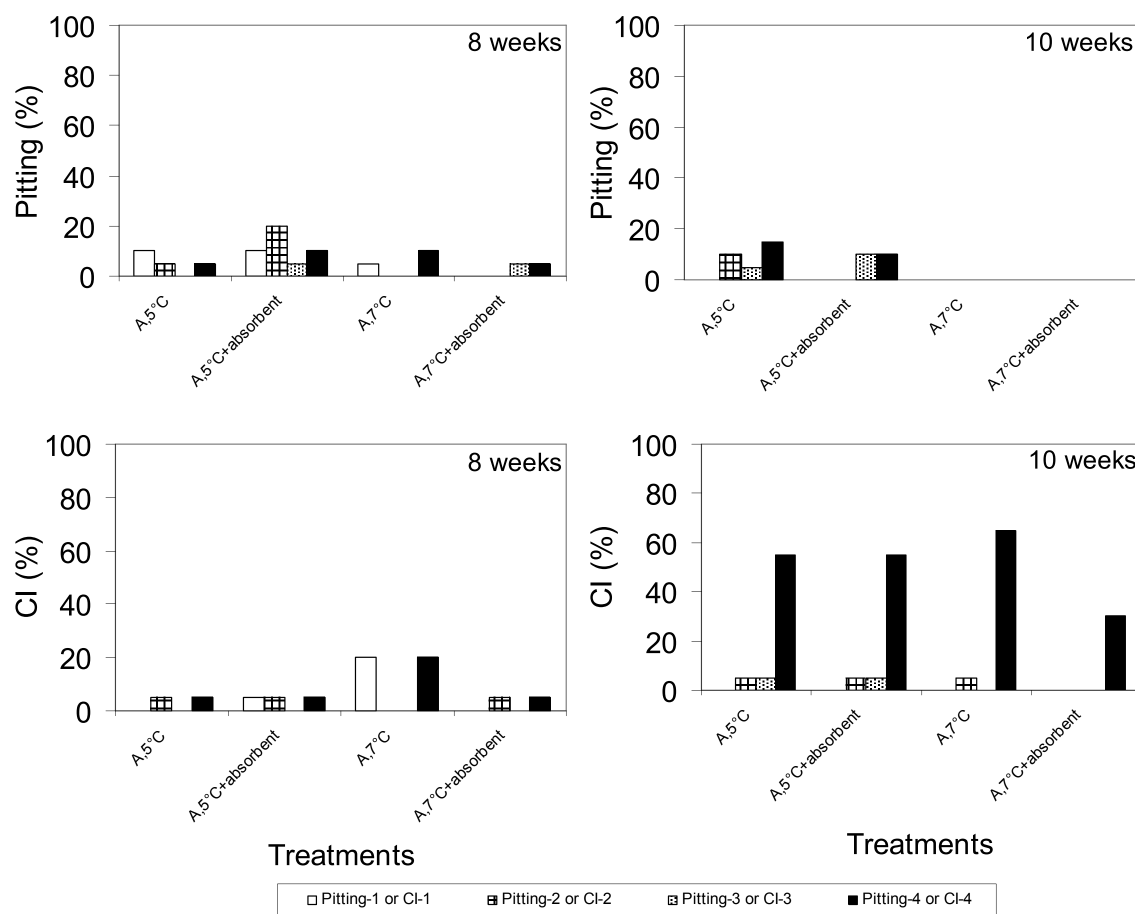


Figure 4.14 Incidence of pitting injury and CI after 8 (left) and 10 (right) weeks of storage plus 3 days shelf life at 20°C for H4 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

Incidence and severity of pitting and CI of H4 fruit increased after 3 days at 20°C following 8 and 10 weeks at 5 and 7°C. CI symptoms were more evident than pitting: although CI symptoms were present at low levels after 8 weeks of storage (Fig. 4.13 and 4.14), after placing the fruit in shelf life for 3 days, the symptoms developed to more severe CI and the proportion of fruit affected was much higher (Fig. 4.14). All treatments showed less than 30% CI at 10 weeks of storage (Fig. 4.13), but after the 3 days shelf life up to 60% of fruit now exhibited more severe CI (Fig. 4.14). Overall, this confirms that a storage temperature of ≤7°C is not suitable for NZ lime storage and demonstrates that any acceptable storage regime must provide very low levels of CI to enable fruit to be marketed with an acceptable ambient shelf life.

4.1.5 Rots under RA

4.1.5.1 Introduction

Fresh fruits are prone to fungal contamination in the field and through the supply chain to the end consumer (Tournas and Katsoudas, 2005). Pathological breakdown leading to decay is one of the major problems that limits the capability for long term storage of citrus fruit (Porat *et al.,* 2004). Fresh lime fruit has a moisture content of 89.3% (w/w) and they contain many nutrients to support growth of pathogens (Ortiz, 2002), but the low pH of the flesh limits their susceptibility to attack by pathogens other than fungi (Tripathi and Dubey, 2004; Tournas and Katsoudas, 2005). The common postharvest fungal diseases of citrus are Alternaria rot (*Alternaria citri*), gray mould (*Botrytis cinerea*), sour rot (*G. candidum*), brown rot (*Phytophthora citrophthora* and *Ph. parasitica*), green mould (*P. digitatum*) and blue mould (*P. italicum*) (Giudice, 2002). In addition, other cases of fungal spoilage of citrus fruits have been attributed to *Fusarium* and *Aspergillus* (Tournas and Katsoudas, 2005). In this research the rots were tentatively defined as early stage *Botrytis*, *P. italicum*, *Geotrichum*, *P. digitatum* and in rare cases, unknown soft rot. The incidence of these was quantified for the different storage regimes and incorporated in the overall measurement of quality (section 3.6).

The pattern and severity of mould infection observed in this work depended on temperature and whether or not fruit were treated with the fungicide TBZ. For untreated fruit, the incidence of infection and the rate of spread of the pathogens tended to increase significantly with storage temperature and time. H1 fruit stored under RA at 2 and 5°C showed less than 5% of *Geotrichum* at 8 weeks of storage and of the fruit stored under RA at 2°C, about 5% were classified as affected by *P. italicum* and about 17% by soft rot at 10 weeks of storage. The H1 fruit stored under RA at 13°C showed about 14 and 11% soft rot at 8 and 10 weeks of storage, respectively, and some 83% were affected by *Botrytis*, but only on the button and at 10 weeks of storage (Fig. 4.15).

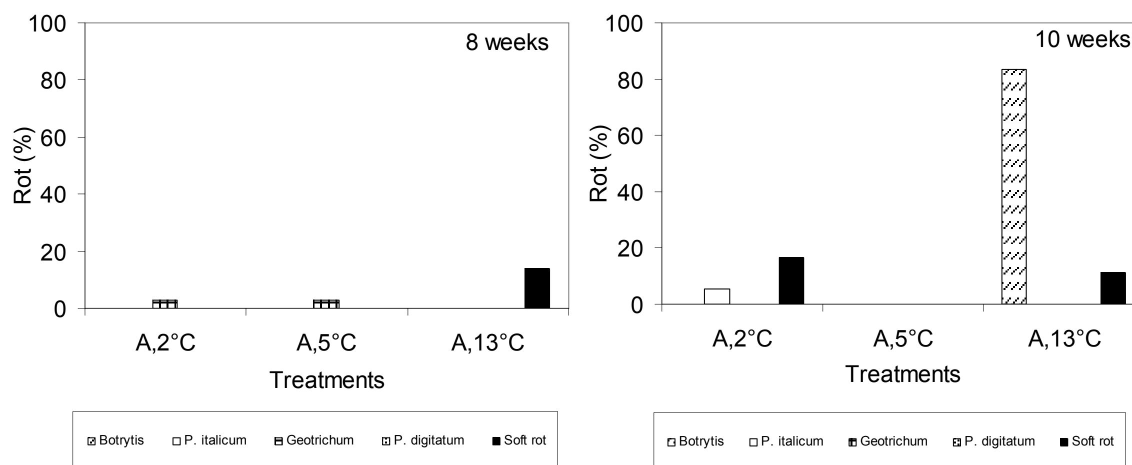


Figure 4.15 Incidence of rots after 8 (left) and 10 (right) weeks of storage for the fruit stored under RA at 2, 5 and 13°C, H1.

The H2 fruit (also untreated with TBZ) stored under RA at 5, 7, 9 and 13°C showed both *Botrytis* and *Geotrichum* following storage for 6 and 8 weeks. The incidence of these infections increased with increasing storage temperatures after 6 and 8 weeks, respectively. However, an unexpected decrease of rot incidence of fruit stored at 13°C was observed when compared to other treatments at 8 weeks (Fig. 4.16).

Given ongoing problems with infection, I resolved to include 1500 ppm of thiabendazole (TBZ) as a pre-storage treatment in cold water. In the case of H3 fruit this treatment gave good protection against soft rots of lime fruit until 10 weeks of storage (Fig. 4.17). However, the fruit stored under RA at 7, 15 and 20°C still showed symptoms of *Botrytis* infection at 10 and 12 weeks (Fig. 4.17). There was no recorded *Botrytis* or soft rot infections for the fruit stored at 5°C even after 12 weeks of storage. It could be concluded that the application of 1500 ppm of TBZ in combination with cold storage at 5°C was effective at reducing storage rots over 12 weeks of storage.

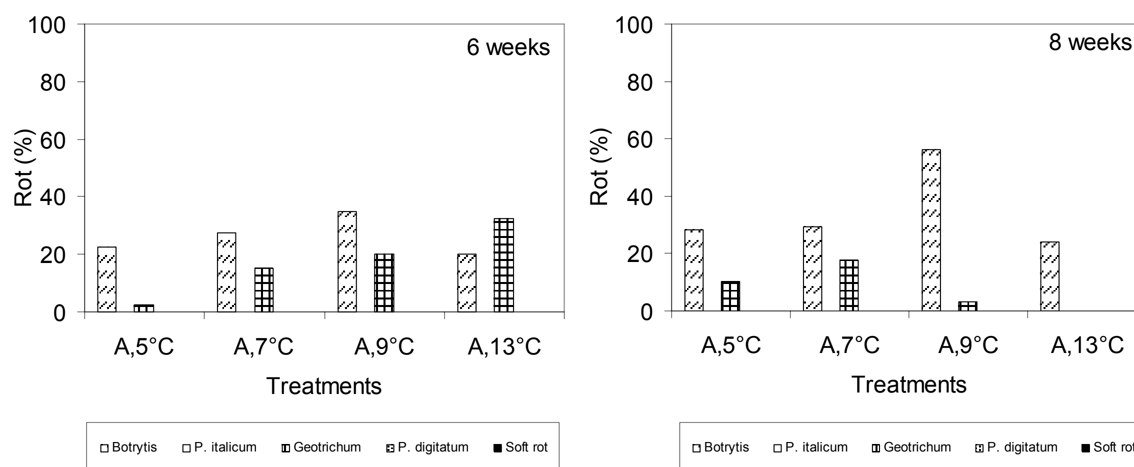


Figure 4.16 Incidence of rots after 6 (left) and 8 (right) weeks of storage for the fruit stored under RA at 5, 7, 9 and 13°C, H2.

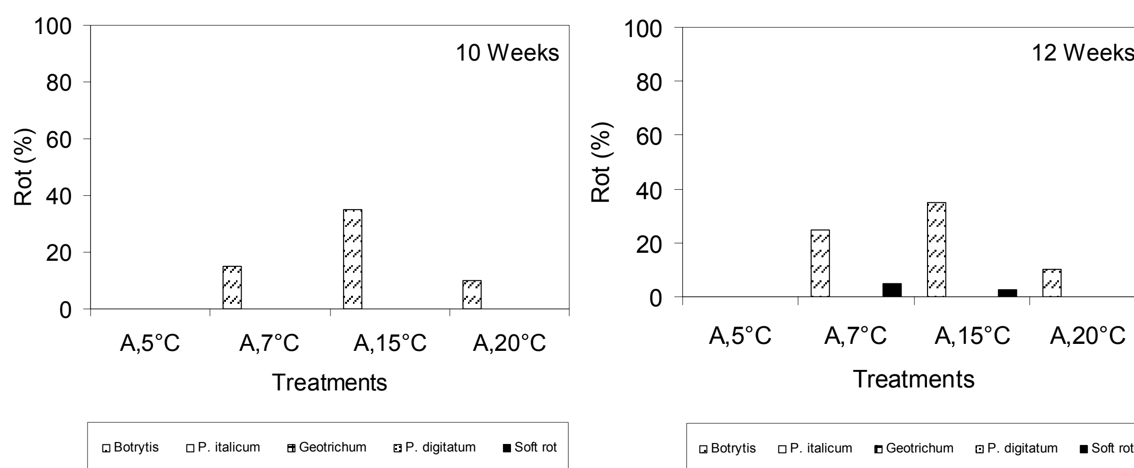


Figure 4.17 Incidence of rots after 10 (left) and 12 (right) weeks of storage for the fruit stored under RA at 5, 7, 15 and 20°C, H3.

4.1.6 Compression firmness (CF) under RA

4.1.6.1 Introduction

Firmness is frequently used as a quality index for fruits and vegetables and is related to the product's maturity, freshness, and extent of bruising or compressive damage (Hahn, 2004). Loss of firmness can be an indicator of the end of shelf-life and a key factor that influences the consumer's acceptance of the product (De Ketelaere *et al.,* 2006). Firmness assessments can be made by compression or puncture tests with various probes. Different applied forces or deformation levels can be selected, depending on the purpose of the measurement and how the quality attributes are to be interpreted (Abbott, 1999). In this research, non-destructive compression firmness (by 2 mm deformation) of a whole lime was measured during storage and shelf life as the indication of fruit firmness. This was presumed to be a reasonable representation of a 'squeeze test' that a consumer may use in selecting fruit for purchase.

4.1.6.2 Association of CF with disorders or mass loss for limes stored under RA

The mean CF of H1 fruit stored under RA at 2, 5 and 13°C for 10 weeks and placed for 3 days shelf life at 20°C decreased from ~ 27 N to 9.5, 13 and 18 N, respectively (Fig. 4.18). The CF of each treatment was significantly different from each other at 10 weeks of storage (Fig. 4.18). Loss of firmness at 2°C was evident after 8 weeks and increased further by 10 weeks. At 5°C, more rapid loss of firmness of lime occurred after 8 weeks of storage.

There appeared to be a link between the extent of CI and loss of firmness as shown in Fig. 4.19. In this figure, overall CI scores at each assessment time (a weighted sum of the percentage of injured fruit for each degree of severity of CI) of the fruit stored at 2 and 5°C were calculated by Eq. 4.1.

$$CIscore = \sum (\%CI1 \times 1) + (\%CI2 \times 2) + (\%CI3 \times 3) + (\%CI4 \times 4) \qquad \textbf{Eq. 4.1}$$

Fruit stored under RA at 2°C had significantly more CI than fruit stored at 5°C. This connection between CI and CF was not unexpected as CI leads to extensive softening of rind tissue and so will decrease fruit resistance to compression. The similar CI levels at 10 weeks for fruit storage at 2 and 5°C were reflected in (relatively) similar final CF values. There was no CI in fruit stored at 13°C and the CF did not decline after 8 weeks of storage (Fig. 4.18).

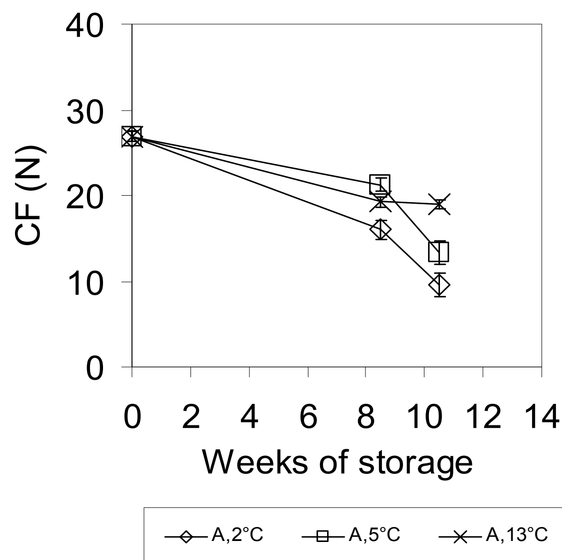


Figure 4.18 CF for NZ limes stored under RA at 2, 5 and 13°C after shelf life for 3 days after 8 and 10 weeks of storage, H1. Vertical bars indicate ± SE (n=36).

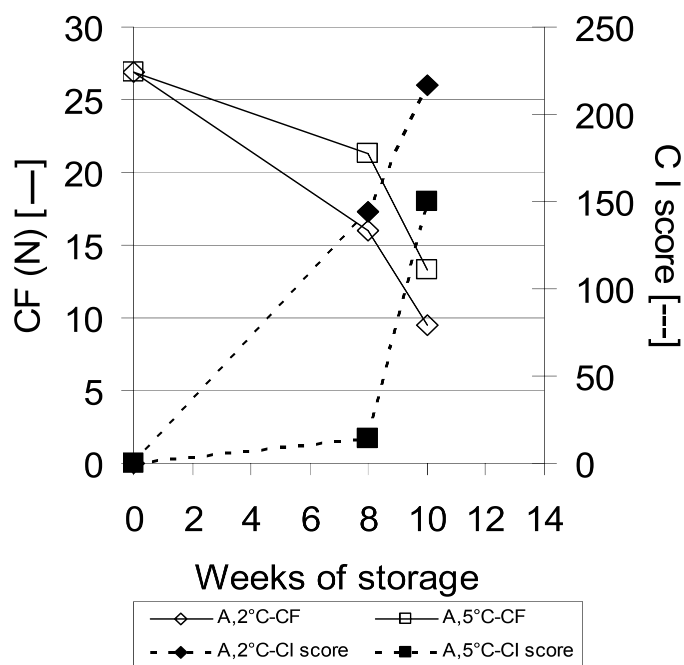


Figure 4.19 Association between CF of the fruit stored for 8 and 10 weeks plus 3 days shelf life at 20°C and CI scores of fruit at 8 and 10 weeks of storage for NZ limes stored under RA at 2 and 5°C, H1.

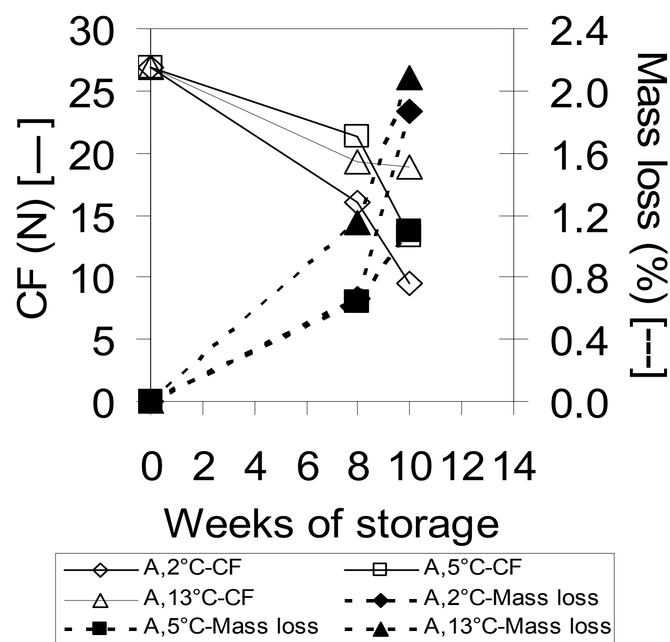


Figure 4.20 Association between CF of the fruit stored for 8 and 10 weeks plus 3 days shelf life at 20°C and all types (CI-1-CI-4) of mass loss at 8 and 10 weeks of storage for NZ limes stored under RA at 2, 5 and 13°C, H1.

Mass loss is another possible cause of loss of CF. The association between CF and percentage mass loss of fruit stored at 2, 5 and 13°C is shown in Fig. 4.20. The fruit stored at 2°C increased in mass loss after 8 weeks and this also coincided with the increase in CI. The overall mass loss in limes was relatively low (appendix I) and the relative changes in mass loss differed from the changes in CF. Mass loss at 13°C was higher as could be expected but did not result in a high firmness loss. Mass loss was lower at 5°C but firmness loss was higher, especially between weeks 8 and 10. Both mass loss and firmness loss were higher at 2°C and overall the data suggest CF was more affected by CI than mass loss.

4.2 Behaviour of lime fruit under CA storage

4.2.1 Introduction

The use of controlled atmospheres (CA) can prolong the storage life of many freshly harvested commodities by reducing the respiration rate and slowing other metabolic processes. This is achieved by both lowering the storage temperature and by restricting oxygen availability and/or elevating carbon dioxide concentration in the storage environment (Mathooko, 1996). CA has been shown to be effective in inhibiting the ripening of fruit, suppressing the production of ethylene, inhibiting certain storage disorders and slowing the growth of decay organisms.

Based on the literature for other citrus, it appeared that CA may possibly be helpful in prolonging the high quality storage life of NZ limes so the most promising conditions identified for citrus storage, namely 10% O_2 with 0 or 3% CO_2 (see section 2.8.3.2) were studied under a range of sub-ambient temperatures (2-13°C) in the 2004 lime season. As, there were also reports of the risks of CI for limes under CA storage and this was a potential concern, so there was the need to check the effects of CA storage on lime disorders.

4.2.2 Colour changes during CA storage

4.2.2.1 Trends in hue in CA storage

Both H° and CS were measured and recorded in the same way as the fruit stored in RA. Different CA storage regimes were investigated for the 2004 main crop (H1) in order to evaluate the relative benefits of 0 and 3% CO_2 with 10% O_2 and the results were confirmed on an experiment in the late crop (H2) in the same year. Only 3% CO_2 with 10% O_2 was used for the second harvest. The CO_2 concentrations and temperature ranges employed are summarised in Table 4.4.

Table 4.4 The temperatures ranges and CA conditions for H1 and H2 used during the year 2004.

Harvest #	CO_2 concentration with 10% O_2	Temperature ranges (°C)
1	0 and 3%	2, 5, 13
2	3%	5, 7, 9, 13

Typical colour profiles for both the green and yellow positions on the fruit stored under CA storage are shown in Fig. 4.21 for H1 fruit. CA storage at 3% CO_2 with 10% O_2 and 5°C delayed degreening on the green side compared to RA, for which yellowing was seen after 8 weeks of storage. The results from this research are similar to Wild *et al.,* (1976) who examined lemons (*C. limon* (L.) Burm. f.) stored at 10°C in air and with CA at 3-5% O_2 and 0.1-0.2% CO_2, with and without C_2H_4 absorbent, for 27 weeks. CA storage successfully reduced the rate of the green colour loss when compared with the air treatments in this early study. However, in the current research project, the fruit stored under CA regimes of 0 or 3% CO_2 with 10% O_2 stored at 2 or 13°C did not show any delay in colour change and the H° decreased rapidly after 8 weeks of storage (Fig. 4.21A). For the yellow side of the fruit the H° of the fruit stored under CA at 3% CO_2 was not significantly different from the fruit stored under RA at 5°C and the 0 and 3% CO_2 CA regimes stored under 2 and 13°C (Fig. 4.21B).

Following from this experiment, only the 3% CO_2 plus 10% O_2 condition was selected for further study with fruit in H2. The results showed that limes stored under this CA condition at 5°C were not significantly different in colour from the fruit stored under RA at 5°C (Fig. 4.22A, B) or CA at 7, 9 or 13°C on either the green or yellow side of fruit after 8 weeks of storage (Fig. 4.22A, B). Overall this suggests there is no major benefit offered by CA storage in delaying degreening.

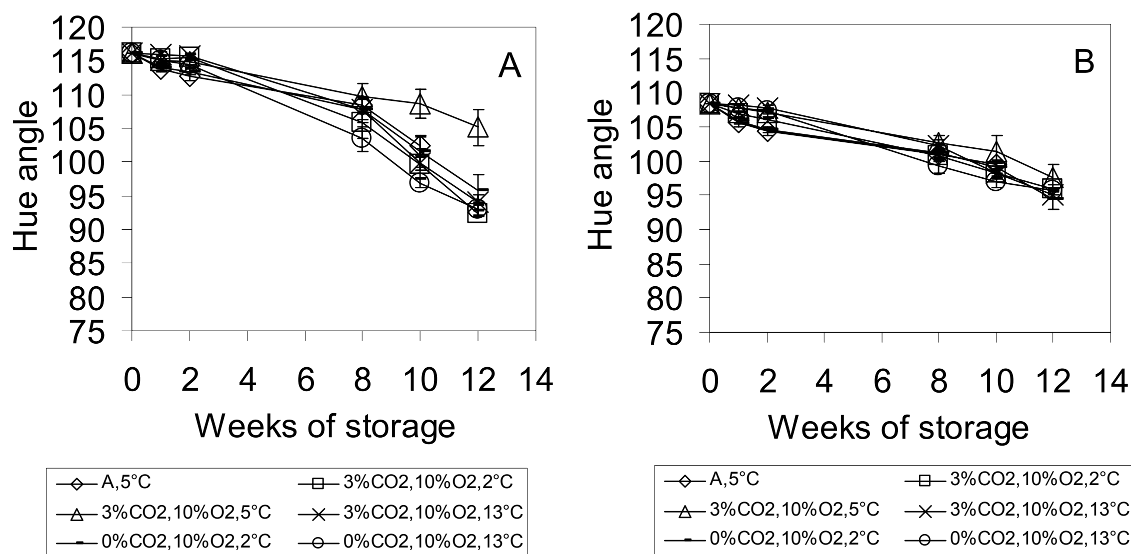


Figure 4.21 Colour change for NZ limes stored under RA at 5°C and 0 or 3% CO_2 with 10% O_2 CA at 2 or 5 and 13°C on the green side (A) or yellow side (B), H1. Vertical bars indicate ± SE (n=18).

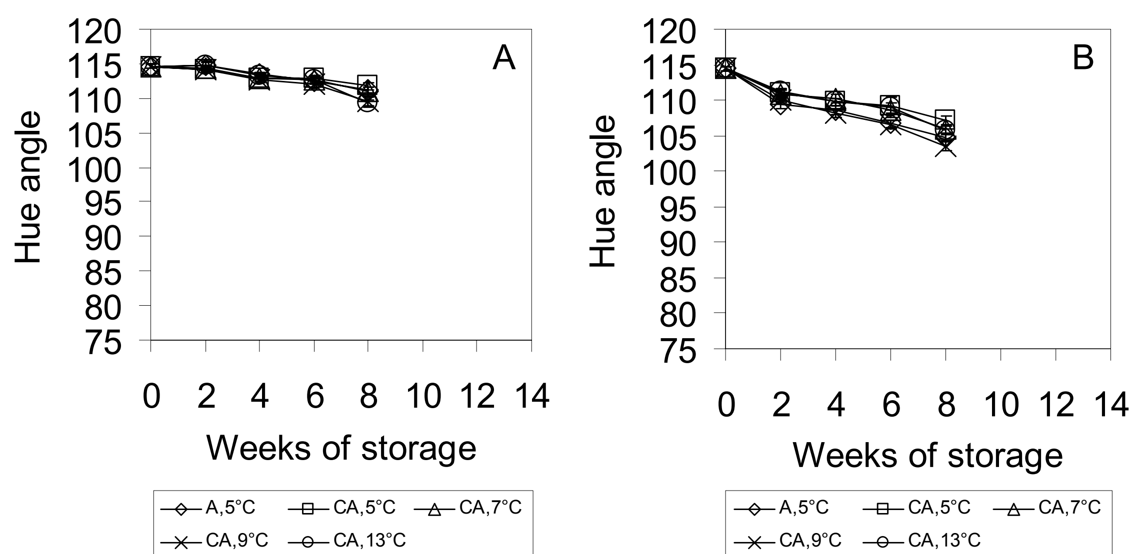


Figure 4.22 Colour change for NZ limes stored under RA at 5°C and 3% CO_2 with 10% O_2 CA at intermediate temperatures ranges 5, 7, 9 and 13°C on the green side (A) or yellow side (B), H2. Vertical bars indicate ± SE (n=40).

4.2.2.2 Colour score under CA storage

CS data generally matched the trends in hue data (Fig. 4.23-4.24). The fruit stored under CA had a CS <50% yellow at 2 weeks of storage and CS <75% at 8 week of storage except for those fruit stored under both CA conditions at 13°C (Fig. 4.23). All H2 fruit

stored under RA at 5°C or CA conditions at all temperatures gave a CS <75% yellow after 8 weeks of storage (Fig. 4.24). The H2 fruit stored under CA at 9 and 13°C showed CS of ~50% or lower at 8 weeks (Fig. 4.24) whereas the CS of the fruit stored under RA at 9 and 13°C at the same time (Fig. 4.9) were >60%. This confirms that when assessed visually based on overall fruit colour, the CA condition slightly delayed colour change of the fruit at 9 and 13°C compared with RA storage.

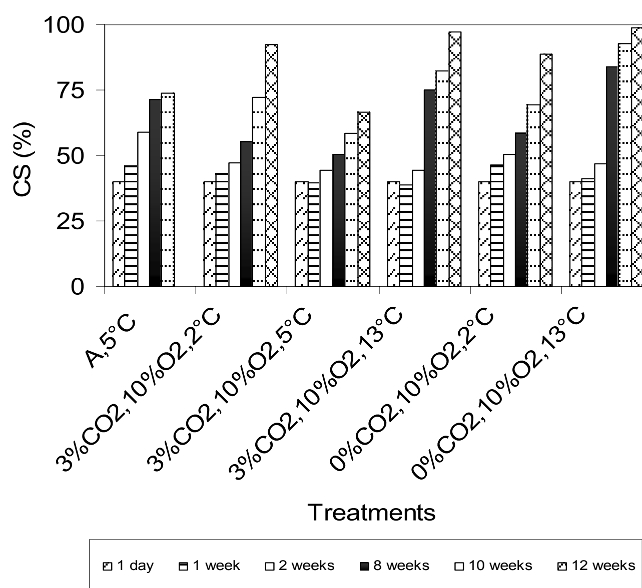


Figure 4.23 CS after harvest for NZ limes stored under RA at 5°C and 0 or 3% CO_2 with 10% O_2 CA at 2 and 13°C or 3% CO_2 with 10% O_2 CA at 5°C until 12 weeks of storage, H1.

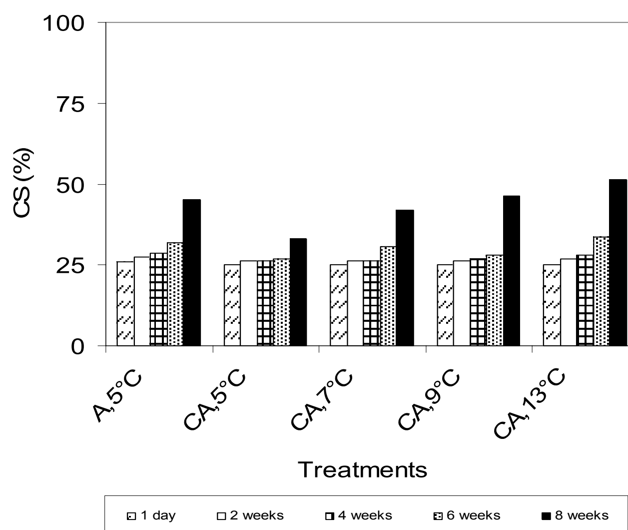


Figure 4.24 CS after harvest for NZ limes stored under RA at 5°C and 3% CO_2 with 10% O_2 CA at 5, 7, 9 and 13°C until 8 weeks of storage, H2.

4.2.3 Disorders under CA

For H1, no significant pitting or CI was seen in any treatment at 13°C for this was not a chilling regime (Fig. 4.25). However, at chilling temperatures, CA appeared to promote CI with a high incidence and severity observed for all 2 and 5°C regimes. There appeared to be no progression from pitting to CI as observed for RA storage (Fig. 4.11), so perhaps the mechanism of CI is different under CA.

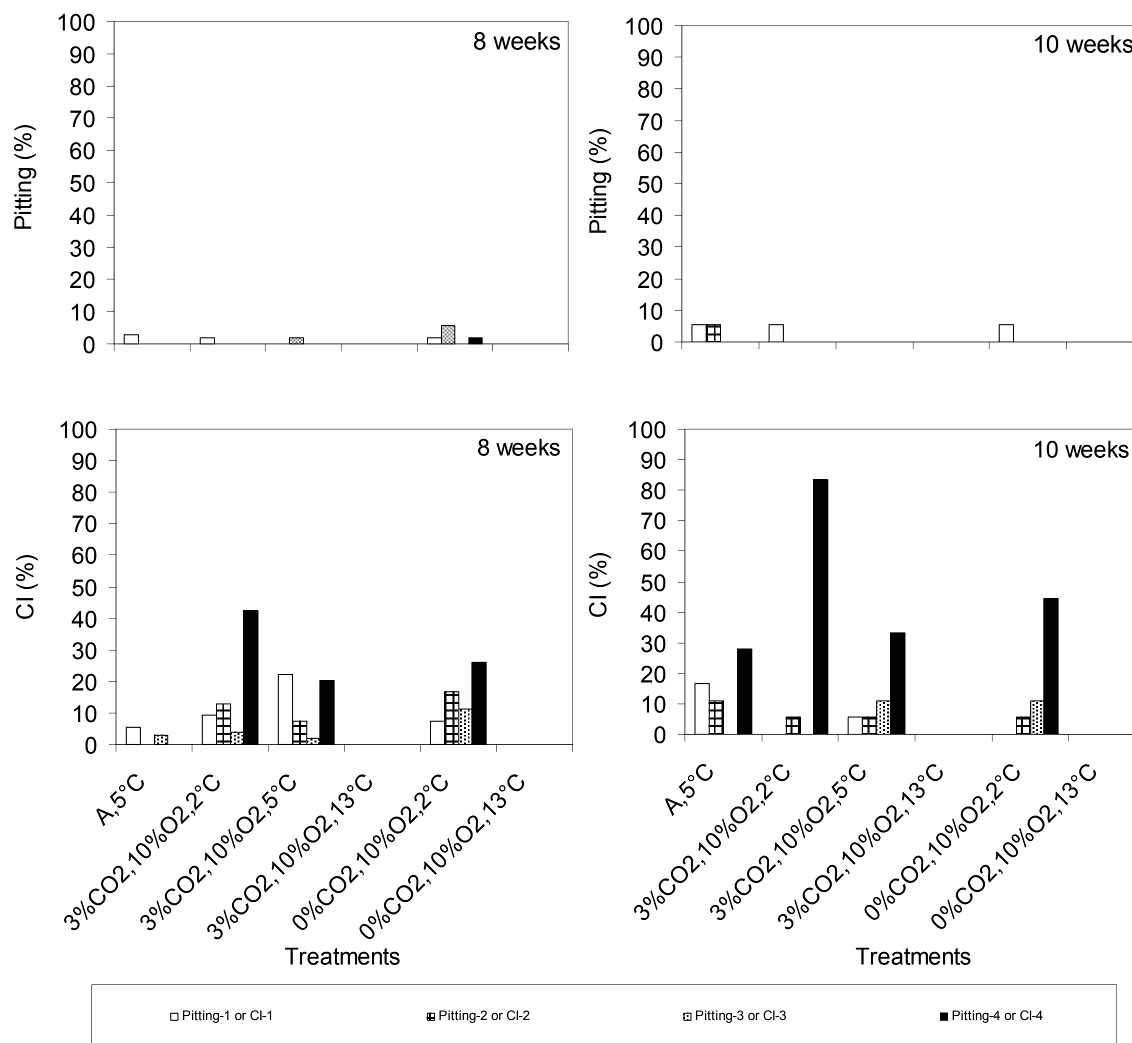


Figure 4.25 Incidence of pitting (top) and CI (bottom) of lime stored under RA at 5°C and 0 or 3% CO_2 with 10% O_2 CA at 2 and 13°C or 3% CO_2 with 10% O_2 CA at 5°C after 8 (left) and 10 (right) weeks of storage, H1 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

In H2, CI was generally less severe when compared with H1 fruit, but more attention was paid to early stages of CI development with 2 weekly monitoring from 6 weeks. Only occasional and mild pitting symptoms (pitting-1 or pitting-2) were first noticed and recorded at 4 weeks of storage (data not shown). The H2 fruit showed more pitting symptoms than H1 fruit at 8 weeks of storage (Fig. 4.25 and 4.26). This may be linked to their being greener and therefore possibly more immature at harvest. However, comparing between the H1 and H2 fruit stored under CA at 5°C for 8 weeks of storage, the percentage of severe CI (CI-4) of H1 fruit was higher than H2 fruit. The fruit stored under RA at 5°C showed more pitting than other CA treatments at 5, 7, 9 and 13°C, respectively at 8 weeks of storage. This means either that CA could protect against pitting for 8 weeks of storage or pitting was not an important mechanism under CA, however clearly the fruit stored under CA conditions suffered from CI. In summary 3% CO_2 with 10% O_2 reduced pitting in lime but could not help to protect limes from CI (Fig. 4.26).

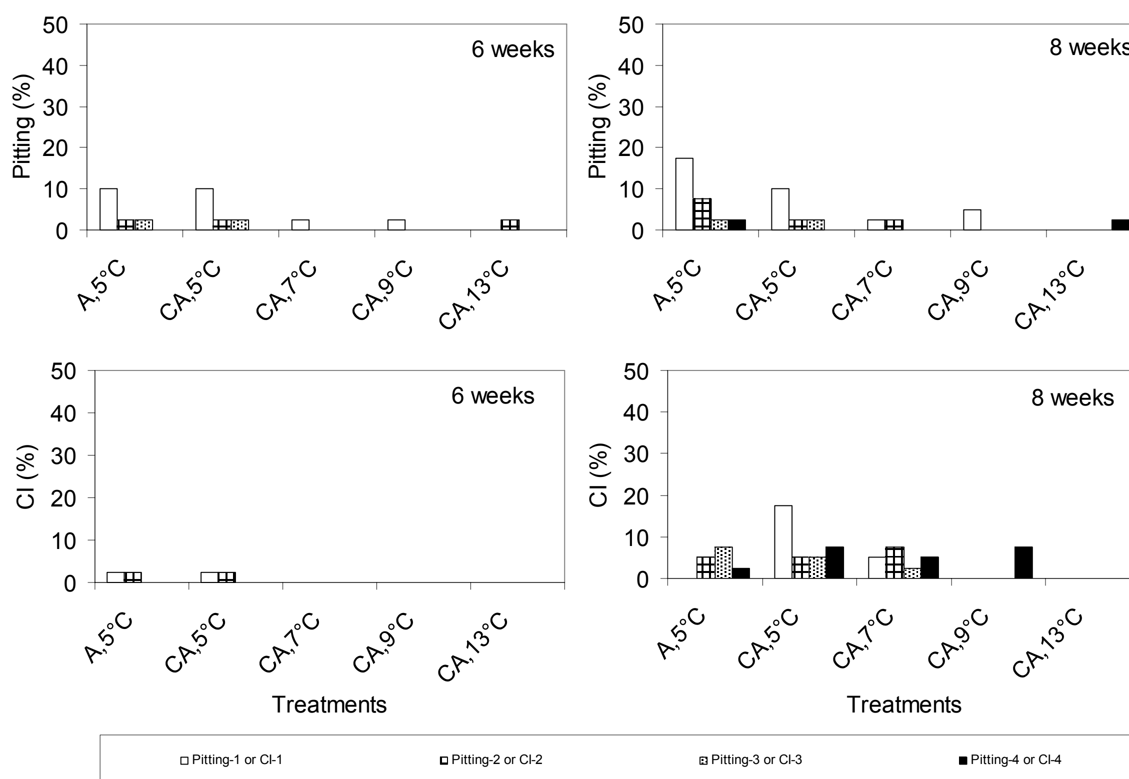


Figure 4.26 Incidence of pitting (top) and CI (bottom) of lime stored under RA at 5°C and 3% CO_2 with 10% O_2 CA at 5, 7, 9 and 13°C after 6 (left) and 8 (right) weeks of storage, H2 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

4.2.4 Rots under CA

There was low incidence of rots for H1 limes stored under RA at 5°C and all CA treatments stored at 2, 5 or 13°C after 8 weeks of storage. All CA treatments increased in incidence of rots at 10 weeks of storage (Fig. 4.27). The CA treatments did not show any protection against rots after 8 or 10 weeks of storage.

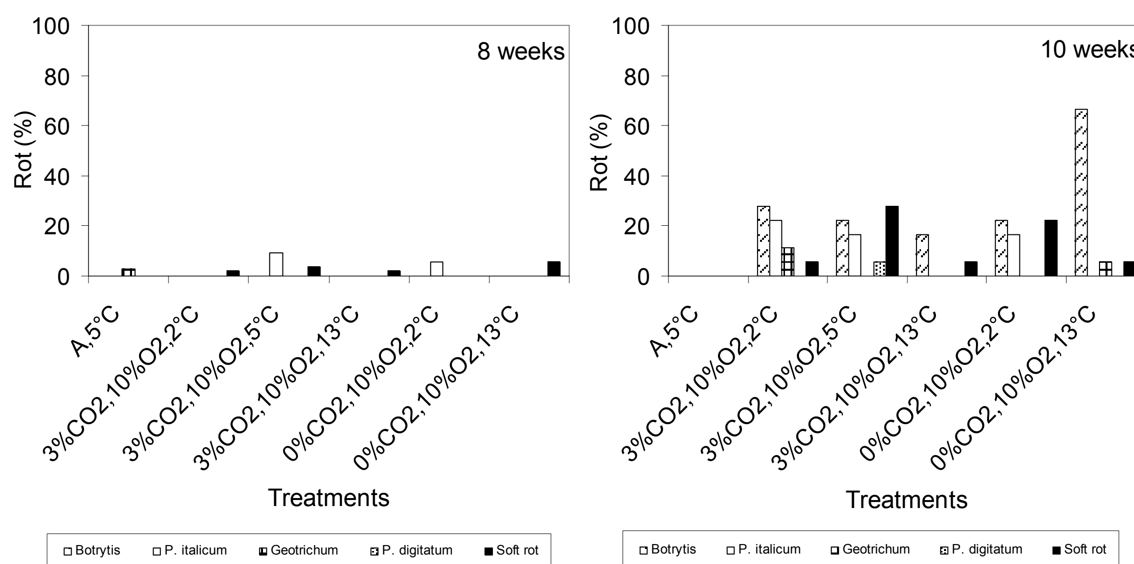


Figure 4.27 Incidence of rots of lime stored under RA at 5°C and 0 or 3% CO_2 with 10% O_2 CA at 2 and 13°C or 3% CO_2 with 10% O_2 CA at 5°C after 8 (left) and 10 (right) weeks of storage, H1.

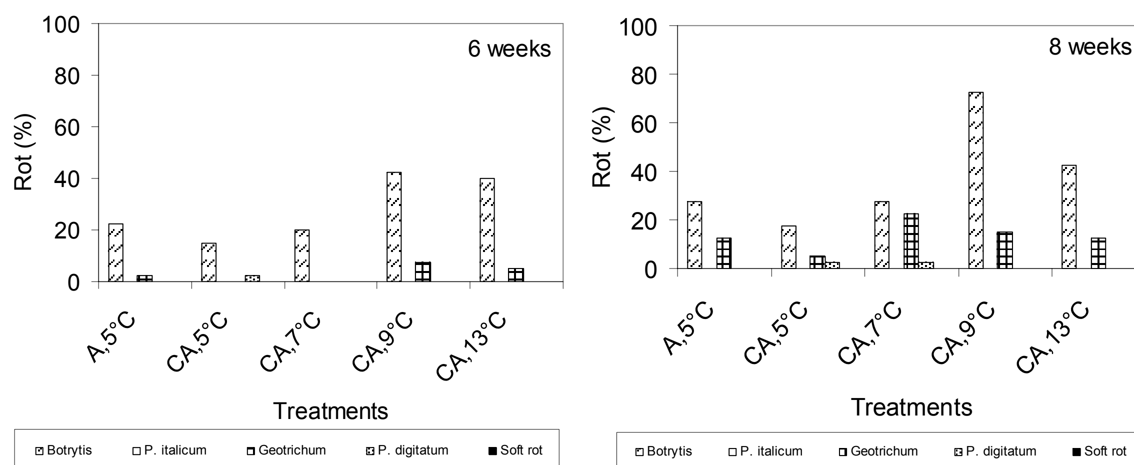


Figure 4.28 Incidence of rots of lime stored under RA at 5°C and 3% CO_2 with 10% O_2 CA at 5, 7, 9 and 13°C after 6 (left) and 8 (right) weeks of storage, H2.

The H2 fruit showed a reasonably high level of rots at 6 and 8 weeks of storage. The fruit stored under RA and CA at 5 and 7°C (Fig. 4.16 and 4.28) both showed similar percentages of rots. The fruit stored under CA conditions at the warmer temperatures at 9 and 13°C showed increased rots after 6 weeks of storage but the incidence was similar to RA storage at these temperatures (Fig.4.16 and 4.28).

Overall the CA treatments do not offer any advantages in terms of their effective control of storage rots of limes.

4.2.5 Compression firmness under CA

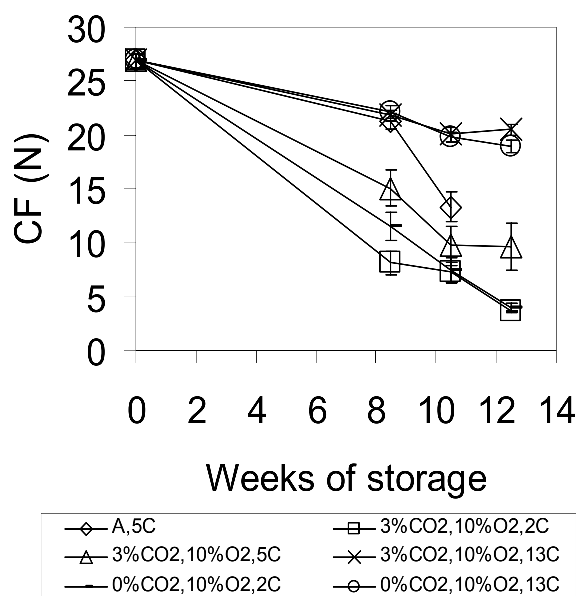


Figure 4.29 CF for NZ limes stored under RA at 5°C and 0 or 3% CO_2 with 10% O_2 CA at 2 and 13°C or 3% CO_2 with 10% O_2 CA at 5°C, H1. Vertical bars indicate ± SE (n=36).

Compression firmness of fruit stored under CA conditions showed a similar trend to fruit stored under RA, i.e. the higher the CI, the greater the loss of firmness. The fruit stored under RA at 5°C and CA at 13°C showed the highest CF at 8 weeks, but by 10 weeks the fruit stored under RA at 5°C decreased in firmness coinciding with the appearance of CI. Only the fruit stored under 13°C treatments showed a constant low rate of firmness loss over 12 weeks of storage (Fig. 4.29); this loss of CF was presumably related to mass loss as noted earlier.

4.3 Discussion

Temperature is the single most significant factor affecting the maintenance of quality of citrus fruits after harvest. The storage life of citrus fruits can be markedly extended if the fruit is stored at the lowest safe temperature that significantly suppresses respiration and fungal development whilst not inducing or promoting CI (Ladaniya, 2004). However citrus fruit are very sensitive to low temperature (Murata, 1997; Ladaniya, 2004; Rivera *et al.,* 2004) and pitting, staining and necrosis of the peel are typical symptoms of CI (Sala and Lafuente, 1999). Spalding and Reeder, (1983) and Arpaia and Kader, (2000) reported that pitting is a chilling injury symptom of lime and this develops into more severe symptoms evident as brown discolouration. CI severity increases with lower temperatures below 10°C (50°F) and longer durations of exposure to these temperatures. Ladaniya, (2004) reported that CI for yellow acid limes (*C. aurantifolia* Swingle) appeared after 30 days at 5.5-7°C, whereas aging of the peel was faster at 9.5-11°C.

In this research, I also observed that the aging of the peel of limes based on degreening and colour score of the fruit stored at 9 or 13°C increased faster than the fruit stored at lower storage temperatures (5 or 7°C) after 6 weeks of storage (Fig. 4.2 and 4.9). The rate of degreening of the fruit stored under RA at 2°C was slow and very similar to the fruit stored under RA at 5°C (Fig. 4.1), but the incidence and severity of pitting and CI of the fruit stored at these low temperatures was high. Peel pitting and browning resulting from CI was noticed during storage at both these low storage temperatures. Peel colour changes of NZ lime fruit can best be delayed by storage under RA at 5°C until 8 weeks, especially when fruit are picked green, however NZ limes will suffer from CI if storage is prolonged over 8 weeks.

Degreening or colour loss in 'Tahiti' lime increased with temperature (Spalding and Reeder, 1983). From this work, lime fruit stored under RA at intermediate temperatures (5, 7 and 9°C) showed the generally expected trend of increasing rate of degreening at higher temperatures (Fig. 4.2). There was not much additional acceleration in the rate of degreening when the temperature was increased at the higher end of the range tested (15 and 20°C) (Fig. 4.3). When other crops such as banana and tomatoes are stored at temperatures higher than 30°C, the degreening was inhibited (Mitcham and McDonald, 1992; Masarirambi *et al.,* 1995; Drury *et al.,* 1999).

Similar to other studies, any storage temperature ≤ 7°C adversely influenced the quality of limes. Storage at 2 and 5°C caused pitting and CI by 6 weeks of storage and storage at 7°C caused CI by 8 weeks of storage. Furthermore, the incidence and severity of pitting and CI increased after the fruit were placed for shelf life after storing the fruit at 5 or 7°C for 8 or 10 weeks (Fig. 4.14) confirming that NZ limes should not be kept at ≤7°C. Temperatures higher than 9°C did not cause any CI during storage (Fig. 4.11-4.12). Other researchers agree that this is the critical temperature for limes. Spalding and Reeder, (1983) report that 'Tahiti' lime stored too long at 7.2°C (45°F) can be seriously affected by CI. Wills *et al.,* (1998b) reported that the lowest safe temperature for lime is 7°C but my results strongly point to 7°C being too low for NZ limes.

Maturity of lime fruit at harvest is important because green limes can be stored for longer than green-yellow limes before becoming unacceptable for sale based on colour. I found the rate of degreening of the green lime (H2 and H5) was slower than the green-yellow stage lime (H1) (see Fig. 4.5B). Ladaniya, (2004) reported that green limes showed lower storage losses when compared to yellow fruit.

The change of CS depended on the storage temperature. Rapid changes in CS occurred above 10°C and were similar in trend to changes in hue (Fig. 4.8-4.9). CS is a less precise measurement than H° but gives a convenient and useful indication of harvest maturity and overall fruit colour. CS appeared linearly related to H° of the fruit with, for example, 25% and 75% CS referring to green and green-yellow limes with hue angles of about 110 and 95° (measured with a spectrophotometer Fig. 4.10), respectively.

Lower storage temperatures also reduced the incidence of rots. Lime stored at 5°C without the fungicide TBZ seemed to have fewer rots than fruit stored at 7, 9 or 13°C (Fig. 4.16) and lime stored at 5°C with the fungicide TBZ showed no rots (Fig. 4.17); the fruit stored under RA at 7, 15 or 20°C typically showed about 10-35% of fruit affecting rots (Fig. 4.17). Storing fruit at 5°C could therefore provide some benefits for lime storage if other techniques can be found to reduce CI. Some techniques that have been reported to reduce CI in citrus for example hot water dipping (Wild, 1993; Rodov *et al.,* 1995; Porat *et al.,* 2000) and intermittent warming (Cohen *et al.,* 1983; Cohen, 1988; Cohen *et al.,* 1990b; Artes *et al.,* 1993; Schirra and Mulas, 1995a; Schirra and Cohen, 1999; Kluge *et al.,* 2003a; Porat *et al.,* 2003).

Rots are an important factor limiting storage life of limes, in common with other citrus fruit. Rots of ‘Tahiti’ lime caused by *P. digitatum* Sacc., *P. italicum* Wehmer, and *Geotrichum* were observed after the fruit suffered from CI (Spalding and Reeder, 1983). In this work, I found *Botrytis*, *P. italicum* (tentative), *Geotrichum*, *P. digitatum* and in rare cases, an unknown soft rot (Fig. 4.15-4.17). The incidence and severity of rots were reduced after the fungicide TBZ was used at different concentrations or in combination with HWD or IW treatments. The effect of TBZ on rots during storage of lime is further discussed in chapter 5.

Loss of firmness of lime stored at 2°C after 3 day shelf life at 20°C was faster than lime stored at 5 and 13°C after 3 days shelf life. Firmness loss of lime stored at 5°C rapidly increased after 8 weeks because of the occurrence of CI. In contrast, the fruit stored at 13°C did not show CI therefore, their firmness after storage for 10 weeks plus 3 days shelf life remained high (Fig. 4.18). This indicated that the loss of firmness appeared to be associated with occurrence of CI (Fig. 4.19). Loss of firmness was indirectly associated to mass loss but I believe this is because CI itself can lead to mass loss. Ganji-Moghadam and Rahemi, (1998) (cited in (Ladaniya, 2004) reported that lime fruit showing the lowest CI also had the lowest mass loss. From my work, I can confirm that fruit stored at 5°C had lower CI symptoms than fruit stored at 2°C and the fruit at 5°C showed less mass loss than at 2°C. The fruit stored at 13°C had higher mass loss than the fruit stored at 2°C but they were firmer than the fruit stored at 2°C (Fig. 4.20).

Two atmosphere modifications were investigated for their effect on lime quality: ethylene adsorption and controlled atmosphere (CA) storage. The use of C_2H_4 absorbent did not show more benefits than storing the fruit under RA (5 and 7°C). It only delayed colour change of the peel at the beginning of lime storage and showed a small benefit in delaying yellowing at the yellow side of the fruit (Fig. 4.6). As noted previously, I do not know what ethylene concentration was achieved in the presence of the adsorbent sachets.

Optimal CA storage for horticultural produce depends on the species, maturity and ripeness stage of the produce, furthermore the temperature and the duration of exposure during storage is also important (Brecht *et al.,* 2003). CA has been reported to reduce decay of fruit and vegetables after harvest (Vigneault *et al.,* 2004) and delay colour changes by slowing the rate of chlorophyll loss or biosynthesis of carotenoids and

anthocyanins in fruit and vegetables. In addition CA storage can delay fruit ripening and softening. CA reduced losses in acidity in fresh fruits such as in 'Golden Delicious' apples (Kader, 1986) and apples (*Malus sylvestris* (L.) Mill.), pears (*Pyrus communis* L.) and kiwifruit (*Actinidia deliciosa*) have been successfully stored in commercial scale CA (Brecht *et al.,* 2003; Lallu and Burdon, 2007).

CA does have some disadvantages. The technique is more expensive to maintain and less easy to manage, and more experienced staff are required. There is also a risk to human health if workers are accidentally exposed to very low O_2 atmospheres. Wills *et al*., (1998c) reported that maintenance of the optimal gas concentration is critical and deviations can affect the quality of commodities. Furthermore, Forney and Lipton, (1990) reported that CA can induce or aggravate disorders such as CI and pitting in many horticultural commodities.

For this work, 3% CO_2 CA at 5°C was applied to 'Tahiti' lime. This treatment delayed colour change on the green side of the green-yellow fruit (H1) after 8 weeks of storage (Fig. 4.21), but did not delay colour change of the greener fruit used in H2 when compared with the control fruit (Fig. 4.22). All (0 and 3% CO_2 with 10% O_2) CA regimes applied to the H1 fruit stored at 2, 5 or 13°C delayed the increase of CS (degreening) by up to 2 weeks when compared with the control fruit stored at 5°C (Fig. 4.23). The 3% CO_2 atmopshere at 5°C was the best treatment to delay green colour loss for up to 12 weeks of storage (Fig. 4.21 and 4.23).

Similar beneficial effects of CA were reported by (Hatton *et al*., (1967) and Spalding and Reeder, (1974), who reported that the 'Tahiti' lime fruit stored at 5% O_2 with 7% CO_2 maintained more acceptable green colour compared to limes stored in air for 60 days; however the fruit had low juice content, thick rinds and a high incidence of decay. I found that during longer storage fruit stored under different (0 or 3% CO_2) CA conditions at 2°C suffered from CI and the green colour rapidly decreased, giving a higher CS after 10 weeks of storage (Fig. 4.23). Sritananan *et al*., (2006) also reported that all their CA storage conditions (combinations of 3 and 5% of O_2 and CO_2 (3% O_2 + 3% CO_2, 5% O_2 + 3% CO_2, 3% O_2 + 5% CO_2 and 5% O_2 + 5% CO_2) reduced loss of chlorophyll. This loss was about 50% from the initial value under CA conditions, whereas for the control fruit the pigment loss about 80-90%. CA also slowed peel colour changes of lime (*C.*

aurantifolia) compared to the fruit stored in air. The results on this work on delaying green colour change of limes is also similar to the response of lemons. When lemons (*C. limon* (L.) Burm. f.) were stored at 10°C in air or CA of 3-5% O_2 and 0.1-0.2% CO_2 for 27 weeks, CA storage reduced the rate of green colour loss in lemons for up to 27 weeks (Wild *et al.,* 1976). The authors noted that C_2H_4 absorbent reduced the rate of ripening and therefore increased resistance to mold incidence under CA. In another study, beneficial effects of several CA conditions in reducing the loss of chlorophyll and ascorbic acid in unirradiated and irradiated Eureka lemons was reported (Shrikhande and Kaewubon, 1974).

CA treatments caused an increase in the severity of CI in H1 (Fig. 4.25) and H2 (Fig.4.26) fruit during low temperature storage compared to control fruit stored at 5°C. In particular, pitting symptoms of lime developed into CI symptoms when the fruit were exposed to CA storage conditions at low temperatures (2 or 5°C). CA treatments also resulted in increased rots of H1 fruit (Fig. 4.27) during storage at 2, 5 or 13°C and an increase in the incidence of rots for H2 fruit was also observed as the temperature was raised. The H2 fruit stored at 7, 9 or 13°C showed some CI symptoms but less than the fruit stored under CA at 5°C (Fig. 4.26). Thus the CA treatments used for lime storage at intermediate temperatures (7, 9 or 13°C) reduced the incidence of CI but increased incidence of rots instead, so there was no benefit of CA for storage of lime. Similar effects of CA were reported by (Hatton *et al*., (1967) and Spalding and Reeder, (1974) who reported that 'Tahiti' lime stored under CA storage showed both increased injury and decay. Spalding and Reeder, (1983) also reported that increasing the storage temperature leads to increased rots during lime storage. Rots also increased after the fruit suffered from CI due to the growth of fungi in damaged tissue. Forney and Lipton, (1990) reported that the effect of CA on fruits and vegetables is variable. A given concentration of CO_2 may result in protection against CI or increase of CI, depending on the physiological condition of the produce.

It was noticed that the greener limes (H2 fruit) were more susceptible to pitting than the green-yellow fruit (H1 fruit). This appeared similar to Cohen and Schiffmann-Nadel, (1978) who reported that the degree of pitting of lemons was related to maturity stage as determined by the colour of the fruit at picking-time. The lemon fruit were less susceptible to pitting caused by CI when more mature fruit were harvested. However in

this work I found that the green-yellow (i.e. more mature) fruit were then more susceptible to CI under CA.

The effects of CA on citrus are clearly complicated. In my work I found that the effects of CA conditions (0 and 3% CO_2 with 10% O_2) applied to lime fruit at chilling (2, 5 or 7°C) and higher (9 and 13°C) temperatures were different. The fruit stored under all CA conditions at low temperatures showed pitting and CI. The incidence and severity of CI of the fruit were increased under all CA conditions compared to RA, however the pitting symptoms were not increased progressively under CA. Rind pitting of lime was low for H1 fruit but more severe CI symptoms were evident, whereas in H2 (greener fruit) more pitting was observed, but still less than the control fruit, and the most severe CI was recorded when the fruit were stored under CA conditions (Fig. 4.25-4.26). There may be different mechanism of CI occurring under CA compared to RA. For example, I noticed that the fruit stored under CA at 9°C showed about 8% CI (Fig. 4.26) whereas the fruit stored under RA did not show any CI (Fig. 4.12).

Various reasons have been proposed to describe the increase in CI of limes (and other fruit) after treatment with high CO_2. Forney and Lipton, (1990) reported that CA does not appear to protect against CI in many crops such as tomatoes, cucumbers, bell peppers and asparagus spears and may lead to additional injury in some cases. Kays and Paull, (2004b) reported that CO_2 may impose a significant stress on harvested products. The effects of CO_2 on the products maybe beneficial or detrimental depending on the nature of the product, the concentration of CO_2 within the tissue, the exposure duration, the internal O_2 concentration and other factors. Shipway and Bramlage, (1973) reported that the effect of CO_2 may lead to accumulation of toxic substances of acetaldehyde and ethanol which can be a cause of CO_2 injury in apples.

Spalding and Reeder, (1983) reported that conditioning ‘Tahiti’ lime by exposing the fruit to 30 or 40% CO_2 for 1 day at 21.1°C (70°F) before storage at 1.7°C (35°F) for 2 weeks caused more rind injury (CI) during cold storage than for nonconditioned fruit. They argued that the greater incidence of CI in the rind in conditioned fruit may be due to injury caused by CO_2 treatments that augmented the CI and they proposed that the fruit may be injured by CO_2 concentrations greater than 10%. This was similar to my results in which CI was worse in H1 fruit stored under CA conditions when compared with RA

(Fig. 4.25). Hatton *et al*., (1967) reported that the 'Tahiti' lime stored under the best CA treatment (5%O_2 and 7%CO_2) of their test still showed the incidence of rind scald (a scald-like rind injury) whereas limes stored under air storage were not affected. This injury was one of the factors, as well as increased decay and reduced juice content, that meant CA storage could not be recommended for limes.

The firmness of fruit stored under CA at low storage temperatures (2 and 5°C) decreased rapidly at 8 weeks of storage because the fruit suffered from severe CI (Fig. 4.25 and 4.29). The dramatic loss of texture in limes seemed directly related to major tissue disruption during CI. Overall, from my results, I can conclude that CA conditions (0 or 3% CO_2 with 10% O_2) did not extend storage life of 'Tahiti' lime after harvest.

Storage of some citrus such as grapefruit, 'Valencia', 'Temple' oranges and 'Persian' limes at 5-15% O_2 reduced the severity of CI of these fruits compared to air storage (Forney and Lipton, 1990) but increase in decay, especially from *Penicillium* was found after 'Tahiti' limes were stored under CA conditions (ranging of <4-19% O_2 and 0->30% CO_2) at 10-21.1°C (Salama *et al.,* 1965). 'Tahiti' lime stored under CA (5% O_2 plus 7% CO_2) also showed increases in decay, rind scald and reducing juice content (Hatton *et al.,* 1967; Hatton *et al.,* 1975). However, some beneficial effects of CA on lemons have been reported for example, the best control of physiological disorders found in 'Primofiori' lemons (*C. limon* Burn) stored under CA condition (12% O_2 and <2% CO_2) at 13°C, >95% RH during 2 months of storage (Artes *et al.,* 1993) and the exposure of CO_2 (40%) for 3, 6 and 9 days reduced membranosis in 'Femminello comune' lemons stored at 0 or 12°C (Bertolini *et al.,* 1991).

A beneficial effect of CA was observed for example the H2 fruit stored under CA at 9 and 13°C showed similar H° to the control fruit stored at 5°C (Fig. 4.22) and similar tendency also observed in the CS of these treatments which reached about 50% at 8 weeks of storage (Fig. 4.24). In contrast, the H° of the fruit stored under RA at 9 and 13°C changed rapidly after 4 weeks of storage compared to the control fruit stored at 5°C (Fig. 4.2). Furthermore the CS of the fruit stored at 9 or 13°C were about 60% at 8 weeks of storage (Fig. 4.9). It is therefore possible to extend storage life and delay degreening of lime under CA at 9 or 13°C if TBZ is used to protect against rots, but only for relatively short periods after harvest (6-8 weeks).

As CA can be both technically more difficult and more costly to implement, other postharvest treatments such as HWD, TC or IW might be preferable options to maintain lime quality. Therefore, I further investigated these treatments to extend lime storage life and compared the results with RA or CA storage. This analysis is the subject of the following chapter.

CHAPTER 5

Intermittent warming and hot water dipping as possible strategies to extend lime (*Citrus latifolia* Tanaka) storage life

5.1 Introduction

In the previous chapter the effects of controlled atmosphere (CA) on storage of NZ limes was studied. The results showed that CA appeared unsuitable for extending lime storage life. Therefore two further techniques, intermittent warming (IW) and hot water dipping (HWD), were identified for the next stage of this study. IW and other variable temperature scenarios are control strategies applied during storage; heat treatments, including HWD, are possible pre-storage strategies to reduce postharvest losses of horticultural products as an alternative or complement to storage intervention (Couey, 1989; Klein and Lurie, 1992a). As previously noted, long-term storage of fruit at low temperature can cause chilling injury (CI) development and increase the product's susceptibility to decay, especially after the fruit are removed to ambient temperatures. An optimum IW treatment should therefore be applied before CI development within the fruit, or at least when this is still at a reversible stage (Schirra and Cohen, 1999). An optimal HWD regime must similarly cause no damage in the fruit and must provide adequate protection against the onset of CI.

In this research, I studied the effect of IW and HWD on the quality of NZ lime fruit, either when employed alone or in combination. The fungicide thiabendazole (TBZ) is widely used to control a range of fungal diseases of citrus fruit but may also provide other benefits. Schirra and Mulas, (1995b) reported that TBZ reduced CI of 'Tarocco' oranges during cold storage at 8°C with a subsequent simulated marketing period for 1 week at 20°C. They also reported that the efficacy of TBZ against fungal diseases but CI was increased when it was used in combination with hot water. I therefore tested TBZ in combination with both IW and HWD. The influence of ethylene removal via inclusion of potassium permanganate absorbent was also investigated.

The objectives of this work were as follows:

1. To determine the effects on lime quality of different IW strategies, including varying the duration of cold storage before warming, the extent of warming, and the number of warming cycles.
2. To compare the postharvest behaviour and determine maximum storage duration for NZ limes after pre-treatment by different HWD conditions in combination with regular air storage (RA) or a range of IW conditions.
3. To determine whether ethylene absorbent or fungicide (TBZ) treatment were necessary or beneficial to retaining fruit quality during extended storage of limes.

5.2 Experimental overview

The influences of several IW conditions with and without ethylene absorbent or fungicide were investigated for H1 - H4 fruit. The cool and warm temperatures, and the duration and frequency of the IW periods are shown in Fig. 3.1. Different HWD conditions were only investigated for H3 and H5; the ranges of temperature and duration of dipping are shown in Fig. 3.1 and the methods of dipping are explained in sections 3.3.2 and 3.3.3. Colour (section 3.4), colour score (section 3.4.5), disorders (section 3.5), rots (section 3.6), compression firmness (section 3.7.1), and respiration rate and ethylene production rate (section 3.7.2) of the limes were monitored over 8 – 12 weeks of storage.

5.3 Intermittent warming (IW)

5.3.1 Trends in hue under IW conditions

The first IW treatment tested was a long-cycle low-temperature treatment of 3 weeks at 2°C followed by 1 week at 13°C. This treatment was designed based on treatments used by Kluge *et al*., (2003a) and Spalding and Reeder, (1983). Results for this first IW experiment (H1, 2004) are shown in Fig. 5.1. The IW treatment maintained the hue of the limes almost as well as constant low temperature storage at 2 and 5°C. IW delayed colour change compared to fruit stored at 13°C for both the green and yellow sides of the fruit during 12 weeks of storage (Fig. 5.1A, B).

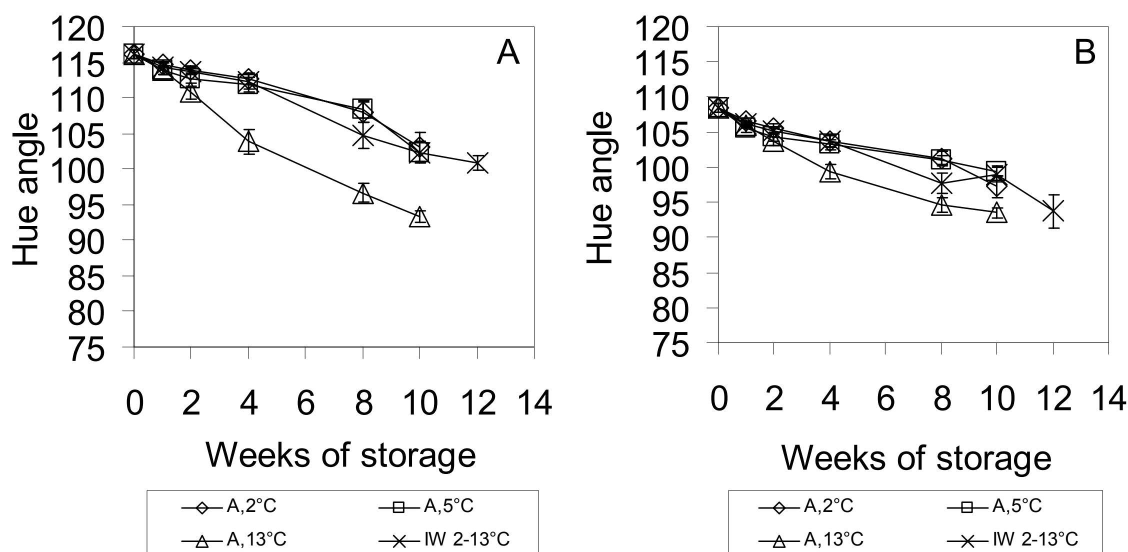


Figure 5.1 Colour change for NZ limes stored under RA at 2, 5 and 13°C and an IW condition at 2°C, for 3 weeks and 13°C for 1 week on the green side (A) or yellow side (B), H1. Vertical bars indicate ± SE (n=18).

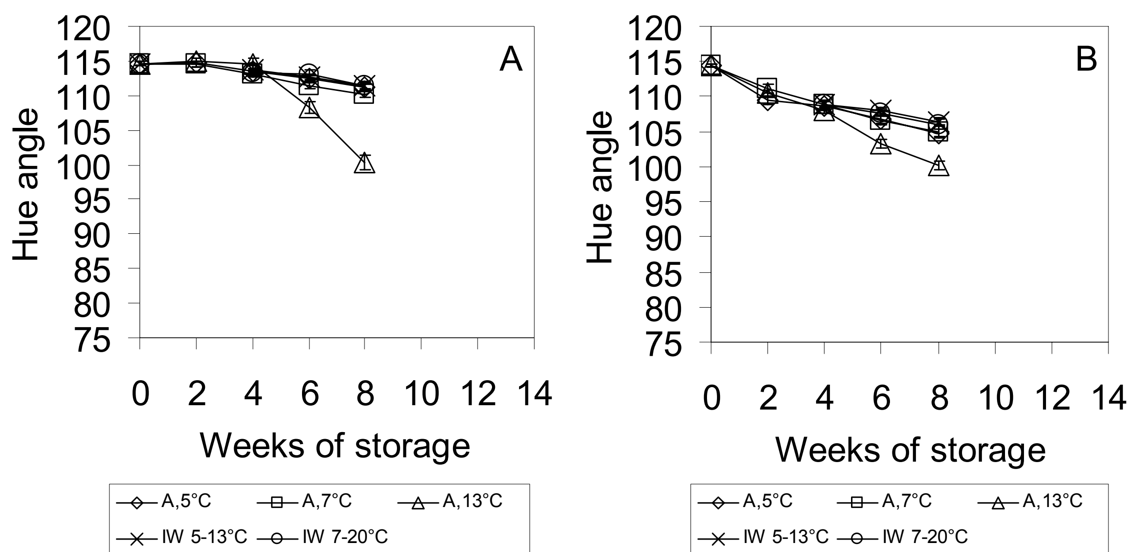


Figure 5.2 Colour change for NZ limes stored under RA at 5, 7 and 13°C and IW conditions at 5°C for 3 weeks and 13°C for 1 week or at 7°C for 3.50 weeks and 20°C for 3 days on the green side (A) or yellow side (B), H2. Vertical bars indicate ± SE (n=40).

For the subsequent experiment (H2, 2004), I tested two long-cycle IW combinations; 5°C, 3 weeks and 13°C, 1 week and 7°C, 3.50 weeks and 20°C, 3 days. The higher baseline temperatures employed in this study were selected based on the appearance of disorders in IW fruit stored at 2°C (see Fig. 5.7). Both these IW conditions significantly delayed yellowing on both the green and yellow sides of the fruit, giving rates of colour changes

similar to the fruit stored at the constant temperatures of 5 or 7°C (Fig. 5.2A, B). The fruit stored at 13°C showed normal rapid yellowing after 4 weeks of storage. The tentative conclusion from this experiment was that IW could delay colour change, but based on the occurrence of increased pitting symptoms during the IW treatment of 5°C, 3 weeks and 13°C, 1 week (see Fig. 5.8), a decision was made to further reduce the time before warming.

For H3 (2005), since chilling disorders were not being well controlled, I introduced two shorter cycle combinations of IW: 5°C, 12 days and 15°C, 2 days, and 7°C, 12 days and 20°C, 3 days. I also included the best of the previous long-cycle treatments for comparison, i.e. 7°C, 3.5 weeks and 20°C, 3 days. All three IW treatments effectively delayed yellowing as well as constant 5 or 7°C storage (Fig 5.3A, B). The fruit stored at 15 or 20°C showed the expected rapid yellowing on both the green and yellow sides of the fruit when compared with the fruit stored under 5 or 7°C and all IW conditions. However, the fruit stored at 5°C retained the highest hue (i.e. was most green) at 12 weeks of storage (Fig 5.3A). There was no apparent effect on the rate or extent of degreening through treating the IW fruit with the fungicide TBZ (Fig. 5.3A, B).

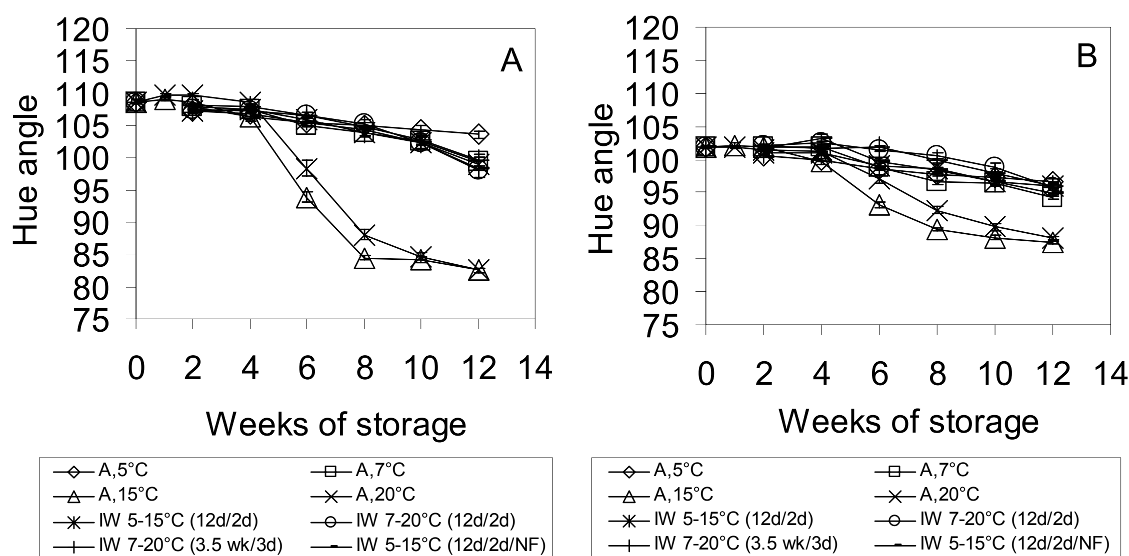


Figure 5.3 Colour change for NZ limes stored under different RA storage at 5, 7, 15 and 20°C and IW conditions at 5°C for 12 days and 15°C for 2 days with and without fungicide or 7°C for 12 days and 20°C for 2 days or 7°C for 3.50 weeks and 20°C for 3 days on the green side (A) or yellow side (B), H3. Vertical bars indicate ± SE (n=80).

In H4 (2006), I tested different numbers of IW cycles to determine if continuous cycling (up to a maximum of six cycles) was necessary. I chose the IW regime of 5°C for 12 days and 15°C for 2 days and compared 1, 2 and 6 warming cycles during storage. The 1- and

2-cycle regimes were followed by constant 5°C storage. I also investigated a step-down treatment of 10°C storage for 2 weeks followed by storage at 5°C and the importance of the duration of storage at 5°C before the first IW period. For the latter, the first IW treatment of 15°C for 2 days was applied after either 12, 16, 20 or 24 days at low temperature to encompass the range of conditions I had studied previously. The change in warming temperature from 13°C to 15°C in the low temperature IW regime was made as literature (Kimpel and Key, 1985; Saltveit, 2005) suggested a minimum change of 10°C might provide improved results. The objective of this investigation was to compare between these six IW regimes and the step-down technique to provide more understanding of the effects of variable temperature-storage on lime quality. During IW storage, the fruit were physically moved to each cold room at the specific temperature and duration of warming cycle for 1, 2 or 6 cycles, respectively (Fig. 3.1).

The hue of fruit from each different IW treatment for each warming cycle was compared with the fruit stored under RA at 5°C and the step-down treatment, as shown in Fig. 5.4 (A, B). Both the 2- and 6-cycle IW programmes resulted in long term delay of colour change on both sides of the fruit; the 1-cycle and step-down techniques were less effective, but in this case were no worse than continuous storage at 5°C with respect to colour change.

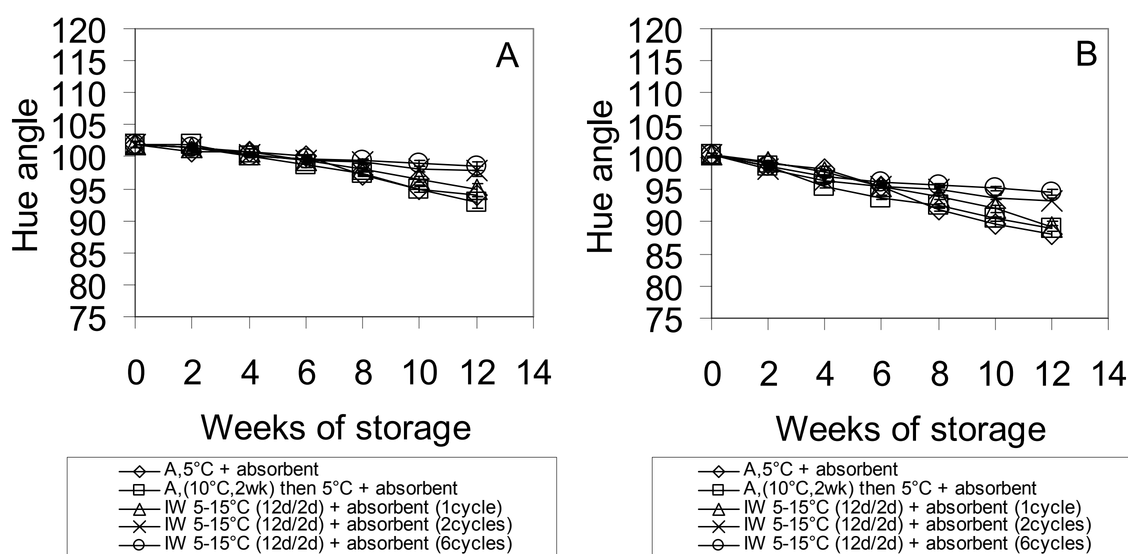


Figure 5.4 Colour change for NZ limes stored under RA at 5°C, a step-down technique by stored the fruit at 10°C for 2 weeks then stored at 5°C until the end of storage and different IW conditions (5°C, 12 days and 15°C, 2 days) regimes plus C_2H_4 ethylene absorbent and different warming frequency (1, 2 or 6 cycles) on the green side (A), or yellow side (B), H4. Vertical bars indicate ± SE (n=60).

The best treatment with respect to fruit colour of the other IW treatments (namely 6 cycles of 12 days at 5°C plus 2 days at 15°C) is compared with the IW treatments of different initial cold storage durations in Fig. 5.5. Shorter durations at 5°C (12 and 16 days) were more effective at delaying colour change over 12 weeks of storage on both sides of fruit whereas longer durations (20 and 24 days before warming) appeared too long for optimal retention of colour. The fruit stored at 5°C consistently gave the lowest H° (i.e. was most yellow) throughout the storage period (Fig. 5.5A, B).

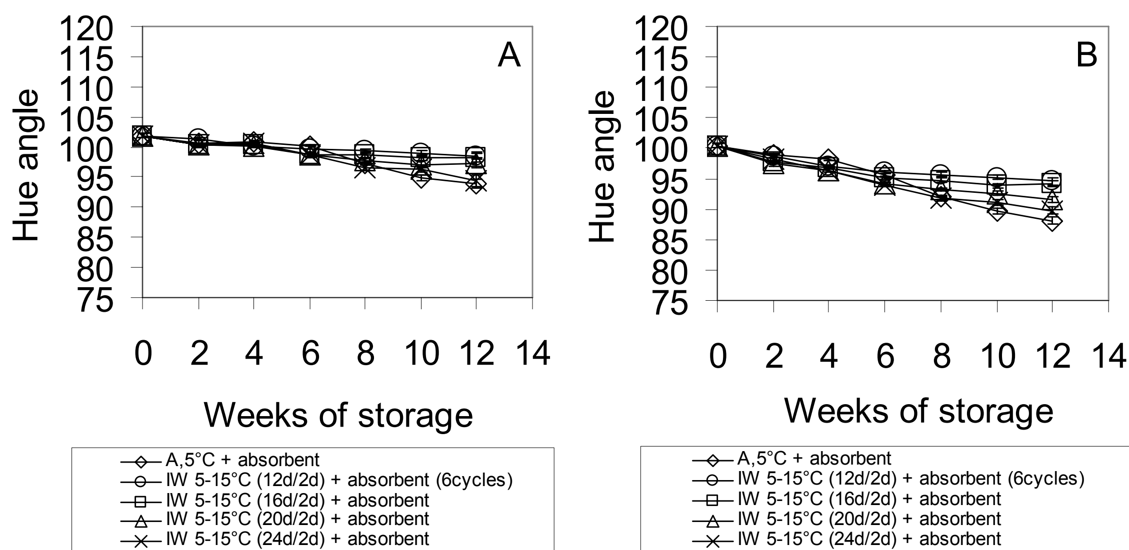


Figure 5.5 Colour change for NZ limes stored under RA at 5°C and different IW conditions (5°C, 12 days and 15°C, 2 days) regimes plus C_2H_4 ethylene absorbent (6 cycles) and other different IW conditions at different durations (16, 20 and 24 days for the first warming at 5°C and 15°C, 2 days) on the green side (A), or yellow side (B), H4. Vertical bars indicate ± SE (n=60).

The change in hue for H4 limes stored under all IW conditions after shelf life (data not shown) was lower (by about 0.5-1.5°) than the hue angle of lime stored in the same conditions at each of 8, 10 and 12 weeks of storage. The greatest change (~ 4 - 7° at 12 weeks) was observed for the step down and 1 cycle treatments and the smallest change for the 2 cycle IW trial (< 1°). Note no 6 cycle fruit were available for shelf life studies but based on the 2 cycle results I expected there would have been very little change in hue for that treatment.

5.3.2 Colour score under IW condition

In addition to hue, the use of a colour grading scheme to quantify colour change was also investigated (see section 3.4.4). The average initial CS of the fruit in H2, 3 and 4 were 25, 25 and 30% yellow, respectively (Fig. 5.6).

Fig. 5.6 illustrates that the effect of different treatments on CS was not as clearly marked as the effect on hue. A reasonable commercial limit for CS (as estimated by the researcher) was judged to be about 75% yellow. For this limit all treatments were effective at enabling 12 weeks of storage. Five treatments had not reached a CS of 50% by 8 weeks, as follows: H2 and H3 fruit stored at 5°C, H2 fruit stored under IW (5°C, 3 weeks and 13°C, 1 week), and H3 fruit both treated and not treated with fungicide and stored under IW (5°C, 12 days and 15°C, 2 days). The use of fungicide during storage with IW (5°C, 12 days and 15°C, 2 days) did not influence colour change as measured by CS (Fig. 5.6).

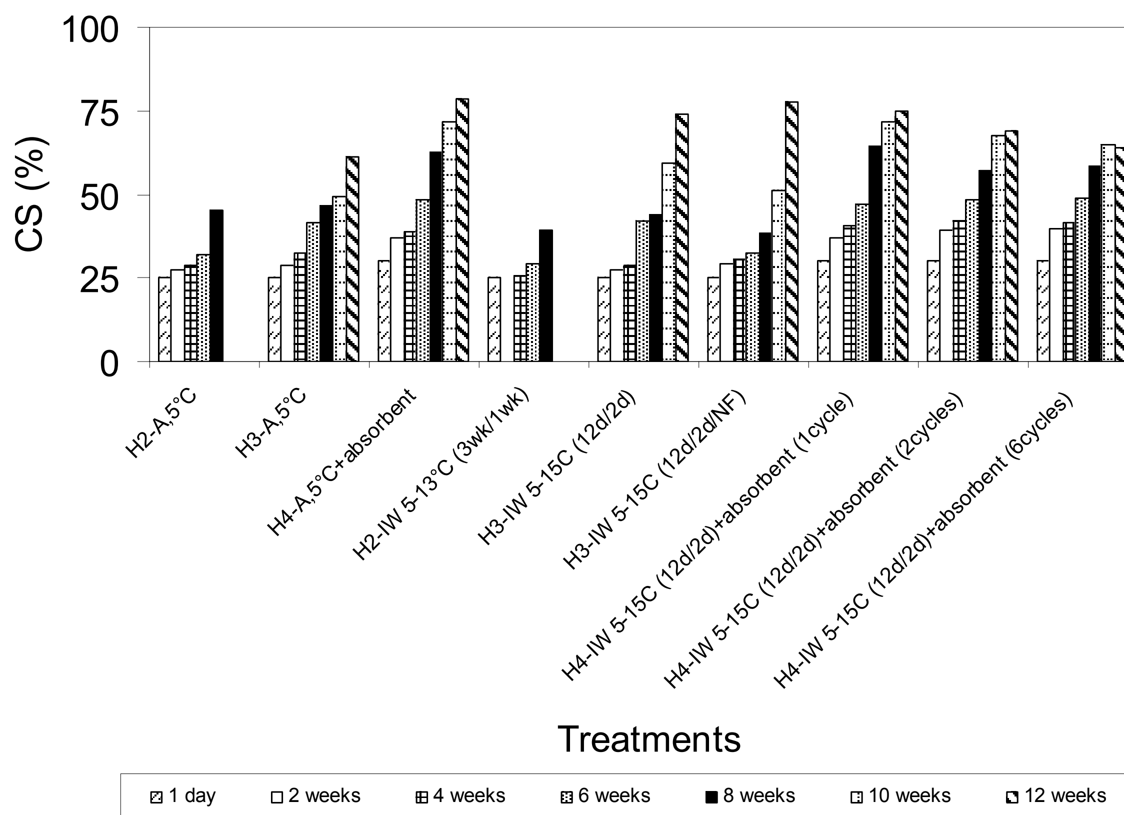


Figure 5.6 Comparison of CS after harvest for NZ limes stored under RA at 5°C and different IW conditions for 1 day, 2, 4, 6, 8, 10 and 12 weeks of storage, H2, 3 and 4.

5.3.3 Disorders under IW condition

The key disorders examined were pitting and chilling injury (CI). For H1, storing the fruit under the long-cycle low-temperature IW treatment (2°C, 3 weeks and 13°C, 1 week) did not protect against pitting when compared with the fruit stored under RA at 2 or 5°C after 8 and 10 weeks of storage (Fig. 5.7 top). Although IW did reduce the severity of CI compared with the fruit stored under RA at 2°C or 5°C, it did not completely protect fruit against CI after 8 or 10 weeks of storage (Fig. 5.7 bottom). Consequently, in later harvests I investigated shorter IW cycle times with higher base storage temperatures.

For H2, the fruit stored under RA at 13°C did not show any pitting or CI and the fruit stored at 7°C showed only very slight CI (< 10% of fruit affected) at 8 weeks of storage. Fruit stored at 5°C showed some pitting and CI at 6 and 8 weeks, however the fruit stored under the IW condition at 5°C for 3 weeks and 13°C for 1 week showed no CI after 8 weeks of storage. Some pitting of the IW fruit was observed at 6 and 8 weeks of storage, but this did not develop to CI within 8 weeks of storage (Fig. 5.8).

For H3, moderate pitting symptoms (although mostly only of pitting-1 severity) were observed for the fruit stored under RA treatments at 5 or 7°C for 8 or 10 weeks, but the fruit stored in the short-cycle IW conditions of 12 days at a cold temperature (5 or 7°C) with 2 days of warming (at 15 or 20°C) showed significantly less pitting. In contrast, long-cycle IW at 7°C for 3.5 weeks and 20°C for 3 days did not protect the fruit against pitting (Fig. 5.9). No CI was observed for the fruit stored under RA at 5°C at 8 and 10 weeks of storage, nor for the fruit stored under all the IW conditions tested for this harvest (data not shown). This is probably because the fruit from this harvest were the best quality fruit of all the experiments, as described in the previous chapter. Furthermore, the use of TBZ might have helped to protect against CI for this harvest.

For H4, the fruit stored under RA at 5°C (with C_2H_4 absorbent) showed a high percentage of pitting by 10 weeks and CI by 12 weeks of storage. Both the incidence and severity of these disorders were reduced by all IW treatments of 1, 2 and 6 cycles and by the step-down treatment. However, the step-down treatment and the 1-cycle IW treatment did not reduce CI as effectively as the 2-cycle IW regime and overall the best treatment for

protecting against these disorders in H4 was the IW treatment of 6 cycles. This effectively protected the fruit against CI for up to 12 weeks of storage (Fig. 5.10).

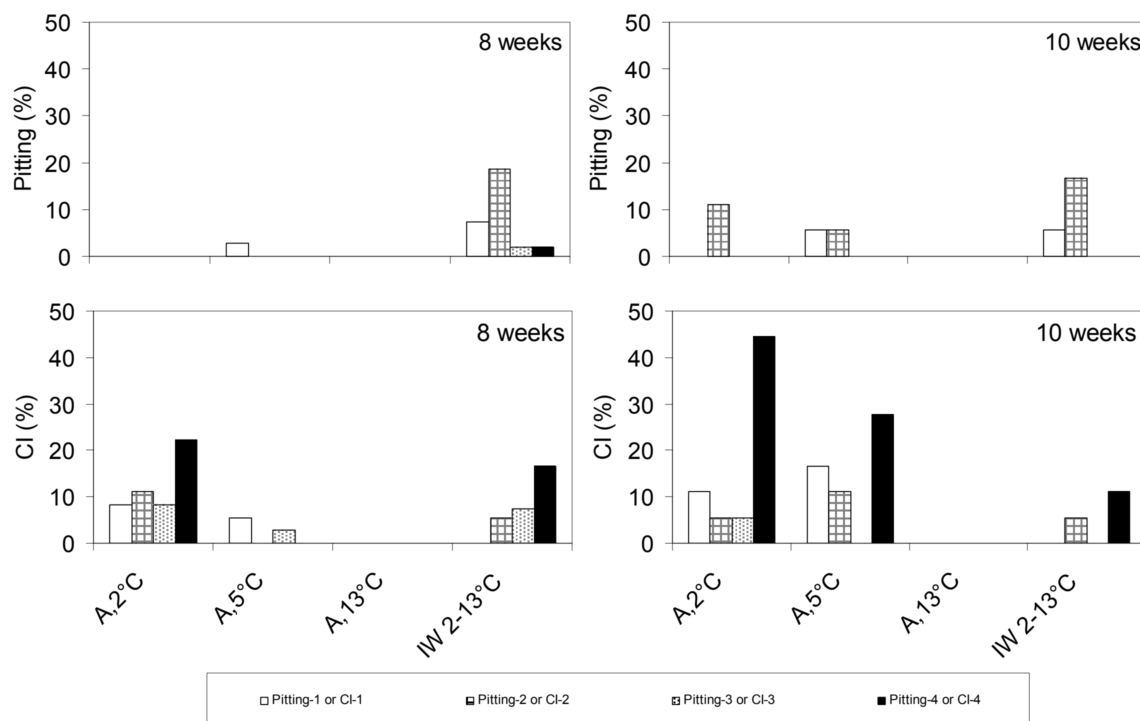


Figure 5.7 Incidence of pitting (top) and CI (bottom) after 8 (left) and 10 (right) weeks of lime stored under RA at 2, 5 and 13°C and an IW condition (2°C for 3 weeks and 13°C for 1 week), H1 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

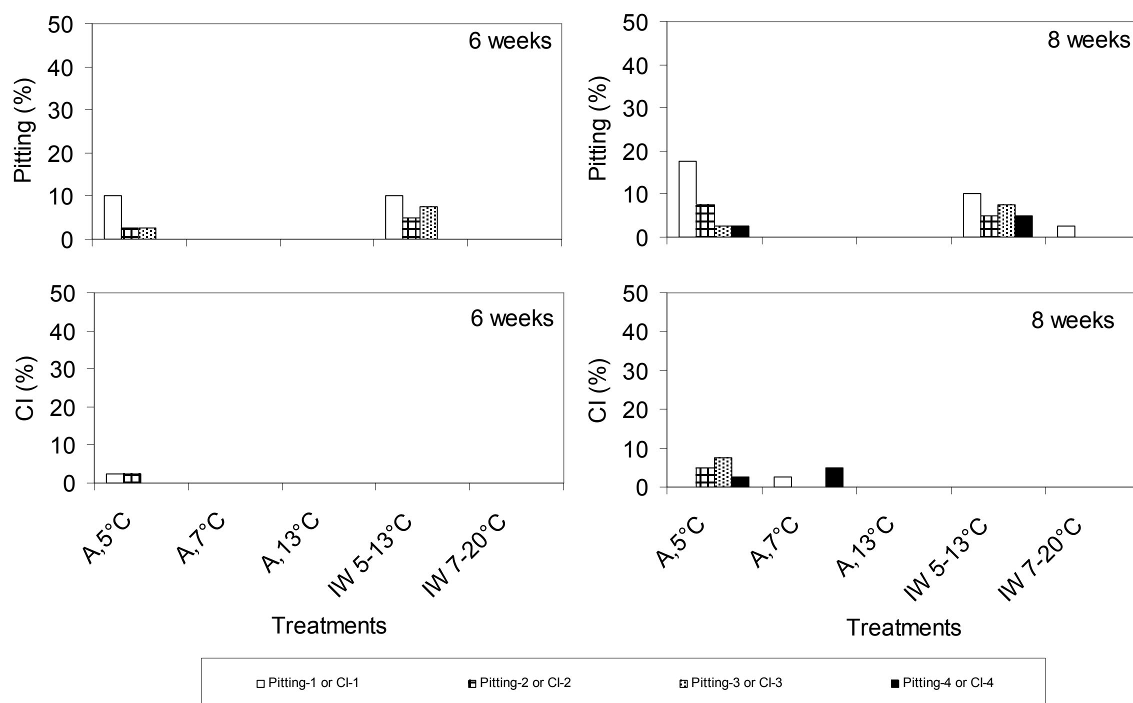


Figure 5.8 Incidence of pitting (top) and CI (bottom) after 6 (left) and 8 (right) weeks of lime stored under RA at 5, 7 and 13°C and IW conditions (5°C for 3 weeks and 13°C for 1 week or 7°C for 3.5 weeks and 20°C for 3 days), H2 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

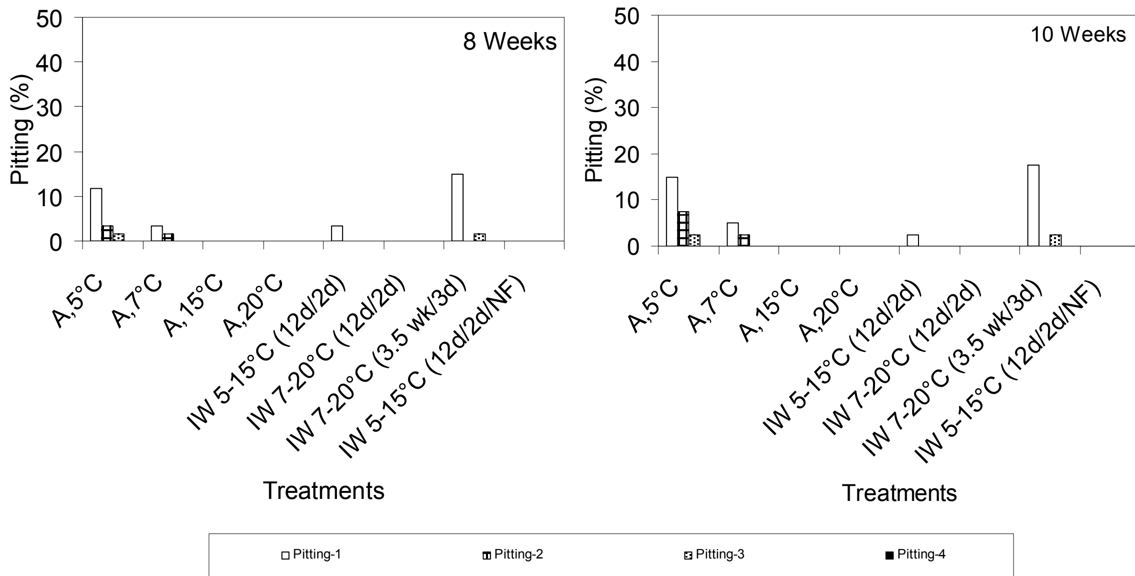


Figure 5.9 Incidence of pitting (no CI) after 8 (left) and 10 (right) weeks of lime stored under RA at 5, 7, 15 and 20°C and IW conditions (5°C for 12 days and 15°C for 2days with and without the fungicide or 7°C for 12 days and 20°C for 2 days or 7°C for 3.5 weeks and 20°C for 3 days), H3 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

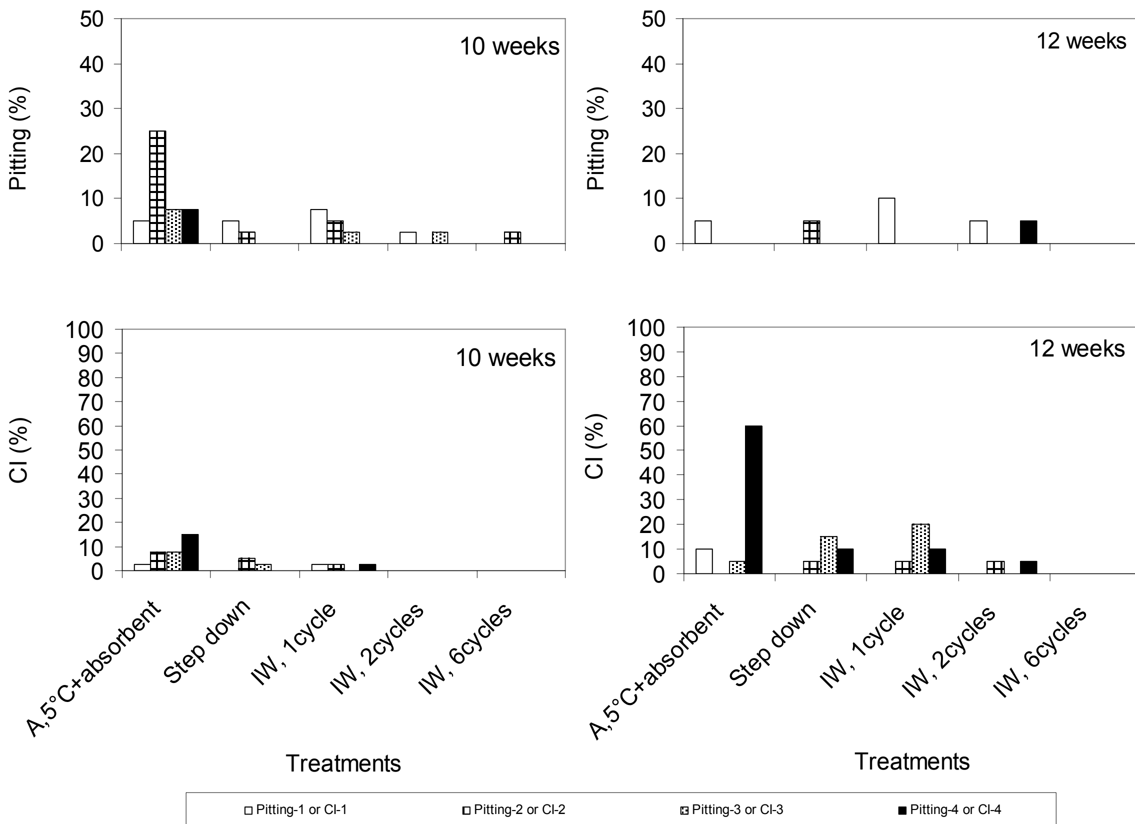


Figure 5.10 Incidence of pitting (top) and CI (bottom) after 10 (left) and 12 (right) weeks of lime stored under RA at 5°C with C_2H_4 absorbent and the step-down treatment (10°C for 2 weeks and then moved to 5°C) and IW conditions (5°C for 12 days and 15°C for 2 days) with different warming durations for 1, 2 or 6 cycles and added with C_2H_4 absorbent, H4 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

5.3.4 Disorders after shelf life

As many storage quality changes become increasingly evident once fruit are warmed to ambient conditions, 20 fruit from each treatment in H4 were placed in a 20°C room for 3 days after 8, 10 and 12 weeks of storage.

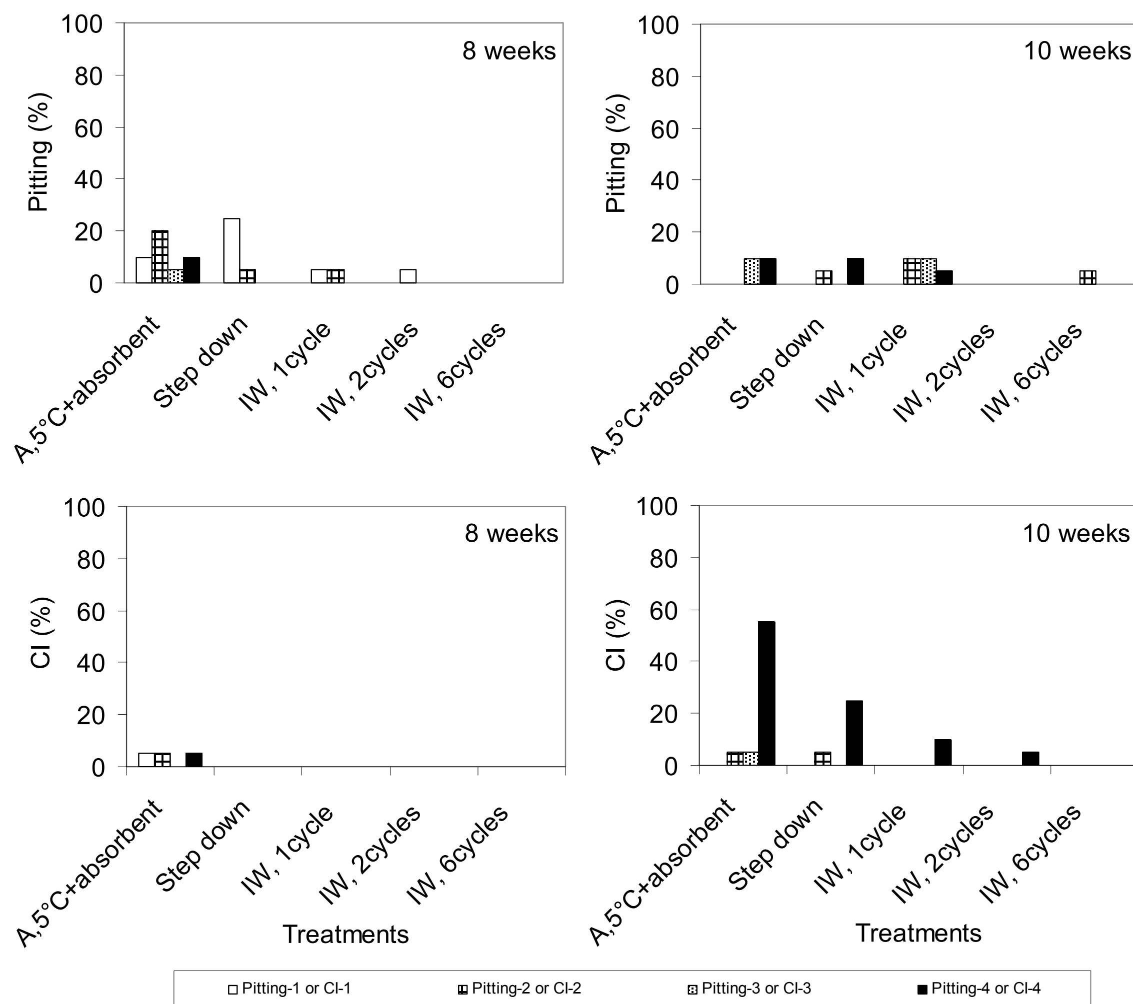


Figure 5.11 Incidence of pitting (top) and CI (bottom) after 8 (left) and 10 (right) weeks of lime stored under RA at 5°C with C_2H_4 absorbent and the step-down treatment (10°C for 2 weeks and then moved to 5°C) and IW conditions (5°C for 12 days and 15°C for 2 days) with different warming durations for 1, 2 or 6 cycles and added with C_2H_4 absorbent, H4 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4). These fruit were placed at 20°C for 3 days on removal from cool store.

As expected, the incidence and severity of disorders increased during this time (Fig. 5.11), especially for CI. Those treatments providing the best quality after 8, 10 and 12 weeks (data not shown for the latter) storage with shelf life were the 6-cycle IW (12 + 2d) and the 2-cycle IW treatments. Both showed some CI, but the incidence was very much

less than for other treatments. Even after 8 weeks of storage, the ambient shelf life period led to the appearance of significant pitting for the 5°C (constant storage) and step-down treatment fruit. After 10 weeks, severe CI was present in those same treatments and the 1- and 2- cycle IW fruit were showing moderate pitting and some severe (about 10% fruit) CI.

5.3.5 Rots under IW condition

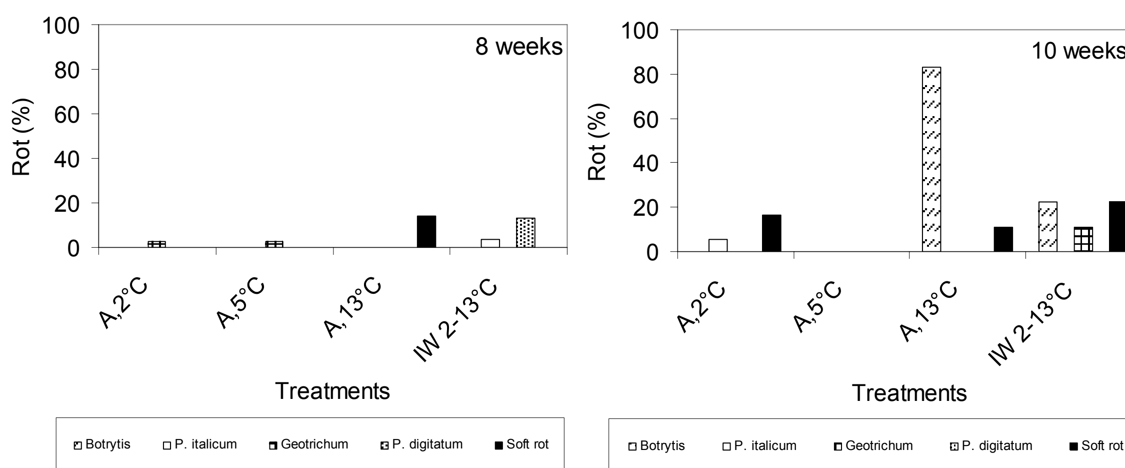


Figure 5.12 Incidence of rots after 8 (left) and 10 (right) weeks of storage for the fruit stored under RA at 2, 5 and 13°C and an IW condition (2°C for 3 weeks and 13°C for 1 week), H1.

Unsurprisingly, rots were worst for H1 fruit stored at the warmest temperature: 13.9% of fruit stored at 13°C showed soft rot at 8 weeks and more than 80% appeared infected with *Botrytis* at 10 weeks of storage. The fruit stored under the IW condition of 2°C for 3 weeks and 13°C for 1 week were not as well protected against rots as the fruit stored under constant low temperature storage at 2 or 5°C (Fig. 5.12). From these results, it was concluded that constant low storage temperatures prevented rots more effectively than storage for any length of time at higher temperatures.

For H2, there were similar levels of rots in all treatments, however *Geotrichum* was noted to be more prevalent (~37%) at the warmest temperature (13°C) than in the fruit stored at 5 or 7°C, which showed *Geotrichum* infections in a range of ~5-30% for 6 and 8 weeks of storage. IW at 5°C for 3 weeks and 13°C for 1 week prevented *Geotrichum* better than the constant temperature storage at 7 and 13°C but could not protect against *Botrytis* development, which was noted at 6 and 8 weeks of storage (Fig. 5.13).

For H3-H5, the fungicide TBZ was applied to the fruit before storage because rots were proving a limited factor for extension of the storage period. However, the application method and concentration of the fungicide used for each harvest were different (as explained in Table 3.2 and section 3.1.3-5).

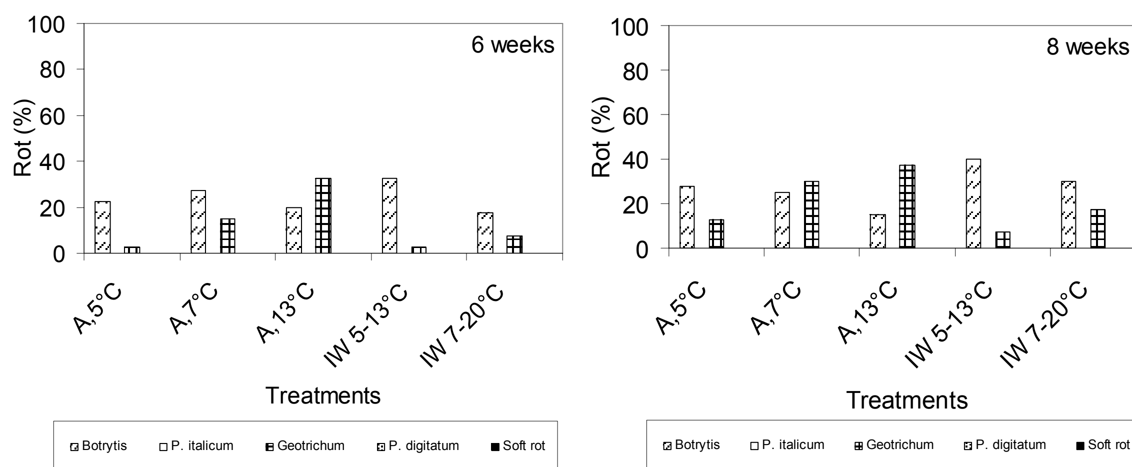


Figure 5.13 Incidence of rots after 6 (left) and 8 (right) weeks of storage for the fruit stored under RA at 5, 7 and 13°C and IW conditions (5°C for 3 weeks and 13°C for 1 week or 7°C for 3.5 weeks and 20°C for 3 days), H2.

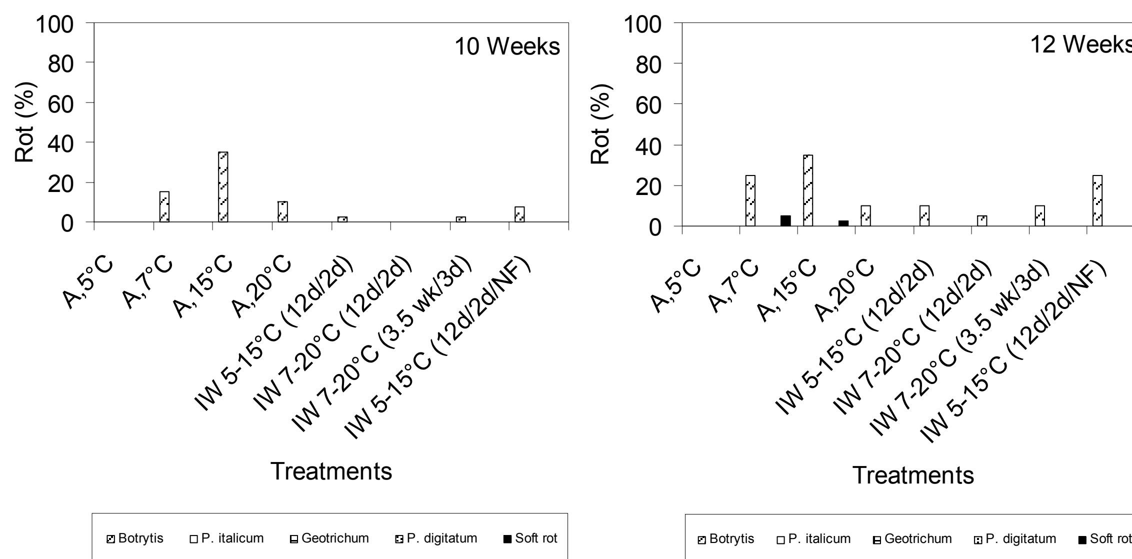


Figure 5.14 Incidence of rots after 10 (left) and 12 (right) weeks of storage for the fruit stored under RA at 5, 7, 15 and 20°C and IW conditions (5°C for 12 days and 15°C for 2 days with and without the fungicide or 7°C for 12 days and 20°C for 2 days or 7°C for 3.5 weeks and 20°C for 3 days), H3.

As expected fungicide treatment reduced the incidence of rots until 12 weeks of storage especially for those tentatively identified as *P. italicum, Geotrichum* and *P. digitatum*, however TBZ still did not completely prevent growth of *Botrytis*. In H3 treatments, rots were again more common at temperatures above 5°C. The fruit stored under RA at 7, 15

and 20°C showed a higher incidence of rots than the IW conditions, however there was little benefit from IW conditions when compared with the fruit stored under RA at 5°C (Fig. 5.14).

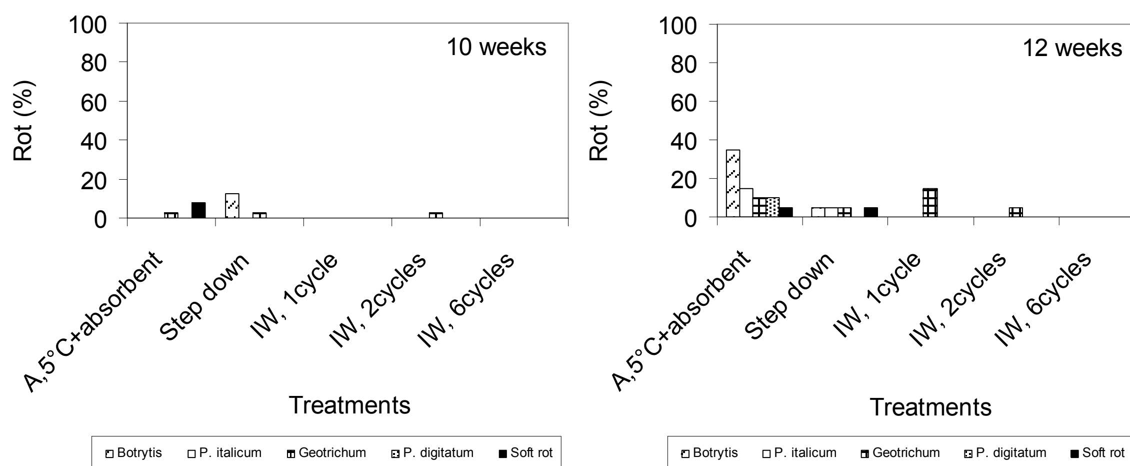


Figure 5.15 Incidence of rots after 10 (left) and 12 (right) weeks of storage for lime stored under stored under RA at 5°C with C_2H_4 absorbent and the step-down treatment (10°C for 2 weeks and then moved to 5°C) and IW conditions (5°C for 12 days and 15°C for 2 days) with different warming durations for 1, 2 or 6 cycles and added with C_2H_4 absorbent, H4.

The fungicide TBZ was also used in H4 treatments (Table 3.2 and Fig. 5.15). The incidence of rots in fruit stored under RA at 5°C with C_2H_4 absorbent was surprisingly high at 12 weeks of storage. No explanation could be found for this observation, which was contrary to previous results.

The step-down and all IW treatments at 1-, 2- and 6-cycles gave a low incidence of rots (Fig. 5.15). The IW treatments applied to H4 fruit were clearly beneficial for storage of limes. These results differed from H1, because the IW treatment for H1 (2°C, 3 weeks and 13°C, 1 week) led to disorders such as pitting and CI (Fig. 5.7) which may have increased the susceptibility to rots after storage (Fig. 5.12); in addition, the fungicide TBZ was not used for H1 fruit (Table 3.2).

The results of the IW conditions for H4 also differed from the IW conditions for H2 with respect to the incidence of rots because the conditions employed for H2 (5°C, 3 weeks and 13°C, 1 week or 7°C, 3.5 weeks and 20°C, 3 days) reduced CI but did not protect against pitting as well as the H4 IW conditions (Fig. 5.8 and 5.10). This, and the fact that TBZ was also not used for H2 fruit (Table 3.2), contributed to a high incidence of rots in

H2 fruit for all the treatments (Fig 5.13). Overall, the incidence of rots in H4 fruit when using IW conditions was similar to the IW fruit for H3 in terms of the protective effect against pitting and CI for the fruit (Fig. 5.9 and 5.10), and also with regard to the lower incidence and severity of rots (Fig. 5.14 and 5.15).

The H4 fruit stored at 5°C (without C_2H_4 absorbent) showed a slightly lower percentage of rots than the fruit stored at 5°C (with C_2H_4 absorbent) (data not shown). Whilst a comparison of H3 and H4 at 5°C might indicate the presence of the C_2H_4 absorbent adversely affected rot control, there seems no obvious reason why this should be so.

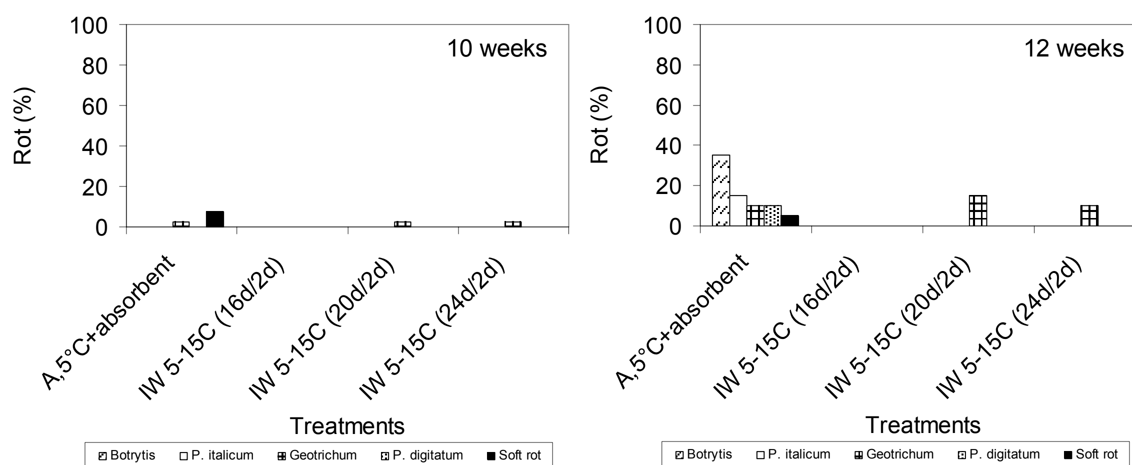


Figure 5.16 Incidence of rots after 10 (left) and 12 (right) weeks of storage for the fruit stored under RA at 5°C with C_2H_4 absorbent and IW conditions (5°C for 16, 20 and 24 days, respectively and 15°C for 2 days with C_2H_4 absorbent), H4.

All the fruit stored under IW conditions at 5°C for 16, 20 or 24 days, respectively, with a warming cycle at 15°C for 2 days (with C_2H_4 absorbent), showed fewer rots than the fruit stored under RA at 5°C. The fruit stored in the short-cycle IW at 5°C for 16 days and 15°C for 2 days were particularly well protected against rots during 12 weeks of storage (Fig. 5.16), similar to the optimal IW condition (6 cycles; 5°C, 12 days and 15°C, 2 days) in the same experiment (Fig. 5.15 and 5.16).

There were no dramatic increases of rots after 8 and 10 weeks plus 3 days shelf life at 20°C for the fruit stored under RA at 5°C with C_2H_4 absorbent and the fruit stored under the step-down treatment, 1-cycle IW, IW (5°C, 20 days and 15°C, 2 days or 5°C, 24 days and 15°C, 2 days). Overall the fruit stored under RA at 5°C plus C_2H_4 absorbent, step-down and IW (5°C, 24 days and 15°C, 2 days) showed higher rots than other treatments

but still lower than 15%, whereas the fruit stored under 2-, 6- cycle IW and IW (5°C, 16 days and 15°C, 2 days) did not show rots after 8 and 10 weeks of storage (data not shown).

5.3.6 Compression firmness

Firmness is not a major quality attribute of lime fruit but was also measured to provide another potential indicator of chilling injury and other changes (such as mass loss) occurring during postharvest storage. Compression firmness of fruit from selected runs in three harvests is compared in Fig. 5.17A-C.

For H2, the fruit stored under RA at 5°C lost firmness after 6 weeks of storage presumably because the fruit showed CI. In contrast the fruit stored under the IW condition (5°C, 3 weeks and 13°C, 1 week) stayed firmer until 8 weeks of storage. These IW fruit showed some pitting but no CI at 6 and 8 weeks of storage (Fig.5.17A and 5.8). The H2 fruit stored under warmer IW conditions (7°C, 3.5 weeks and 20°C, 3 days) showed significantly more weight loss than the other treatments after 4 weeks of storage (5.3% compared to < 2% see appendix I) and this could have contributed to their lower firmness (Fig. 5.17A).

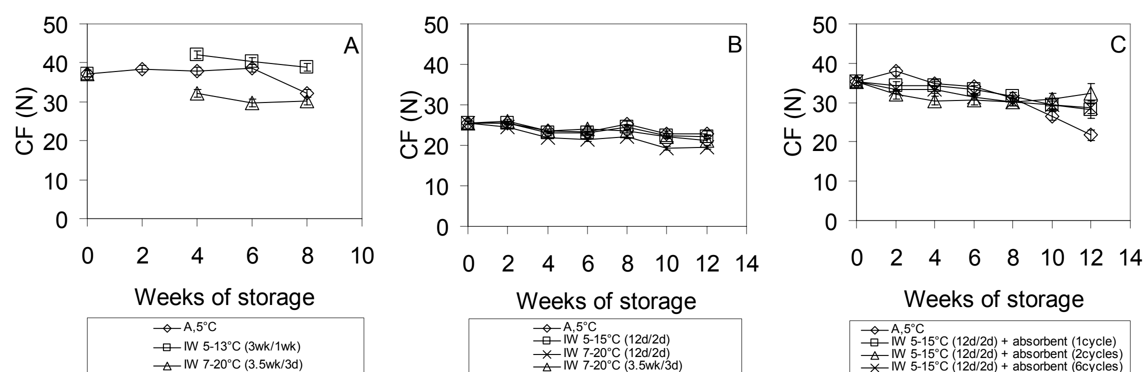


Figure 5.17 CF for NZ limes (A) stored under RA at 5°C and IW conditions (5°C for 3 weeks and 13°C for 1 week or 7°C for 3.5 weeks and 20°C for 3 days, H2. Vertical bars indicate ± SE (n=80); (B) stored under RA at 5°C and IW conditions (5°C for 12 days and 15°C for 2 days or 7°C for 12 days and 20°C for 2 days and 7°C for 3.5 weeks and 20°C for 3 days, H3. Vertical bars indicate ± SE (n=80); (C) stored under RA at 5°C and IW conditions (5°C for 12 days and 15°C for 2 days) regimes plus C_2H_4 ethylene absorbent and different warming frequency (1, 2 or 6 cycles), H4. Vertical bars indicate ± SE (n=60).

For H3, only the fruit stored under the short-cycle, warmer IW condition (7°C, 12 days and 20°C, 2 days) showed significantly more firmness loss than other treatments (Fig. 5.17B), although the effect was small. This may be due to higher mass loss (about 3% after 12 weeks) for these treatments, as although there were differences in the incidences of pitting (higher in the long cycle IW; 7°C for 3.5 weeks and 20°C for 3 days), no CI (Fig. 5.9) or rots (*Botrytis* at the button) (Fig. 5.14) were observed in these fruit.

For H4 the firmness of the control fruit stored at 5°C was slightly higher than fruit subjected to IW conditions (1-, 2- and 6-cycles) up to 6 weeks. Thereafter the firmness of the control fruit declined such that by 10 weeks, it was significantly lower than the IW fruit. In contrast, the IW fruits maintained their firmness until 12 weeks of storage (Fig. 5.17C). Firmness loss appeared related to both mass loss and onset of CI, with the latter causing more rapid loss in firmness late in storage, presumably due to softening and weakening of peel and related structures.

5.3.7 Respiration and ethylene production rate

Respiration and ethylene production are important indicators of physiological status in many fruit. These parameters were measured to identify possible patterns in the metabolic response of H4 limes stored at 5°C (with and without C_2H_4 absorbent) and IW (5°C, 12 days and 15°C, 2 days with C_2H_4 absorbent) at different warming frequencies (1-, 2- or 6-cycles).

Initial testing showed that internal fruit temperatures had equilibrated to about 15°C within 1 hour of removal from 5°C storage (data not shown). Thus the respiration and ethylene production rates of fruit stored at 5°C (with and without IW and C_2H_4 absorbent) were measured after equilibration in a 15°C room for 1 hour whereas the IW fruit stored at 15°C were measured directly at this temperature. The fruit stored at 5°C (with and without C_2H_4 absorbent) were sampled at 6, 10 and 12 weeks. The fruit stored under 1-cycle IW were first sampled at 5°C and measured at 13 days (i.e. the day before the shift to 15°C) and then sampled at 15°C and measured again at 14 days; further samples at 5°C were taken (but measured at 15°C) at 6, 10 and 12 weeks. The fruit stored under 2-cycle IW were sampled at 5°C and 15°C at 13 and 14 days, and 27 and 28 days, respectively, and then sampled at 5°C at 6, 10 and 12 weeks (but measured at 15°C). The fruit stored

under 6-cycle IW were sampled at 5°C at the day before 2, 4, 6, 10 and 12 weeks and sampled at 15°C at 2, 4, 6, 10 and 12 weeks.

Respiration rates at 15°C of the fruit stored at constant temperature (5°C) were higher than the fruit stored under IW treatments (5°C, 12 days and 15°C, 2 days) (Fig. 5.18). The inclusion of C_2H_4 absorbent appeared to reduce the respiration rate of the fruit stored at 5°C but the fruit stored at 5°C (with and without C_2H_4 absorbent) still showed the highest respiration rate of about 33 and 40 ml CO_2 kg^{-1} hr^{-1} at 42 days, possibly indicating the fruit were stressed from continuous exposure to the cold storage temperature. The respiration rate then decreased steadily to about 25 ml CO_2 kg^{-1} hr^{-1} by 70 days.

The 6- and 2-cycle IW fruit (5°C, 12 days and 15°C, 2 days) showed very similar trends and maintained a low respiration rate. After a period of 2 days at 15°C, the respiration rate increased from that of the fruit just removed to 15°C and the extent of increase appeared to decline with prolonged storage. However the 1-cycle IW fruit behaved differently to other IW fruit (2- and 6-cycle). At 2 weeks the respiration rate at 5 and at 15°C was similar to the other IW treatments at 15°C. By 6 weeks (when they had been stored continuously at 5°C for 4 weeks) the respiration rate of 1 cycle fruit was significant higher than the other IW fruit and by 12 weeks (i.e. 10 weeks of storage at 5°C after their only warming) their respiration rate had became similar to that of the control fruit stored at 5°C.

Ethylene production was very low but showed similar trends to the respiration rate data (Fig. 5.18 and 5.19). For example after 6 weeks the control (constant 5°C) gave the highest ethylene production rate, which then declined; ethylene production in fruit with C_2H_4 absorbent was lower, in agreement with respiration rate data. The 2 and 6 cycles IW fruit gave consistently low ethylene production, but in this case no increase in 1 cycle IW (Fig. 5.19) ethylene production rate was observed late in storage (in contrast to the trend in respiration rate). The ethylene production rate of the control fruit stored at 5°C without C_2H_4 absorbent increased rapidly at 84 days and was possibly linked to the development of rots (Fig. 5.15). Overall the fruit stored under all IW treatments (5°C, 12 days and 15°C, 2 days) at 1, 2 and 6 cycles plus C_2H_4 absorbent produced less than 0.5 µl C_2H_4 kg^{-1} hr^{-1} of ethylene during storage.

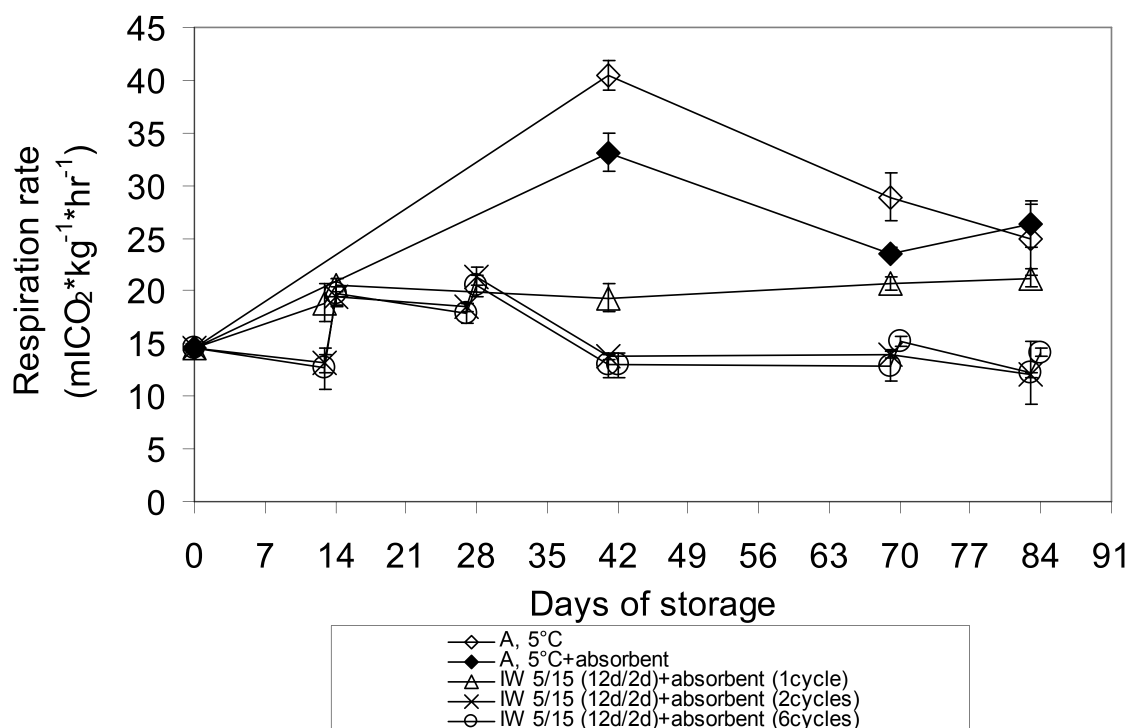


Figure 5.18 Respiration rate (measured at 15°C) of limes stored under RA at 5°C (with and without C_2H_4 absorbent) and different IW conditions (5°C, 12 days and 15°C, 2 days) regimes plus C_2H_4 absorbent and different warming frequency (1, 2 or 6 cycles), H4. Vertical bars indicate ± SE (n=3).

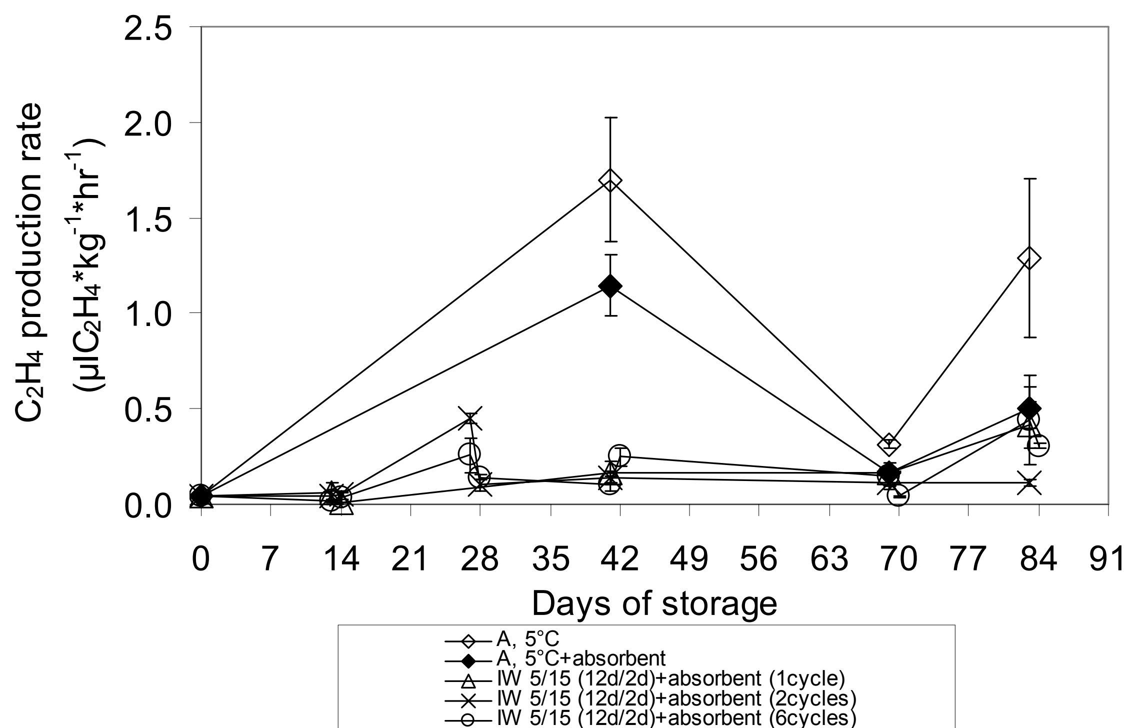


Figure 5.19 Ethylene production (measured at 15°C) rate of limes stored under RA at 5°C (with and without C_2H_4 absorbent) and different IW conditions (5°C, 12 days and 15°C, 2 days) regimes plus C_2H_4 absorbent and different warming frequency (1, 2 or 6 cycles), H4. Vertical bars indicate ± SE (n=3).

5.4 Hot water dipping (HWD)

All fruit used in H3, H4 and H5 for HWD treatments were dipped into hot water (with or without fungicide, TBZ) in a water bath which was set up for the desired temperature as shown in Chapter 3 (section 3.3.2 and 3.3.3). The ranges of temperature of hot water, time of dipping and storage temperature are shown in Fig. 3.1.

In a preliminary trial the flesh surface and core temperatures of lime fruit stored at the ambient temperature (15°C) and pre-treated by HWD at 47 and 52°C for 2, 4 and 6 min were monitored. The resulting temperature profiles are shown in Fig. 5.20 and 5.21, respectively. The temperatures were measured every 30 seconds using Gemini data loggers (Tinytag®, (Energy Engineering Ltd, NZ) and the data were downloaded by the Gemini Logger Manager 2.2 software.

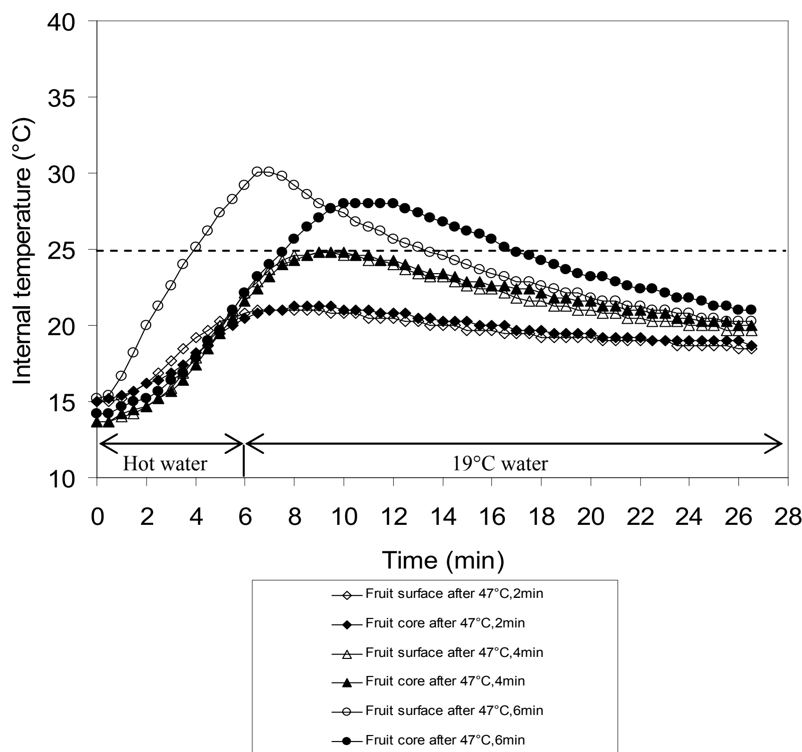


Figure 5.20 Temperature profiles for the outer flesh surface and core of limes during dipping into hot water at 47°C for 2, 4 and 6 min then into 19°C water.

Before HWD, the temperature of the internal flesh surface (i.e. flesh temperature directly under the peel) and the core were about 15°C. The fruit internal surface temperatures after HWD at 47°C for 2, 4 and 6 min reached maximum values of 21, 24.8 and 30.1°C at 6.5, 9 and 6.5 min, respectively. The core temperature changes lagged behind the surface

temperature and reached maximum values of 21.3, 24.8 and 28°C at 8, 9 and 10 min, respectively (Fig. 5.20). Similar trends were observed for the fruit subject to HWD at 52°C. Internal surface temperatures of HWD fruit at 52°C for 2, 4 and 6 min reached maximum values of 22.4, 27.4 and 33.3°C at 7.5, 7.5 and 6.5 min, respectively. On the other hand, core temperatures reached maximum values of 23.2, 27.4 and 31.7°C at 7.5, 8.5 and 9.5 min, respectively (Fig. 5.21).

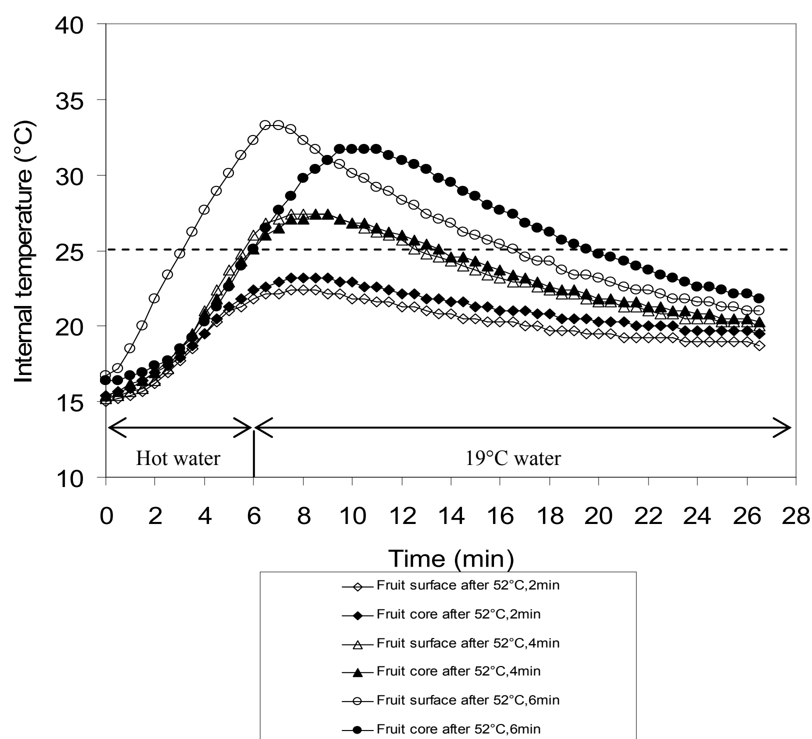


Figure 5.21 Temperature profiles for the outer flesh surface and core of limes during dipping into hot water at 52°C for 2, 4 and 6 min then into 19°C water.

As expected, there was a more rapid change in temperature at the fruit surface than the core. Internal temperatures of the HWD fruit at 47 or 52°C for 2 min were never higher than 25°C, whereas fruit dipped for 4 and 6 min showed outer flesh temperatures higher than 25°C for periods of time ranging from 1–14 min. The outer surface (flavedo) of the fruit was assumed to be similar in temperature to the water but I did not test it. The internal temperatures decreased once the fruit were dipped into 19°C water. The rate of decrease in temperature of the fruit surface and core was slower than for heating (Fig. 5.20 and 5.21), reflecting the low temperature driving force under these conditions.

5.4.1 Trends in hue in HWD conditions

For H3, changes in hue for both the green and yellow positions of limes pre-treated with (52-53°C) HWD for 2 minutes, with and without storage under IW (5°C, 12 days and 15°C, 2 days), and with and without C_2H_4 absorbent, were measured.

The fruit stored under RA at 15°C showed rapid yellowing after 4 weeks of storage on both green and yellow sides of the fruit (Fig. 5.22). All other treatments slowed degreening to a similar extent, but there was an indication after 10 weeks of storage that pre-treatment by HWD at 52-53°C for 2 minutes and then storing the fruit under the IW condition (5°C, 12 days and 15°C, 2 days) did not maintain the colour of the limes as well as either only pre-treating the fruit by HWD or IW alone (6-cycle; Fig. 5.4).

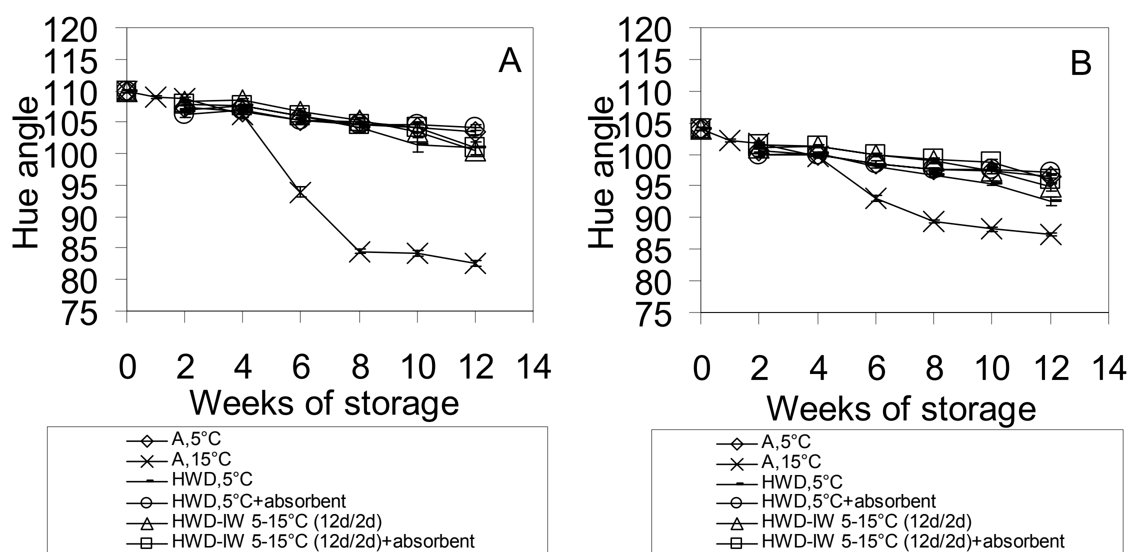


Figure 5.22 Colour change for NZ limes stored under RA at 5 and 15°C and pre-treated limes with 52-53°C HWD then stored under RA at 5°C with and without the IW condition (5°C for 12 days and 15°C for 2 days) and/or with and without C_2H_4 absorbent on the green side (A) or yellow side (B), H3. Vertical bars indicate ± SE (n=80).

For H5, changes in hue for both green and yellow sides of lime stored after different HWD (42 to 57°C) regimes of different dipping durations (2, 4 and 6 minutes) were investigated (Fig. 5.23 to 5.29). In the following figures, profiles of colour change of the fruit in H5 are presented for different sets of HWD treatments and compared with the fruit stored under RA at 5°C or 13°C.

All the fruit stored under RA, with and without HWD at 5°C, showed more rapid yellowing after 8 weeks of storage (Fig. 5.23). The HWD fruit (47°C for 2 and 4 min) showed slower changes in hue until 12 and 10 weeks of storage, respectively, but dipping the fruit at 47°C for 6 min or at 52°C for 2 or 4 min had no beneficial effect with respect to delaying degreening. In fact, the fruit dipped at 52°C for 6 min showed skin damage and accelerated yellowing at 12 weeks of storage. Overall the hue of the fruit dipped at 47°C for 2 min was the highest of all HWD treatments after 12 weeks storage at 5°C (Fig. 5.23A, B).

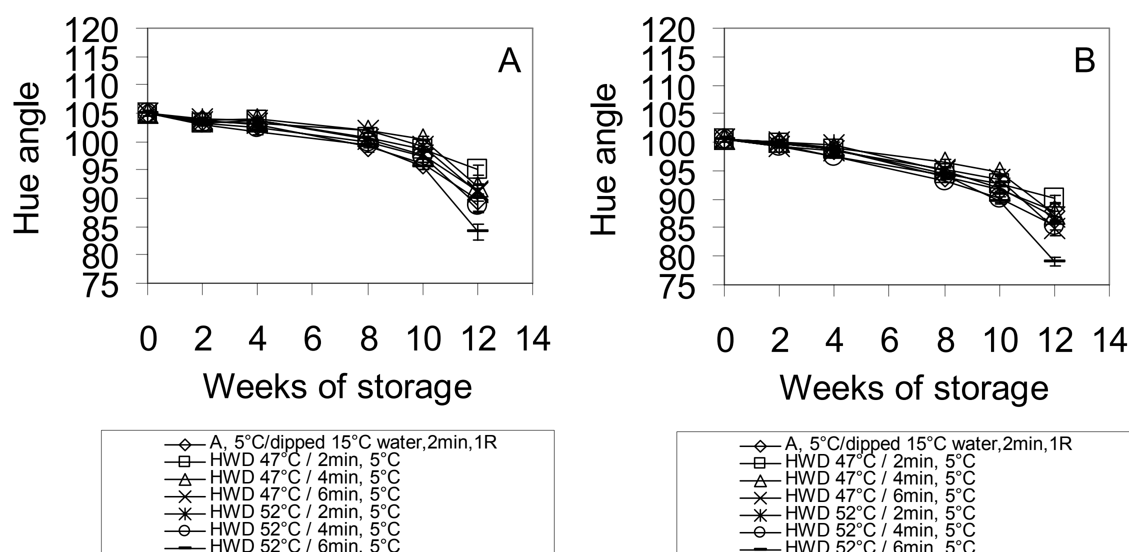


Figure 5.23 Colour change for NZ limes stored under RA at 5°C and pre-treated limes with different HWD conditions (47°C or 52°C for 2, 4 or 6 min, respectively) then stored at 5°C on the green side (A) or yellow side (B), H5. Vertical bars indicate ± SE (n=60).

This experiment was also designed to identify the safe range of temperatures for HWD. I expected that temperatures lower than 47°C might be acceptable for lime but have limited beneficial effects, while temperatures above 52°C might possibly show negative effects on fruit quality, especially for longer durations of dipping. Therefore, HWD treatments of 42°C for 2 min and 57°C for 6 min were chosen as extremes and the treatments of HWD at 47°C and 52°C for 4 min were repeated without the fungicide TBZ because I expected that these treatments might be in a safe range to maintain fruit quality and might also protect against rots. Unfortunately the lack of fruit during the 2006 season limited the extent of replication per treatment to only one replication in some treatments (Fig. 3.1 and 5.24A, B).

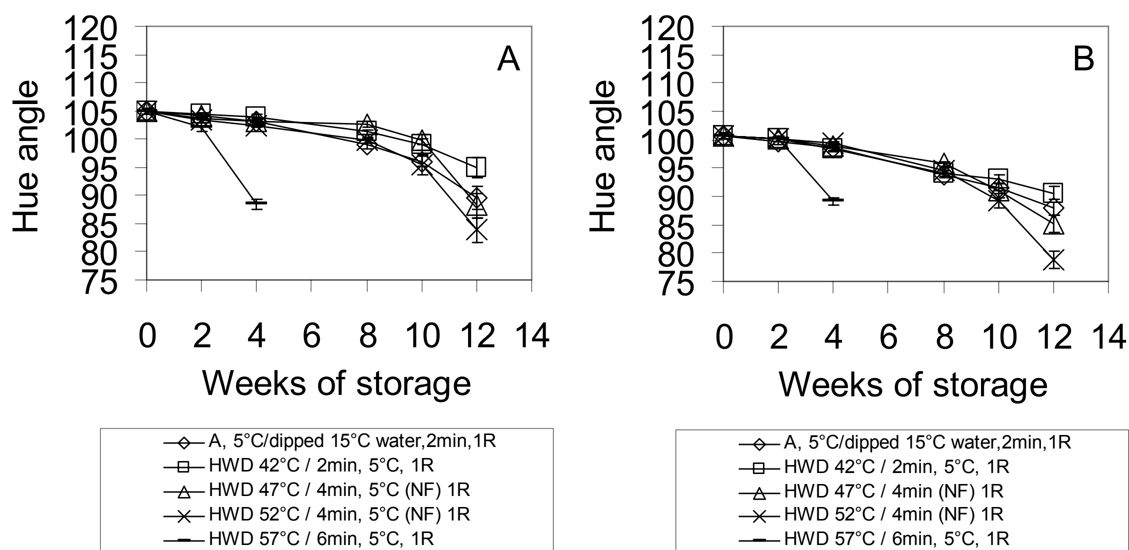


Figure 5.24 Colour change for NZ limes stored under RA at 5°C and pre-treated limes with HWD at 42°C for 2 min, 47 and 52°C for 4 min without fungicide, 57°C for 6 min then stored under RA at 5°Con the green side (A) or yellow side (B), H5. Vertical bars indicate ± SE (n=60).

The results suggested that the best treatment with regard to colour maintenance was the HWD treatment at 42°C for 2 min with fungicide; this treatment maintained a high hue (green colour) until 12 weeks. Fruit stored after HWD at 47°C for 4 min without fungicide maintained their colour well until 10 weeks, but after that time the hue decreased rapidly (Fig. 5.24A, B). Dipping the fruit at 57°C for 6 min damaged the fruit, which become brown after only 2 weeks of storage. It could be concluded that effective treatments were HWD at 42°C for 2 min with the fungicide and/or HWD at 47°C for 4 min without the fungicide (Fig. 5.24A). The fruit stored after HWD at temperatures higher than 47°C all showed adverse effects on colour during long term storage.

The fruit dipped at 42, 47 and 52°C for 2 min were compared to the control fruit stored at 5°C and the results showed that HWD at 42 and 47°C for 2 min delayed colour change of the fruit better than the either control or the HWD at 52°C for 2 min (Fig. 5.25A, B).

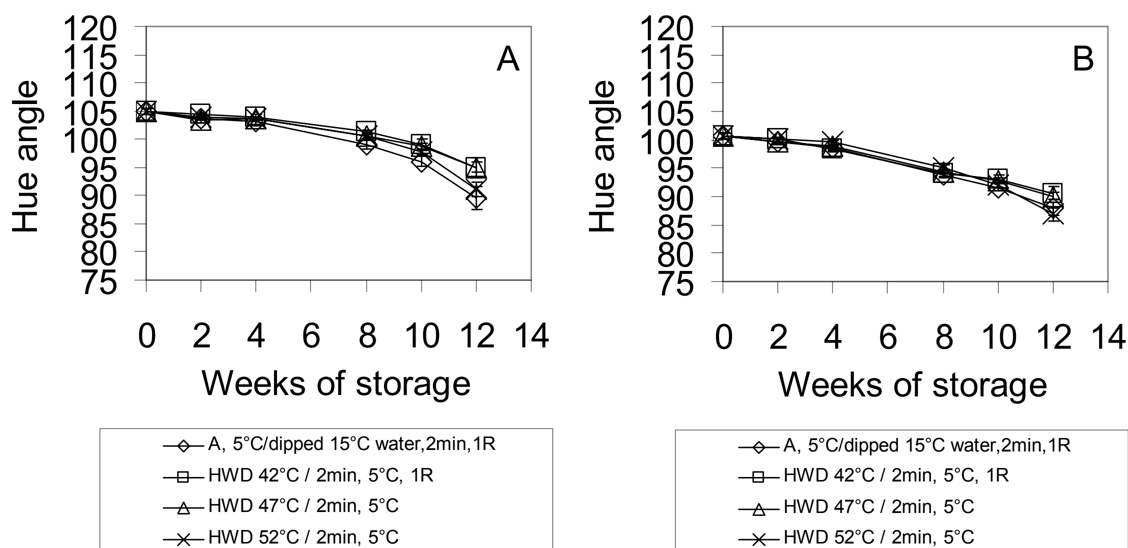


Figure 5.25 Colour change for NZ limes stored under RA at 5°C and pre-treated limes with HWD at 42, 47and 52°C for 2 min then stored under RA at 5°C on the green side (A) or yellow side (B), H5. Vertical bars indicate ± SE (n=60).

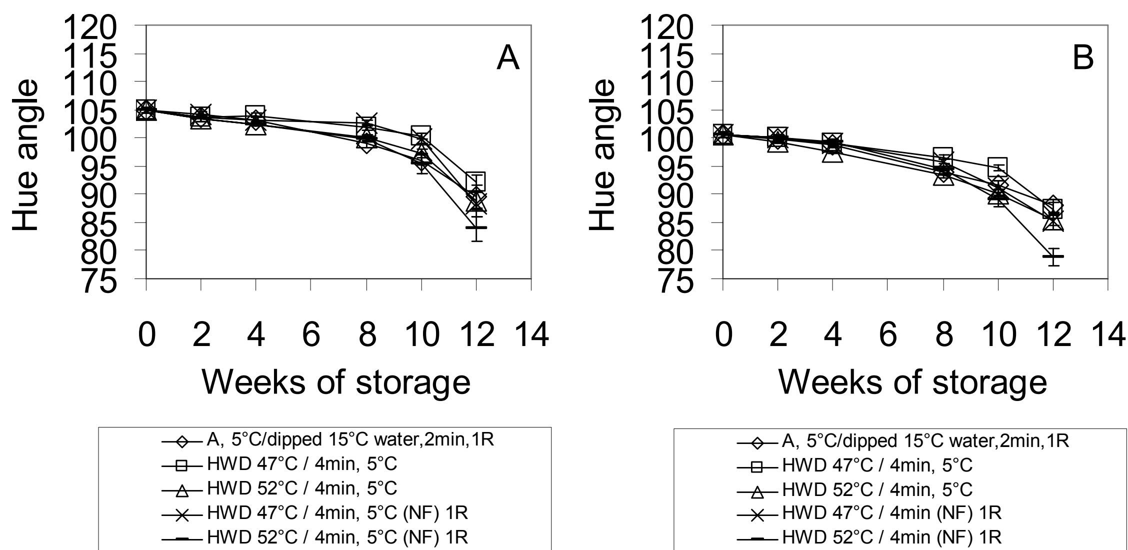


Figure 5.26 Colour change for NZ limes stored under RA at 5°C and pre-treated limes with different HWD conditions at 47 and 52°C dipped for 4 min with and without fungicide then stored under RA at 5°C on the green side (A) or yellow side (B), H5. Vertical bars indicate ± SE (n=60).

The fruit dipped at 47°C for 4 min with and without the fungicide showed similar H° on the green side (Fig. 5.26A) The presence of TBZ appeared beneficial in delaying yellowing, especially on the yellow side of the fruit, after HWD at 47°C for 4 min throughout 12 weeks of storage (Fig. 5.26B). Overall it appeared that the fruit dipped with TBZ changed less in hue than the fruit dipped in hot water only.

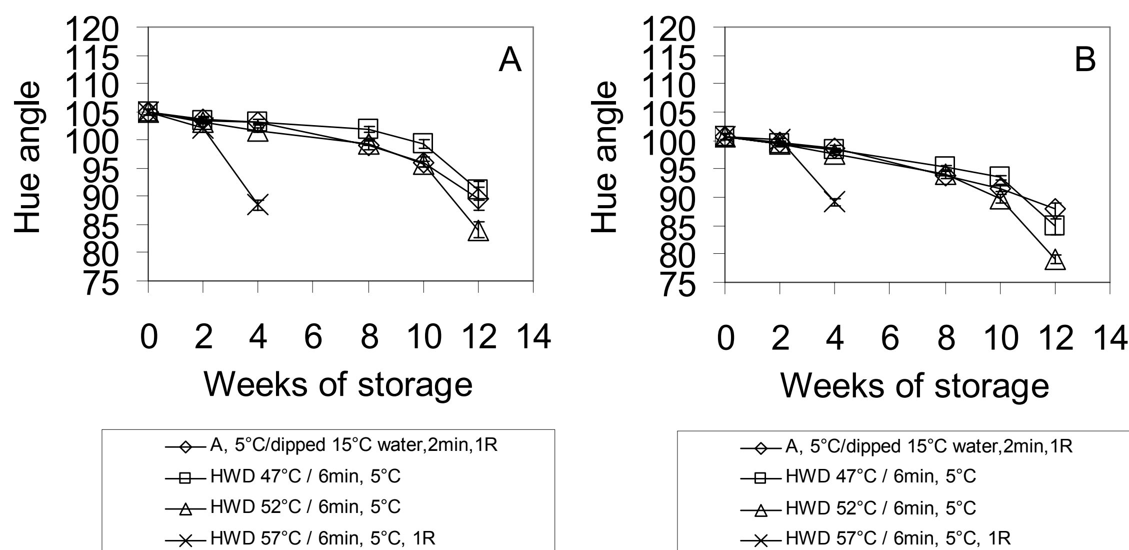


Figure 5.27 Colour change for NZ limes stored under RA at 5°C and pre-treated limes with different HWD conditions at 47, 52 and 57°C dipped for 6 min then stored under RA at 5°C on the green side (A) or yellow side (B), H5. Vertical bars indicate ± SE (n=60).

Fruit dipped at high temperatures for long periods suffered from heat injury (Fig. 5.30) and subsequently showed CI and brown coloration of the skin during storage at low temperature (Fig. 5.31-5.32). The effect of this on hue was visible progressively earlier as the dipping temperature increased (Fig. 5.27A, B), where the use of a 6 min dip at 57°C led to a loss of green colour within 4 weeks. The fruit dipped at 47°C for 6 min degreened rapidly after 10 weeks of storage (Fig. 5.27B). Therefore HWD at too high a temperature (>47°C) or for too long a period (>4 min) does not help to maintain fruit colour and can damage the fruit surface, reducing the overall fruit quality after storage.

When fruit were stored at a warmer temperature (13°C) after dipping at 47 or 52°C for 6 min, this pre-treatment with HWD was never beneficial in delaying change in hue (Fig. 5.28A, B).

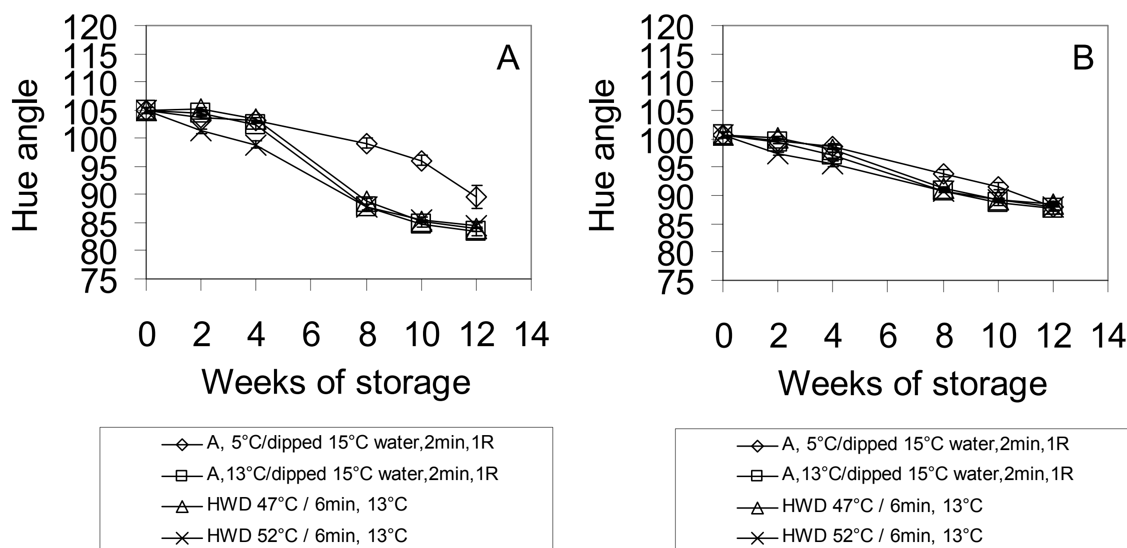


Figure 5.28 Colour change for NZ limes stored under RA at 5 and 13°C and pre-treated limes with different HWD conditions at 47 and 52°C dipped for 6 min then stored under RA at 13°C on the green side (A) or yellow side (B), H5. Vertical bars indicate ± SE (n=60).

5.4.2 Colour score under HWD conditions

For H3, the effect of HWD on CS was similar to the effect on H° (e.g. compare Fig. 5.22 and Fig. 5.29). In particular, the fruit pre-treated with HWD and stored under IW, with and without C_2H_4 absorbent, developed a higher CS than the control and the HWD treatments without IW (Fig. 5.29). This confirmed there was no benefit of these combined postharvest storage regimes.

For H5, the CS of the fruit stored under HWD at 57°C for 6 min was significantly different from the other treatments after 2 weeks of storage because of the severe heat injury to the fruit. Differences between CS for all other treatments were not as clearly marked as the effect on hue (Fig. 5.23 to 5.28).

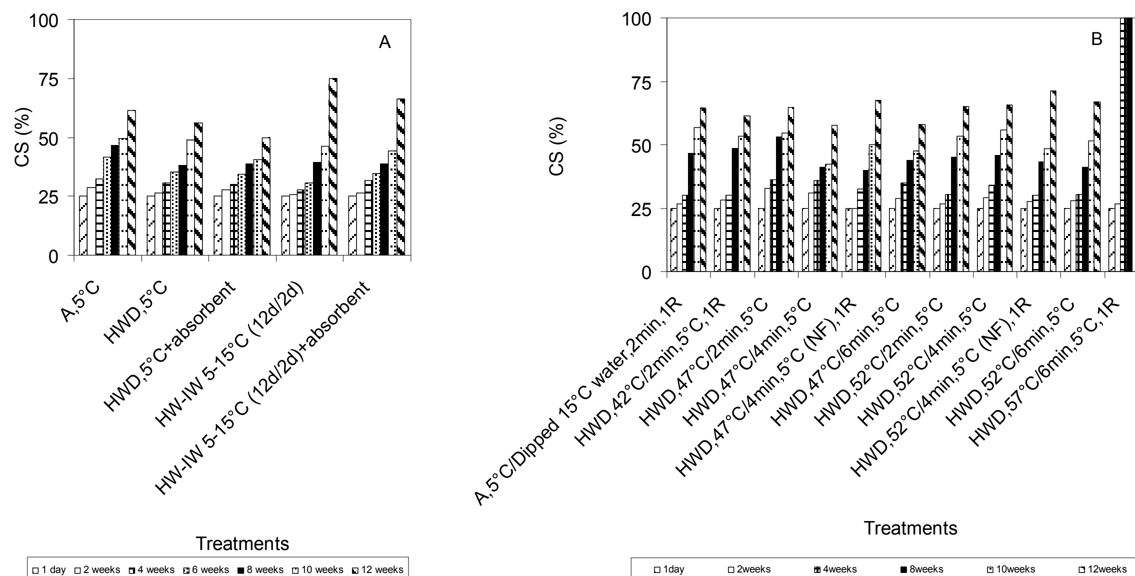


Figure 5.29 CS after harvest for NZ limes stored under RA at 5°C and pre-treated limes with 52-53°C HWD condition dipped for 2 min, with and without C_2H_4 absorbent and the same HWD condition plus IW condition (5°C for 12 days and 15°C for 2 days) with and without C_2H_4 absorbent then stored under RA at 5°C from 2, 4, 6, 8, 10 and 12 weeks of storage, H3 (A) and limes stored under RA at 5°C and pre-treated limes with several HWD conditions, H5 (B).

5.4.3 Disorders during storage following HWD

The H3 fruit stored after HWD at 52-53°C for 2 min showed some heat injury after 1 day of storage (Fig. 5.30A) and minor heat injury symptoms were more obvious for all similar HWD treatments after 2 weeks of storage (Fig. 5.30B). Although heat injury was sometimes very obvious (e.g. for HI-4 fruit) it was still only seen in less than 5% of the fruit. However, it was concluded that the HWD condition at 52-53°C for 2 min with TBZ (200 ppm) was not suitable for pre-treatment of limes before storage.

As previously noted, the control fruit stored under RA at 5°C showed significant pitting, but no CI after 8 and 10 weeks of storage (see section 4.1.3.2). The fruit stored after HWD at 52-53°C for 2 min with IW (5°C, 12 days and 15°C, 2 days) did not develop CI during 10 weeks of storage but did show some pitting. Fruit stored after HWD (without IW and without C_2H_4 absorbent) showed significant CI at 8 weeks of storage. This suggested that the C_2H_4 absorbent may help to protect against CI although it did not prevent pitting (Fig. 5.31).

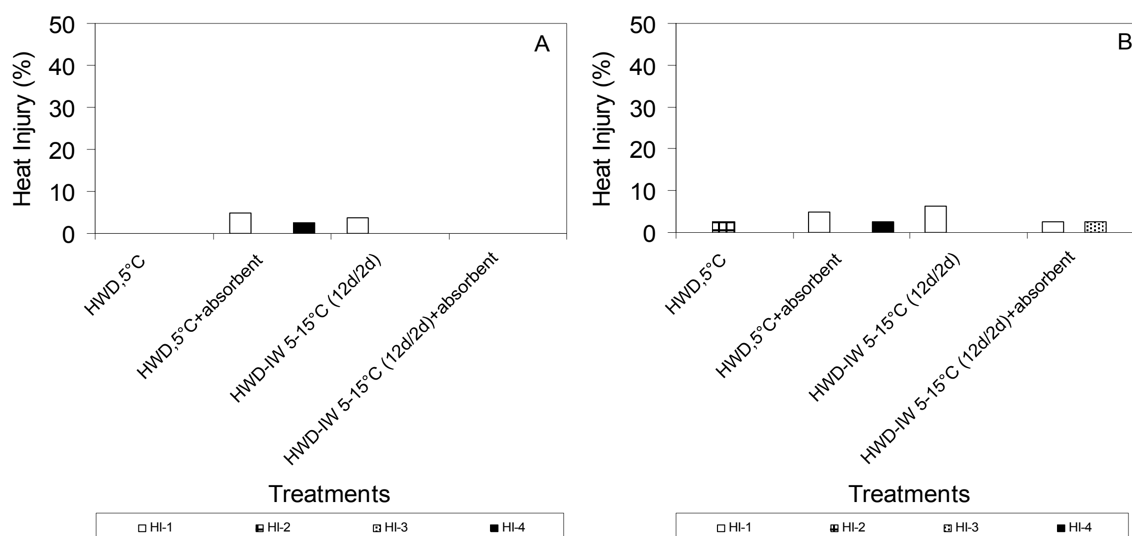


Figure 5.30 Incidence of heat injury after 1 day (A) and 2 weeks of storage (B) of lime pre-treated with HWD at 52-53°C with and without C_2H_4 or lime pre-treated with HWD at 52-53°C with and without C_2H_4 and stored under IW condition (5°C for 12 days and 15°C for 2 days), H3 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

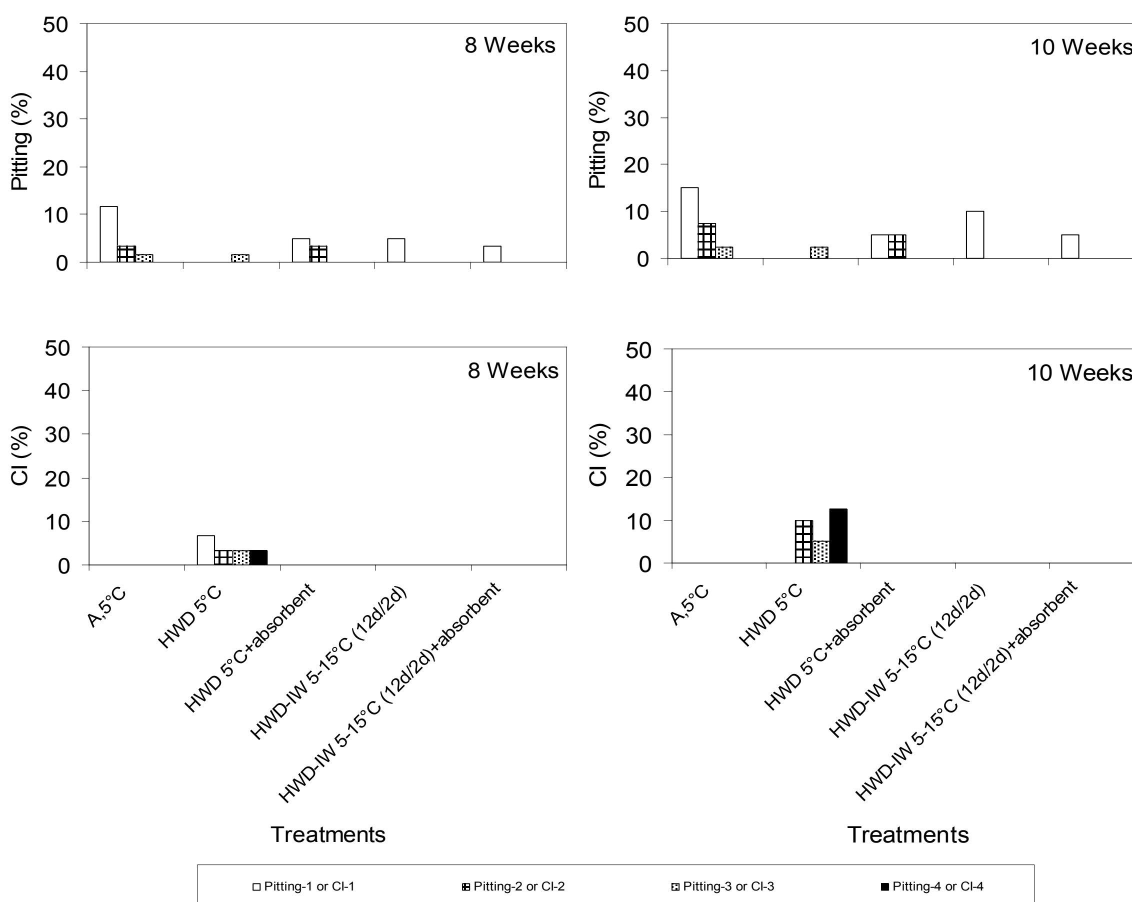


Figure 5.31 Incidence of pitting and chilling injury after 8 (left) and 10 (right) weeks of storage of lime stored under RA at 5°C or limes pre-treated with HWD at 52-53°C with and without C_2H_4 or lime pre-treated with HWD at 52-53°C with and without C_2H_4 and stored under IW condition (5°C for 12 days and 15°C for 2 days), H3 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4).

For the wider range of conditions evaluated in H5, there was little pitting up to 10 weeks of storage except for the 57°C for 6 min HWD treatment. Fruit in this treatment exhibited significant pitting and heat injury early in storage. Specific data are shown in appendix II-Fig A2 for pitting and Fig A3 for heat injury.

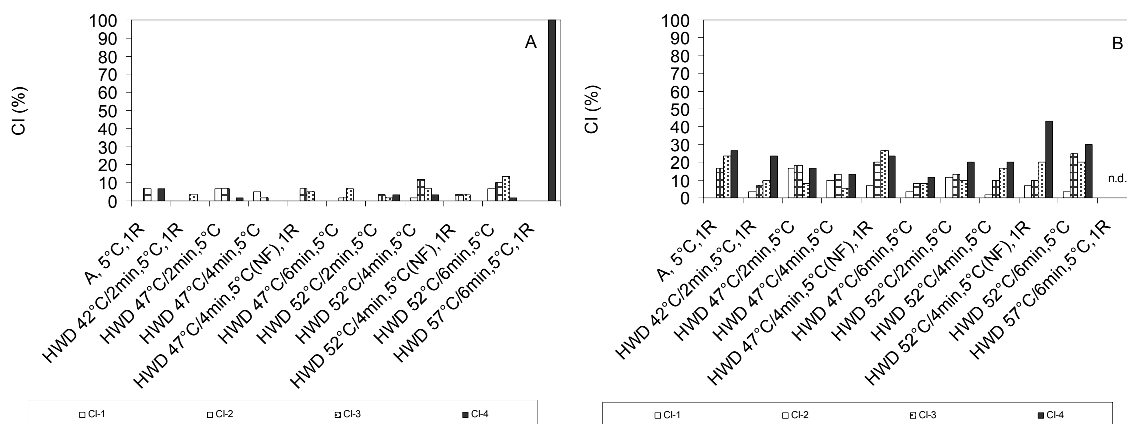


Figure 5.32 Incidence of chilling injury after 8 (A) and 10 (B) weeks of storage of lime stored under RA at 5°C or pre-treated limes with different HWD conditions (47°C or 52°C for 2, 4 or 6 min, respectively) with fungicide or pre-treated limes with HWD at 42°C for 2 min, 47 and 52°C for 4 min without fungicide and 57°C for 6 min with fungicide then all treatments were stored under RA at 5°C, H5 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4). n.d. = no data.

The fruit dipped at 42°C for 2 min showed decreased yellowing (Fig. 5.24A, B) and did not show pitting until 12 weeks of storage (appendix II-Fig A2), however they did show minor CI at 8 weeks (Fig. 5.32A) and this increased in severity and incidence by 10 weeks of storage (Fig. 5.32B).

The fruit stored after HWD at 47 or 52°C for 4 min with or without fungicide showed no or only low pitting (level of < 10%) by 10 weeks (appendix II-Fig A2). These fruit showed CI at 8 weeks of storage (Fig. 5.32) and the fruit without fungicide showed more severe CI at 10 weeks of storage (Fig. 5.32B). The fruit pre-treated with HWD at temperatures higher than 47°C showed heat injury symptoms at 4 weeks of storage (appendix II-Fig A3-B).

In summary, dipping the fruit at 47°C for 2-6 min with fungicide (TBZ) was safe with respect to heat injury for the fruit (appendix II-Fig A3); these fruit also showed only minor pitting symptoms (appendix II-Fig A2) but the treatments were still ineffective

overall for preventing CI (Fig. 5.32A, B). I did not study the shelf life of limes after pre-treatment by HWD because these treatments offered no benefit over IW treatments.

5.4.4 Rots under HWD conditions

Some rots appeared on the fruit pre-treated with HWD at 52-53°C with and without C_2H_4 absorbent at 10 weeks of storage whereas fruit pre-treated with HWD followed by IW (5°C, 12 days and 15°C, 2 days; with and without C_2H_4 absorbent) did not show rots at this time. All HWD treatments (regardless of subsequent storage conditions) led to the development of some rots by 12 weeks of storage whereas control fruit stored at 5°C did not develop rots. However, the incidence of rots after 12 weeks was low (<10%) in all treatments (Fig. 5. 33).

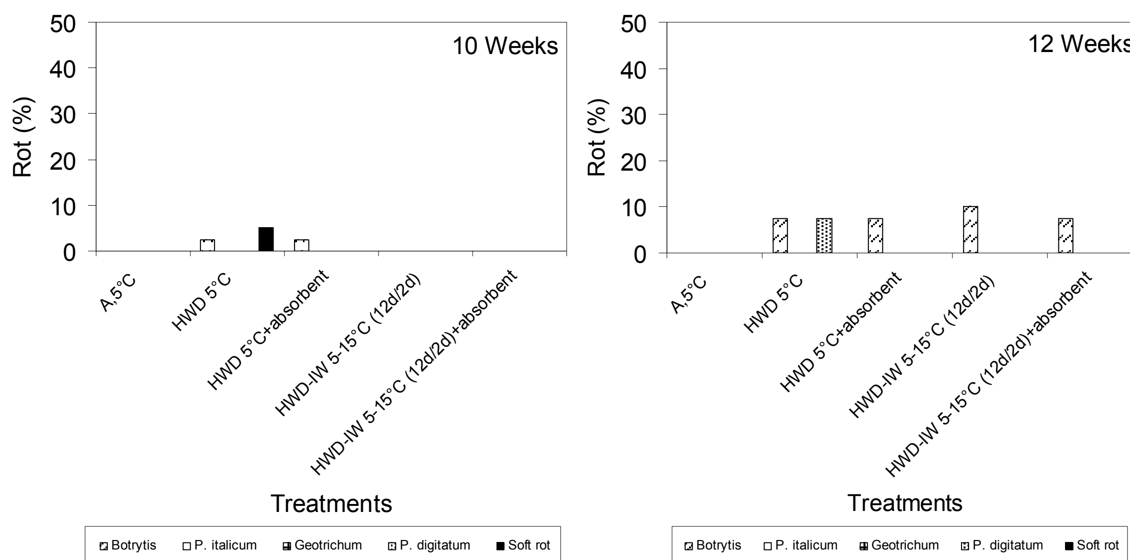


Figure 5.33 Incidence of rots after 10 (left) and 12 (right) weeks of storage for limes stored under RA at 5°C or limes pre-treated with HWD at 52-53°C with and without C_2H_4 or lime pre-treated with HWD at 52-53°C with and without C_2H_4 and stored under IW condition (5°C for 12 days and 15°C for 2 days), H3.

In H5, dipping the fruit at 42°C for 2 min and 47 and 52C° for 4 and 6 min, respectively, and then in fungicide (1,200 ppm for 3 min) in 15°C cool water (Table 3.2) gave good protection against rots until 10 weeks (Fig. 5.34 and 5.35). The combination of TBZ treatment and low incidence of pitting and CI presumably contributed to this. In contrast fruit dipped at 52°C for 6 min showed significantly more rots at 12 weeks as these fruit suffered from pitting at 2 weeks (appendix II-Fig. A2) and CI at 8 weeks (Fig. 5.32).

The role of fungicide is illustrated in Fig. 5.35. Fruit dipped at 52C° for 4 min without TBZ did not develop rots by 10 weeks of storage. No HWD treatments, even when TBZ was included, gave complete protection against rots at 12 weeks but the best treatment was at 42°C for 2 min (with fungicide) (Fig. 5.35). Note that data for fruit pre-treated with HWD 57°C for 6 min are not shown in Fig. 5.32 because all the fruit had to be thrown away after 8 weeks of storage because of severe CI.

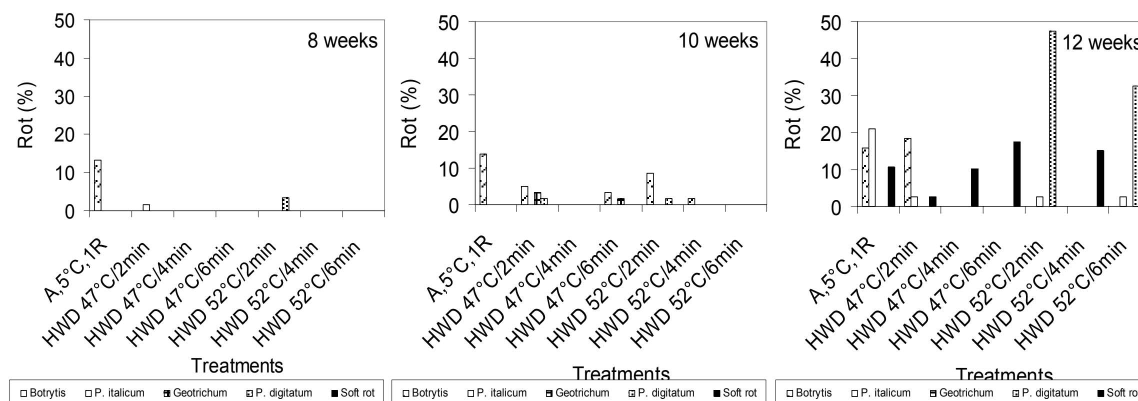


Figure 5.34 Incidence of rots after 8 (left), 10 (middle) and 12 (right) weeks of storage for limes stored under RA at 5°C or pre-treated limes with different HWD conditions (47°C or 52°C for 2, 4 or 6 min, respectively) then stored at 5°C, H5.

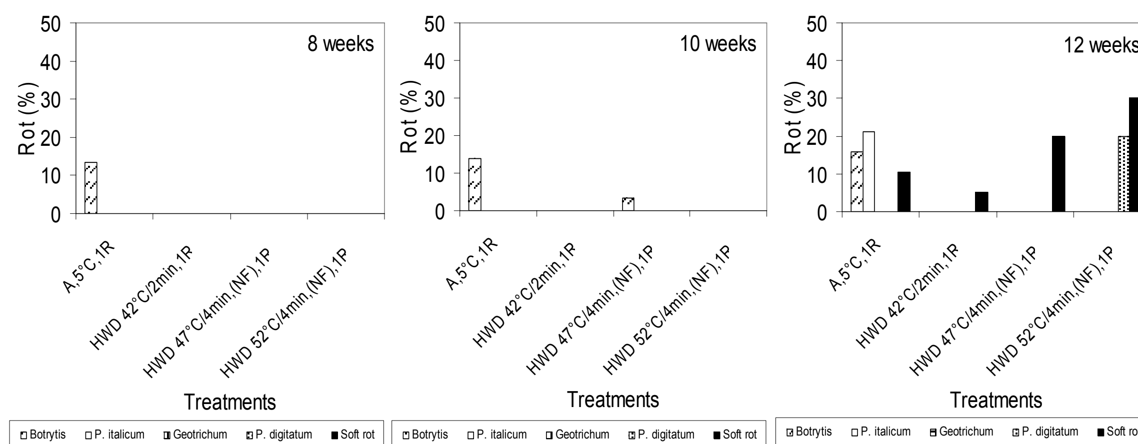


Figure 5.35 Incidence of rots after 8 (left), 10 (middle) and 12 (right) weeks of storage for limes stored under RA at 5°C or pre-treated limes with HWD at 42°C for 2 min or 47 and 52°C for 4 min without fungicide then stored under RA at 5°C, H5.

5.4.5 Compression firmness under HWD conditions

Treatment by HWD at 52-53°C for 2 min accelerated subsequent softening of H3 fruit, unless these were stored under IW or treated with an ethylene absorbent (Fig. 5.36). This loss of firmness coincided with the development of CI (Fig. 5.31). For H5 fruit, firmness was not measured as this was judged to give no better indication of CI or overall quality than the other measures employed.

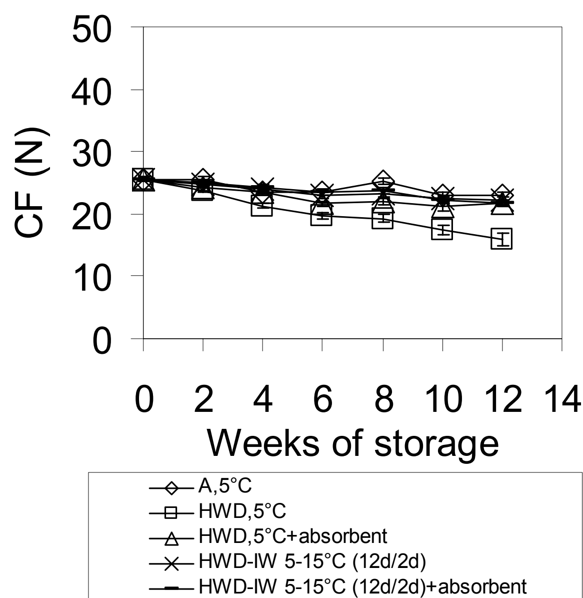


Figure 5.36 CF for NZ limes stored under RA at 5°C and pre-treated limes with 52-53°C HWD stored under RA at 5°C with and without C_2H_4 absorbent or IW condition (5°C for 12 days and 15°C for 2days) with and without C_2H_4 absorbent, H3. Vertical bars indicate ± SE (n=80).

5.5 Overall acceptability of limes after storage

In order to summarise the complex effects described in this and the previous chapter, an "acceptability index" was developed. The criteria considered for the index were disorders (i.e. pitting and CI), colour score and rots, with the thresholds for each shown in Table 5.1. Fruit meeting these criteria compare favourably to the best batches of limes available in local supermarkets in NZ. Fruit with CI-2 to 4 are sometimes seen in the marketplace in NZ but usually (though not always) only as lower-priced fruit, however fruit of poor colour are frequently encountered.

Table 5.1 Application of the criteria of lime acceptability

Criteria	Acceptable level
Pitting	1-2
Chilling injury	1
Colour score	up to 75%
Visually obvious rots	none

Fig. 5.37 summarises the best and worst treatments from H2, H4 and H5 based on application of the criteria in Table 5.1.

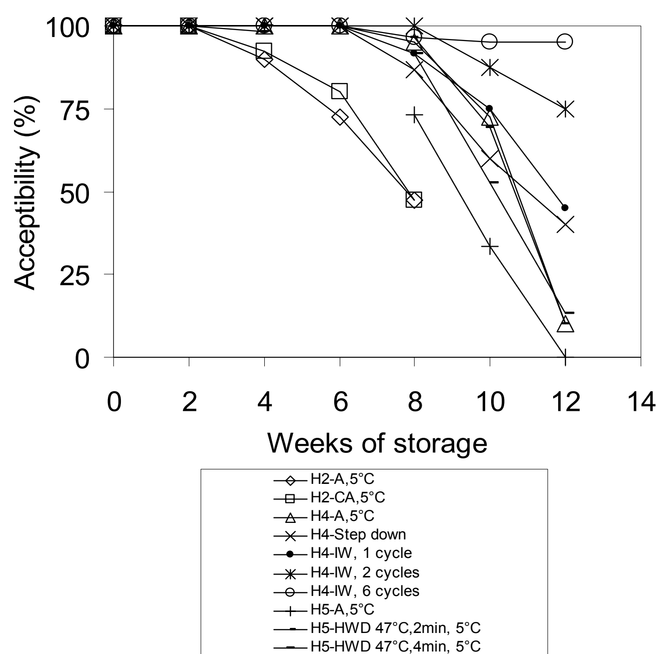


Figure 5.37 Acceptability of limes after storage under RA at 5°C, H2, 4 and 5 compared with limes stored under CA (3% CO_2/10% O_2) at 5°C, H2, limes stored under the step-down treatment (10°C, 2 weeks then stored under 5°C plus C_2H_4 ethylene absorbent until the end of storage, H4, limes stored under IW conditions (5°C, 12 days and 15°C, 2 days) regimes plus C_2H_4 ethylene absorbent and different warming frequency (1, 2 and 6 cycles) ,H4 and limes pre-treated with HWD at 47°C for 2 and 4 min, respectively and then stored under RA at 5°C, H5.

Initially all fruit were 100% acceptable in all experiments. There were differences between the same treatments in different harvest seasons, partly due to differences in the initial colour quality of the limes. As an example, the quality of fruit stored under RA at 5°C in H2 decreased from 100% acceptability to 90% at 4 weeks of storage whereas the H4 fruit stored under the same condition reached this lower quality level after 8 weeks of storage. The H4 fruit were dipped in the fungicide TBZ (1,200 ppm for 3 min) before storage, however the fruit lost quality rapidly from 95% acceptability at 8 weeks to just

10% acceptability at 12 weeks of storage. The 5°C H2 fruit declined from 90% acceptability at 4 weeks to less than 50% acceptability at 8 weeks.

Although many treatments, including storage in RA at 5°C, could provide adequate quality up to 8 weeks, few postharvest treatments provided extended high quality storage life. There were no benefits from CA and the step-down treatment for storage of H2 and H4 limes, respectively. However, there were clear benefits from the best treatments in H4 and H5 when compared to their control treatments. In particular, the H4 fruit stored under IW conditions for 2- and 6-cycles showed high acceptabilities of about 75 and 95%, respectively, after 12 weeks of storage. The 1-cycle IW fruit provided ~75% acceptably fruit after 10 weeks but under 50% by 12 weeks. The H5 fruit stored after HWD at 47°C for 2 and 4 min showed moderate acceptability with index values of approximately 53 and 70% after 10 weeks of storage (Fig. 5.37). The performance of the fruit from the 6-cycle (short-cycle) IW regime was outstanding and this is clearly the best of all postharvest treatments evaluated in this research.

5.6 Discussion

In this research, the quality of lime fruit during storage under several HWD and IW conditions was observed in order to determine the best strategy to prolong the fruit's high quality storage life. In addition, the effect of an ethylene absorbent was studied and the use of the fungicide TBZ was evaluated as an additional pre-storage intervention. A step-down technique (temperature conditioning) was also tested as a simpler and more convenient technology to compare with IW.

The main factors that limit storage and shelf life of lime fruit are degreening of lime peel, pitting and CI, and occurrence of rots. Several authors (Kluge *et al.,* 2003a; Thompson, 2003; Ladaniya, 2004; Murata, 1997) reported that green colour of lime peel is retained better at 4°C than the recommended RA storage condition (9-10°C), but the fruits might suffer from pitting which can shorten their storage life. I chose 5°C as the primary control for storage treatments in this work. In my work, I attempted to investigate several postharvest regimes that could delay or maintain both storage life and shelf life of lime by reducing these deteriorative factors as much as possible. The effects of key strategies tested are discussed in the following sections.

Thiabendazole (TBZ)

The fungicide TBZ is well known to control a wide range of fungal diseases for citrus fruit and is reported to have a secondary beneficial effect on CI (at least for oranges, during cold storage and shelf life). The efficacy of TBZ on fungal diseases and CI was also increased when it was used in combination with hot water (Schirra and Mulas, 1995b).

From my tests in H3 fruit, the use of TBZ in combination with IW and HWD for the fruit showed that, as expected, the fungicide (applied at 1,500 ppm for 3 min in 20°C cold water) was very effective at reducing rots for different RA or IW treatments until 12 weeks of storage (Fig. 5.14). Rodov *et al*., (1995) similarly reported that the use of fungicides (imazalil and thiabendazole, at 1,000 ppm) with HWD (53°C, 2-3 min) protected four citrus fruits: grapefruit (*C. paradisi* Macf., cv. March), lemon (*C. limon* Burm., cv. Eureka), oroblanco (*C. grandis* Osb. x *C. paradisi* cv. Oroblanco, syn. Sweety) and kumquat (*Fortunella margarita* Swingle cv. Nagami) against decay. However, they also found that the risk of heat damage during the hot water dip may increase when the fungicide was added. These finding are supported by my work: the use of fungicide (200 ppm for 2 min) with HWD reduced rots in fruit stored under IW (Fig.5.33) but also appeared to exacerbate heat injury for the H3 fruit (Fig. 5.30). The use of TBZ did however appear to help to protect against CI for the H3 fruit stored under RA at 5°C and for several IW treatments (Fig. 5.9).

The effects of TBZ on lime quality when applied in cold water were further investigated in the H4 and H5 fruit. For H4 fruit, I found that the use of TBZ (1,200 ppm for 3 min in 15°C cold water) with all IW conditions of the fruit reduced CI (Fig. 5.10) and effectively reduced rots (Fig. 5.15 and 5.16) when compared with the control fruit stored at 5°C until 12 weeks of storage. For H5, dipping fruit at 42 and 47°C for 2 and 4 min, respectively, and treating them with TBZ (1,200 ppm for 3 min) at 15°C were the most effective treatments to protect against rots until 10 weeks (Fig. 5.34 and 5.35). The presence of the fungicide was also beneficial in delaying yellowing, especially on the yellow side of fruit (Fig. 5.26). Dipping the fruit at 47°C for 2-6 min followed by cooling in 15°C water containing the fungicide did not lead to heat injury (appendix II-Fig A3) and fruit showed only minor pitting (appendix II-Fig A2), but they were not protected against CI (Fig. 5.32).

Overall, the effects of the fungicide TBZ against rots and CI on lime during storage are broadly similar to other reports for citrus fruits. For example, Schirra *et al*., (1998) dipped 'Tarocco' oranges for 3 min with and without thiabendazole (TBZ) at 200 ppm in water (50°C) or 1,200 ppm (19°C, room temperature) and stored the fruit at 3°C for 6 weeks, followed by 1 additional week at 20°C. They concluded that hot water dipping at 50°C reduced CI and the postharvest treatment with 200 ppm TBZ at 50°C was more effective in reducing CI symptoms. However in my work, the use of TBZ (200 ppm for 2 min) on lime at 52-53°C led to about 10% CI at 10 weeks of storage whereas the fruit stored at RA at 5°C did not show CI, probably because the temperature of the water was too high for the limes and this led to greater susceptibility to CI after storage at low temperature (5°C). The fruit pretreated with HWD at 52-53°C with fungicide plus C_2H_4 absorbent or with IW condition (5°C, 12 days and 15°C, 2 days) were protected against CI up to 10 weeks of storage (Fig. 5.31). This was similar to Schirra and Mulas's (1995) report that pre-treatment with HWD 52°C for 3 min for 'Fortune' mandarin was more effective in control of CI and decay during storage under IW condition (2°C, 4 days and 10°C, 3 days) when compared with the fruit stored continuously at 2°C.

As already described in chapter 2, despite the efficacy of the fungicide, many consumers would prefer the use of non-chemical disease suppression technologies.

<u>Intermittent warming</u>

IW has been a common research procedure used to reduce CI in various horticultural products and is recommended for lemons, grapefruits and mandarins (Porat, 2004). As one example, IW (2°C, 3 weeks and 11°C, 1 week) could retain green colour, reduce CI and incidence of decay development, and extend postharvest storage for 'Oroblanco' citrus fruit. Although IW did not affect juice total soluble solids and acid composition, it did affect fruit taste and the amount of off-flavour volatiles from juices. However, the taste of IW fruit was still acceptable after 16 weeks of storage (Porat *et al.,* 2003). For my work I did not specifically analyse for the taste and off-flavour volatiles of the lime juices after storage and shelf life, but I did observe that the juices from fruit stored under IW treatments were very acceptable in colour and smell.

Despite this growing positive evidence from the research literature Artes *et al*., (1993) and Kluge *et al*., (2003a) also stated that this technique needs more practical research to

confirm the apparent benefits. They reported that while IW effectively reduced CI in many citrus fruits, the identification of optimum conditions has proved difficult as optimal temperatures, IW frequency and durations may vary from cultivar to cultivar, with stage of fruit maturity, and for different planting and growing conditions. For example, Kluge *et al.*, (2003a) reported that both the temperature and the exposure time to this temperature affect the occurrence of CI symptoms on limes. Furthermore, some treatments designed to control one disorder may make another problem worse e.g. they reported that *P. digitatum* was found in nearly 40% of 'Tahiti' lime after they had been warmed at 38°C for 24 hour every 14 days.

IW may also not be easy to manage in a large scale commercial coolstore, where repeatedly increasing and decreasing the storage temperature is usually a slow and lengthy process or else expensive to achieve quickly (e.g. by forced draft heating or cooling). In addition, too many or too lengthy warming cycles may increase deterioration and development of rots compared with continuous constant low temperature storage (Porat, 2004).

From my work, treating limes with some IW treatments was proved effective for maintaining quality. Shorter-cycles (12 days) at low temperature (5°C) in combination with 2 days at high temperature (15°C) delayed colour change prior to the development of some minor CI (Fig.5.10). The duration of each cold storage period (12, 16, 20 and 24 days) at 5°C before the warming cycle at 15°C for 2 day was tested and the results clearly demonstrated that shorter-cycle IW treatments (12 and 16 days) were more effective at delaying colour changes (Fig. 5.5) and protecting against rots (Fig. 5.15 and 5.16) than the long-cycle IW combinations (20 and 24 days before warming) at 15°C for 2 days. The fruit stored under our best IW conditions (i.e. 2 or 6 cycles IW) retained quality and showed a high level of acceptable fruit (Fig. 5.37). However as noted above, the fungicide TBZ is still required in order to protect against rots and to aid in reducing CI development. The 2- and 6-cycle IW regimes (5°C, 12 days and 15°C, 2 days) delayed degreening on the green and yellow side of fruit better than other treatments (Fig. 5.4). The 6-cycle IW (5°C, 12 days and 15°C, 2 days) was the best treatment to delay colour change (Fig.5.4 and 5.5), and protected against fruit chilling disorders (Fig.5.10) and rots (Fig. 5.15) until 12 weeks of storage.

This work has also provided important new knowledge on how the number of cycles of IW influences the quality of limes. The 6-cycle regime was more effective than 2- or 1-cycle and the data obtained can be compared with that of Kluge and co-workers (2003).

Kluge *et al*., (2003a) stored 'Tahiti' lime under a constant temperature at 5°C compared with three different IW conditions (IW-1; 20°C, 2 days and 5°C, 7 days, IW-2; 20°C, 2 days and 5°C, 14 days and IW-3; 38°C, 1 day and 5°C, 14 days) (with and without 1.0 µl l^{-1} of 1-Methylcyclopropene (1-MCP)). They found no CI in fruit after 30 days of storage (similar to my work) in all harvests but they did find 40-58% CI of fruit stored at 5°C after 60 days of storage. In contrast, I found less than 20% CI in the fruit stored at 5°C in my work at 8 weeks (56 days) of storage (Fig. 5.7-5.10).

Kluge *et al*., (2003a) also reported that the occurrence of CI symptoms was reduced to 12.5-20% by their IW treatments but all their fruit showed CI after 90 days of storage. They also found no differences in physicochemical characteristics between the fruit stored at 5°C continuously and the fruit stored under IW treatments (IW-1 and IW-2). The more extreme IW-3 treatment led to adverse effects on fruit quality such as a reduction in acidity (TA, % citric acid) during storage and also loss of ascorbic acid content, with declines of approximately 30 and 50% at 30 and 60 days, respectively. In addition, a significantly higher respiration rate and production of ethanol and acetaldehyde were observed under IW-3 compared to constant temperature storage at 5°C and the other IW conditions. Thus Kluge *et al.* (2003)'s IW regimes were able to achieve gains with respect to improved colour but overall were not really successful in providing high levels of acceptable fruit. From my results, I concluded that the correct selection of the number of cycles and duration of warming was very important. I could not retain quality of fruit by only using 1-cycle IW, but I could retain quality better using only a 2-cycle regime. Quality retention was the best when the 6-cycle IW was used. This I hypothesise to be associated with tissue repair processes facilitated during warming periods during IW storage.

Respiration rate was measured to provide an indication of the physiological state of the fruit. All previous studies that cite respiration rate for limes quote values between 3 and 16 ml CO_2 kg^{-1} hr^{-1}. Arpaia and Kader, (2000) reported that the ranges of respiration rate of lime at 10, 15 and 20°C were 3-5, 5-8 and 6-10 ml CO_2 kg^{-1} hr^{-1}, respectively. Win *et*

al., (2006) reported that the respiration rate of untreated lime (*C. aurantifolia*, Swingle) stored under ambient conditions (24–31°C and 73–81% RH) was about 11.2 ml CO_2 kg^{-1} hr^{-1}. Kluge *et al*., (2003b) reported that 'Tahiti' lime stored continuously at 5 and 10°C and then placed in simulated marketing condition at 20°C for 3 days exhibited respiration rates of about 13 and 15 ml CO_2 kg^{-1} hr^{-1} at 30 days, respectively, and that this was reduced to about 5 and 8 ml CO_2 kg^{-1} hr^{-1} at 60 days of storage, respectively. Kluge *et al.* (2003) also reported that the fruit stored under three IW conditions (IW-1 – IW-3, as previously explained), exhibited respiration rates of about 13, 12 and 15 ml CO_2 kg^{-1} hr^{-1} at 30 days, respectively, and 5, 6 and 14 ml CO_2 kg^{-1} hr^{-1} at 60 days of storage, respectively.

Similar respiration rates for 'Tahiti' lime were also obtained by Jomori *et al*., (2003). They reported that the respiration rates of the fruit treated with 0 µl l^{-1} 1-MCP and stored continuously at 5 and 10°C plus 3 days shelf life at 20°C were 13 and 15 ml CO_2 kg^{-1} hr^{-1} at 30 days, respectively, and about 6 and 8 ml CO_2 kg^{-1} hr^{-1} at 60 days, respectively, whereas the fruit treated with 1 µl l^{-1} then stored at 5 and 10°C plus 3 days shelf life exhibited lower respiration rates about 10 and 15 ml CO_2 kg^{-1} hr^{-1}, respectively at 30 days and about 7 ml CO_2 kg^{-1} hr^{-1} at 60 days of storage. Other researchers have also reported an overall reduction in respiration rate after long term storage.

The respiration rates measured in the present study were broadly similar to these other studies (10-20 ml kg^{-1} hr^{-1}) for the IW treatments, but were surprisingly high under constant cold storage (30-40 ml kg^{-1} hr^{-1}). This may be because the respiration rate was measured within only a few hours of warming the fruit whereas other researchers may have allowed some time for temperature equilibration. There may be a period of stress-induced respiration increase and if so, the magnitude of this effect may be valuable to investigate further.

Ethylene production of limes is generally very low (< 0.3 µl kg^{-1} hr^{-1}) (Arpaia and Kader, 2000; Win *et al.,* 2006). Arpaia and Kader, (2000) reported that the rate of ethylene production was lower than 0.1 µl kg^{-1} hr^{-1}, while Win *et al.* (2006) reported that the ethylene production rate of untreated lime (*C. aurantifolia*, Swingle) stored under ambient conditions (24–31 °C and 73–81% RH) was about 0.3 µl kg^{-1} hr^{-1} at the initial stages of their storage.

Ethylene production rates from limes in our study were similar and low (< 0.5 µl kg^{-1} hr^{-1}) under IW (plus C_2H_4 absorbent) but were slightly higher under constant cold storage (1.1–1.7 µl kg^{-1} hr^{-1}). Even the fruit stored at 5°C with C_2H_4 absorbent showed a higher rate (about 1.1 µl C_2H_4 kg^{-1} hr^{-1}) at 42 days, possibly stress caused by moving the fruit between cold rooms might be a reason for this increased ethylene production. In addition the development of rots (Fig. 5.15) may have contributed to increased ethylene production in fruit stored at 5°C without C_2H_4 absorbent.

The effect of IW on respiration rate and ethylene production rate is interesting to explore further. Cabrera and Saltveit, (1990) studied the ethylene production rate of cucumber fruit (*Cucumis sativus* L. cv. Poinsett 76) stored for 6 days under IW (2.5°C, 3 days and 12.5°C, 18h) compared to the fruit stored under constant temperature at 2.5°C (chilled) and 12.5°C (control). They found that the ethylene production rates of the fruit stored at a constant 2.5 and 12.5°C were between 5 and 10 nl C_2H_4 kg^{-1} hr^{-1} and 10 and 20 nl C_2H_4 kg^{-1} hr^{-1}, respectively. The IW fruit showed about an 18-fold higher ethylene production rate during the warming period at 12.5°C (for 18 h) compared to the ethylene production rate during the cooling period at 2.5°C for 3 days. The ethylene production rate of IW fruit reduced rapidly within 6 h once the fruit were moved to the cool temperature (2.5°C) again. When all the fruit were transferred to 20°C after 6 days, the fruit continuously stored at 12.5°C showed a slight increase in the rate of ethylene production of about 20 nl C_2H_4 kg^{-1} hr^{-1}. The continuously chilled and IW fruit showed a higher increase of ethylene production rate from 5.2 to 61 nl C_2H_4 kg^{-1} hr^{-1} and from 6.7 to 35 nl C_2H_4 kg^{-1} hr^{-1} after 9 h at 20°C, respectively.

The authors also studied multiple cycles of warming of the cucumber fruit and measured respiration and ethylene production rates. They found that the respiration rate of the fruit increased from 9 to 25 mg CO_2 kg^{-1} hr^{-1}, or about 3-fold, during the first warming period, whereas the increase was only 2-fold during the second (10 to 18) and third (12 to 22) warming periods.

Fernández-Trujillo *et al*., (1998) studied the respiration and ethylene production rates at two maturity stages, firm-breaker (FB) and firm-mature (FM), of ‘Paraguayo’ peaches (*Prunus persica* L. Batsch) under constant temperature storage at 2°C, 3-cycles of an IW condition (2°C for 6 days and 20°C for 1 day), and constant temperature storage at 20°C

(both for normal postharvest ripening and post-storage ripening). They found both the respiration and ethylene production rates behaved similar to the control fruit (2°C) during the cooling period, but increased during warming periods (20°C, 1 day) (especially the first and second warmings) and dropped again after the fruit were transferred to the cooling periods for both FM and FB fruit. They found both the respiration and ethylene production rates in control FM fruit during ripening at 20°C were higher than the IW fruit (during ripening at 20°C) after two weeks of storage with the differences being more evident for ethylene production. In FB fruit, these differences were only observed in respiration rate after 2 weeks of storage of ripening at 20°C. A reduction in ethylene production of the control fruit occurred after the maximum CI levels were observed at the third week of storage and during the post-storage ripening after 4 weeks at 2°C.

Artes *et al*., (1998) studied the respiration and ethylene production rates of tomato (*Lycopersicon esculentum* Mill. cultivar 'Durinta') fruit at breaker stage stored continuously at 9 and 20°C and under IW condition (9°C for 6 days and 20°C for 1 day). They found that the fruit stored at 9°C and transferred to 20°C after 2 or 3 weeks of storage showed increased respiration and ethylene production rates compared with the fruit stored continuously at 20°C. In the fourth week of storage, a peak of ethylene production was slightly delayed in fruit stored at 9°C compared to those fruit stored at 20°C. The fruit stored under the IW condition showed similar metabolic rates to the fruit stored continuously at 9°C, but during the warming periods both the respiration rate and ethylene production rate were increased at all warming periods. The increased rate of respiration during the first warming seemed similar to the second warming but was higher than during the third warming. The authors reported that ethylene production during successive warming periods was stimulated to similar degrees and were in a range from 3 to 4.5 µl C_2H_4 kg^{-1} hr^{-1}.

The patterns of metabolic response during the warming periods of limes stored under IW conditions in my work were therefore similar to other fruit described above in that the respiration rate of limes was also increased during the warming periods. The 2- and 6-IW cycle fruit showed increased of respiration during the first (14 days) and the second (28 days) warming periods, while the fruit stored under 6-cycle IW showed only slightly increased respiration rate at subsequent warming periods (at 70 and 84 days of storage). The 2- and 6-cycle treatments led to overall suppression of the respiration rate but the 1-

cycle effect was gradually lost which supports the hypothesis that a single warming is not enough to retain fruit quality during long term storage. In contrast, the patterns of ethylene production rate of limes stored under IW conditions were different to these other fruit as our limes did not show any increase in ethylene production at the first warming nor did they show a clear pattern thereafter.

<u>Temperature conditioning or step down technique</u>

Temperature conditioning (TC) is a technique commonly used in commercial practice in which the storage temperature is reduced in a stepwise manner to provide gradual acclimation of the fruit to the final storage temperature (Hatton, 1990; Woolf *et al.,* 2003). In this study only a single step-down TC treatment was tested (due to logistical constraints) in which the fruit were stored at an intermediate temperature (10°C) before continuous storage at 5°C.

This step-down technique was not successful because there was a rapid colour change after 4 weeks of storage, especially on the yellow side of the fruit (Fig. 5.4B). As only one condition was investigated, it is not possible to draw any conclusions from this experiment. However, the initial period of time at this temperature (10°C) was perhaps too long (2 weeks) therefore, the second step of storage temperature at 5°C was too late to maintain fruit quality during storage (Fig. 5.4). I conclude that the step-down technique had no overall benefit but as it possibly showed some protection against disorders (Fig. 5.10) and rots (Fig. 5.15) further investigation is warranted.

Spalding and Reeder, (1983) conditioned 'Tahiti' limes stored in flats using polyethylene liners at 7.2°C (45°F), 10°C (50°F), 12.8°C (55°F), 15.6°C (60°F) and 21.1°C (70°F) for 1 week prior to storage at 1°C (35°F) for 2 weeks and compared these to non-conditioned fruit (first chilled at 1°C for 2 weeks then held at 7.2, 10, 12.8, 15.6 or 21.1°C). They found the colour was still acceptable for both groups at the end of the tested periods at 3 weeks but the conditioned fruit developed less CI than non-conditioned fruit. The limes conditioned at 10, 12.8 and 15.6°C for 1 week showed the lowest amount of CI. There was no significant difference in the colour changes between the conditioned and non-conditioned fruit stored at the same temperatures. Decay development was severe in the non-conditioned fruit held at 21.1°C after chilling. This result is similar to my results in

terms of the protection against CI and colour change provided by the step down treatment for the first 3-4 weeks of storage.

Porat *et al.* (2003) also reported that TC (16°C, 1 week before continuous storage at 2°C) effectively reduced the CI in 'Orablanco' citrus fruit during long term storage (16 weeks) to only 5% after 8 weeks of storage, but there was no longer-term benefit in reducing CI after that. The TC treatment also reduced the incidence of decay development during storage, similar to the results of my work.

<u>Hot water dipping</u>

Hot water treatment is a relatively simple technique to apply commercially although it does require additional time, energy and cost. Hot water is effective for raising the temperature rapidly, although this can lead to heat injury if the temperature is too high or the exposure period is too long. Tian *et al*., (1996) reported that the safe zone of HWD treatment occurs in a narrow range of temperature and time, therefore successful implementation requires great care and research to identify the appropriate conditions.

HWD has been widely applied to extend citrus storage life. McLauchlan *et al*., (1997) reported that prestorage treatments of 'Eureka' lemons by HWD at 47-53°C for 1-3 min significantly reduced the incidence of CI after storage at 1°C for 42 days, however browning of the fruit surface occurred after dipping the fruit at 53°C for 3 min. Nafussi *et al*., (2001) reported that the application of HWD at 52-53°C for 2 min prevented decay in lemon fruit inoculated with *P. digitatum* for at least one week. Schirra and D'hallewin, (1997) studied the effects of HWD at 50, 52, 54, 56 and 58°C for 3 min on commercially ripe 'Fortune' mandarins before storage at 6°C for 30 days and 3 days shelf life at 20°C. They found that the HWD at 50-54°C reduced CI and levels of decay during both cold storage and shelf life whereas the HWD at 56-58°C showed no benefits compared with control fruit. HWD at 50 and 52°C did not cause any adverse effects on the rind surface during both storage and shelf life but higher temperature of 54–58°C induced heat damage and promoted rind browning, with the extent and amount of damaged fruit increasing as the temperature of hot water was elevated.

Chilling injury and decay of several citrus fruits (e.g. late-ripening mandarin (*C. reticulata*, Blanco cv. 'Fortune'), grapefruit (*C. paradisi* Macf., cv. March), lemon (*C.*

limon. Burm., cv. Eureka), oroblanco (*C. grandis* Osb. x *C. paradisi*, cv. Oroblanco, syn. Sweety) and kumquat (*F. margarita* Swingle, cv. Nagami)) have all been reported to be effectively reduced by the application of HWD treatments ranging from 47°C (for 6 min) to 53°C (for 2-3 min) at both low and optimal temperature storage (Wild, 1993; Rodov *et al.,* 1995; Gonzalez-Aguilar *et al.,* 1997).

With regard to rind colour, the use of heat treatment by hot drench brushing at 60°C for 10 s significantly delayed degreening of 'Oroblanco' citrus, especially at the stylar and stem ends of the fruit, when compared to control fruit washed at room temperature, until the end of storage periods (15 weeks). Heat treatment at lower temperatures (52, 56°C) did not consistently inhibit yellowing, e.g. HWD at 52°C for 2 min only inhibited yellowing of the fruit in combination with the use of individual seal-packing (Rodov *et al.,* 2000). Heat treatments have also been reported to delay degreening of broccoli by using HWD at 43-55°C for up to 10 min (Forney, 1995; Tian *et al.,* 1996; Tian *et al.,* 1997).

For my work, the application of HWD at 52-53°C for 2 min with and without C_2H_4 absorbent did not delay colour changes when compared with the control fruit stored at 5°C (Fig. 5.22) and some fruit showed heat injury (Fig. 5.30). The fruit dipped at 52-53°C for 2 min with IW (5°C, 12 days and 15°C, 2 days), with and without C_2H_4 absorbent, did not develop CI during 10 weeks of storage but did show some pitting. Fruit stored after HWD (without IW and without C_2H_4 absorbent) showed significant CI at 8 weeks of storage (Fig. 5.31). Therefore, I conclude that the HWD at 52-53°C was not suitable for pre-treatment for NZ limes. The other HWD treatments were further investigated and for H5 limes it was clearly demonstrated that a temperature higher than 47°C for longer than 4 min may damage the fruit skin (appendix II-Fig A3) and did not help to maintain green colour (Fig. 5.23 and 5.27). The range of safe and effective HWD (in terms of reducing CI and degreening) from my work was 42-47°C for 2 to ≤ 4 min.

<u>Comparing IW and HWD</u>

Both IW (Cohen *et al.,* 1983; Cohen, 1988; Cohen *et al.,* 1990a; Artes *et al.,* 1993; Schirra and Mulas, 1995a; Schirra and Cohen, 1999; Porat *et al.,* 2003) and HWD (Rodov *et al.,* 1995; Gonzalez-Aguilar *et al.,* 1997; Schirra *et al.,* 1997; Porat *et al.,* 2000; Nafussi *et al.,* 2001) treatments offer longer-term, higher quality storage life and protect citrus

fruit against chilling disorders. The influence of these different treatments on fruit ripening depends on the exposure temperature and time (Lurie, 1998; Paull and Jung Chen, 2000; Vicente *et al.,* 2006). Even though both techniques are related to the application of heat to the fruit, the mode of action is likely to be quite different. The fruit pre-treated with HWD will be more influenced by the very rapid heating of the fruit peel generated by the large temperature difference between the water and fruit whereas the fruit stored under IW condition will be more influenced by the more gradual change in temperature profile, which will also be more evenly distributed through the whole fruit. The peel of the fruit is the most affected tissue in both treatments with the potential for damage as the temperature is increased.

One manifestation of the tissue response may be the generation of heat shock proteins (HSPs). Several environmental factors influence the induction and increase of HSPs which are a group of conserved proteins identified in both prokaryotes and eukaryotes (Kimpel and Key, 1985; Sun *et al.,* 2002; Wang *et al.,* 2006). Eukaryotes frequently produce a small set of HSPs as part of their response to abiotic environmental stress (Heikkila *et al.,* 1984; Sabehat *et al.,* 1996; Kotak *et al.,* 2007) and prior exposure of susceptible plant tissue to a number of abiotic stresses including extreme temperatures (heat and cold shock), ethanol, osmotic shock and salinity can all increase chilling tolerance (Saltveit, 1991; Wang *et al.,* 2004; Saltveit, 2005). Development of acquired thermotolerance is associated with HSP synthesis and the disappearance of HSP is associated with the loss of thermotolerance (Vierling, 1991; Lurie, 1998; Bowen *et al.,* 2002).

HSPs are induced by about a 8-10°C jump in temperature above the normal growing temperature (Kimpel and Key, 1985; Saltveit, 2005) and there is a decrease in synthesis of the normal complement of cellular proteins (Kimpel and Key, 1985). Heat treatment influences many aspects of fruit ripening such as ethylene production, cell wall degradation, changes in gene expression, and protein synthesis (Lurie, 1998; Vicente *et al.,* 2006). During application of a high temperature regime, the expression of fruit ripening related genes decreases or disappears while the accumulation of the genes corresponding to HSPs increases (Picton and Grierson, 1988; Lurie, 1998; Vicente *et al.,* 2006).

In my work, I noted a sharp decline in internal temperatures from the surface to the core during HWD. The temperature probe that I used to record "surface temperature" was actually located in the albedo zone; the flavedo would presumably have experienced even higher temperature changes during HWD. Heat injury was caused in the flavedo under the most extreme HWD temperatures. I noted that HWD at 42°C for 2 min was as beneficial in delaying colour changes as HWD at 47°C for 2 min. My data imply that only the flavedo needs to be warmed in order to delay subsequent colour loss.

I have demonstrated that a range of optimal HWD and IW conditions showed some beneficial effects to retain quality and extend storage life of lime. However, when I consider these regimes based on the rate of temperature change in the fruit during storage the mechanism might be different. The rate of temperature changes in HWD is much faster than IW because the fruit is in contact with hot water directly, and the heat capacity of water is much higher than air, so the temperature of the outer tissue increased rapidly after dipping. If the temperature of the flavedo increases by 8-10°C this may induce HSPs (Lurie, 1998; Bowen *et al.,* 2002).

The temperature of the fruit stored under IW condition can be gradually adapted to the storage environment during warming for a period of time. In this work the range of selected IW regimes provided about a 10°C change (e.g. 5°C ↔ 15°C or 7°C ↔ 20°C IW treatments) so it is also possible that the beneficial effects relate to the induction of HSPs.

The induction of HSP synthesis can occur when plants experience either abrupt or gradual increase in temperature (Vierling, 1991; Wahid *et al.,* 2007). HSPs are synthesised at higher temperatures in soybean seedlings (*Glycine max* L. Merrill, cultivar Tracy) exposed to a rapid heat shock or gradual temperature increase (3°C per hour). However the seedlings showed differences in the pattern of synthesis of different HSPs depending on whether there was rapid or gradual temperature exposure. The tissue was able to survive and keep synthesising proteins at higher temperatures after a gradual temperature increase than if it had been moved rapidly to these temperatures. Three HSP (80, 75 and 16 kD) were found at higher temperatures after exposure to a gradual temperature increase as compared to a rapid heat shock. The synthesis of three non-HSP of 44, 42 and 38 kD was also sustained longer at higher temperatures after a gradual increase in temperature than after a rapid heat shock. In general, DNA transcription was sustained at

temperatures 6 to 9°C higher after a gradual temperature increase than after a rapid heat shock (Altschuler and Mascarenhas, 1985). A gradual increase in temperature induces thermotolerance and this effect is beneficial under natural environments (Vierling, 1991; Wahid *et al.,* 2007). Thermotolerance is potentially a vital strategy achieved by reprogramming gene expression, allowing plants to cope with heat stress (Wahid *et al.,* 2007).

Ferguson *et al*., (1998) and Woolf and Ferguson, (2000) proposed that the development of thermotolerance in fruit subjected to both low and high temperature postharvest treatments (which reduce CI and heat injury) is affected by prior exposure to temperature fluctuations during natural daily temperature cycles. Fruit exposed to direct sunlight can reach daily flesh temperatures above 40°C even when the ambient temperature never exceeds 30°C. These high temperatures can result in differences in internal quality properties such as sugar content, firmness, oil levels and differences in mineral content. Fruit which have experienced different temperature histories will respond differently to postharvest low temperature storage or to heat treatments used for insect disinfestation.

Although IW has been shown to be effective in reducing CI of some fruits and vegetables, the mechanism of this effect is not well understood (Wade, 1979). Heat treatments applied to the fruit after harvest or responses to natural heating (sunlight) of the fruit occurring during the preharvest influence the induction of thermotolerance in the fruit and may lead to consequential changes in the longevity of the fruit after harvest. I may hypothesise that IW treatments (in which the fruit are cooled and warmed periodically) may have the same protective effect as seen in fruit responding to sunlight in the field, if the temperature during any warming period is high enough to induce HSPs. However, other explanations about the effects of IW have been reviewed. IW may increase synthesis of unsaturated fatty acids during a rapid readjustment of metabolism and this may increase resistance to CI (Wang and Baker, 1979; Wang, 1982). Wang and Baker, (1979) found that IW increased the proportion of unsaturated polar lipids in cucumbers and sweet peppers and reduced the deterioration of these products at low temperatures. Wade, (1979) and Wang, (1990) reported that the warming period may also allow the tissues to metabolize toxic substances accumulated during the cold cycle or warming may allow tissues to restore any substances that were depleted or were not able to be

synthesized during the cold cycle. It is postulated that chilling-induced damage to tissues may also be repaired during the warming cycle of IW.

In summary, based on the previous reviews, the IW technique may protect the fruit against disorders and allow the tissue of fruit to repair chilling-induced damage to fruit membranes or metabolic pathways and may also induce HSPs during implementation. From this work, I found that the 1 and 2 cycles IW were sufficient to reduce CI. If a single warming induces HSPs, then I would expect it to be as effective as a brief HWD, as from the published evidence, HWD induce HSPs which also contribute to thermotolerance in plants. HWD has been reported to reduce yellowing in broccoli (Forney, 1995; Tian *et al.,* 1996; Tian *et al.,* 1997). However, the authors did not test whether the mechanism of protection relied on HSP induction. In terms of the protection of CI by HWD in produce such as grapefruit (Porat *et al.,* 2005), avocado (Woolf, 1997; Woolf and Lay-Yee, 1997) and tomatoes (Ilic and Fallik, 2005), the authors agreed that the induction of HSPs after hot water treatment induced protection against CI. However, in contrast with their results, I found HWD was ineffective at protecting limes from CI during storage.

In this work I found that IW treatments (especially the 2 and 6 cycles) effectively delayed degreening and also reduced CI in limes. It is possible that both HWD and IW induce HSPs which may be associated with slowed yellowing in the produce. However, in the case of CI protection, HWD was ineffective for limes whereas IW was effective, provided at least 2 cycles of IW were applied. Therefore in limes, a correlation between HSP induction and delayed degreening may be valid but the benefit for CI seems not to depend on HSPs because HWD did not protect against CI and IW required 2 cycles to protect against CI. This suggests that protection against CI in limes requires repair processes rather than induction of HSPs.

Overall IW was better for maintaining lime quality than HWD or combination of HWD and IW. The combination of these two techniques was not more effective for storage of lime in this work possibly because the only time this was tested was in H3 fruit (Fig. 3.1) which stored particularly well. It is possible that the application of HWD at an optimal temperature, followed by an optimal IW treatment, could lead to further extension of storage life. The IW also showed better results than the step down technique that was

tested. However, the step down technique is very simple to apply and showed some promising results, offering protection against some disorders and rots. By careful control of the temperature and duration of TC, it may be possible to identify a TC regime that can deliver acceptable fruit after storage for 12 weeks. If so, TC may then prove more practical for lime storage at a commercial scale than IW.

CHAPTER 6

Kinetics and mechanism of colour change

6.1 Introduction

Colour is a key quality factor governing consumers' purchasing decisions of limes. The degreening of limes is due to chlorophyll loss and a similar process occurs in other products such as bananas, gold kiwifruit, and tomatoes. In some cases, additional pigments are formed (e.g. lycopene in tomatoes) while in others, the degreening process is largely an 'unmasking' of pigments that are already present.

Degreening is typically a triphasic process: there is an initial slow phase of colour change (as typically quantified by the hue angle), a second fast stage, then a final stage of slow colour change as the final hue is approached. This results in a sigmoidal curve and the complete colour change process can often be modelled using sigmoidal functions such as the logistic equation (Thorne and Segurajauregui Alvarez, 1982; Tijskens and Evelo, 1994; Hertog *et al.,* 2004). For later harvested fruit, the profile of colour change commonly only involves the latter two stages and this can be modelled by an exponential decay model, as described for gold kiwifruit (Schotsmans *et al.,* 2008) and tomatoes (Hertog *et al.,* 2004).

These modelling approaches may also be applicable to other colour parameters, such as colour scores (CS) or one or more of the L*, a*, and b* components. The influence of temperature on the rate of colour change is of particular importance in postharvest horticulture and is frequently quantified using the Arrhenius equation (Tijskens and Evelo, 1994; Ávila and Silva, 1999; Chen and Ramaswamy, 2002; Hertog *et al.,* 2004; Chutintrasri and Noomhorm, 2007; Cruz *et al.,* 2007). The benefit of describing colour change mathematically is that the progress of degreening and the final fruit colour can then be predicted for a known temperature profile, providing a tool for quality management across the supply chain.

Individual fruit will have slightly different characteristic curves for colour change. This biological variability has been investigated by Hertog *et al*., (2004) and others. It has been shown that often the concept of “biological age” can be applied to better understand the causes and extent of variation between fruit samples. For example, by adjusting the time value in the logistic equation, curves for individual fruit (or fruit batches) can often be overlaid to give a single generic curve that reasonably describes the colour change for all fruit (Tijskens and Evelo, 1994; Hertog *et al.,* 2004).

Useful information on the changes that occur in particular pigments may also be obtained from spectral reflectance data measured using a spectrophotometer (Merzlyak *et al.,* 2002; Merzlyak *et al.,* 2003). Such approaches are used in applications ranging from describing colour changes in individual fruit to remote sensing.

In this chapter the lime degreening process reported in Chapters 4 and 5 was further analysed. The objectives were:

1. To characterise the kinetics of change of hue for limes stored under RA at different temperatures.
2. To determine the goodness of fit of the applied models and the possibility of describing the influence of temperature by the Arrhenius equation. If so, these models could then be applied to gain further insight into the efficacy of different postharvest treatments.
3. To determine if the concept of biological age could be applied to different stages of ripening as indicated by the change in hue of the most yellow and most green sides of the fruit.
4. To characterise the changes in chlorophyll concentration and investigate their relationship to changes in hue and spectral reflectance.

6.2 Methodology: Use of the logistic equation to describe degreening

The colour of lime fruit in this work was represented by the hue angle (H°, degree) and colour score (CS). In the first instance the logistic equation (Thorne and Segurajauregui Alvarez, 1982; Tijskens and Evelo, 1994; Hertog *et al.,* 2004) shown in Eq. 6.1 was used to describe degreening of limes using the hue:

$$H(t) = H_{+\infty} + \frac{H_{-\infty} - H_{+\infty}}{1 + e^{kt(H_{-\infty} - H_{+\infty})} \dfrac{H_{-\infty} - H_0}{H_0 - H_{+\infty}}}$$ **Eq. 6.1**

Eq. 6.1 describes hue at a given time (*t,* in days) as a function of $H{+\infty}$ and $H{-\infty}$, the minimum and maximum hue (at plus and minus infinite time), respectively. H_0 is the initial hue value and k is the temperature-dependent rate constant (with the unit of time^{-1}).

The temperature-dependence of k was described by the Arrhenius equation in Eq. 6.2.

$$k = k_0 e^{\frac{Ea}{RT}}$$ **Eq. 6.2**

where k_0 is preexponential Arrhenius constant (time^{-1}), E_a is the activation energy (J mol^{-1}), R is the universal gas constant = 8.314 J mol^{-1} K^{-1}, T is temperature (absolute, in K). The activation energy can be obtained from the slope of the linear portion of a plot of *ln k* vs *1/T*.

In this work, models were fitted by using the Solver™ function within Microsoft® Excel to minimise the sum of residual squares, i.e. min $\Sigma(H°_{experimental} - H°_{predicted})^2$.

6.3 Variability in colour change profiles: examination of the role of biological age

6.3.1 Individual vs. mean hue data

In most cases, the mean hue angle for batches of fruit was analysed. However it is also important to understand the variability within a population. This is illustrated by hue data for 24 individual fruit from H5 as shown in Fig 6.1A.

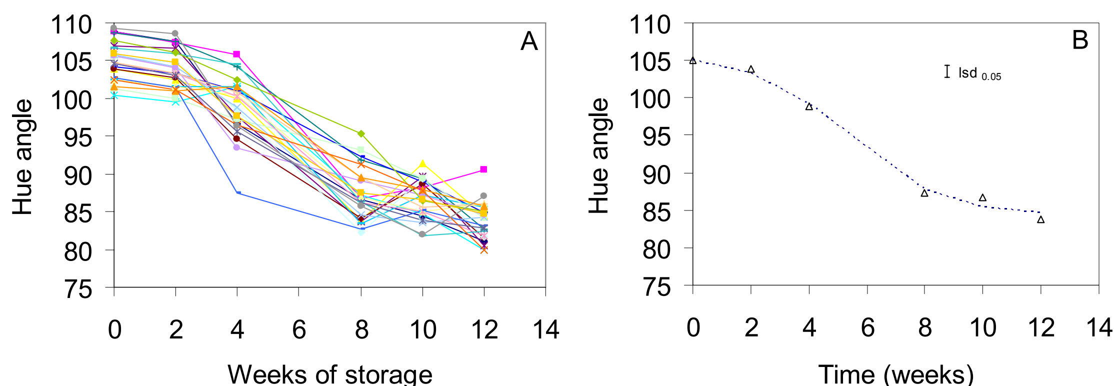


Figure 6.1 H° on the green side of 24 lime fruit from H5 stored under RA at 13°C: Individual hue profiles for 24 lime fruit (A) and the mean (Δ) and predicted (dashed lines) hue of this sample fitted with the logistic model, Eq. 6.1, ($LSD_{0.05}$ = 1.5738, n = 24) (B).

The change of H° showed the expected pattern of an initial slow phase for the first 2-3 weeks of storage, followed by a phase of more rapid change until about 8 weeks; the change in hue was slow again thereafter until the end of storage at 12 weeks (Fig. 6.1A, B).

The mean hue of these 24 fruit were calculated and then fitted with the logistic model. The agreement between the mean experimental values and fitted data in Fig. 6.1B confirms that the logistic model is a reasonable model for describing changes of lime colour. However although the fruit all followed the same overall pattern with regard to hue there were some significant variations between them. For example it might be expected that the initially most green fruit would remain the most green throughout the storage period. However, careful study shows that this was not the case. Some of those fruit that were initially most green were the most yellow at the end of storage and *vice versa*. This was illustrated by ranking the fruit at t=0 (1=most green, 24 = least green) and comparing the initial ranking with that of the same fruit at week 12 (Fig. 6.2 and appendix I, Table A1). There was no clear relationship between the initial and final rankings.

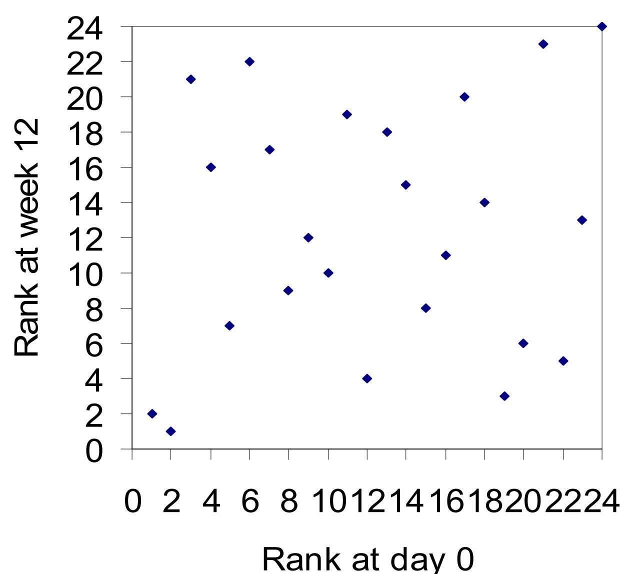


Figure 6.2 Ranking of H° of the 24 green fruit at initial day 0 compared with the final H° of the same fruit at 12 weeks of storage.

From Fig. 6.1A it is also observed that sometimes an increase in hue was measured at later time measurements. This probably reflects the practical difficulty of measuring in exactly the same location on the skin surface. However considering both figures above, it is clear that differences between curves can arise due to differences in initial hue, in the point at which the colour change moves from the initial slow stage to the fast stage (i.e. the fruit's 'biological age'), and in the rate (i.e. the *k* value) at which the process proceeds. The *k* values calculated for the individual hue profiles of this fruit sample (reported in Table A.1; see appendix I) show several orders of magnitude variation between the slowest and highest rates of colour change but the average of these is reasonably close to the rate constant fitted to the mean data in Fig. 6.1B (0.05 and 0.03 d^{-1}, respectively). Some very high and low *k* values were obtained as these values were particularly influenced by the nature of the colour change occurring between 4 and 10 weeks. If data had been collected more frequently during this time then lower variance in the individual *k* values could be expected.

The influence of both the *k* value and the time at which the rapid phase of colour change commences are illustrated in Fig. 6.3 by comparing actual hue profiles for four fruit with the mean value model from Fig.6.1B. In Fig 6.3 the background (grey) lines show the

logistic model fitted to the mean data plotted with different adjusted times (*tadj*) added to shift the curves left or right to provide a range of initial hue values at t = 0. This represents the application of the concept of "biological age" (Hertog *et al.,* 2004). The four lime fruit hue profiles were selected from those 24 fruit as examples of the change in H° of two initially high hue (dark green) fruit and two initially low hue (less green) fruit compared with the average of the 24 fruit.

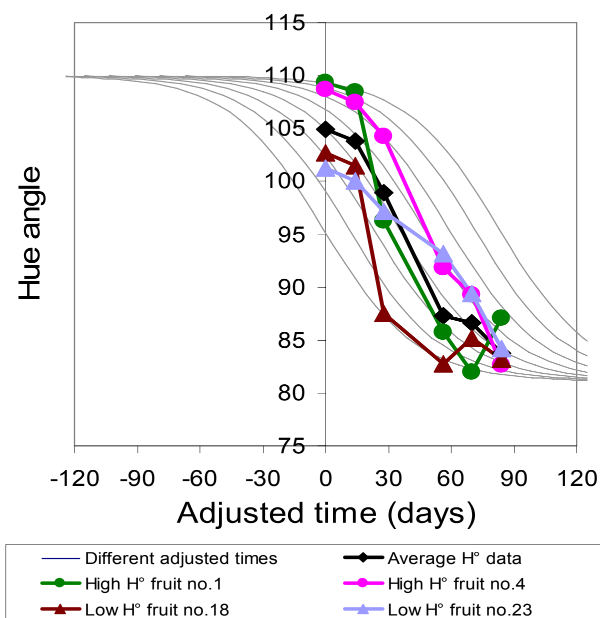


Figure 6.3 Comparisons between the high (●) and low (▲) hue angle fruit sets with the average (♦) hue data of 24 fruit.

If the fruit only differed in 'biological age', then fruit with different initial hues would be expected to reasonably closely follow the respective background line to which they are initially closest (as is the case for the averaged data set; black line). The fact that the individual fruit lines move markedly relative to these background lines suggests that a fruit's *k* value is also affected by fruit maturity as well as storage temperature. The impact of variability in *k* on the colour profile must therefore also be factored into models of colour change when it is important to describe the behaviour of individual fruit.

6.3.2 Further consideration of mean hue data

In the analysis above individual fruit were shown to exhibit a continuous change in hue that follows a logistic curve. If the more yellow side of the fruit is considered to be tissue that is simply further along the degreening process than the greener parts of the fruit, an

additional means of investigating the biological variation and also the influence of temperature on the colour change is to examine if the green side behaves in the same fashion as the yellow side, but simply later in time. The mean hue values of fruit batches sampled at different times and temperatures were calculated and modelled using the logistic equation as described above. Examples of the analysis are shown in Fig. 6.4 and 6.5.

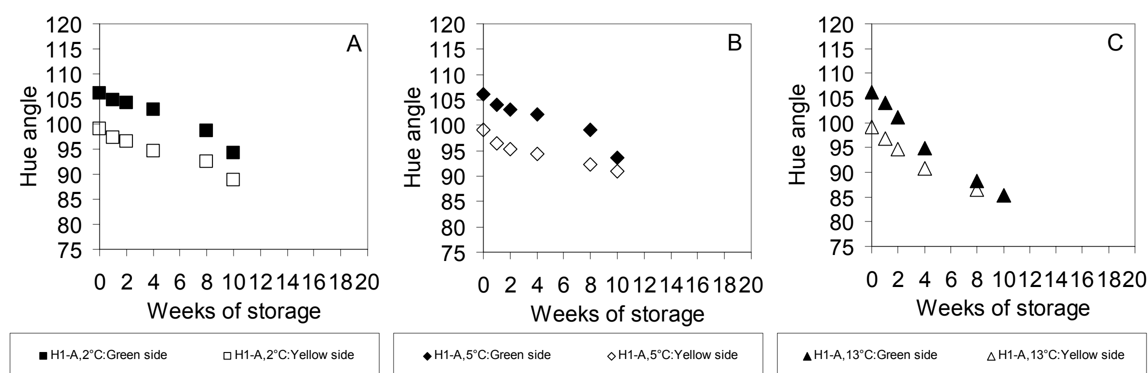


Figure 6.4 H° of lime stored under RA at 2 (A), 5 (B) and 13°C (C) on the green (closed symbol) and yellow side (open symbol), H1. (average of 18 fruit, independent sampling at 8 and 10 weeks). The data were converted from CR-200 data to spectrophotometer data by the formula Eq. 3.1: y = 0.9136x, (y= $H°_S$ and x= $H°_C$).

Obviously the H° on the green (closed symbol) side of limes stored under RA at 2°C (Fig. 6.4A), 5°C (Fig.6.4B) and 13°C (Fig. 6.4C) was higher than on the yellow side (open symbol) throughout 12 weeks of storage. By adding an adjustment time to the yellow side data, it is possible to overlay the green and yellow hue profiles. The Solver™ function within Microsoft® Excel was used to calculate the best fit time adjustment for each of the yellow side data in order to overlay these with the green side data.

With time adjustment, the green and yellow data sets matched closely as shown in Fig. 6.5 and the combined data sets were quite well described by a single logistic equation. This appears to confirm that the most yellow part of the fruit, which commenced degreening prior to harvest, continued to change colour at a consistent temperature-dependent rate thereafter.

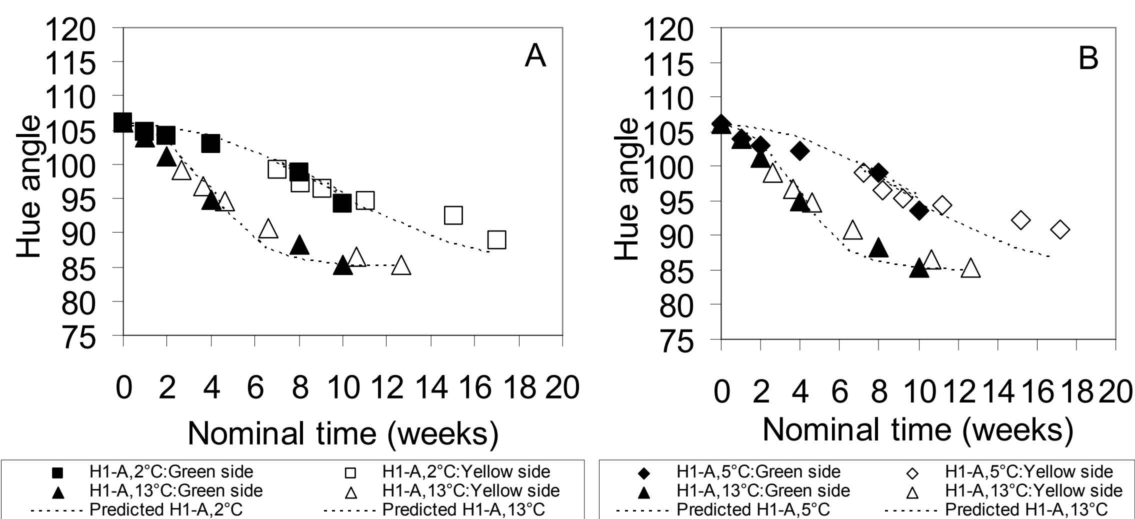


Figure 6.5 H° of combined data sets of limes stored under RA at 2 and 13°C (A) and RA at 5°C and 13°C (B) on the green (closed symbol) and yellow side (open symbol) , H1. Comparisons between yellow sides of H1 limes stored under RA at 2 (□) and 13°C (Δ) (A) and yellow sides of limes stored under RA at 5°C (◊) and 13°C (Δ) (B) were overlaid on each of their green sides of lime stored under 2 (■), 5 (♦) and 13°C (▲), respectively. Predicted H° (dashed lines) for limes stored at 2, 5 and 13°C are also presented.

A single logistic equation was fitted to each of the combined data sets of H1 limes on the green and yellow sides stored at 2, 5 and 13°C, respectively. The coefficients (*k* value) of each combined data set are shown in Table 6.1.

Table 6.1 The coefficients (*k* value) of the combined data sets of H1 limes stored at 2, 5 and 13°C, respectively

Combined data sets of stored limes	*k* value (d^{-1})
at 2°C	0.0021
at 5°C	0.0021
at 13°C	0.0050

The same approach was then applied for H3 limes stored under RA at 5 and 15°C (Fig. 6.6). In this case the H° data were measured by the spectrophotometer.

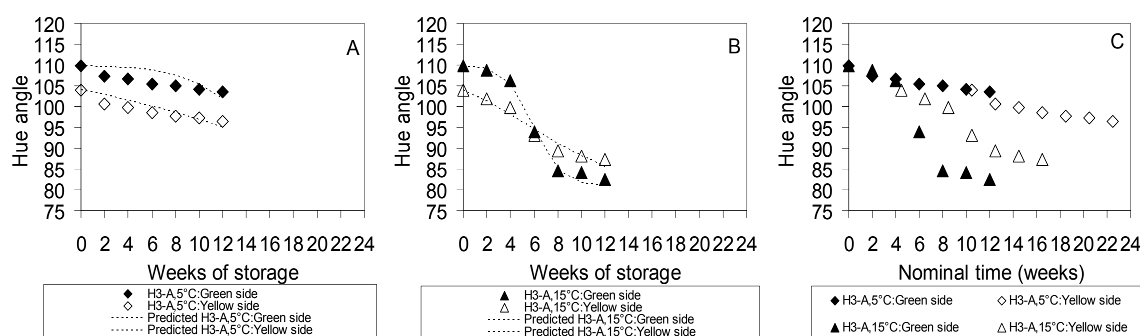


Figure 6.6 H° and predicted H° (dashed lines) of lime stored under RA at 5 (A) and 15°C (B) on the green (closed symbol) and yellow side (open symbol), H3. (average of 60 fruit at 0 day, 80 fruit at 2-4 weeks, 60 fruit at 6-8 weeks and 40 fruit at 10-12 weeks). The data at 0 day was converted from CR-200 data to spectrophotometer data by the formula: y = 0.9136x, (y= $H°_S$ and x= $H°_C$). Comparisons between yellow sides of limes stored under 5 (◊) and 15°C (Δ) were overlaid on each of their green sides of lime stored under 5 (♦) and 15°C (▲) (C).

When a logistic model was fitted separately to the green and yellow hue data a fairly good fit was obtained for H° of the fruit stored at 5 and 15°C, respectively (Fig. 6.6A, B). However, in this case, although there was reasonable coherency between the green and yellow hue data for 5°C, the curves for degreening of the yellow and green sides at 15°C were quite different and could not be fitted by a single logistic model when the adjustment function was applied (Fig. 6.6B). The shape of the curves suggests that chlorophyll (and possibly other pigments) on the green side was degraded faster than on the yellow side. The average initial colour score of the H3 limes was 25% yellow which means that the fruit were quite green at the beginning of storage; in contrast the fruit in H1 were initially 40% yellow. This presumably implies a difference in fruit maturity that might have contributed to this effect.

To further understand these differences between batches the modelling exercise was repeated again for H5 fruit stored under RA at 5 and 13°C.

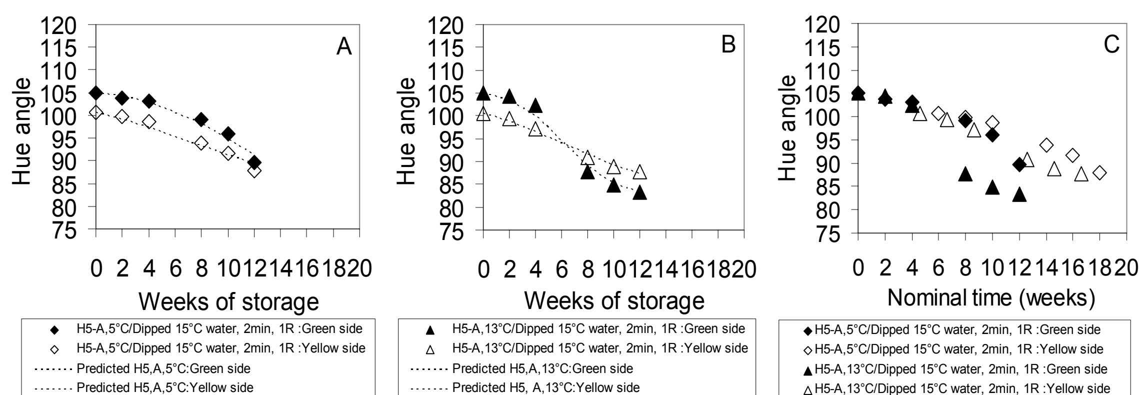


Figure 6.7 H° and predicted H° (dashed lines) of pre-treated limes (dipped in 15°C water, 2 min) then stored under RA at 5 (A) and 13°C (B) on the green (closed symbol) and yellow side (open symbol), H5. (average of 24 fruit). Comparisons between yellow sides of limes stored under 5 (◊) and 13°C (Δ) were overlaid on each of their green sides of lime stored under 5 (♦) and 13°C (▲) (C).

The logistic model was again fitted to the mean green and yellow H° data. Similar results to H3 fruit were observed as the predicted data of each individual H° data set agreed with the experimental data sets (Fig. 6.7A, B). These profiles showed similar trends to the H3 fruit stored under RA at 5 and 15°C, as shown in Fig. 6.6 A, B and C. However the H5 fruit stored under RA at 5°C showed CI symptoms at 8 weeks of storage (Fig. 5.32) whereas the H3 fruit stored under RA at 5°C showed only pitting symptoms (Fig. 5.31). This CI may have influenced the pattern of degreening in the H5 limes as the hue on the green side decreased rapidly after 8 weeks of storage (Fig. 6.7A). In contrast the H3 fruit exhibited a stable and slower pattern of hue loss until 12 weeks (Fig. 6.6A).

After applying a time adjustment and overlaying the yellow and green sides of H5 lime, there was poor matching of the two H5 curves (Fig. 6.7C), similar to the behaviour observed for H3 (Fig. 6.6C). Thus it appears the concept of 'biological age' can not be applied in a simple manner to these fruit. It appears the degreening process is regulated in different ways between the green and yellow sides, possibly as a result of production practices or environmental effects (e.g. variations in temperature or sunlight exposure whilst the fruit is on the tree) which may have contributed to this difference. For example, perhaps the yellow side of the fruit may have some protective processes delaying colour loss on the tree, which may be less marked in the fully green side. This was further explored through analysis of the spectral data for these fruit and is discussed in section 6.6 below.

6.4 Temperature kinetics of degreening

6.4.1 Hue angle

To investigate the influence of temperature on the degreening process the mean hue values of fruit batches stored at different constant temperatures and sampled at different times were calculated and modelled using the logistic equation as described above. The logistic model fitted quite well the mean hue data for the green sides of H1 limes stored under RA at 2 and 13°C (Fig. 6.8A) or 5 and 13°C (Fig. 6.8B), and for H5 limes stored under RA at 5 and 13°C (Fig. 6.8C) until the end of their respective storage periods.

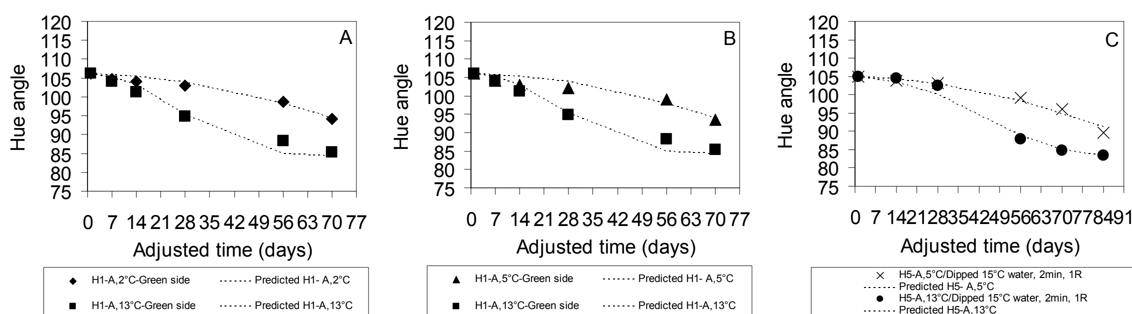


Figure 6.8 H° on the green side of H1 (A & B) or H5 (C) lime and predicted H° (dashed lines) of H1 lime stored under RA at 2 (♦) and 13°C (■) (A) or 5 (▲) and 13°C (■) (B) and H5 limes stored under RA at 5 (×) and 13°C (●) (C), fitted with the logistic model, Eq. 6.1.

Similar levels of agreement between the modelled and experimental data were obtained for all other RA treatments and the *k* values of each fitting of the individual green-side mean H° data sets of fruit stored under a range of temperatures from 2 to 20°C from H1 to H5 was used to obtain Arrhenius plots of each run.

Fig. 6.9 presents Arrhenius plots for three individual harvests (H1, H2 and H3) and the combined data sets with and without the data at 20°C are shown in Fig. 6.10A and B, respectively.

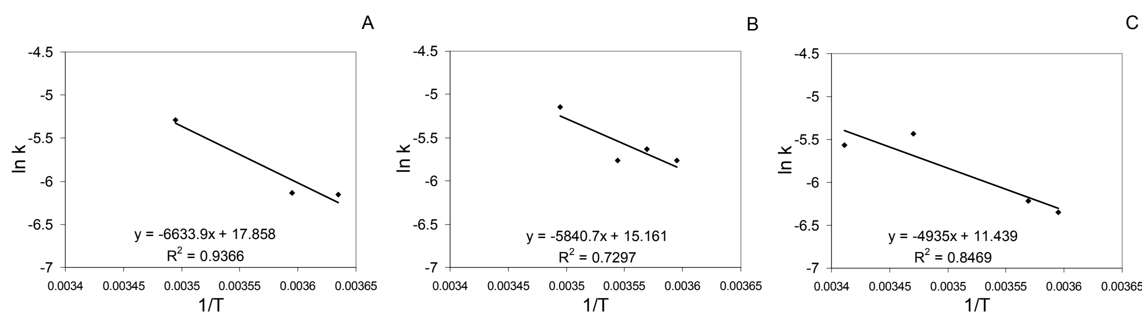


Figure 6.9 Arrhenius plots of H° of lime (♦) for three individual harvests stored at different storage temperatures; H1 at 2, 5 and 13°C (A), H2 at 5, 7, 9 and 13°C (B) and H3 at 5, 7, 15 and 20°C (C).

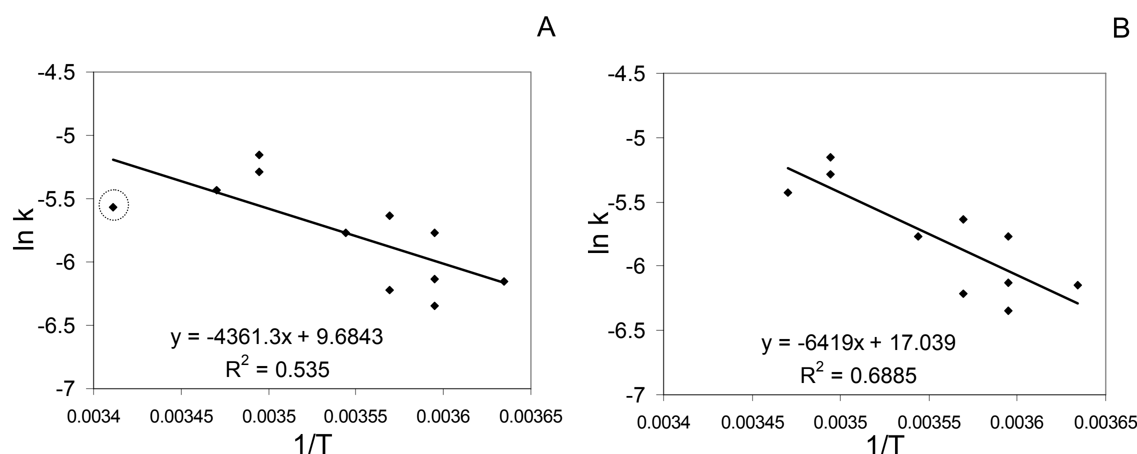


Figure 6.10 Arrhenius plots of H° of lime (♦) for the combined data sets stored at different storage temperatures from 2, 5, 7, 9, 13, 15 and 20°C (note data measured at 20°C is illustrated in a dotted circle) (A) and without 20°C (B).

The estimated activation energies (E_a) for H1, H2 and H3 were 55, 49 and 41 kJ mol^{-1}, respectively (Fig. 6.9A, B and C). The calculated E_a of the combined 3 harvests with the data of H3 at 20°C included was 36 kJ mol^{-1}; if the H° data at 20°C for H3 were excluded, the E_a was 53 kJ mol^{-1} (Fig. 6.10A, B). The reason the latter scenario was examined was because the *k* value obtained for H3 at 20°C was very low. As this was the only data set at this temperature, caution must be exercised. It is well known that warmer temperatures reduce the rate and extent of degreening. In tropical regions where the average temperatures remains high all year, chlorophyll levels remain high for oranges and mandarins and the fruit peel stays green. But when temperatures of air and soil decrease below 15°C, chlorophyll is degraded and chloroplasts are converted to

chromoplasts containing yellow, orange or red pigments. At temperatures above 35°C or below 15°C, carotenoid synthesis is reduced (Erickson, 1967; Davies and Albrigo, 1994).

Overall the E_a values estimated for lime degreening fall within the typical range of E_a of enzymatic reaction in other food systems of ~40-130 kJ mol^{-1} (Robertson, 1993) and lie between values reported for other fruit degreening systems: Hertog *et al*., (2004) estimated E_a for hue change to be 138, 169 and 170 kJ mol^{-1} in three tomato cultivars (‘Style’, ‘Tradiro’ and ‘Quest’), respectively, stored for 3 weeks at 12, 15 and 18°C while Chen and Ramaswamy, (2002) estimated E_a for colour change (based on measurement of L, a and b) of banana stored at 10, 16, 22 and 28°C for 18 days to be 36, 29 and 21 kJ mol^{-1}, respectively.

From Fig. 6.9 and 6.10, it is also obvious that there was considerable spread of the data (especially considering that the y axis has a logarithmic scale). This may possibly be due to different climate and production practices between the seasons or harvests. The Arrhenius model provided a reasonable fit to the data in each case, explaining ~54-94% of the variation in different experiments, but the modelling process was hindered by the limited range of temperatures evaluated in each run. As noted, the 20°C data for H3 had a marked influence on the estimated E_a (Fig. 6.10A, B). An unpublished study at Massey University by Jenkins (2007) on lime degreening across a wider range of temperatures was initiated to provide further information on lime degreening. This suggested that a square root model (following Mawson (2006)) would provide a better fit to the degreening rate and that at 20°C significant deviation from the Arrhenius relationship can be expected. This was also the conclusion of Mawson, (2006) for the contribution of chlorophyll loss to colour change in tomatoes.

In summary the modified square root model may be more appropriate than Arrhenius to describe temperature dependence of lime colour change for both high (≥ 20°C) and low temperature storage. For temperatures below 20°C, the value of E_a estimated in Fig. 6.10B of ~53 kJ mol^{-1} appears reasonable if the Arrhenius model is applied.

6.4.2 Colour score

Colorimeters and spectrophotometers are relatively expensive machines and are not always convenient to use in an orchard, where no experienced staff may be available to conduct measurements. In such circumstances a grading chart is commonly used to assess colour quality. Many fresh products are assigned their quality score for a range of attributes by using grading charts because these are cheap, robust and suitable for use throughout both local or global supply chains. They are (commonly) non-destructive and require no calibration. They are also not sensitive to differences in instrumentation systems (such as between different models, age, service histories, etc) as can occur with objective quality measurements. On the other hand, grading charts are subjective and generally lack precision (Pranamornkith *et al.,* 2006). To further understand the relationship between the CS and hue data, the temperature kinetics of degreening as estimated by the CS based on a grading chart were compared to those based on spectrophotometric measurement of hue.

Fruit were randomly placed in treatment groups and each fruit in a group was assigned a score of 0, 25, 50, 75 or 100% yellow according to a pre-prepared grading chart (section 3.4.5). Fruit were stored under various temperature and atmosphere regimes (Fig. 3.1) and were regularly assessed for colour score. An average score for each treatment sample was calculated at each sample time and the modified logistic equation (Eq. 6.3) was fitted to these data.

$$CS(t_{ref}) = \frac{CS\max}{1+e^{(\beta - kt_{ref})}} \qquad \textbf{Eq. 6.3}$$

wherein Eq. 6.3 CS_{max} is the maximum colour score (in %) and refers to the CS at infinite time (in our case = 100, assuming fruit would finally be completely yellow). The value of β at a constant temperature is a function of the maximum and initial colour change as given by Eq. 6.4 and k is the temperature-dependent rate constant (with the unit of time^{-1}); t is the time (d).

$$\beta = \ln\left(\frac{CS\max}{CS(t_0)} - 1\right) \qquad \textbf{Eq. 6.4}$$

Examples of the model fitting for limes stored under RA at 2, 5, 7, 9 13, 15 and 20°C are shown in Fig. 6.11.

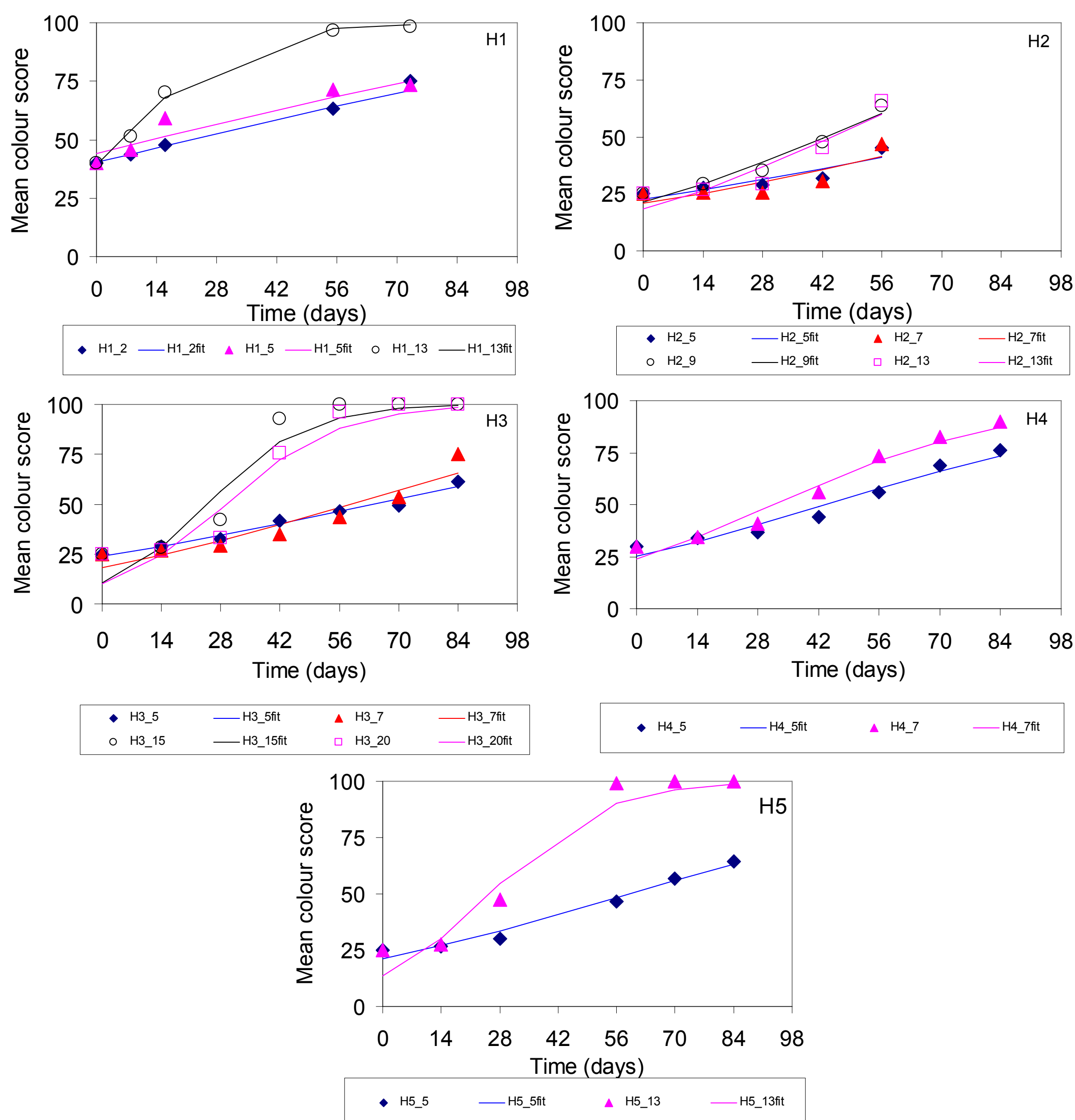


Figure 6.11 Mean CS (symbols) and predicted CS (solid line) of H1-H5 lime fruit stored under RA at different storage temperatures from 2 to 20°C, fitted with the logistic model, Eq. 6.3.

Overall there was a good fit between the model of the CS data of H1 to H5 fruit and the observed data points. To provide a comparison with the lime fruit this alternative logistic model was also used to fit the digitised data for colour development of dark lemons stored at different temperatures at 2, 5, 8 and 14°C (Cohen and Schiffmann-Nadel, 1978). The original data were digitised using the program Techdig (version 1.1b) and the lemons were divided into groups according to their colour at picking-time: dark green = grade 1; light green = grade 2; yellow-green (silver) = grade 3; yellow = grade 4; ripe, beginning to bronze = grade 5. These data were also described well by the logistic equation (Fig 6.12).

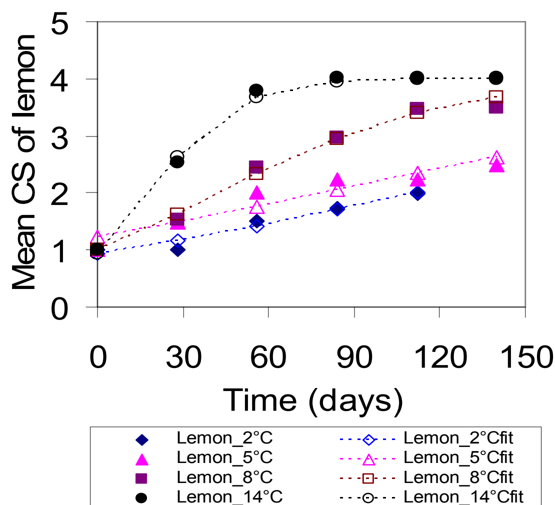


Figure 6.12 Digitised data of colour development of dark lemons stored at different temperatures at 2, 5, 8 and 14°C in Fig. 1 of Cohen and Schiffmann-Nadel (1978) by Techdig software and the data were fitted by the logistic model Eq. 6.1.

Fig. 6.13 presents the Arrhenius plots for rate of change in lime CS from all harvests (H1 - H5) in my work using *k* values estimated for mean CS values (as per Fig. 6.9) compared to the Arrhenius plot of the colour development of dark lemons stored at 2, 5, 8 and 14°C (Cohen and Schiffmann-Nadel, 1978). Although there is some scatter in the data, as could be expected, it is clear that the two processes are similar and show a broadly similar dependence on temperature.

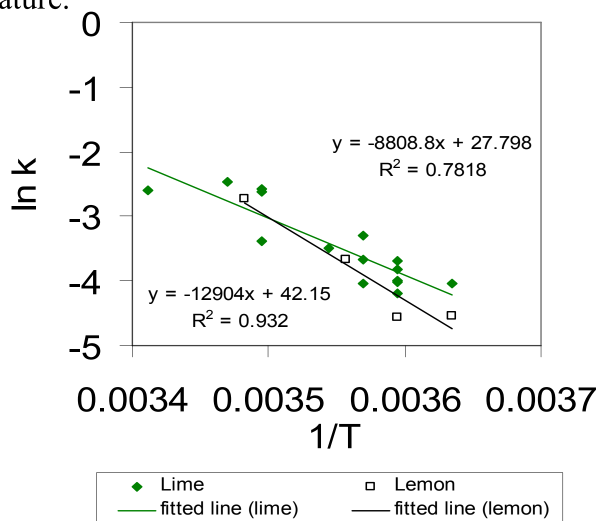


Figure 6.13 Arrhenius plots of CS of lime (♦) for five harvests stored at different storage temperatures; H1 at 2, 5 and 13°C, H2 at 5, 7, 9 and 13°C, H3 at 5, 7, 15 and 20°C, H4 at 5 and 7°C and H5 at 5 and 13°C compared to Arrhenius plot of digitised data of colour development of dark lemons (□) stored at different temperatures at 2, 5, 8 and 14°C in Fig. 1 of Cohen and Schiffmann-Nadel (1978).

The *k* values for the change in both CS of limes and the colour development of lemons were used to estimate E_a values for each case (Fig. 6.13). The values calculated for E_a of H° and CS models were of similar magnitude at 53 and 86 kJ mol^{-1}, respectively (note that both the E_a for change in H° and CS were calculated by excluding the H° and CS data at 20°C based on arguments given above) (Fig. 6.9-6.10 and 6.13). The estimated *k* values for lime and lemon colour changes were similar at 86 and 107 kJ mol^{-1}, respectively (when the CS data of both fruits were scored at storage temperatures between about 2 to 15°C). The higher E_a value for this parameter is probably due to the use of categorical data in estimating the mean CS. Thus fruit that was 90% yellow would be scored as 100% yellow by CS but would be part of a continuous scale when measured by the colorimeter or spectrophotometer. As these instruments are more sensitive and better able to differentiate differences in colour, so the colour measurement and derived data are expected to be more accurate. However, the broad agreement obtained in these analyses suggests it may be possible to quantitatively interpret values of colour obtained from simple grading chart data.

As noted, the E_a of CS of lime (86 kJ mol^{-1}) was slightly lower than the E_a obtained from the digitised data for colour development of dark lemons (107 kJ mol^{-1}). This suggests colour development of lemon may be slightly more responsive to temperature than lime, and may reflect that these lemons were grown in what is presumed to be a warmer and more sunny environment (Israel) than that in NZ.

6.5 Applying degreening models in the prediction of colour change by IW

Since under IW fruit spend some time at both cold and warm temperatures, a key advantage of having a model for temperature dependence of colour change is that I can compare the actual change in hue angle or colour score with that predicted for fruit at the appropriate combination of temperatures. This provides a means of quantifying the effectiveness of IW on delaying degreening of lime and may help to identify if the fruit physiology is altered from that at constant temperature storage.

Using the fitted *k* values from experiment 3 (H3), a comparison was made between the expected and actual colour changes for limes stored under different RA and IW conditions. Both predicted H° and CS vs time are compared with actual H° and CS in Fig. 6.14 and 6.15, respectively.

6.5.1 H° and Time

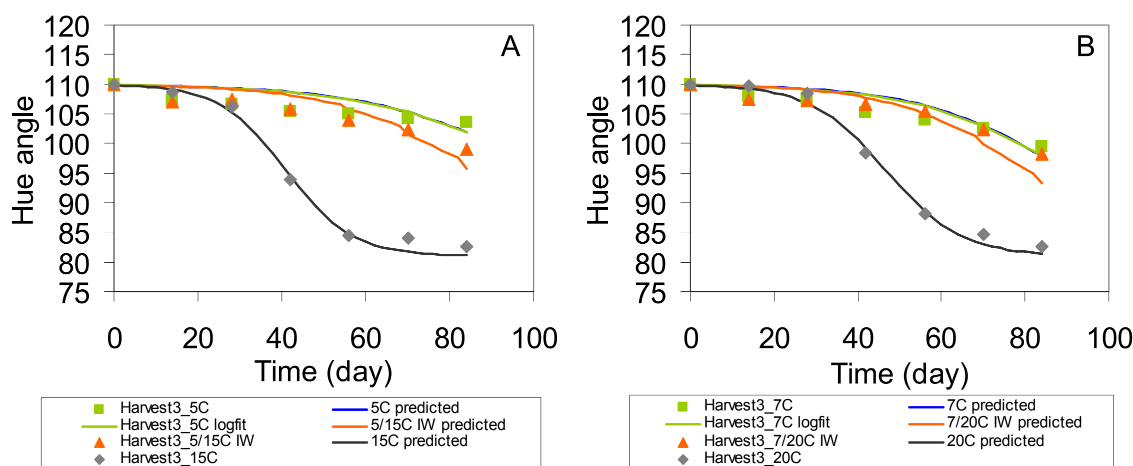


Figure 6.14 Comparison of H° of stored lime experimental & calculated under RA at 5 and 15°C with stored lime under IW at 5°C for 12 days and 15°C for 2 days (A) and H° of stored lime experimental & calculated under RA at 7 and 20°C with stored lime under IW at 7°C for 12 days and 20°C for 2 days (B) (H3).

Predicted H° data from the model for H3 fruit stored under RA at constant temperatures 5, 15, 7 or 20°C were similar to the measured data. For both IW treatments (5°C, 12 days and 15°C, 2 days, Fig. 6.14A; and 7°C, 12 days and 20°C, 2 days, Fig. 6.14B) the model predicted a slightly more accelerated degreening than measured. In particular, for IW at 7-20°C the fruit retained their green colour at the end of storage better than predicted. Unfortunately as no further data was available, apart from this small beneficial effect it can only be concluded that IW data generally follow the trend that is predicted by the model. This suggests that the response of fruit to IW is just that expected with regard to the influence of temperature on colour. The beneficial effect of IW on CI is however much more marked as was noted in the previous chapter.

6.5.2 CS and Time

When the mathematical model was used for predicting the expected CS a poor fit was obtained (Fig. 6.15A, B). In this case the fruit colour changed more slowly than expected in the early stages of storage and more rapidly than expected in the latter stages. At the higher temperatures the CS was not consistently well predicted throughout the storage period (particularly up to 40 d) and this no doubt has influenced the poor fit of the variable-temperature model.

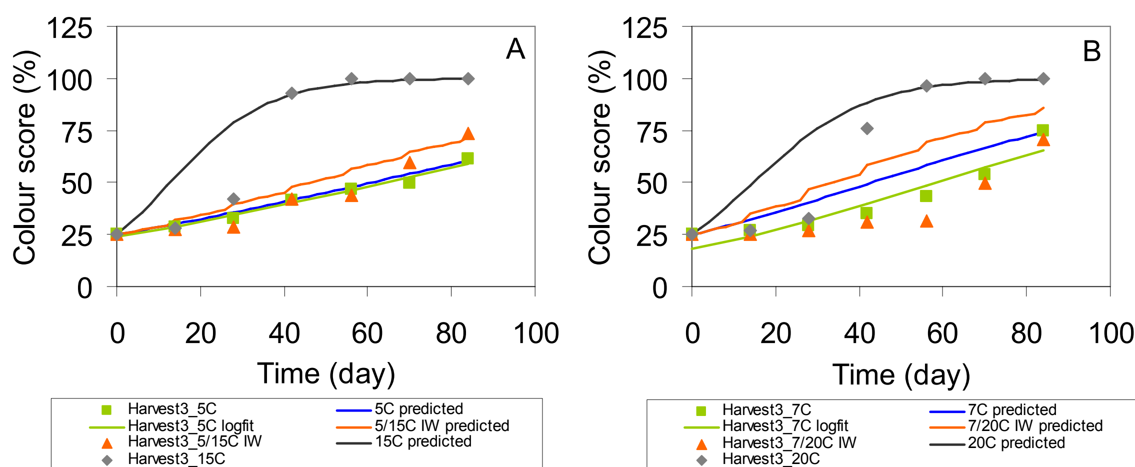


Figure 6.15 Comparison of CS of stored lime experimental & calculated under RA at 5 and 15°C with stored lime under IW at 5°C for 12 days and 15°C for 2 days (A) and CS of stored lime experimental & calculated under RA at 7 and 20°C with stored lime under IW at 7°C for 12 days and 20°C for 2 days (B) (H3).

In summary, the kinetic models developed for hue do appear useful for predicting fruit colour under varying temperature regimes. Models for CS appear to have more limited utility, especially when fruit are stored at temperatures near ambient (15 or 20°C); however it is possible that collecting further data at more frequent intervals during storage may assist in improving the accuracy and usefulness of these models.

6.6 How is colour related to pigment concentration?

6.6.1 Introduction

The rind of citrus fruits consists of flavedo and albedo and contains more bitter components and pectin than other parts of the fruit. The exocarp or flavedo, which is the coloured portion of the peel, contains chlorophyll and carotenoid pigments in chloroplasts or chromoplasts, and oil glands formed by special cells that produce terpenes and oils. Chlorophyll and carotenoids are also located in plastids in juice vesicles (pulp) (Baldwin, 1993). These pigments are fragile molecules and easily modified and these modifications can alter the colour, commercial value, and also the nutritive quality of the fruit and its products (Schoefs, 2002).

During the early stages of citrus fruit development the flavedo is dark green and thereafter, as fruit mature, chlorophyll is gradually lost and chloroplasts are transformed into carotenoid-rich chromoplasts (Goldschmidt, 1988). The decline of chlorophyll in the rind takes several months (during growth and ripening on the tree) and the onset of carotenoid accumulation almost coincides with the disappearance of chlorophyll (Eilati *et al*., 1969; Gross, 1987).

Rind colour of citrus is significantly affected by climate. Warm temperatures interfere with both loss of chlorophyll and accumulation of carotenoids. On the other hand, cool temperatures enhance the desired colour changes. Thus, fruit stay greener and are more pale in the tropics (Davies and Albrigo, 1994; Spiegel-Roy and Goldschmidt, 1996). A characteristic carotenoid complex is responsible for typical colour of each citrus species and hybrid; peel and pulp pigments show certain differences. The total amount of carotenoids in yellow citrus fruits (pummelo, grapefruit, lemon and lime) is low and most of these belong to the colourless carotenoids. Large amounts of a complex mixture of carotenoids are contained in orange-coloured citrus fruits (orange, sour orange and mandarins). Cryptoxanthin or β-citraurin are examples of these compounds that are present in small amounts but have a high colouring quality in oranges (Yokoyama and Keithley, 1991; Spiegel-Roy and Goldschmidt, 1996).

Plant tissues show the colour of the predominant pigment but may contain also many other coloured molecules which will only be visible when the dominant pigment disappears (Schoefs, 2002). Thus the loss of chlorophyll causes a shift in colour from brilliant green to a wide variety of colours (yellow, brown, orange) in ripe and senescent tissues. The degradation pathway of chlorophyll (*a*) involves 5 possible steps. Step one involves the cleavage of the phytol ($C_{20}H_{39}$) chain of chlorophyll *a* (blue green) by the enzyme chlorophyllase, resulting in the formation of chlorophyllide (blue green). Step two is the acidic removal of the Mg^{2+} atom from chlorophyll *a* to form pheophytin (olive brown). In step three chlorophyllide or pheophytin are converted to pheophorbide (olive brown) through the loss of its magnesium ion or its phytol chain, respectively. The pheophorbide is then converted to a fluorescent compound (FC) (colourless) by the enzyme dioxygenase (step four) and the final step is the conversion of the FC into a non-fluorescent compound [rusty pigment (RP) 14]. This final step results in a shift of the system of delocalized double bonds, leading to a loss of fluorescence (Heaton and Marangoni, 1996).

A study on the carotenoid composition of green lime (*C. aurantifolia*) peel revealed that this lime species contained mainly chloroplast pigments: lutein (46%), (9Z/9'Z)-lutein (3.4%), (13Z/13'Z)-lutein (1.8%), β-cryptoxanthin (3.4%), (Z)-β-cryptoxanthin (0.9%), α-carotene (8.5%) and β-carotene (24.2%) (Agocs *et al.,* 2007). Lutein, violaxanthin and neoxanthin are the most abundant xanthophylls in photosynthetic plant tissues, and they are key components of the light-harvesting complexes (DellaPenna and Pogson, 2006).

The objective of the work reported in this section was to use spectral reflectance data and measurements of chlorophyll concentration to explore how changes in pigment concentration influenced the observed colour of the fruit. Spectral data were collected as outlined in section 3.4.3 and chlorophyll was extracted and quantified as described in section 3.8.3.

6.6.2 The relationships between hue, colour score and chlorophyll

In this work, 42 limes were prepared for measurement of peel colour on the green and yellow sides and chlorophyll content from the peel on the green side of fruit during

storage at 13°C. The skin colours of fruit were measured at 0, 2, 4, 8, 10 and 12 weeks of storage and the pigment content of fruit was measured at 2, 4 and 8 weeks of storage.

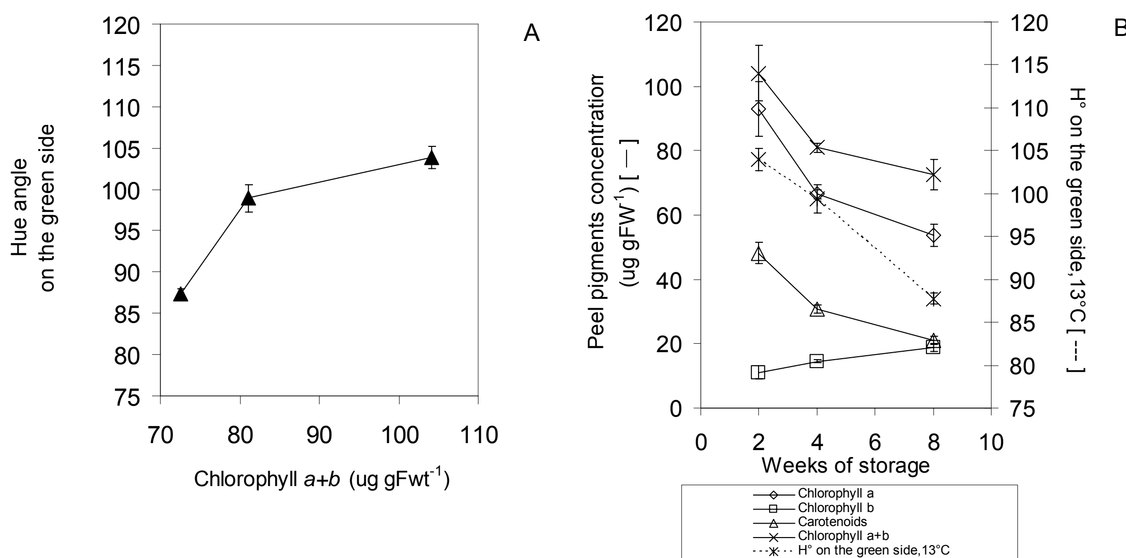


Figure 6.16 Relationship between H° on the green side and chlorophyll *a+b* of lime stored at 13°C (A) and pigments on lime peel changes of stored lime at 13°C at 2, 4 and 8 weeks of storage compared to H° of stored lime at 13°C for 2, 4 and 8 weeks of storage (B). (Vertical bars indicate ± SE (n=6).

The fruit became yellow (although not fully) after 6 weeks of storage (H° < 95°) and Fig. 6.16A, B shows that chlorophyll *a+b* contents decreased as the hue angle of these fruit also decreased. There was a non-linear relationship between hue and chlorophyll *a+b* content. Fig. 6.16B indicates that chlorophyll *a* and other pigments declined continuously with hue, but not chlorophyll *b*. The decrease in chlorophyll content of lime in this work is consistent with data reported for 'Mexican' lime stored at ambient temperature (24-31°C) (Win *et al.,* 2006). A slight increase in chlorophyll *b* content and decrease in carotenoid content in my work after storage were observed. The decline in carotenoid content of lime was also similar to the research of Gross *et al*., (1983) who studied the changes of carotenoid content in pummelo, *C. grandis* 'Goliath', during four stages of development of the fruit: green, colour break, almost ripe, and fully ripe. These authors found that the total coloured carotenoid content decreased from 26 µg g^{-1} in the green fruit to 5 µg g^{-1} in the fully ripe fruit. A decrease of carotenoid content in lemons was also observed by Yokoyama and Vandercook, (1967) who demonstrated that the total carotenoid content of the peel of mature-green lemons was 2.1 µg g^{-1} (as β-carotene) wet

weight and decreased to 1.4 μg g^{-1} (as β-carotene) in the yellow lemons. They reported that the carotenoids in lemons decreased in amount as the chlorophyll disappeared.

As expected, the relationship between CS and lime pigments was opposite to the relationship between H° and these pigments (Fig. 6.16 and 6.17).

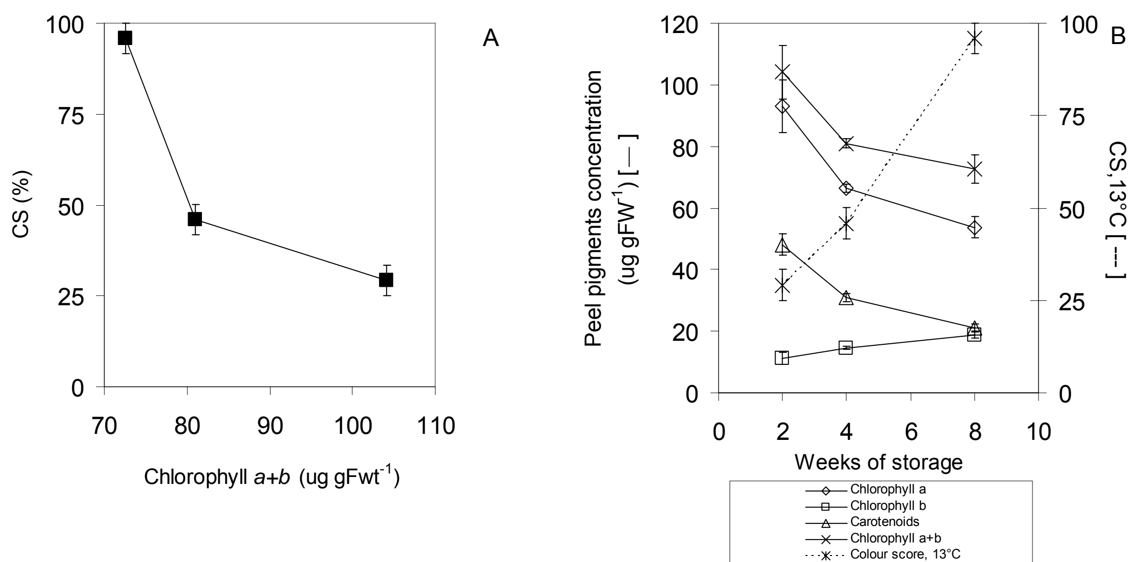


Figure 6.17 Relationship between CS and chlorophyll *a+b* of lime stored at 13°C (A) and pigments on lime peel changes of stored lime at 13°C at 2, 4 and 8 weeks of storage compared to CS of stored lime at 13°C for 2, 4 and 8 weeks of storage (B). (Vertical bars indicate ± SE (n=6).

6.6.3 Relationship between reflectance spectra (%), chlorophyll content and hue angle of lime fruit stored under different conditions

6.6.3.1 Introduction

Appearance is one of the most important quality measurements of fruit and vegetables and is assessed instrumentally by measuring electromagnetic (usually optical) properties. Optical properties indicate the response of matter to visible light wavelengths (400–700 nm, sometimes given as 380–770 nm). Ultraviolet (UV, 4–400 nm) and near infrared (NIR, 700 or 770 to 2500 nm) have also been used for this assessment (Abbott, 1999). Valuable information relating to the pattern of pigment changes and their condition in

plant tissues can be provided with this non-destructive optical technique (Penuelas and Filella, 1998; Abbott, 1999; Merzlyak *et al.,* 2003). Visible and near-infrared reflectance techniques can be used to assess both the biomass and the physiological status of plants (Penuelas and Filella, 1998). Visible light from violet to red, occupies only a narrow portion of wavelengths in the total electromagnetic spectrum (Gunasekaran *et al.,* 1985; Penuelas and Filella, 1998). Penuelas and Filella, (1998) reported that narrow-bandwidth spectroradiometers can detect reflectance in the visible and near-infrared parts of the spectrum, although most researchers report only on the properties of leaves. Penuelas and Filella (1998) reported that leaf reflectance is low because of absorption by photosynthetic pigments such as chlorophylls and carotenoids in the visible spectrum (400-700 nm). There are no strong absorption characteristics in the near-infrared domain (700-1300 nm). The authors also indicated the main wavelengths used in physiological reflectance studies include 430 and 445 nm for carotenoids and 531 and 570 nm for xanthophylls and 550-680 nm for chlorophylls. Gitelson *et al*., (1996) reported that the 'red edge' range of the reflectance spectrum was 680-750 nm and Penuelas and Filella, (1998) reported that the red edge was the wavelength of maximum slope in the increase of reflectance from red to near-infrared, which has been reported to be a good indicator of chlorophyll content at the leaf level and also at the canopy level.

The use of reflectance spectral features can be beneficial to estimate the pigment content in produce (Sims and Gamon, 2002; Merzlyak *et al.,* 2003). For example, Merzlyak *et al*., (2003) developed non-destructive techniques by using the reflectance spectral data for pigment analysis in five apple cultivars. They established that the *in vivo* absorption maxima for carotenoids were at 480, 455 and a shoulder at 425 nm, and for chlorophyll the absorbance maxima were in the ranges between 550-650 nm and 690-705 nm. They also found that the reflectance in the wavelength between 520-530 nm was the best measure of carotenoid concentration in the presence of chlorophyll.

6.6.3.2 Reflectance spectra on the green and yellow side of limes

From those 42 fruit prepared for colour measurement and pigment content analysis (see section 6.6.2), six lime fruit were taken for pigment analysis at each measurement period. Therefore there were 24 fruit left intact from the beginning to end of storage and the reflectance spectra of these 24 fruit are presented in Fig. 6.18 from day 0 until 12 weeks

of storage. The fruit were stored at 13°C allowing the colour of lime peels to change from green to fully yellow following the normal process without showing any CI symptoms. Reflectance spectral data were obtained from the spectrophotometer (see section 3.3.4.3). These changes are very similar to those reported for reflectance spectra of lemon during ripening (Merzlyak *et al.,* 1999) and for green to green-yellow apples (Merzlyak *et al.,* 2003).

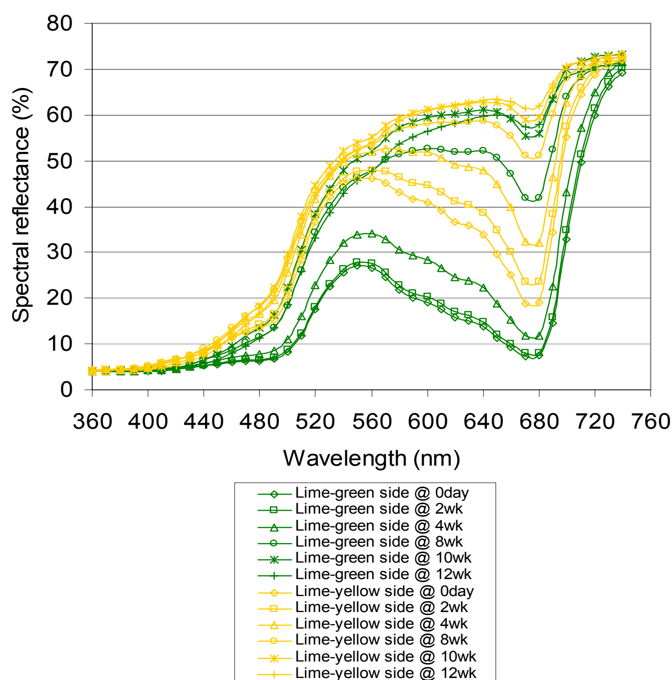


Figure 6.18 Average (n=24) reflectance spectra (%) of limes stored under RA at 13°C for 0, 2, 4, 8, 10 and 12 weeks of storage on the green and yellow side.

There was generally a steady increase in spectral reflectance on both the green and yellow sides of the fruit (Fig. 6.18), representing the loss in pigments such as chlorophyll and carotenoids (see Fig. 6.16 and 6.17). The hue angle changes and increase in % reflectance at 680 nm (R680) from day 0 of these 24 fruit are shown in Fig. 6.19A and B, respectively. The rate of degreening on the green side was faster than the yellow side from 4 weeks of storage (Fig. 6.19A). Detailed analysis of the increase in % R680 calculated from % R680 of each week – % R680 at 0 day confirms this trend as the rate of change of reflectance was also faster on the green side than on the yellow side, which is consistent with a more rapid loss of chlorophyll (Fig. 6.19A and B). The most green (high H°) and most yellow (low H°) of H5 lime fruit at harvest (T = 0), respectively are illustrated in Fig. 6.20.

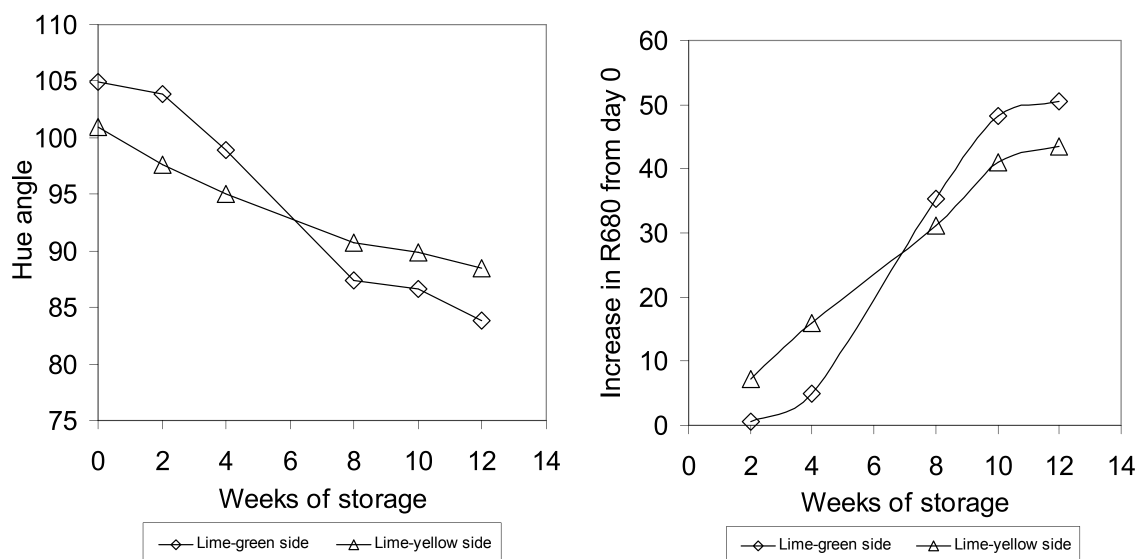


Figure 6.19 Average (n=24) hue angle changes of limes stored under RA at 13°C for 0, 2, 4, 8, 10 and 12 weeks of storage on the green (◊) and yellow side (Δ) (A) and increase in % reflectance at 680 nm from 0, 2, 4, 8, 10 and 12 weeks of storage on the green (◊) and yellow side (Δ) (B).

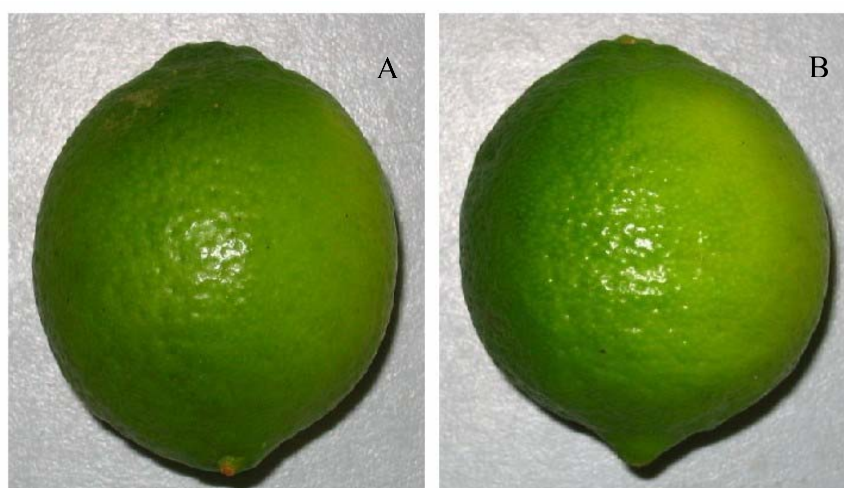


Figure 6.20 Examples of most green A; ($H°_S$ = 105°) and most yellow fruit B; ($H°_S$ = 101°) used to examine changes in spectral reflectance during storage at 13°C, H5.

I identified the five greenest and yellowest fruit at harvest and Fig. 6.21A shows how mean spectral reflectance of the greenest fruit samples changed during storage for both the greenest and most yellow sides of the fruit compared to the changes for the most yellow fruit (Fig. 6.21B). The final spectral reflectance profiles are nearly identical for

both the green and the yellow side of the fruit at 12 weeks of storage (Fig. 6.21A, B), coinciding with the fruit being fully yellow.

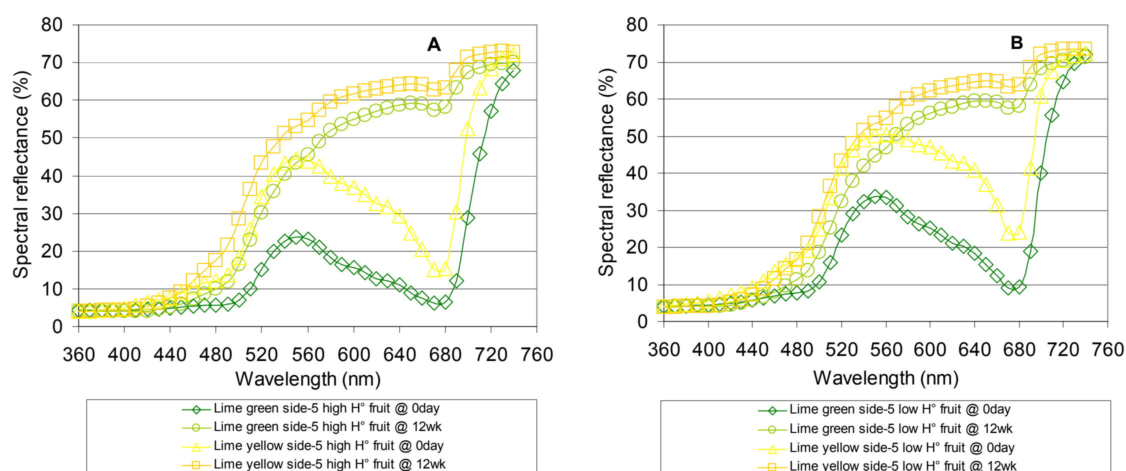


Figure 6.21 Average reflectance spectra (%) of limes stored under RA at 13°C for 0 day and 12 weeks of storage on the green and yellow side of fruit for the high H° value set (A) and the low H° value set (B).

In order to look more carefully at the pigments responsible for this change, I prepared difference spectra between the most green fruit and most yellow fruit (from those 24 fruit) at harvest and after storage. The difference spectra in Fig. 6.22 - 6.24 were calculated as the difference between two selected spectra as follows. Firstly, for the 5 most green fruit at harvest (Fig. 6.22A), the difference in reflectance at each wavelength between the most green and most yellow sides of the fruit at harvest (Fig. 6.22A ◊) was compared with the difference between the initially most green side at harvest and the yellow side after 12 weeks (Fig. 6.22A □). The former indicates at which wavelengths and to what extent, degreening had already occurred on the fruit at harvest. The latter describes the maximum changes in reflectance that occurred over 12 weeks of storage. I also made the same comparison for the most yellow fruit at harvest (Fig. 6.22B), comparing the yellow and green sides of these fruit at harvest (○) and the yellow side after 12 weeks of storage with the green side at harvest (Δ).

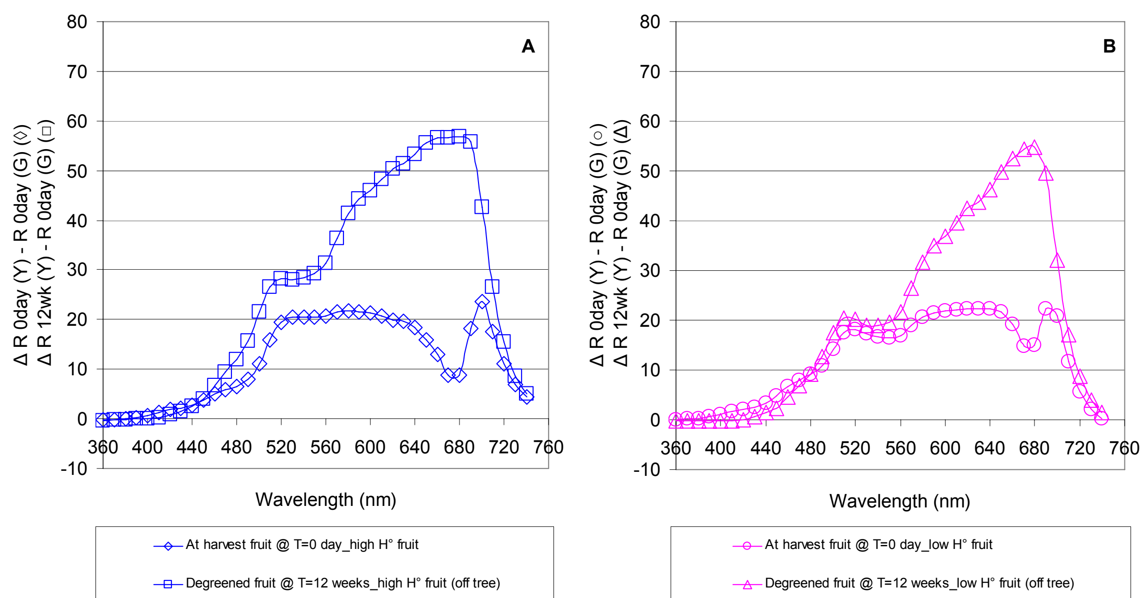


Figure 6.22 Difference of reflectance spectral data of lime stored under RA at 13°C for the "at harvest fruit" at T = 0 day (◊) and the "degreened fruit" at T = 12 weeks (□) stored at 13°C of the high H° value set (A) and the "at harvest fruit" at T = 0 day (○) and the "degreened fruit" at T = 12 weeks (Δ) stored at 13°C of the low H° value set of lime (B) NB: (Yellow at 0 day – Green at 0 day = "at harvest fruit" @ T=0) and (Yellow at 12 weeks – Green at 0 day = "degreened fruit" @ T=12 weeks).

For the initially most green side (Fig. 6.22A) the difference spectra profiles were less similar, overlapping only in the ranges 360-440 nm and above 710 nm. Most pigments loss therefore occurred in the region 480-710 nm, with a large loss of pigments that absorb at 680 nm. This means that the high H° value set (Fig. 6.22A) showed the most difference for these wavelengths which relate to degradation of carotenoids (at 480, 455 nm or 520-530 nm) (Merzlyak *et al.,* 2003), xanthophylls (531 and 570 nm) and chlorophyll (550-680 nm) (Penuelas and Filella, 1998).

Fig 6.22B shows that the difference spectra reflectance profiles of the most yellow side at harvest, even though this was quite 'green' (H° = 101°; Fig. 6.20B), were similar for those regions between 360 and 540 nm and above 710 nm. So major pigment changes on the most yellow side were associated only with those pigments that contribute to the reflectance in the range 540-710 nm and particularly at 680, as expected, where chlorophyll absorbs strongly. This means that the yellowest fruit at harvest did not show any further changes in spectral reflectance between 360-540 nm from day 0 to 12 weeks of storage (Fig.6.22B). This is the region in which the carotenoids (+ xanthophylls)

absorb maximally suggesting ripening-related carotenoid changes were already completed on the tree for these fruit (Penuelas and Filella, 1998; Caffarri *et al.,* 2001; Merzlyak *et al.,* 2003).

6.6.3.3 Comparison of differences in reflectance spectral data of the high H° fruit set versus the low H° fruit set at T = 0 and T = 12 weeks

Fig. 6.23A and B compare difference spectra from Fig. 6.22 at T=0 and 12 weeks, respectively. This indicates those spectral regions that are most different between the most green and most yellow fruit sets. Fig. 6.23A shows that at harvest the most significant difference between these fruit groups appears to be only around chlorophyll adsorption 680 nm with possibly a significant difference around 540 – 560 nm. Fig. 6.23B shows the mean difference between the most green and most yellow sides of the overall most green and most yellow fruit at 12 weeks, when the fruit were (100%) yellow. There is little difference in final chlorophyll reflectance (680 nm) but important differences between 500-680 nm. This suggests the major differences in pigment composition between these two extreme groups of fruit (classified with respect to hue) are in the carotenoids (520-530 nm) (Merzlyak *et al.,* 2003) and xanthophylls (531 and 570 nm). Chlorophyll does absorb between 550-680 nm (Penuelas and Filella, 1998) and may also contribute to observed differences between the more green and more yellow fruit, but overall these important spectral differences may explain why their kinetics of change in hue appear to be different (see section 6.4.1).

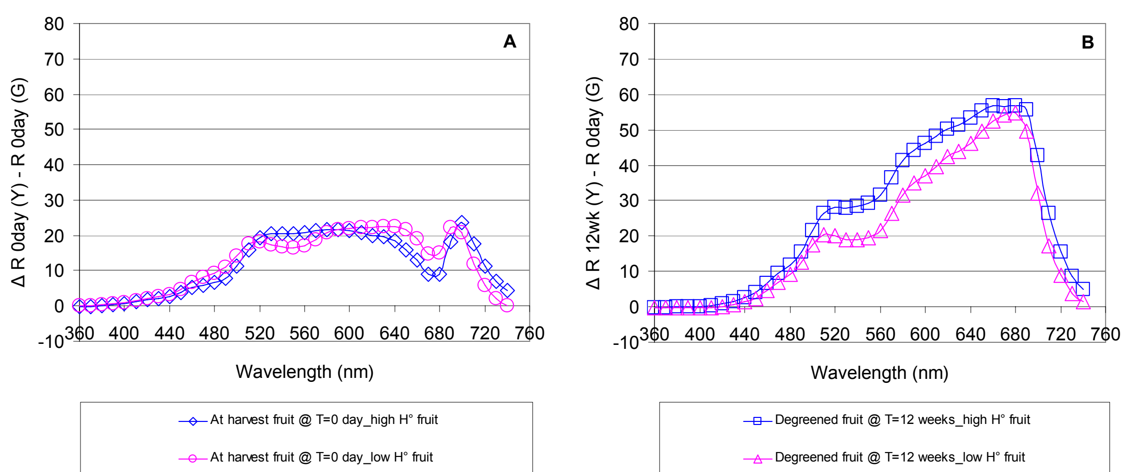


Figure 6.23 Difference of spectral reflectance data of "at harvest fruit" at T = 0 day of the high (◊) and low H° set (○) (A) and "degreened fruit" at 12 weeks stored at 13°C of the high (□) and low H° set (Δ) (B).

6.6.3.4 Implications of difference spectra for the underlying pigment changes during postharvest storage

The changes in spectral reflectance during storage are shown in Fig. 6.24 from $t = 0$ d for the green and yellow sides. Fig. 6.24A shows differences were evident in the reflectance spectra between 430-720 nm of the green side of green fruit stored from 0 day to 12 weeks. There were few differences in the spectra during storage until 2 weeks and increasing differences appeared thereafter from 4 to 12 weeks. At 520-530 nm and 531 or 570 nm where the carotenoids and xanthophylls absorb respectively, there were significant changes of the spectra between 4 and 12 weeks of storage on the green side of the initially most green fruit. This suggests the green fruit decreased in carotenoid and xanthophyll content during this storage period. Significant losses of chlorophyll were also observed for these green fruit at wavelengths between 550-650 and 680-720 nm.

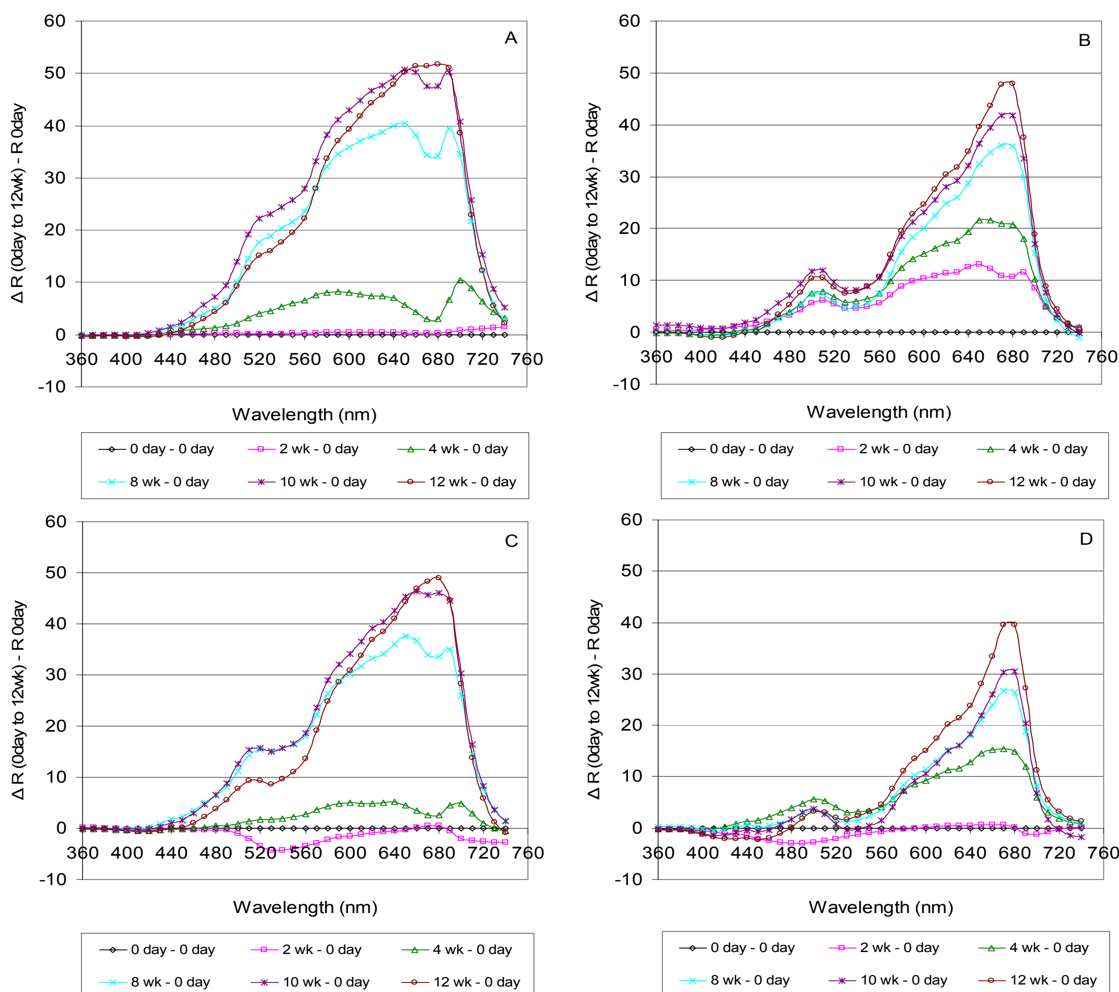


Figure 6.24 A-D Range of pigments changing of different spectra from 0 day until 12 weeks of storage of average of five high H° limes on the green (A) and yellow side (B) and average of five lowest H° limes on the green (C) and yellow side (D).

Fig. 6.24B shows differences also developed in the reflectance spectra on the yellow side of green fruit between 430-720 nm. There appeared to be only a small subsequent change in carotenoid or xanthophyll content as indicated by reflectance at 520-530 nm or 531 or 570 nm, respectively, when compared to the green side of the same fruit (Fig. 6.24A). Chlorophyll losses were usually slow for the initial 2 weeks of storage except on the yellow side of the high H° fruit which started to lose chlorophyll from the start of storage. It should be noticed that the rates of chlorophyll loss after 8 weeks were approximately identical for all fruit, on both (i.e. the green or yellow) sides.

The pattern of pigment changes of the green side of the most yellow fruit (Fig. 6.24C) was similar to the greenest fruit (Fig. 6.24A) except at 2 weeks. These imply that carotenoid synthesis continued during the first 2 weeks of storage.

There was a similar trend in changes of difference spectra between the yellow side of the more yellow fruit (Fig. 6.24D) and the yellow side of the greener fruit set (Fig. 6.24B) except at 2 weeks of storage. The difference spectra of the yellow fruit set showed little change between 0 and 2 weeks of storage (Fig. 6.24D) whereas the difference spectra of the greener fruit set showed a significant difference (most notably at 520 – 560 nm) between 0 and 2 weeks of storage (Fig. 6.24B). The differences in spectral data of each yellow side also suggested that the loss of pigments absorbing between 430-700 nm for the yellow fruit were lower than that occurring in the greener fruit (Fig. 6.24B, D).

The losses of carotenoids and xanthophylls on the green side of both the high and low H° fruit set (Fig. 6.24A, C) appeared higher than the yellow side of the high and low H° fruit set (Fig. 6.24B, D), respectively. The high H° fruit set on both the green (Fig. 6.24A) and yellow (Fig. 6.24B) sides appeared to showed greater loss of pigments than the low H° fruit set on both the green (Fig. 6.24C) and yellow (Fig. 6.24D) sides, respectively. On the yellow side, the high and low H° fruit set showed the smallest changes of pigments related to carotenoids and xanthophylls between 520-530 nm and 531 or 570 nm, respectively, after storage for 12 weeks, however the high H° fruit set on the yellow side lost more chlorophyll (680-700 nm) than the lower H° fruit set on the same side (Fig 6.24B and D).

Overall the largest difference was the lower loss of chlorophyll in the yellower fruit than in greener fruit (Fig. 6.24B and D). Also, the overall change in pigments for the greener fruit was higher than for the yellow fruit (Fig. 6.24A and C). These major trends were as expected.

Sims and Gamon, (2002) developed several spectral indices for estimation of pigment content of a wide range of leaf species. The authors reported that in most cases these indices have been tested for only one or a small number of related species. In their work, a reflectance index (the ratio of R800/R680) was used as a simple means for estimation of chlorophyll content in leaf samples. Other authors have also found this ratio is a good predictor of the chlorophyll content (Merzlyak *et al.,* 1998; Merzlyak *et al.,* 2003). Sims and Gamon, (2002) suggested that it is not clear whether these indices can be applied across species with varied characteristics of leaf structure. It is also imperative to verify for any new fruit species that the precise reflectance ratio chosen is a reasonable predictor of pigment concentration. For example, Merzlyak *et al*., (2003) also suggested that a ratio of R800/R700 was directly proportional to total chlorophyll content in apple fruit. Thus, in this work I tested spectral indices R800/R680 (based on the earlier figure (Fig. 6.18) and R800/R700 as proxies for chlorophyll content in limes.

Fig. 6.25A shows the association between chlorophyll *a* content and the reflectance index on the green side of lime stored at 13°C. Average concentrations of chlorophyll *a* were 93, 66 and 54 µg gFW^{-1} at 2, 4 and 8 weeks of storage, respectively. The concentration of chlorophyll *a* decreased as the reflectance indices decreased, although the relationship was not linear. The ratio R800/R680 was confirmed to be a good descriptor for chlorophyll *a* in limes (Fig. 6.25A); in contrast the index R800/R700 did not change with chlorophyll content (Fig. 6.25B).

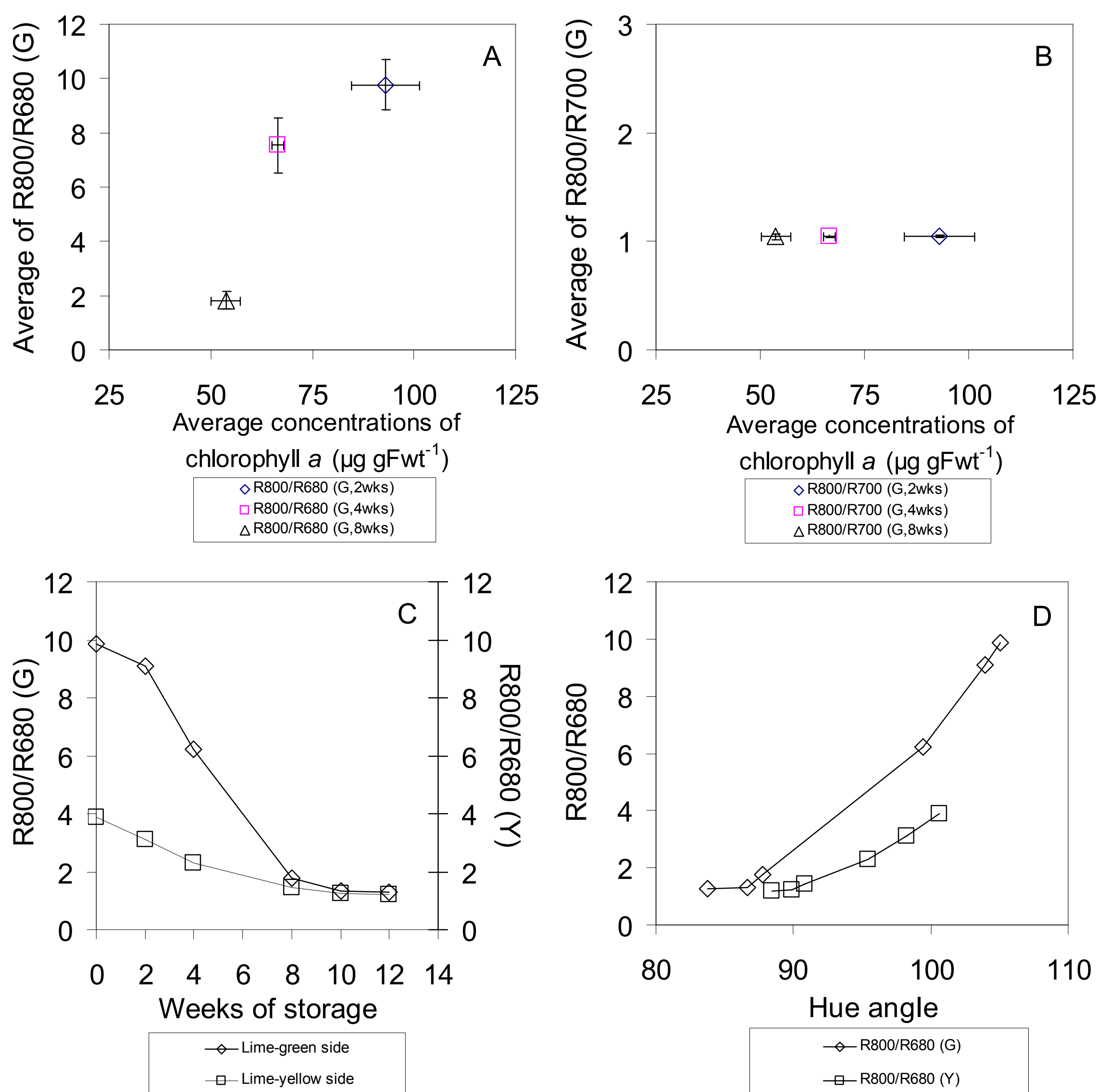


Figure 6.25 Average of reflectance index (R800/R680,G) (A) and (R800/R700,G) (B) plotted vs. average of chlorophyll *a* content of lime stored under RA at 13°C for 2 (◊), 4 (□) and 8 (Δ) weeks of storage. Horizontal and vertical bars indicate ± SE of the X and Y axis, respectively (n=6). Reflectance index (R800/R680) plotted vs. weeks of storage of lime stored at 13°C (C). The index R800/R680 (G ◊) is plotted on the left scale for the green side of lime and the index R800/R680 (Y □) is plotted on the right scale for the yellow side of lime. Reflectance index (R800/R680) plotted vs. hue angle of lime stored under RA at 13°C for 0, 2, 4, 8, 10 and 12 weeks of storage on the green (◊) and yellow side (□) (D).

Further analysis of the relationship between the reflectance indices and times of storage and hue angle values is shown in Fig 6.25C and D, respectively. In Fig. 6.25C the reflectance indices on both the green and yellow sides confirm the decrease of chlorophyll content in lime peel during storage until 12 weeks. This index gave a similar profile to that of hue angle for both the green and yellow sides stored at 13°C for 12 weeks (Fig. 6.7B). The value of this index on the yellow side at 0 weeks of storage was approximately the same as that on the green side at 4-6 weeks of storage.

Fig. 6.25D shows the relationship between reflectance index and hue angle. This makes it clear that the relationship differed for the green and yellow sides of the fruit and that fruit of the same hue angle can have quite different reflectance ratios. For example, even though the H° of lime on the green side at 4 weeks of storage (H°=99.39°) and yellow side at 0 weeks of storage (H°=100.61°) were quite similar, the reflectance indices were quite different. Therefore although the reflectance ratio is useful to describe pigment change during ripening, I need to use more complete spectral reflectance data to truly understand pigment changes during ripening. I can not necessarily describe the colour changes of lime fruit at different stages of ripening by the same model.

The reflectance index R800/R680 is associated with chlorophyll as described previously. I also tested other indices R800/R520 and R800/R480 that are associated with carotenoid content changes by the same procedure. In this case R800/R520 showed a slightly better correlation with carotenoid content than R800/R480. This index also does not require modification for a chlorophyll effect as suggested by Merzlyak *et al*., (2003), because there is very little light is absorbed by chlorophyll *a* and *b* in plants in the spectral region between 500-600 nm (Berg *et al.,* 2002). For these reasons I chose this index to describe carotenoid content changes for limes.

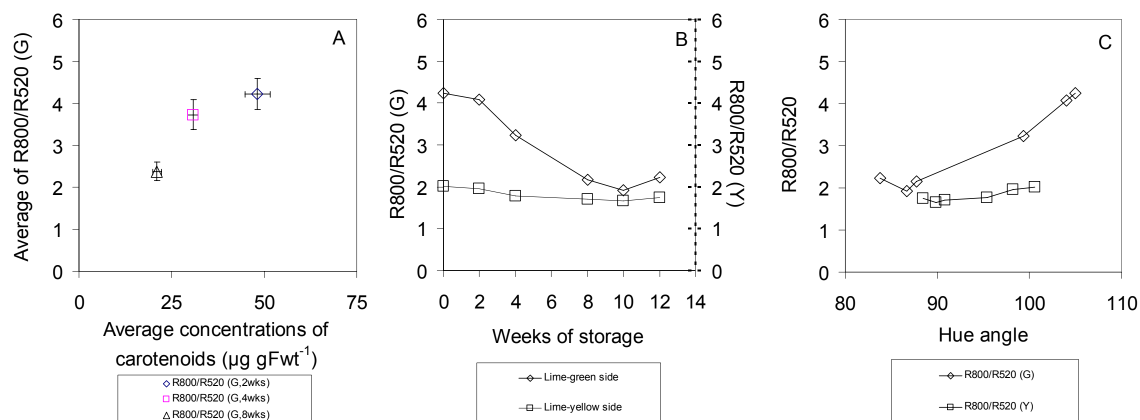


Figure 6.26 Average of reflectance index (R800/R520,G) (A) plotted vs. average of carotenoid content of lime stored under RA at 13°C for 2 (◊), 4 (□) and 8 (Δ) weeks of storage. Horizontal and vertical bars indicate ± SE of the X and Y axis, respectively (n=6). Reflectance index (R800/R520) plotted vs. weeks of storage of lime stored at 13°C (B). The index R800/R520 (G ◊) is plotted on the left scale for the green side of lime and the index R800/R520 (Y □) is plotted on the right scale for the yellow side of lime. Reflectance index (R800/R520) plotted vs. hue angle of lime stored under RA at 13°C for 0, 2, 4, 8, 10 and 12 weeks of storage on the green (◊) and yellow side (□) (C).

Fig. 6.26A indicates that there was not a linear relationship between carotenoid content and the reflectance index R800/R520, similar to what I found for the association between chlorophyll *a* content and the reflectance index R800/R680. When the reflectance index (R800/R520) of the green and yellow sides was plotted against storage duration at 13°C (Fig.6.26B) or hue angle (Fig.6.26C) I again found that the reflectance ratio changes were different between the greener and yellower sides.

The association of these selected indices (R800/R680 and R800/R520) with the pigment changes of lime on both green and yellow sides stored at 13°C is shown in Fig. 6.27A and 6.27B, respectively. The best treatments from H4 (6 cycles IW at 5 and 15°C plus C_2H_4 absorbent) and H5 (HWD at 47°C for 4 min prior to storage at 5°C) were compared with the normal fruit (H5) stored under RA at 13°C until 12 weeks of storage (Fig. 6.27A and B).

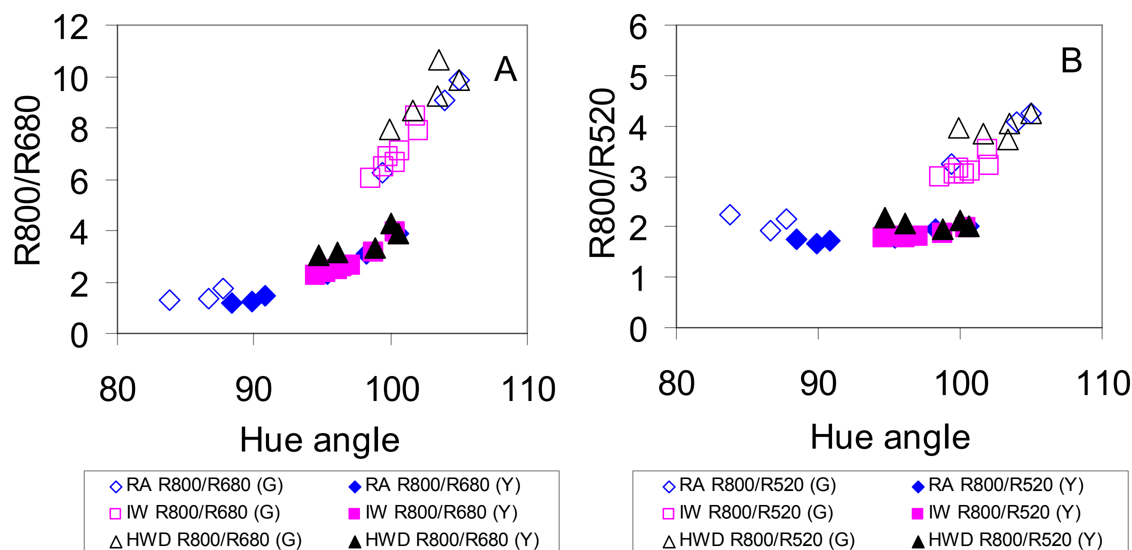


Figure 6.27 Reflectance indices (R800/R680) (A) and (R800/R520) (B) plotted vs. hue angle of lime stored under RA at 13°C on the green (◊) and yellow side (♦), IW condition (5°C, 12 days and 15°C, 2 days) for 6 cycles plus C_2H_4 absorbent, on the green (□) and yellow side (■) and HWD treatment by dipped the fruit at 47°C for 4 min then stored at 5°C, on the green (Δ) and yellow side (▲).

Fig. 6.27A and B show that the relationship between hue and pigments (as judged by spectral ratio) is the same regardless of the storage treatments applied. Therefore HWD and IW treatments have not appeared to have altered the pattern or mechanism of pigment change; they have just reduced the rate of change in pigments. (Note a single point relating to a fruit showing CI, for the HWD treatment, has been removed from the analysis.) However, although the changes in R800/R680 and R800/R520 are marked for the green side of limes, the ratio changes much less for the yellow side of lime fruit (closed symbols), suggesting these simple ratios are most useful for the analysis of the greener side of lime fruit.

6.7 Do changes in pigment differ between IW and HWD?

The same RA, IW and HWD treatments also were compared with regard to the difference of reflectance spectra (%) at 12 week and 0 weeks of storage on the green side (Fig. 6.28A) and yellow side (Fig. 6.28B). On the green side, the fruit stored at 13°C showed significant changes between the final and the initial pigments concentration whereas the fruit stored under IW and HWD showed only small changes of pigment composition after

storage for 12 weeks. Similar trends of the difference spectra for all treatments on the yellow side were also obtained after 12 weeks of storage. The fruit stored under HWD showed some disorders after 8 weeks of storage, so the reflectance spectra were different from the fruit stored under IW. The discolouration on the peel of these HWD lime can be seen as browning between 500-580 nm. Overall, IW delayed the change of reflectance spectra at all wavelengths during long term storage.

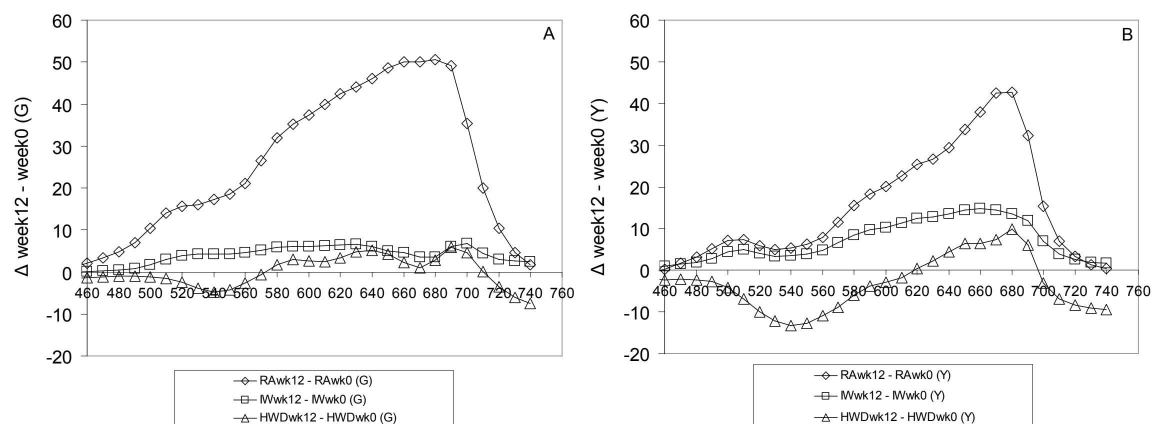


Figure 6.28 Difference of average reflectance spectra (%) at 12 week and 0 weeks of storage of limes stored under RA at 13°C (RA) (◊), IW condition (5°C, 12 days and 15°C, 2 days) for 6 cycles plus C_2H_4 absorbent or IW fruit (IW) (□) and HWD treatment by dipped the fruit at 47°C for 4 min then stored at 5°C (Δ), on the green (A) and yellow side (B).

6.8 Discussion

Lime colour changes were initially thought to follow the biological age concept described by Hertog *et al*., (2004). The logistic equation was used to describe the colour change and fitted the observed colour profiles appropriately for both H° and colour score (CS) at a given temperature and for a given fruit side (green or yellow). As colour score (CS) can be an easier assessment to make of lime colour than the use of H° or analysis of pigment changes, particularly in less developed commercial settings, further evaluation of the options for employing CS data are recommended.

I investigated the differences in degreening between the initially more green and yellow sides of the fruit. This showed the change of colour on the green side did not follow that of the yellow side (e.g. see Fig. 6.6), except in H1 (see Fig. 6.5), when the two profiles were manipulated using a time adjustment factor. Thus although the biological age

concept initially seemed to be an appropriate concept to explain lime colour changes for the more mature fruit of H1, this was not the case for the less mature fruit of later harvests. Two important factors appeared to contribute to this biological variance. Firstly the rate constant (*k,* dependent on temperature) varied significantly between experiments and between fruit in any given sample (see e.g. appendix III); and secondly, the nature of pigment formation and degradation appeared different on different sides of the fruit, as indicated by the spectral reflectance data analysis.

The Arrhenius model was acceptable to describe the effects of temperature on degreening kinetics of lime for both H° data measured by the two colorimeters (both chromameter and spectrophotometer) and CS data based on a grading chart. The activation energies (E_a) for degreening based on H° and CS during air storage were estimated to be 53 and 86 $kJ.mol^{-1}$ respectively, typical of other food deterioration reactions (between 42-126 kJ mol^{-1}) (Robertson, 1993) or fruit ripening (Chen and Ramaswamy, 2002; Hertog *et al.,* 2004). Following Arrhenius kinetics, the effect of a range of temperatures from 2 to 13°C on colour change was generally predictable. However at the higher temperatures evaluated (15 to 20°C) the effect of temperature appeared to differ. It is possible degreening is slightly slowed at 20°C. This could be further explored using either linked first order formation and degradation mechanisms (Tijskens, 2004) or a model similar to that for temperature dependence of microbial growth (square root model; (Mawson, 2006)). Each has potential advantages and disadvantages and an evaluation of these could be included in a wider investigation of models of colour change in such fruit.

The benefit of a kinetic model is that it permits us to predict changes of lime H° or CS depending on temperature. Comparison between calculated (i.e. expected) and experimental (actual) H° and CS of limes stored under different RA and IW conditions were done based on these Arrhenius models. Overall, I found that the degreening of lime stored under IW condition and characterised by hue was only slightly slower than I expected by using the model to predict these values (Fig. 6.14). Thus there was no strong evidence to suggest IW alters the mechanism of degreening and any such effect was nowhere near as dramatic as the beneficial effect of IW on chilling injury (CI).

The relationship between colour change (H° and CS) and pigment concentration (chlorophyll *a*, *b*, *a*+*b* and carotenoids) changes of the fruit stored at 13°C was studied (Fig. 6.16 and 6.17). As expected I found that the concentration of chlorophyll *a*, *a*+*b* and carotenoids decreased coincident with the decrease of H°, whereas the chlorophyll *b* concentration slightly increased (Fig. 6.16). These changes were largely consistent with other published work.

In my work, the reflectance spectral data provided similar useful information on the lime colour change process. The decrease of chlorophyll and carotenoid content in lime peel after storage (Fig. 6.16) followed the changes in spectral data as plotted in Fig. 6.18. The steady increase in spectral reflectance on both green and yellow sides represented a loss in pigments of lime after storage. I found that the end points of spectral reflectance on the green and yellow sides of lime were consistent at the end of storage. I also found that most pigment changes occurred in the region 480-710 nm, with a large loss of chlorophyll, which absorbs at 680 nm.

After I compared the spectral reflectance of "at harvest" fruit at t = 0 day and the "degreened" fruit at t = 12 weeks, I found that the degradation of pigments could be separated into changes associated with carotenoids which absorb at 480, 455 nm or 520-530 nm (Merzlyak *et al.,* 2003), xanthophylls which absorbs at 531 and 570 nm, and changes associated with chlorophyll between 550-680 nm (Penuelas and Filella, 1998).

From previous reviews, xanthophylls (particularly lutein) may be lost during degreening of limes during storage, especially for the high H° green fruit (Fig. 6.22A). In contrast, the low H° fruit, which were actually also quite green, appeared to show very little loss of pigments at the spectral band of 360-540 nm and above 710 nm (Fig. 6.22B). This suggested that the loss of carotenoids or xanthophylls has probably already occurred on the tree however, these fruit still showed a major postharvest loss of chlorophyll (550-650 nm and 690-705 nm) (Fig. 6.22B).

When plants are under stress or during leaf senescence, the chlorophyll content tends to decline more rapidly than the carotenoid content (Gitelson and Merzlyak, 1994; Merzlyak *et al.,* 1999; Sims and Gamon, 2002) and such changes in physiological status can be assessed by the use of visible reflectance techniques (Penuelas and Filella, 1998). This is

a fast and non-destructive technique that can also be used to estimate pigment concentrations (Merzlyak *et al.,* 2003). Sims and Gamon, (2002) estimated leaf pigment content by developing a large number of spectral indices for a wide range of species of leaf. Merzlyak *et al*., (1998) used reflectance spectroscopy to study light-induced pigment breakdown in situ in leaves (wax flower (*Hoya carnosa* R. Br.)) and ripening fruits of apple (*M. domestica* Borlh. Cv. Zhigulevskoe) and lemon (*C. limon* Burm. cv. Pavlovsky). They found that the relative rates of loss of chlorophyll and carotenoid could be deduced from changes in reflectance ratios. Li *et al*., (1997) used similar techniques to develop an optical chlorophyll sensing system to detect changes in chlorophyll content of ripening bananas.

Reflectance spectra can be used more specifically to estimate changes in pigment concentration by establishing that certain ratios correlate with pigment concentration. It is important to verify the right ratio to use for any particularly fruit species. Merzlyak *et al.*, (2003) used R800/R700 to describe chlorophyll content in apples; in limes, this ratio showed no relationship with chlorophyll content but R800/R680 was correlated with chlorophyll content (Fig. 6.25). R800/R680 is one of the spectral indices used for estimation of chlorophyll content in many species (Sims and Gamon, 2002). If I displaced the curves of this reflectance index, the curve for the yellow side overlapped the green at about 4 weeks of storage (Fig. 6.25C) which was similar to the trend for hue angle shown in Fig. 6.7. However while R800/R680 can be used to describe the trends in colour or hue angle, it could not indicate the exact value of pigment concentration or hue angle; at a similar hue angle on the green and yellow side the index values were quite different (6.25D).

I have shown that the index R800/R520 is correlated with carotenoid contents in limes (Fig. 6.26A, B and C). However, the change in this spectral index with respect to hue angle was also different for the different sides of the fruit, confirming that the pigment changes occurring after harvest are different as a result of the prior changes that have occurred on the tree. Thus, the yellow side is not simply the same as the green side will become at a later time. These differences may be the result of sun or ultraviolet exposure or temperature differences that affect lime peel and subsequently influence the pigment concentration after harvest. These external effects on pigment contents of lime peel suggest deeper physiological investigation of these mechanisms would be justified.

Analysis of the changes in reflectance indices (R800/R680 for chlorophylls and R800/R520 for carotenoids) further suggested that although HWD or IW delayed fruit ripening, they did not fundamentally alter the mechanism of colour change of the peel of limes. However, the reflectance spectra showed the treatments did have specific effects on pigments; for example the fruit stored under HWD treatment showed evidence of browning whereas the IW fruit overall showed little change in colour of the peel compared to other treatments.

In summary, both the logistic hue model incorporating Arrhenius kinetics and the reflectance spectra analysis are useful tools for describing the degreening process of limes. I could further extend analysis to other degreening processes to look for similarities and differences with respect to enzyme activities or chloroplast structure to better understand the physiology of this process and its regulation under both constant and variable temperature conditions.

However through the analysis I have also demonstrated that the biological age concept suggested by other authors (Tijskens and Evelo, 1994; Hertog *et al.,* 2004), and that has been successfully applied for tomatoes, does not apply so well for fruit such as limes that do not ripen evenly all around. I have shown that the pigment (chlorophyll and carotenoid) changes were different on each side of the fruit (Fig. 6.25D and 6.26C). Therefore even using an overall colour index obtained using a camera (Bunnik *et al.,* 2006) may not improve the utility of biological age models. Determining whether there are physiological explanations (such as pigment protection in the sun-exposed side of fruit on the tree) could be fertile ground for further research. Finally it is also important to note that there is a need to further examine the relationship between actual pigment concentrations and hue, e.g. by the application of colour mixing models employed in other fields of technology such as printing and dyeing.

CHAPTER 7

Phytochemical composition of lime (*Citrus latifolia* Tanaka) fruit during storage

7.1 Introduction

There is a large body of published information on the health-protective effects of plant foods such as fruits, vegetables, nuts and whole grains. Consuming fruits and vegetables provides both vitamins and phytochemicals, and citrus fruit are a very good source of these health-promoting compounds (Craig, 1997; Baghurst, 2003).

The purpose of this part of the research programme was to characterise the vitamin C and phytochemical compounds of limes at harvest and determine the impact of storage regime and storage time on their postharvest retention.

7.2 Methodology

The vitamin C content of harvest 3 (H3) fruit was analysed by HPLC as detailed in section 3.8.2. These fruit were of the highest overall quality obtained in this study so were expected to provide the best indication of the effect of storage on nutrient composition. The fruit were stored under different air (RA) or intermittent warming (IW) regimes either with no pretreatment or with hot water dipping (HWD).

Analysis of flavonoids of harvest 1 (H1) and H3 fruit was conducted using HPLC as described in section 3.8.1. The H1 fruit were the most yellow fruit of all harvests (40% yellow) before storage. These fruit were selected for analysis because they included a wide range of fruit qualities after storage. The analysis was repeated for the best quality fruit in H3.

7.3 Effect of different postharvest treatments and storage on vitamin C content of lime

At harvest, the H3 fruit contained approximately 33.6 mg vitamin C.100 ml^{-1} lime juice (Fig. 7.1). The fruit stored under RA at 20°C showed an initial loss of 27% of vitamin C after 4 weeks but thereafter vitamin C was retained. Fruit stored under cooler temperatures (5-15°C) showed lower losses of vitamin C and at 12 weeks their compositions were not significantly different from day 0 ($p < 0.05$) (Fig. 7.1A). Fruit stored under a range of IW conditions retained vitamin C as well as 5°C stored fruit and while they lost approximately 12% vitamin C after 12 weeks of storage, this was not significantly different from day 0 ($p < 0.05$) (Fig.7.1B). HWD followed by storage at 5°C (without C_2H_4 absorbent) led to a more rapid loss of vitamin C in the first 4 weeks and about 46% overall loss of vitamin C after 12 weeks of storage (Fig.7.1C). Possibly, this was associated with ethylene given off by damaged tissue since the loss appeared to be prevented by inclusion of an ethylene absorbent (Fig. 7.1C). The vitamin C contents of the fruit after HWD with inclusion of an ethylene absorbent or HWD followed by IW condition (5°C, 12 days and 15°C, 2 days), with and without ethylene absorbent, were all similar to the fruit stored under RA at 5°C at both 4 and 12 weeks. Generally the fruit dipped with HWD did not maintain their vitamin C content as well as storing the fruit under IW conditions or under RA at 5°C (Fig. 7.1A, B and C).

After these experiments were completed I found that ca. 14 % vitamin C in citrus may be in the form of dehydroascorbate (Lee and Coates, 1999) which would not be detected efficiently with this method. My data are therefore a slight underestimate of total vitamin C, but since all samples were treated uniformly, the conclusions still stand.

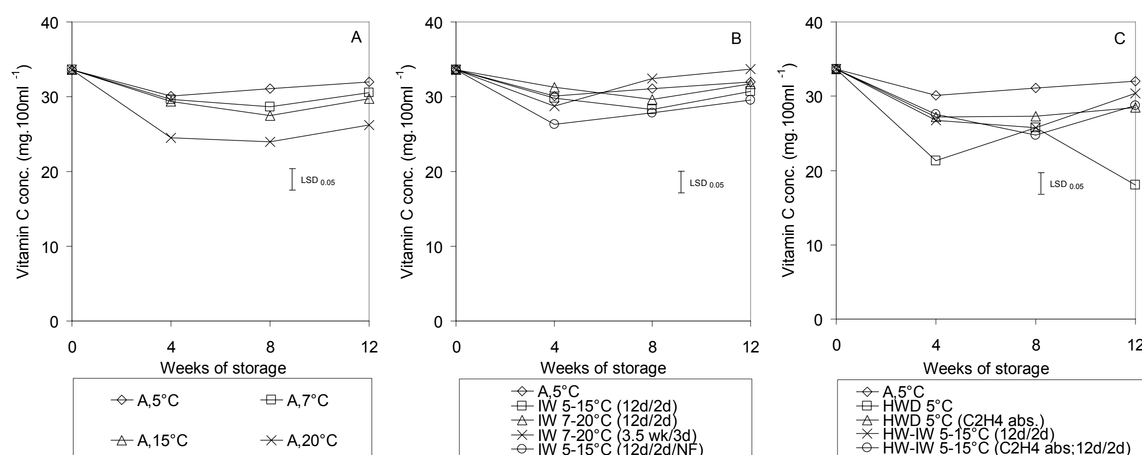


Figure 7.1 Retention of vitamin C in H3 fruit stored under RA at 5, 7, 15 and 20°C (A), IW conditions (5°C, 12 days and 15°C, 2days with and without fungicide; 7°C, 12days and 20°C, 2 days; 7°C, 3.5 weeks and 20°C, 3 days) (B) and HWD conditions (C) at 0, 4, 8 and 12 weeks of storage, respectively ($LSD_{0.05}$ = 2.988, n=2).

The behaviour of the HWD fruit may indicate that the HWD temperature employed was too high. Pretreatment at 52-53°C for 2 min caused heat injury on the peel (Fig. 5.30) and subsequently chilling injury was observed during storage (Fig. 5.31).

7.4 Effect of postharvest techniques on lime phytochemicals after storage

There have been few studies on retention of phytochemicals in lime fruit under different storage conditions. I have therefore characterised their composition at harvest and studied changes in the juice composition for a range of storage regimes.

7.4.1 Flavonoids of lime and their identification

There were 22 flavonoids observed after the juice samples were analysed by HPLC. A typical chromatogram showing peaks 1-22 at three different wavelengths of 280, 313 and 350 nm is shown in Fig 7.2.

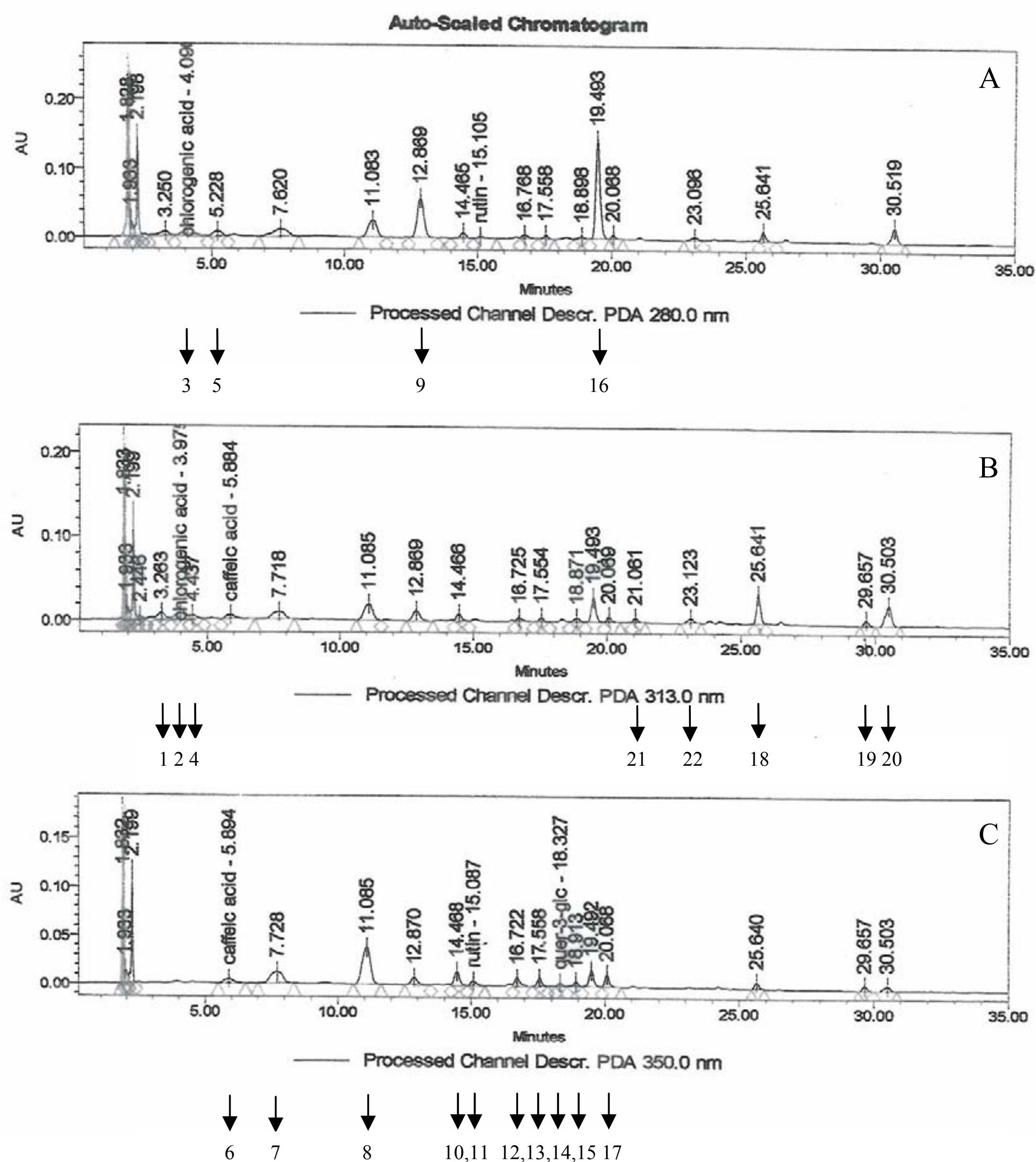


Figure 7.2 Three standard chromatograms of 22 flavonoid peaks in a lime juice sample showing the characteristic patterns of elution observed at (A) 280 nm, (B) 313 nm and (C) 350 nm.

Preliminary identification of the lime flavonoid peaks was undertaken by liquid chromatography-mass spectrometry (LCMS) by Nigel Joyce, PFR, Lincoln, New Zealand. LCMS could identify 10 of the peaks. Retention times and the wavelength (λ max) at which the highest absorbance was observed (280, 313 or 350 nm) of the 22 peaks measured are shown in Table 7.1. Ion fragment data are supplied in appendix IV Table A.2.

Table 7.1 Retention times and maximum wavelength for 22 peaks based on data from all days of flavonoid analysis.

Peak	Flavonoids	Common name	RT	λ max			References
				280	313	350	
1			3.26		√		
2			3.98		√		
3	Caffeic acid-O-glucoside(formic adduct)		4.09	√			
4			4.44		√		
5			5.22	√			
6			5.89			√	
7	Apigenin-6,8-di-C-glucoside	Vicenin-2	7.73			√	(1)
8	Chrysoeriol-6,8-di-C-glucoside[1]	Stellarin-2	11.09			√	(1)
9	Eriodictyol-7-O-rutinoside	Eriocitrin	12.87	√			(1, 2)
10	Quercetin-3-O-rutinoside	Rutin	14.47			√	(1)
11			15.09			√	
12	Chrysoeriol-6-C-glucoside (pos.Diosmetin)		16.72			√	
13	Naringenin-7-O-rutinoside	Narirutin	17.56			√	(1)
14			18.33			√	
15	Isorhamnetin-3-O-rutinoside		18.91			√	
16	Hesperetin-7-O-rutinoside	Hesperidin	19.49	√			(1, 2, 3)
17	Diosmetin-7-O-rutinoside	Diosmin	20.07			√	(1)
18			25.64		√		
19			29.66		√		
20			30.50		√		
21			21.06		√		
22			23.12		√		

[1] Based on mass of ion fragments and relative retention time to Apigenin 6,8-di-C-glucoside (Gil-Izquierdo *et al.,* 2004).

RT = Retention time

References:

(1) Gattuso *et al.* (2007)

(2) Miyake *et al.* (2006)

(3) Nielsen *et al.* (2006)

7.4.2 Principal components analysis (PCA) of changes in composition of flavonoids after storage

PCA was undertaken as an initial means to identify possible patterns and associations in the flavonoid composition in response to different postharvest treatments. PCA was conducted on log-transformed values of peak area of 20 flavonoid peaks. (Peak 19 was missing in most samples, and peak 21 had many missing points so these were not included). Genstat software (9th Edition; Lawes Agricultural Trust, Rothamsted Experimental Station, VSN International Ltd, UK) was used for the analysis. The flavonoid data were analysed in conjunction with Dr. Duncan Hedderley and Dr. Andrew McLachlan biometricians at PFR, Palmerston North, NZ.

The flavonoid data were analysed from day 0 (M0) until 3 months (M3) of storage for limes stored under RA at 2, 5 and 13°C (with and without GA_3), limes stored under high CO_2 (3% CO_2 and 10% O_2) at 2, 5 and 13°C, limes stored under low O_2 (0% CO_2 and 10% O_2) at 2 and 13°C, and limes stored under IW condition (2°C, 3 weeks and 13°C, 1 week), (Fig. 7.3 A-J).

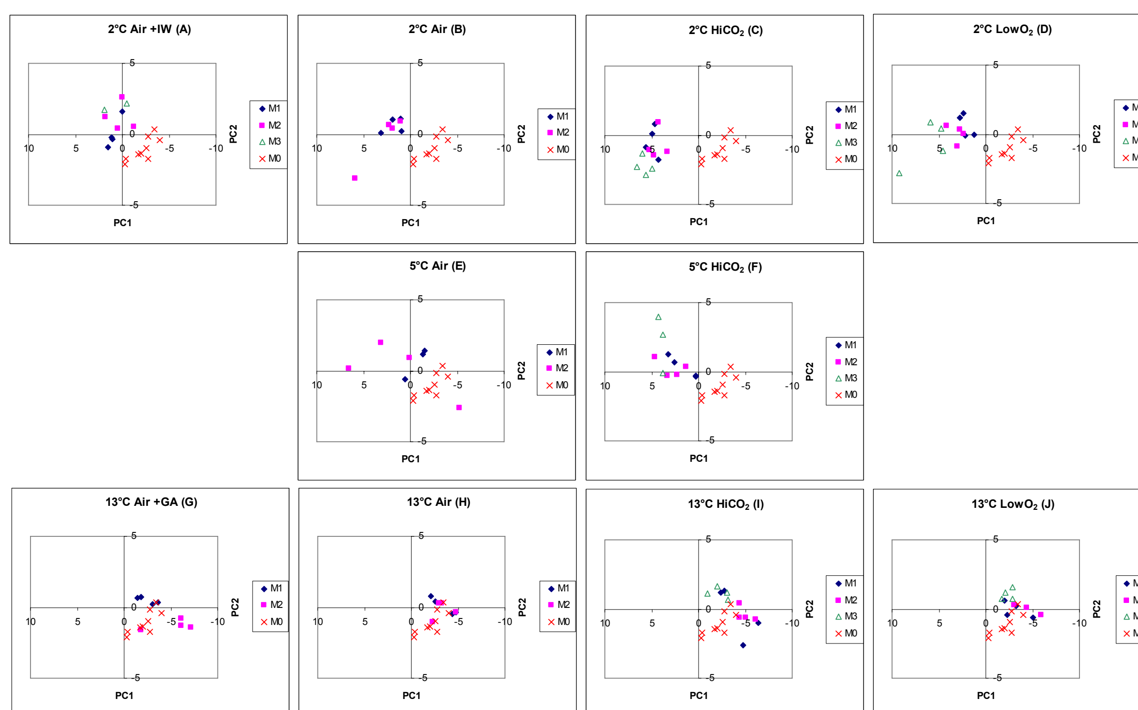


Figure 7.3 PCA of changes in composition of flavonoids after storage for 0 days to 3 months for limes stored under RA at 2, 5 and 13°C (with and without GA), limes stored under high CO_2 (3% CO_2 and 10% O_2) at 2, 5 and 13°C, limes stored under low O_2 (0% CO_2 and 10% O_2) at 2 and 13°C, and limes stored under IW condition (2°C, 3 weeks and 13°C, 1 week), H1.

Each point on the graph represents the flavonoid composition of one replicate batch of limes at one harvest time.

For the fruit stored under all storage conditions at 2 and 5°C (Fig. 7.3A to F) it is apparent that the flavonoid composition profile moved from a position just to the right hand side of the PC1 axis to the left hand side of PC1 (i.e. from negative to positive values) at both 1 month (M1) and 2 months (M2) (Fig. 7.3B and E) or 1 month (M1) and 3 months (M3) (Fig. 7.3A, C, D, F), respectively. All these treatments, regardless of the specific conditions, showed CI (see Fig. 4.10, 4.25 and 5.7); (Fig.7.3A to F). On the other hand, none of the fruit stored at 13°C (Fig. 7.3G to J) showed CI and the flavonoid composition profile for these fruit remained largely unchanged on the right hand side of the PC1 axis. Overall, the shift of flavonoid data on PC1 from the right to the left hand side appeared to be associated with CI of fruit during storage. The fruit stored under CA at 2°C with high CO_2 or low O_2 (Fig. 7.3C and D) were more severely effected by CI than the fruit stored under RA at 2°C (Fig. 4.25). This is supported by the trends in flavonoid composition, as the average flavonoid composition for the fruit stored under CA at 2°C (high CO_2 and low O_2) at 1 to 3 months (Fig. 7.3C and D) moved to the left hand side sooner (after 1 month) and more markedly than the fruit stored under RA at 2°C (Fig. 7.3B). CI was delayed in the fruit stored under RA at 2°C with IW (Fig. 7.3A) and changes in PC1 were less marked. The results indicate that IW delayed the changes of flavonoid composition better than other treatments at 1 month, but because the storage temperature (2°C) was too low the fruit were still affected by CI, especially after storage for 2 or 3 months (Fig. 7.3A).

The association between PC1 values and CI scores of H1 fruit was analysed and is shown in Fig. 7.4, using a simple weighted average to represent increasing severity of CI. PC1 values were averaged from four replications of each treatment and overall CI scores at each assessment time were calculated by Eq. 4.1 as described in chapter 4.

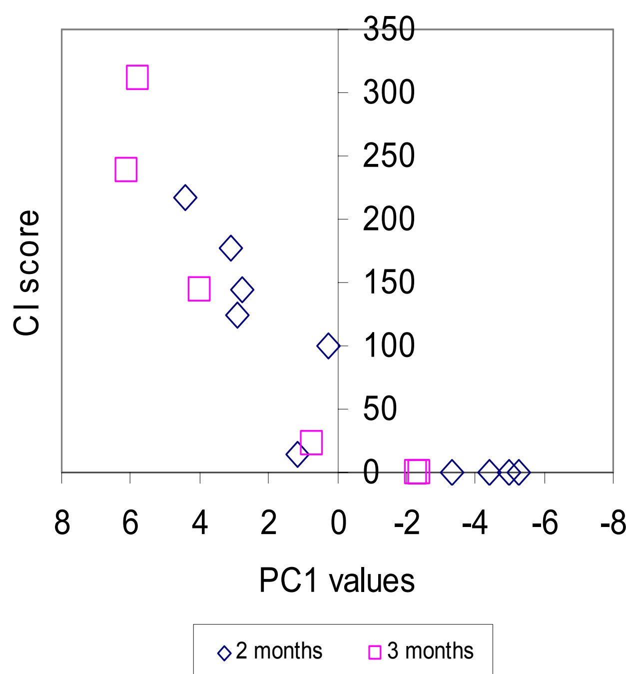


Figure 7.4 Association between PC1 values and weighted sum of CI scores for lime fruit, H1.

The CI scores of H1 fruit were strongly correlated with PC1 values (Fig. 7.4). In the absence of CI, PC1 remains between 0 and -6, and an increasing CI score is associated with an increasing trend to higher, positive PC1 values. Obviously, the detail of this relationship depends on the way CI scores are weighted, but the principle is clear: more positive PC1 values represent more severely chilling injured fruit.

Interpreting changes in PC2 values biologically is more problematic. The flavonoid composition of limes stored under CA at 2°C with high CO_2 at 3 months (Fig. 7.3C) had lower PC2 scores whereas limes stored under CA at 5°C with high CO_2 at 3 months had higher PC2 scores (Fig. 7.3F). Limes stored under RA at 2°C with IW conditions (Fig. 7.3A) at 1-3 months remained at the centre of the PC2 scale at 1 month but moved to positive values at 2 and 3 months. PC2 is therefore not responding to CI in a simple manner.

The trends in flavonoid composition of limes stored at an intermediate temperature (13°C) under RA, with and without GA_3, or CA with either high CO_2 or low O_2, are indicated in Fig. 7.3G, H, I and J, respectively. The major flavonoids of lime stored under RA at 13°C with and without GA_3 were grouped by PCA on the right hand side of PC1

and/or around the centre of PC2 scale and did not change much according to the time of storage. This suggests that since these fruit do not develop CI (Fig. 7.3G and H), there is little effect of storage on the overall composition of their flavonoids. After 3 months of storage under CA (at low O_2 alone or in combination with high CO_2), there was a slight tendency for an increase in PC2 values. There was also a tendency for PC2 values to increase from the use of CA (either low O_2/high CO_2 or low O_2 alone) at 13°C (Fig. 7.3I and J). However, since there were no data for flavonoid composition at 3 months for fruit stored under RA at 13°C with and without GA_3 (Fig. 7.3G and H), I do not know if this effect is due to the storage atmosphere or time.

In order to try to extract more information from the PC1 and PC2 scores, the latent vector scores were examined for each of the 20 flavonoids.

7.4.3 Latent vector analysis

Latent vector analysis was undertaken as a means to determine which flavonoids were driving the changes in PC1 and PC2 scores. The latent vector plot is shown in Fig. 7.5.

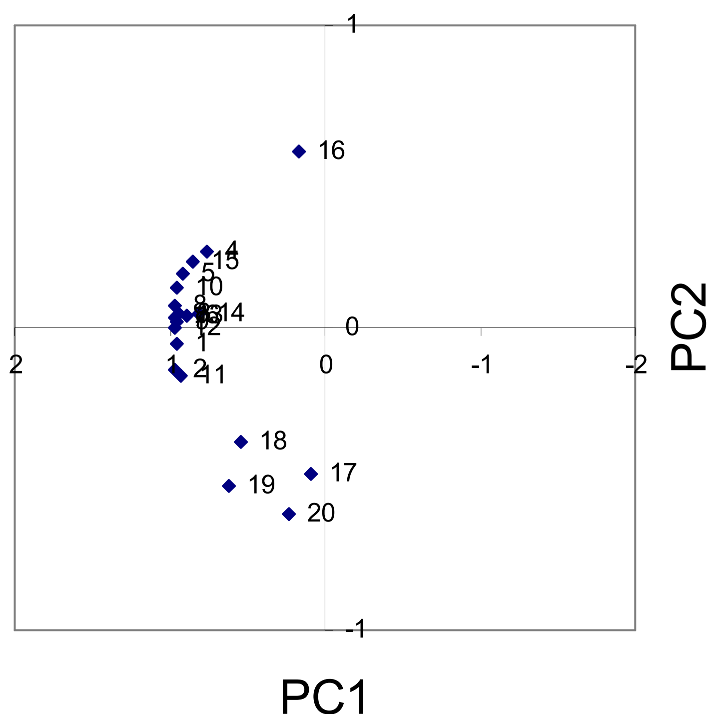


Figure 7.5 Latent vector analysis of changes in flavonoid composition of 20 separate compounds, H1.

Latent vector analysis ascribes to each data point (flavonoid peak in this case) how much of an effect it is contributing to the overall variability in the data set. I can see that the latent vectors for peaks 1-15 are all similar, with positive latent vectors for PC1 (Fig. 7.5). This means that if these compounds increase in concentration in any particular stored sample, they will move the data point representing the average flavonoid composition towards more positive values (see Fig. 7.3), which is what happens when CI is worse. These are the compounds that consistently increase in concentration under severe chilling and this increase is largely prevented by IW (see Fig. 7.6).

PC2 is strongly influenced by peaks 16-20. However, peaks 17-20 had many missing values and peak 16 showed an unusual pattern of change, especially at 3 months of storage. Peak 16, hesperidin (HSP), is the largest peak observed (Fig. 7.2) and changes in its concentration were quite different from peaks 1-15. In general, it did not markedly increase with chilling, but did increase under IW (see Fig. 7.8A). Peaks 17-20 were not always present, were generally lower in concentration, and sometimes disappeared over time during storage.

7.5 Changes in composition of selected lime flavonoids

7.5.1 Introduction

In this research, the flavonoids neoponcirin (NPO), isorhoifolin (IRF) and naringin (NRG) (see Table 2.2) were not found in my samples. However, five known compounds among the lime flavonoids were selected from the 22 peaks in Table 7.1 for further analysis. Four were chilling-affected flavonoids: vicenin-2 (apigenin-6,8-di-C-glucoside: peak 7), eriocitrin (eriodictyol-7-O-rutinoside: peak 9), rutin (quercetin-3-O-rutinoside: peak 10), and narirutin (naringenin-7-O-rutinoside: peak 13). The fifth was the most abundant flavonoid, hesperidin (hesperetin-7-O-rutinoside: peak 16). Characteristic changes in these selected flavonoid compounds are presented in Fig. 7.6-7.8.

7.5.2 Changes of composition of individual flavonoids

The four chilling-affected flavonoids showed consistent patterns of change in apparent concentration (as measured by peak height) in H1 limes (Fig. 7.6). The flavonoid content stayed stable for up to 2 months at 13°C (Fig. 7.6A to D), but increased during storage at 2°C. IW (2°C, 3 weeks and 13°C, 1 week) reduced subsequent accumulation of each flavonoid (Fig. 7.6A, B, C and D).

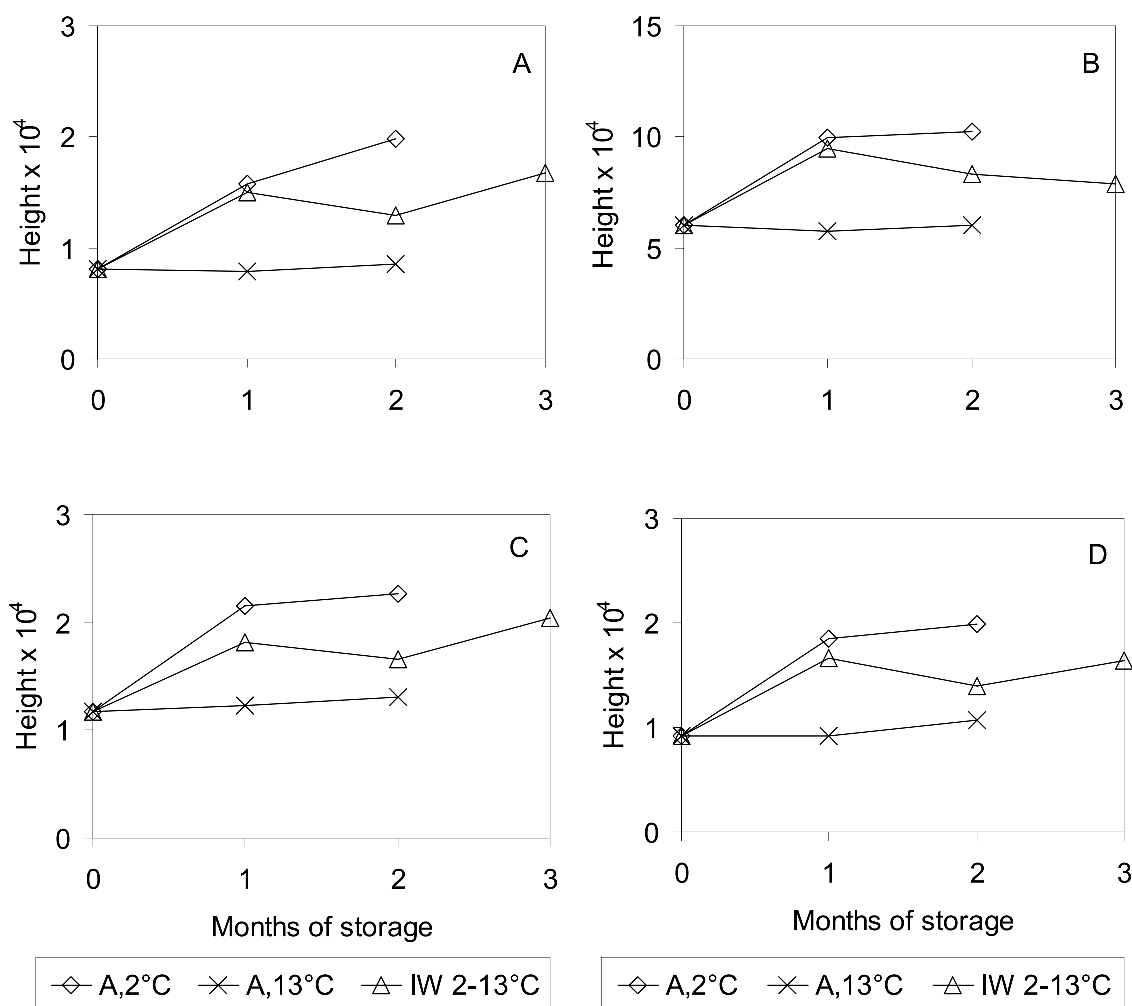


Figure 7.6 Characteristic changes in peak height for selected lime flavonoids in fruit stored under RA at 2 or 13°C, or IW at 2°C for 3 weeks and 13°C for 1 week: peak 7 (Apigenin-6,8-di-C-glucoside) (A), peak 9 (Eriodictyol-7-O-rutinoside) (B), peak 10 (Quercetin-3-O-rutinoside) (C) and peak 13 (Naringenin-7-O-rutinoside) (D), H1.

There were similar trends in flavonoid composition in H3 fruit (compare Fig. 7.6A, B, C and D with Fig. 7.7A, B, C and D), although the storage temperatures were higher and the changes in composition were less marked.

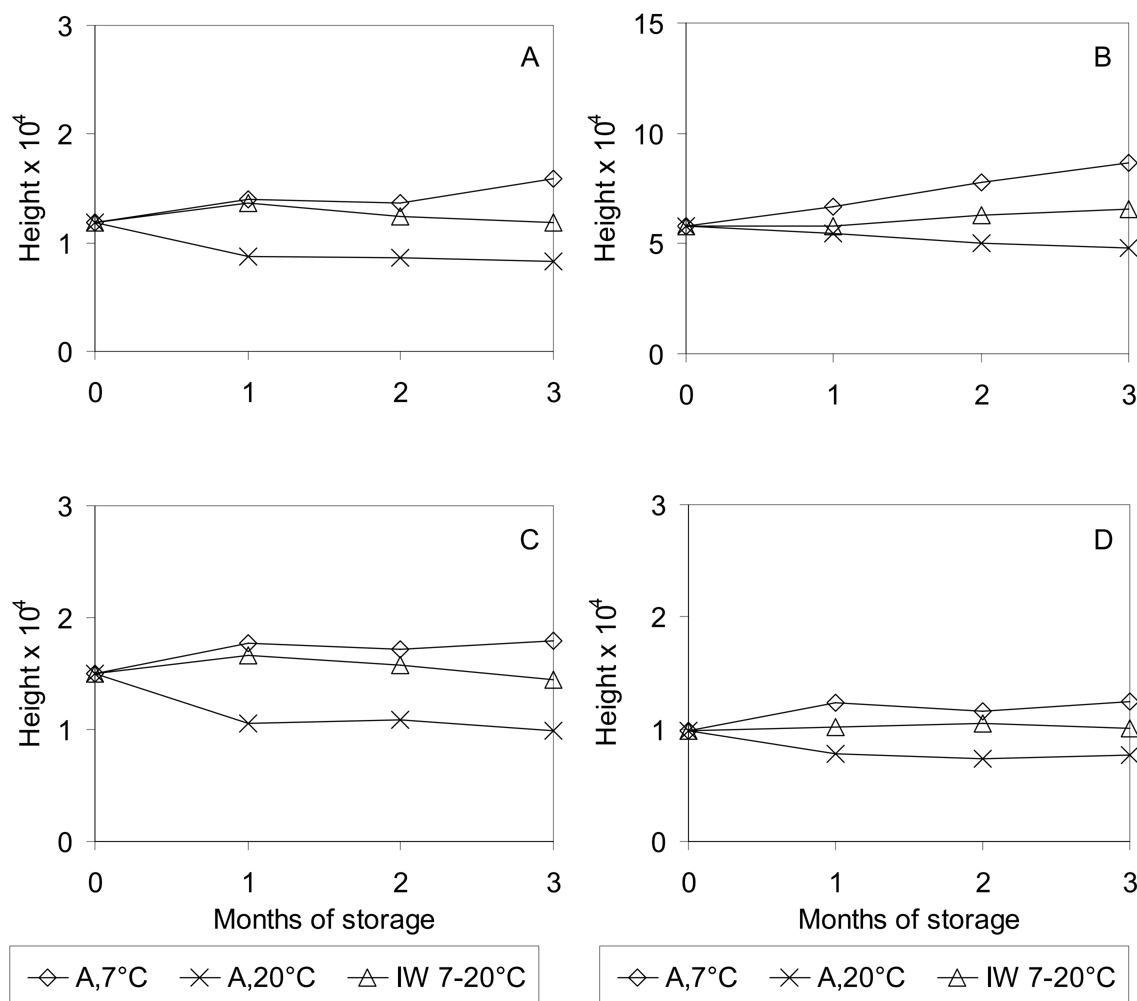


Figure 7.7 Characteristic changes in peak height for selected lime flavonoids in fruit stored under RA at 7 or 20°C, or IW at 7°C, 12 days and 20°C, 2 days: peak 7 (Apigenin-6,8-di-C-glucoside) (A), peak 9 (Eriodictyol-7-O-rutinoside) (B), peak 10 (Quercetin-3-O-rutinoside) (C) and peak 13 (Naringenin-7-O-rutinoside) (D), H3.

In H3, the constant non-chilling temperature evaluated was 20°C and the chilling temperature was 7°C. As for H1, there was a consistent increase in flavonoid content over 3 months of storage at the lowest temperature and in this case a slow and continuous decrease at the highest temperature. Fruit stored under a rapid cycling IW condition (7°C, 12 days and 20°C, 2 days) exhibited an intermediate pattern with an initial increase in the concentration of each flavonoid after 1 month, as in H1, but with little change thereafter (Fig. 7.7A-D).

7.5.3 Changes in hesperidin concentration

The main flavonoid peak in my study was hesperidin and the changes in HSP content for different storage regimes in H1 and H3 are compared in Fig. 7.8.

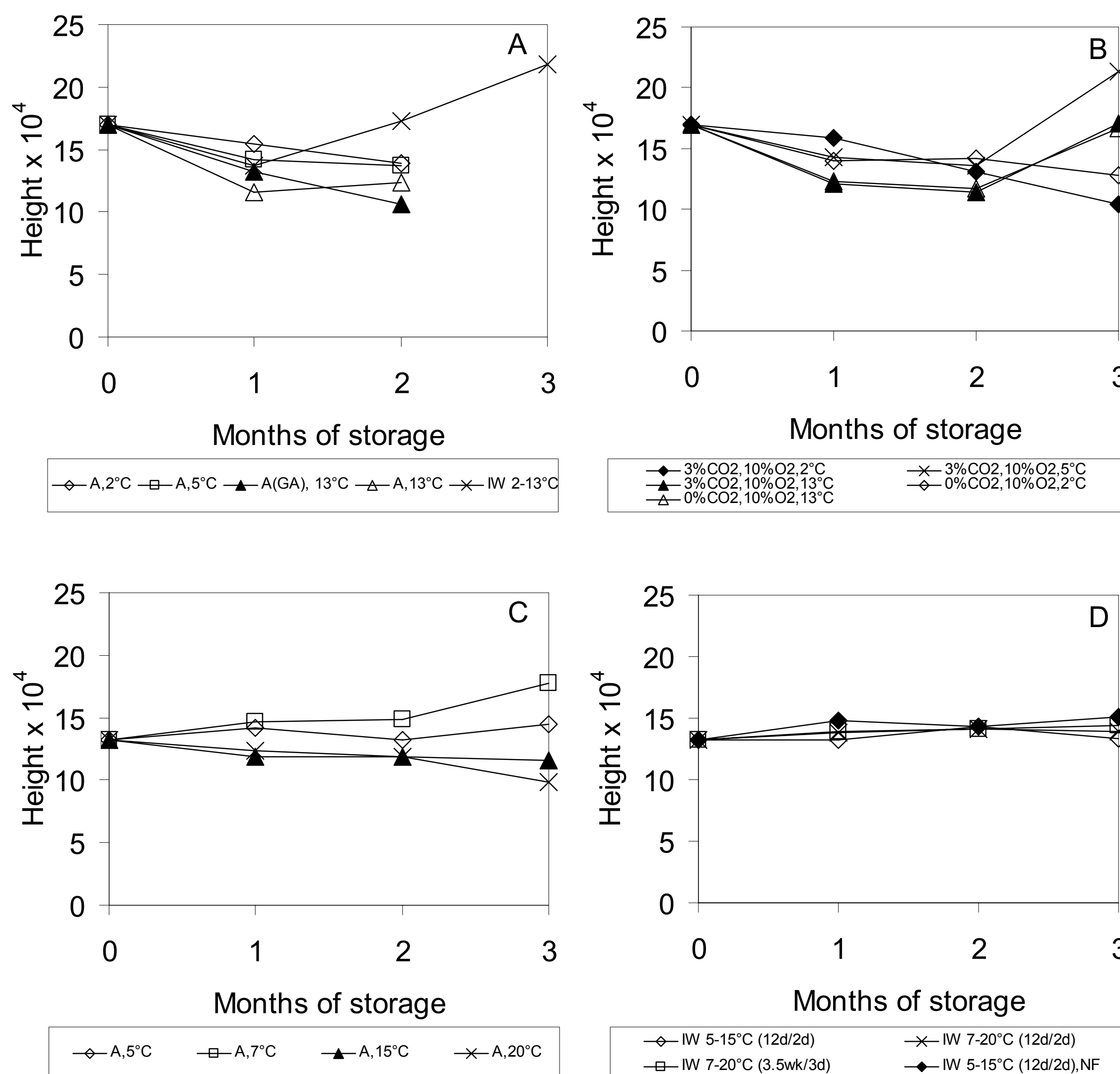


Figure 7.8 Characteristic behaviour of Hesperetin-7-O-rutinoside (peak 16) in H1 limes stored under RA at 2, 5 and 13°C (with and without GA_3). IW condition at 2°C, 3 weeks and 13°C, 1 week (A), or CA (0 or 3% CO_2 with 10% O_2) at 2, 5 and 13°C (B), and H3 limes stored under RA at 5, 7, 15 and 20°C (C), or IW conditions (5°C, 12 days and 15°C, 2 days with and without fungicide), (7°C, 12 days and 20°C, 2 days or 7°C, 3.5 weeks and 20°C, 3 days) (D).

There was generally a slight reduction in HSP content under non-chilling temperatures (Fig. 7.8A, C) and a small increase at the mid-chilling temperature (7°C) (Fig. 7.8C). The

fruit stored under either high CO_2/low O_2 or low O_2 at non-chilling temperature (13°C) showed an increase of HSP at 3 months of storage (Fig. 7.8B). In contrast, the fruit stored under CA (high CO_2/low O_2 or low O_2) at a chilling temperature (2°C) showed a reduction of HSP during storage for 3 months (Fig. 7.8B). Slow-cycling IW at low temperatures showed an increase of HSP after 1 month of storage (Fig. 7.8A). Fast- or slow-cycling IW (5↔15°C or 7↔20°C) at higher temperatures appeared to prevent any change in HSP concentration during storage (Fig.7.8D).

7.6 Discussion

Citrus fruit and their products are significant sources of dietary vitamin C and flavonoids. Variability of vitamin C content in fresh fruit is commonly attributed to differences in variety, cultural practice, maturity stage, climate, fresh fruit handling and storage conditions (Nagy, 1980). Vitamin C from fruits and vegetables is commonly regarded as one of the most important vitamins for human nutrition (Lee and Kader, 2000) and citrus are among the best sources of this vitamin (Hernández *et al.,* 2006).

From this research I found that 'Tahitian' lime grown in NZ contains approximately 34 $mg.100ml^{-1}$ (Fig. 7.1). This figure is similar to other reports. For example, the initial ascorbic acid value of Brazilian 'Tahiti' limes before storage was ~ 31 $mg.100ml^{-1}$ (Kluge *et al.,* 2003a) while Nagy, (1980) and Ziena, (2000) reported vitamin C contents in the ranges of 39-62 $mg.100\ ml^{-1}$ and 15-45 $mg.100\ ml^{-1}$ of lime juice, respectively. Jomori *et al.* (2003) reported that 'Tahiti' limes contained about 22-29 $mg.100ml^{-1}$ vitamin C regardless of storage temperature or treatment with 1-MCP, and Ohnmar *et al.* (2006) reported very similar concentrations for the West Indian lime (*C. aurantifolia*, Swingle) grown in Thailand. Given the wide range of climatic, cultural, varietal and storage handling practices between these countries, it is interesting that there is such close agreement regarding the vitamin C content.

Lee and Kader, (2000) reported that the most important factor to maintain vitamin C of fruit and vegetables after harvest is temperature management, because vitamin C loss can be accelerated at higher temperatures and with longer storage durations. These losses are also increased by low RH, physical damage and CI. Nagy, (1980) reported that vitamin C

can be lost from all citrus fruits if the fruit are stored at high temperatures. The range of temperatures and extent of vitamin C loss depended on the type of citrus fruit.

In this work, limited loss of vitamin C was only observed during continuous storage at 20°C; there was little change in vitamin C content of the fruit during cold storage between 5 and 15°C.

It would be unfortunate if IW and HWD treatments led to a loss of this valuable nutrient and given the elevated temperatures that can be employed in such treatments this possibility existed and required to be investigated. Kluge *et al*., (2003a) reported that 'Tahiti' lime fruit stored under an extreme IW treatment (IW-3; 38°C, 1 day and 5°C, 14 days) lost approximately 30 and 50% of ascorbic acid from at-harvest values after 30 and 60 days, respectively. However in this work the fruit stored under a range of IW conditions maintained their ascorbic acid content as well as the fruit stored under low temperature storage. For example, the fruit stored under both lower and higher temperature IW conditions (5°C, 12 days and 15°C, 2 days and 7°C, 3.5 weeks and 20°C, 3 days) retained vitamin C as well as 5°C stored fruit until 12 weeks of storage (Fig. 7.1B). This is similar to the findings of Kluge *et al*., (2003a) at their lower temperature regimes. In their study, ascorbic acid content of the fruit stored under RA at 5°C and IW-1; 20°C, 2 days and 5°C, 7 days, and IW-2; 20°C, 2 days and 5°C, 14 days (with and without 1.0 µl l^{-1} of 1-methylcyclopropene (1-MCP)) were not significantly different in their vitamin C concentration after 30 and 60 days, respectively.

Artes *et al*., (1993) also reported that the ascorbic acid content of 'Primofiori' lemons could be maintained by IW storage. They used five IW conditions (2°C, 1 week and 13°C, 3 weeks; 8°C, 1 week and 13°C, 3 weeks; 2°C, 2 week and 13°C, 2 weeks; 2°C, 3 week and 13°C, 1 weeks; 8°C, 3 week and 13°C, 1 weeks) and their results suggested that the best treatment was IW of 2°C, 2 week and 13°C, 2 weeks. This gave an apparent increase in ascorbic acid content (14%) whereas the rest of the IW treatments retained ascorbic acid at the harvest levels. This optimal IW treatment also prevented *Alternaria* rot, peteca (a collapsing of the fruit surface and associated disorganized tissue), oleocellosis and rind pitting and reduced the incidence of 'red blotch' disorder. Vitamin C content was also reported to be maintained in peaches (*P. persica*, cv. Zhonghuashoutao) when the fruit were stored under IW (1°C, 24 days and 22°C, 1 day) (Ruoyi *et al.,* 2005).

The adverse effect of elevated temperature was however evident in HWD fruit. In this work, HWD at 52-53°C led to a significant loss of vitamin C (approximately 37% and 46% after 4 weeks and 12 weeks of storage, respectively) unless IW was applied during subsequent storage or an ethylene absorbent was applied (Fig. 7.1C). This is possibly because the pretreatment by HWD at 52-53°C for 2 min caused heat injury of the lime peel (Fig. 5.30) and subsequent CI during storage (Fig. 5.31) but it is significant that IW mitigated this loss of vitamin C. Overall, it appears that providing the exposure to elevated temperatures (≥20°C) is limited, vitamin C loss is minimal and IW treatments in the warm temperature range of 15-20°C can be applied without loss of nutritional quality.

Flavonoids are regarded as one of the most important groups of dietary phenolics in plants (King and Young, 1999) and flavonoids in fruit and vegetables are implicated as active antioxidants (Lurie, 2003). Flavonoids also contribute to taste and colour of plants and may have specific roles as regulators of gene expression (Ooghe *et al.,* 1994; Dugo *et al.,* 2005). *Citrus* species contain a variety of flavonoids that possess a wide range of properties and positive health benefits on humans (Craig, 1997; Dugo *et al.,* 2005). In this work, changes of flavonoid compositions of H1 and H3 limes stored under several postharvest conditions were studied qualitatively. The major flavonoids found in my study are the same types of flavonoids as previously reported by Mouly *et al*., (1994) who demonstrated that the major flavanone glycosides in lemon and lime juices were eriocitrin and hesperidin at about 47-94 mg l^{-1} and 84-196 mg l^{-1}, respectively.

The H1 fruit exposed to extreme chilling temperature developed severe CI and showed significant changes in flavonoid composition. H3 fruit were exposed to less severe regimes and showed lesser CI symptoms. The pattern of flavonoid composition changes H3 fruit was consistent with the H1 group but the changes were less marked in some key compounds. Because IW was effective in minimising CI, I am now able to say that the flavonoid composition will remain similar to the composition at harvest if fruit are stored well, therefore the health benefits of optimally-stored limes are likely to be similar to those in freshly harvested limes. IW treatments thus both extend the storage life of lime fruit and preserve the nutritional quality of the fruit during storage.

In order to understand more clearly the changes in enzyme and regulatory gene expression that may be altering the flavonoid composition during chilling injury, it is now possible to do microarray studies on *Citrus* species (Pons *et al.,* 2005). This was not attempted in my present study but could be useful to further elucidate the dual role of IW in protecting against CI and loss of key nutrients.

Since there was a characteristic change in flavonoid composition induced by CI, it may also be possible to turn this observation into a method for detecting early CI during storage. For optimal management of fruit quality it would be desirable to identify when there is a risk of injury before CI disorders (appear on the rind). This could also be further investigated. However it is also possible that the chlorophyll fluorescence technique may have greater potential as a rapid, non-invasive indicator of stress (Schreiber *et al.,* 1994; DeEll *et al.,* 1995). For example, changes in the fluorescence signal occur in response to O_2 stress in apples (*M. domestica*) cv. Cortland, Delicious, Golden Delicious, Honeycrisp, Jonagold and McIntosh (DeLong *et al.,* 2004; DeLong *et al.,* 2007) and kiwifruit (*A. deliciosa*) during CA storage (Lallu and Burdon, 2007). This observation has led to the development of a new technology called HarvestWatch^{TM} that uses electronic sensors to measure fluorescence emitted from chlorophyll when excited by light (DeLong *et al.,* 2004; DeLong *et al.,* 2007; Lallu and Burdon, 2007).

Obenland and Neipp, (2005) demonstrated that chlorophyll fluorescence imaging could be used as an indicator to detect and localise early rind injury in green lemons (*C. limon* (L.) Burn) after HWD treatment at 55°C for 5 min. They found that chlorophyll fluorescence imaging could identify areas of early rind injury before visual symptoms were detected. They also suggested that this technique will be useful for other forms of postharvest rind disorders in citrus and may facilitate the study of the biochemical principle of the disorders. Nedbal *et al*., (2000) also demonstrated that chlorophyll fluorescence could be used to distinguish between healthy and damaged or infected fruit as an early detection system before the damage could be observed visually.

In summary, I can conclude that all storage conditions of < 20°C and IW regimes with limited time (< 12d overall) at 20°C do not result in any significant changes in major nutrients in ‘Tahitian’ lime fruit. Thus there is good prospect for increasing storage life without compromising the health benefits of citrus fruit.

CHAPTER 8

Overall discussion and conclusions

The objective of this study was to determine what storage conditions and pretreatments would permit long term storage of NZ limes with minimal loss of quality. Low temperature storage, use of controlled atmosphere (CA) environments, and treatment of fruit with physiologically active agents such as gibberellic acid (GA_3) were examined for their ability to slow degreening, whilst the use of fungicide (thiabendazole, TBZ), hot water dipping (HWD), temperature conditioning (step down technique) and intermittent warming (IW) were further investigated to protect the fruit against rots and CI during cold storage.

Although many consumers may prefer non-chemical treatments of their foods, the use of the fungicide TBZ is an effective means of reducing rots of limes. The effectiveness of this fungicide on limes was increased when it was used in combination with HWD but it still did not completely prevent growth of *Botrytis*.. TBZ has also been regarded to have a secondary effect to protect limes against CI: in this respect my results showed similar beneficial effects of TBZ in combination with HWD against CI as was seen on 'Tarocco' oranges (Schirra and Mulas, 1995b), and on 'Marsh' grapefruit (Wild, 1993). However, the use of this fungicide can adversely affect lime skin because it appears to exacerbate heat injury at higher HWD temperatures. Even though the use of the fungicide can protect lime against rots and CI, there is a global trend towards reducing postharvest chemical treatments. In this research, I used the fungicide because I wanted to identify the effects of different postharvest regimes on lime colour changes and I did not want the results to be confounded by disease effects.

I have defined an effective range of HWD temperatures for slowing yellowing of limes during storage for 12 weeks. Fruit can be safely dipped at temperatures between 42 and 47°C for 2-4 min. If higher temperatures for HWD were used, or longer durations of dipping, heat injury on the fruit skin resulted with subsequent disorders such as pitting or

CI and rots after long storage periods. Lower temperatures were largely ineffective for colour maintenance.

I have therefore confirmed the effectiveness of 'mild' HWD treatments in delaying subsequent colour change and my temperature data suggest that only the flavedo needs to experience these warm temperatures.

Hot water rinsing and brushing (HWRB) is used commercially and the effective range of temperatures is between 48 and 63°C for very short periods of 10-25 seconds. This technique has been reported to protect against pathogens that cause surface decay. This technique is easy to use and has a short operating time, and it is very efficient in heat transfer. Furthermore, the cost of this technique is lower than commercial vapour heat treatment (Fallik, 2004); therefore this technique might be considered for lime fruit, and would require optimisation to determine the precise time and temperature combinations that are effective. Since the range of HWD temperatures tested here in combination with low temperature storage (5°C) was still not enough to retain lime quality for 10-12 weeks of storage, therefore other postharvest treatments were investigated.

Postharvest treatments investigated in this work were cold storage, controlled atmospheres (CA), intermittent warming (IW) and temperature conditioning (TC). Advantages and disadvantages of these techniques are explained as follows.

A range of constant regular air (RA) storage from 2 to 20°C was investigated for lime. I found that low temperature storage alone (about 9-10°C), as was recommended by Murata, (1997) and Ladaniya, (2004) did delay 'Tahiti' lime quality losses, reducing mass loss, delaying degreening, maintaining fruit firmness and reducing rot development when compared with the fruit stored at room temperature (20°C) in my trials. However, the use of low temperature storage alone was not enough to maintain the quality of lime for long storage periods (greater than 8 weeks). Ladaniya, (2004) reported that limes showed some undesirable yellow colour after 3 or 4 weeks at 9-10°C. In my tests, storage temperatures that were too low or too high reduced lime fruit quality during storage. If the temperature was too low, it caused disorders such as pitting or chilling injury (CI) whereas if the temperature was too high, colour loss and mass loss were too high.

I demonstrated that different ranges of storage temperatures influenced fruit quality. Low storage temperature below 5°C slowed the rates of quality change for several indices and a range of temperatures from 5 to 13°C gave predictable effects for fruit quality, particularly colour change. These low storage temperatures also slowed mechanisms of fruit ripening (e.g. texture, respiration and ethylene production rate). At the higher temperatures evaluated (15 to 20°C) colour change was fast and followed different kinetics. Therefore I selected the lowest temperature (5°C) that best maintains lime colour change for further analysis. This selected storage temperature was tested in combination with other storage conditions such as controlled atmosphere storage or IW to extend storage life and shelf life of lime.

Controlled atmosphere storage (CA) is a powerful postharvest treatment used commercially for apples and pears in particular. In my work, CA (0 or 3% CO_2 with 10% O_2) was not a suitable treatment for lime under low storage temperature (2 or 5°C). Many researchers have reported beneficial effects of CA on extending the normal storage life and reducing the rate of degreening or chlorophyll loss in lemons or oranges, and therefore reducing the ripening-related increase in disease susceptibility (Monzini and Gorini, 1973; Shrikhande and Kaewubon, 1974; Wild *et al.,* 1976; Bertolini *et al.,* 1991; Artes *et al.,* 1993). However storage of lemons under CA without ethylene absorption led to high mold incidence (Wild *et al.,* 1976) and Alternaria (*A. citri* Ell. & Pierce) rot and red blotch in lemons stored under CA (Artes *et al.,* 1993). Furthermore, lemons stored under some CA conditions can suffer from membranosis and rind pitting (two types of CI) (Bertolini *et al.,* 1991). The few studies on ‘Tahiti’ limes showed that the fruit developed severe injury and decay during CA storage (Salama *et al.,* 1965; Spalding and Reeder, 1974; Hatton *et al.,* 1975). However, beneficial effects of CA on retarding loss of green colour or reduced loss of chlorophyll and colour changes have been reported (Spalding and Reeder, 1974; Sritananan *et al.,* 2006). My results confirmed the effects of CA on delaying degreening of limes when the CA fruit were stored at 9 or 13°C compared with RA storage, if TBZ was used to protect against rots.

However the CA treatments did not delay overall lime quality losses beyond 8 weeks. All CA treatments at low temperatures (below 7°C) led to severe CI and the development of rots. Furthermore, this technique is more complicated to implement and needs significant

infrastructure investment before it can be applied commercially. This confirmed that CA storage is not the best treatment to retain quality of lime fruit during storage.

In my work I found that storing fruit at 5°C could give some benefits for lime storage if other techniques could be found to reduce CI. Sala and Lafuente, (1999) reported that even though the low storage temperature has limitations resulting in CI, low storage temperature continues to be the most useful method to maintain and prolong the postharvest life of horticultural products during storage and has potential as a treatment for fruit fly disinfestation. But in order to get most benefits from low temperature storage for limes, treatments that reduce CI must be used.

Beneficial effects of IW and TC on citrus are well known. In my work, I concluded that IW is the most effective storage regime for NZ limes. The benefits of this technique have been demonstrated for many citrus fruit cultivars (Cohen *et al.,* 1983; Cohen *et al.,* 1990a; Cohen *et al.,* 1990b; Artes *et al.,* 1993; Schirra and Mulas, 1995a; Schirra and Cohen, 1999; Porat *et al.,* 2003) including lime (Kluge *et al.,* 2003a; Kluge *et al.,* 2003b). Two recent reports on 'Valencia' oranges, 'Murcott' tangor and 'Tahiti' lime stored under TC and IW treatments have been released in 2006-07 (Kluge *et al.,* 2006; Kluge *et al.,* 2007). They demonstrated that 'Tahiti' lime and 'Murcott' tangor could be stored at 1°C for 90 days with no CI under IW condition (1°C for 6 days and 25°C for 1 day) whereas the control fruits of lime and 'Murcott' tangor showed CI after 30 and 45 days, respectively. The control group of 'Valencia' oranges was affected by CI after 45 days of cold storage but the fruit stored under IW and TC condition (37°C for 2 days then stored at 1°C) showed reduced incidence of CI and extended storage life. The authors concluded that efficient reduction of CI in these citrus varieties can be obtained by IW treatment and that this afforded better protection to the fruit than the TC treatment. The IW treatment is the most efficient treatment to reduce CI and extend storage life of 'Tahiti' lime and 'Murcott' tangor, with satisfactory quality retained for 90 days at 1°C.

The results from my work also confirmed that IW treatment at 5°C for 12 days and 15°C for 2 days effectively reduced degreening and incidence of CI in lime fruit. The most effective treatment involved continuous temperature cycling throughout storage (i.e. 6-cycle IW) but I found that just two cycles of IW was almost as good for maintaining green colour and reducing CI. I also tested the effects of different delays before the first

warming periods from 12 days at 5°C to 16, 20 and 24 days respectively, and I found that as long as the first warming cycle was applied within 20 days, it was effective at reducing degreening and CI. Even though the use of continuous cycles of IW was the best postharvest regime for lime, it may be difficult for practical implementation at a commercial scale.

In this work I also tested the combination of a pre-treatment by HWD at 52-53°C for 2 min before storing the fruit under IW (5°C for 12 days and 15°C for 2 days) compared to application of RA, HWD or IW alone for lime storage. The combined treatments showed similar results on retaining fruit quality compared with application of the IW alone. I noticed that the combined treatment controlled rots (see Fig. 5.33) better than IW alone; the latter showed about 2-3% rots at 10 weeks (Fig. 5.14) while combined treatments showed only limited rots (~ 10% fruit affected) at 12 weeks. Therefore I concluded that there is not much additional benefit in applying the HWD before IW for lime storage.

There are two practical ways to implement IW. One is to move the fruit from a low temperature (5°C) room to a different room at the warm temperature (15°C) and then move the fruit back after warming. The second approach would be to leave the fruit in the same cold room and change the temperature in the room from 5 to 15°C in a periodic cycle, using forced air to accelerate temperature equilibration. Moving the fruit between rooms would come at a significant labour and logistical cost.

My results show that rapid changes in temperature are effective in protecting the fruit against storage disorders and maintaining fruit quality. I do not know if a more gradual temperature change would be as effective. I do not know if a 10°C increase is absolutely required, or of it is enough to raise the fruit to a 'safe' temperature such as 9°C for a few days in order to allow the tissue of the fruit to recover from a stress condition. I do not know whether beneficial IW treatments lead to HSP induction or whether the warmer temperatures just allow the tissues to metabolise excess intermediates accumulated during chilling or replenish substances which were not able to be synthesised during chilling. This technique may permit repair of chilling-induced damage to fruit membranes and organelles or metabolic pathways (Wang, 1990).

Selecting storage containers for lime is also important during the practical implementation of IW for lime storage. A retail package or a bin may be used to store limes during IW. The use of retail packages may cause many problems such as condensation, which encourages the development of rots and if disorders such as pitting or CI occur during storage, this will lead to higher costs for repacking the fruit before sale. Alternatively, after picking the fruit from an orchard the fruit may be put directly into a bin for pre-treatments or cold storage. In this case IW would be easier to imlement. Storing the fruit in the bin will be more practical because the air movement around the fruit in a cold room can be achieved more easily than when fruit are packed in a retail package and this will enhance heat transfer to (and from) the fruit.

An important issue when fruit are transferred from a cold room (5°C) to a 15°C room is condensation. Warm air holds more water vapour than cold air. For example, the maximum amount of water vapour that can be held in air at 5°C is about 6 g water vapour per kg dry air whereas at 15°C the saturation humidity ratio of water in air is about 11 g water vapour per kg dry air. Thus if fruit are moved from 5°C to 15°C there will be some amount of water that will condense on the fruit surface. This issue can be solved by reducing the relative humidity (RH) in the 15°C room to about 50% RH so that the maximum amount of water vapour will then be less than 6 g water vapour per kg dry air ;and condensation will not occur. However, reducing the RH in the storage room will cause mass loss in the produce. In the case of lime this should not be a big problem when compared with other perishable products. Motlagh and Quantick, (1988) found no significant reduction in weight loss (ranging from 18-21% weight loss) between Brazilian limes (*C. aurantifolia* cv. Persian) stored at 'temperate ambient' conditions (15-20°C, 40-60% RH) compared to the fruit treated with 1.5, 2.0 and 2.5% of a permeable sucrose ester coating material (Pro-long) up to 13 days of storage. Case hardening (a symptom that occurs when the rind's moisture loss exceeds the rate of moisture transfer from pulp to rind) was reported in the control fruit after 13 days. In my case, I stored the fruit in the 15°C, 50% RH for only 2 days and this would lead to little moisture loss during this time because the rate of water loss of lime is intrinsically low.

As the 2 cycle IW was almost as effective as the 6 cycles in my tests, this might be more adaptable to practical management of lime storage. The IW treatments used by Kluge *et al.* (2006) and Kluge *et al.* (2007) may be more difficult to implement because storing the

fruit in the IW condition at 1°C for 6 days and 25°C for 1 day for 3 months meant that the fruit needed to go through 12 cycles of IW, involving significant costs in transferring the fruit and significant risks from condensation or human error.

Since two IW cycles provided fruit of high acceptability after storage, it may be possible to do the first warming of IW fruit in bins in a controlled temperature room and use the second IW period to pack fruit into retail packages. The fruit should then store well at 5°C before final retail sale for a further 9-10 weeks. Of course the retail package would have to support ongoing respiration during extended storage.

Significant changes in flavonoid composition occurred in H1 fruit only after the fruit suffered from severe CI. The pattern of flavonoid composition changes in H3 was similar to and consistent with the H1 group but CI was less marked. Because IW was effective in minimising the severity and incidence of CI, flavonoid composition will remain similar to the composition at harvest if fruit are well stored. Therefore I now can indicate that the health benefits of well-stored limes are likely to be similar to those in freshly harvested limes. IW treatments extended storage life of lime fruit and preserved the nutritional quality of the fruit during storage.

I found some biological variation in colour change of limes during storage even though they were harvested from the same tree and at the same time. Lime colour changes sometimes followed the biological age concept (Hertog *et al.,* 2004). Application of the biological age concept to limes stored at 13 (or 15°C) revealed that (for H1 lime fruit (40% yellow)), the green and yellow side data sets matched closely with the time adjustment concept (see Fig. 6.5) and this confirmed that the most yellow side continued ripening at the same rate as pre-harvest ripening. However, when greener fruit (25% yellow of H3 and H5) were tested, I found that post-harvest changes of the yellow side did not match those of the green side when the adjustment function was applied (see Fig. 6.6 and 6.7). Instead, the green side degreened faster than the yellow side for both harvests. It appeared that for these fruit the degreening process on the green and yellow sides are different, possibly as a result of production practices or environmental effects (e.g. sun exposure) therefore, the concept of ‘biological age’ could not be applied in a simple manner to these fruit.

The logistic equation fitted appropriately the changes in lime colour change for both H° and colour score (CS) and it can therefore be used to predict lime colour change (degreening). The activation energy (E_a) was calculated using the Arrhenius equation for degreening based on H° and CS during air storage and was estimated to be ~36-53 and 86 kJ mol^{-1}, respectively which is within the range of E_a of enzymic reactions of other food deterioration processes (between 42-126 kJ mol^{-1}) (Robertson, 1993). Higher E_a values have been reported for colour change in tomatoes (138-170 kJ mol^{-1}) and dark lemons (107 kJ mol^{-1}, based on my calculations from digitised data); but lower values are reported for bananas in the temperature range 10-28°C (21-36 kJ mol^{-1}) (Cohen and Schiffmann-Nadel, 1978; Chen and Ramaswamy, 2002; Hertog *et al.,* 2004).

I used the Arrhenius equation to predict H° and CS of limes stored under different IW conditions and found that the fruit colour change (especially for hue values) could be explained well by the model. The physiological changes under IW were similar to expected data calculated by the model. In other words, IW treatments may not alter the mechanism of lime degreening during storage but the IW treatments were more effective than the integrated storage temperatures in terms of the CI protection; i.e. IW must have induced some protective mechanisms against CI.

CS was shown to be a simple and efficient colour assessment which is easier to apply than measurements by a colorimeter or pigment analysis, but which was equally useful in predicting and describing degreening.

Loss in pigments of lime can also be assessed by using spectral reflectance techniques. This technique provided information about reflectance spectrum of lime peel from 360-740 nm for the spectrophotometer used in this work. From these spectra, “difference spectra” can be calculated showing the change in reflectance at each wavelength during storage. Because the pigments found in the peel have characteristic reflectance spectra, I can deduce changes in specific pigments from these “difference spectra”. For lime, I found that most absorption occurred in the region 480-710 nm which fits with the presence of carotenoids and xanthophylls (which absorb between 460-560 nm), and chlorophyll (which absorbs between 550-705 nm).

After I compared the differences between reflectance spectral data of the high H° fruit set versus the low H° fruit set, I found that the low H° fruit began to lose their chlorophyll at the beginning of storage from 0 day (Fig. 6.23A). I also found that the greener fruit (high H° fruit set) lost more chlorophyll and also lost more carotenoids and xanthophylls than the lower H° fruit set after 12 weeks of storage (Fig 6.23B).

Yellower fruit at harvest also continued to synthesise carotenoids and xanthophylls for a short period after harvest, before losing both these pigments and chlorophyll, whereas greener fruit simply lost pigments from the moment of harvest and this effect was faster for postharvest colour change than in yellower fruit. The yellow fruit had presumably already lost some chlorophyll on the tree.

The index R800/R680 has been used by other authors to describe changes in chlorophyll content and hue angle changes especially in green tissue. My more detailed analysis showed that this is an over-simplification: fruit with similar colours (same H°) may have different R800/R680 (Fig. 6.25D). R800/R680 and R800/R520 were as effective as hue in differentiating between the more complete yellowing that occurred under RA compared with HWD or IW fruit (Fig. 6.27), but only on the green side of the fruit. On the yellow side, a marked change in hue was not accompanied by a large change in R800/R680, or R800/R520.

Storing the fruit under 6 cycles IW (5°C, 12 days and 15°C, 2 days) or pretreating the fruit with HWD (47°C for 4 min) delayed changes in difference reflectance spectra compared to storing the fruit at 13°C. HWD often led to browning and IW reduced pigment change throughout the recorded spectrum (Fig. 6.28).

All pre-treatment and postharvest treatments were tested between the years 2004-2006 with improved treatments planned and implemented in each successive year. This means that there may be confounding effects from the different seasons and growing regions, which could influence postharvest yellowing. Nevertheless by including 5°C storage treatment in every trial, I have been able to allow for maturity differences when assessing optimal treatments.

My present best IW treatment (2-6 cycles (5°C and 15°C) with ca. 12 days – 20 days between the 2 day IW periods) is now ready for use as a starting point for subsequent research. There are three logical areas for future research:

● The TC (or step down technique in this work) was found to be an easier technique to implement during handling than IW. In my test, TC (10°C for 2 weeks then stored at 5°C) showed some promising results but was not as successful as IW. Further research is required to determine whether there are better TC regimes that can extend storage life.

● I have no information on the mechanism by which IW has successfully extended storage life. In order to distinguish between hypotheses such as induction of HSPs or simple repair of incipient membrane damage, it would be good to analyse changes in gene expression during cold and warm cycles of IW. Citrus microarrays are beginning to emerge around the world (Pons *et al.,* 2005) and it would be possible to extract RNA from my samples and use the microarrays to indicate major changes in gene expression in particular metabolic pathways.

● Chlorophyll fluorescence has been used as a technique for the early detection of some disorders of fruits during storage. It might be used to detect 'stress' in time for the storage conditions to be ameliorated before disorders develop. This technique would also help to identify stress during storage and facilitate the determination of optimal conditions for lime storage.

The key achievements of this work are the extension of storage life of 'Tahiti' lime achieved by using the best intermittent warming (IW) regime, which retained fruit quality for 10-12 weeks of storage. Furthermore, I also found that the fruit stored under IW condition retained their nutritional quality at a similar level to the fruit at harvest. I also identified the best postharvest regime for lime among several postharvest regimes (controlled atmosphere (CA), hot water dipping (HWD), temperature conditioning (TC) and IW) and I have given some suggestions about the implementation of IW for lime storage in extended scale. In addition, the mathematical model used in this work describes the effect of temperature on colour change of limes and therefore provides a means of determining the effectiveness of IW on delaying degreening of lime. Therefore this work has improved the prospect of long-term commercial storage of lime with minimal storage

losses which will have the benefit of smoothing out production peaks or enabling export by sea freight, thereby allowing growers to obtain higher returns from sales of their fruit.

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Appendix I

Weight loss can be severe if the produce is held in a warm temperature for too long or if the fruit are stored under IW and the fruit are transferred too often. The H2 fruit stored under IW condition at 7°C for 3.5 weeks and 20°C for 3 days showed a high percentage weight loss (~5-8%) after 4 weeks. The percentage weight loss of this treatment was higher than the H3 fruit because in H2, we transferred the fruit to a shelf life room for 3 days at 20°C during warming periods whereas in H3, we warmed the fruit in a controlled temperature room at 20°C and at a higher RH.

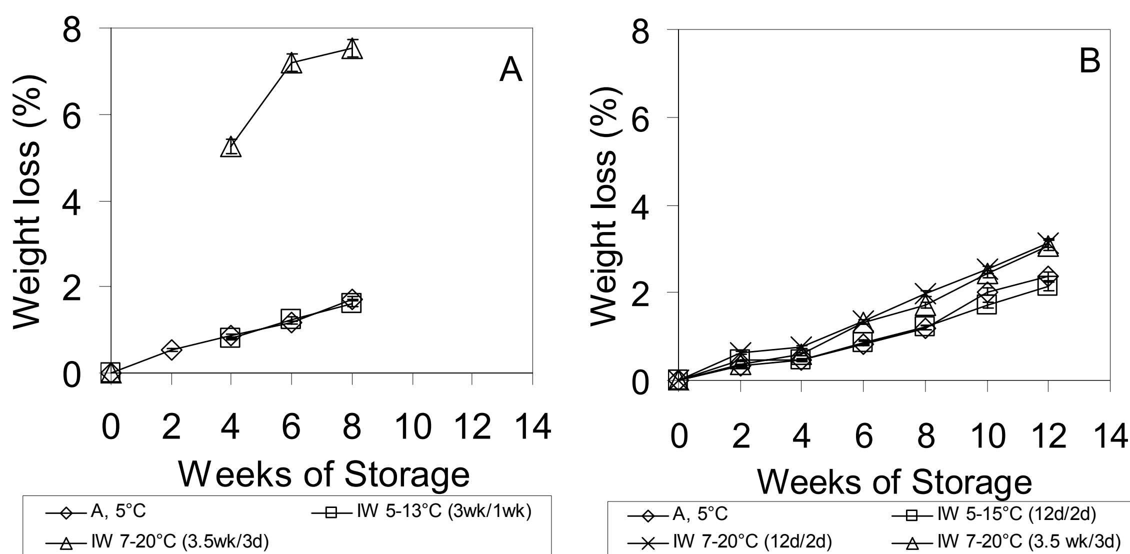

Figure A1 Weight loss (%) for NZ limes stored under RA at 5°C and IW conditions (5°C for 3 weeks and 13°C for 1 week or 7°C for 3.5 weeks and 20°C for 3 days, H2. Vertical bars indicate ± SE (n=80) (A), weight loss (%) for NZ limes stored under RA at 5°C and IW conditions (5°C for 12 days and 15°C for 2 days or 7°C for 12 days and 20°C for 2 days and 7°C for 3.5 weeks and 20°C for 3 days, H3. Vertical bars indicate ± SE (n=80) (B)

Overall, weight loss of lime fruit was generally less than 4% which is well tolerated by fresh produce.

Appendix II

The incidence of pitting of H5 limes was uniformly low. Only one treatment showed significant pitting within the first 2 weeks of storage, namely HWD at 57°C for 6 min which showed significant pitting after 2 weeks (Fig A2-A).

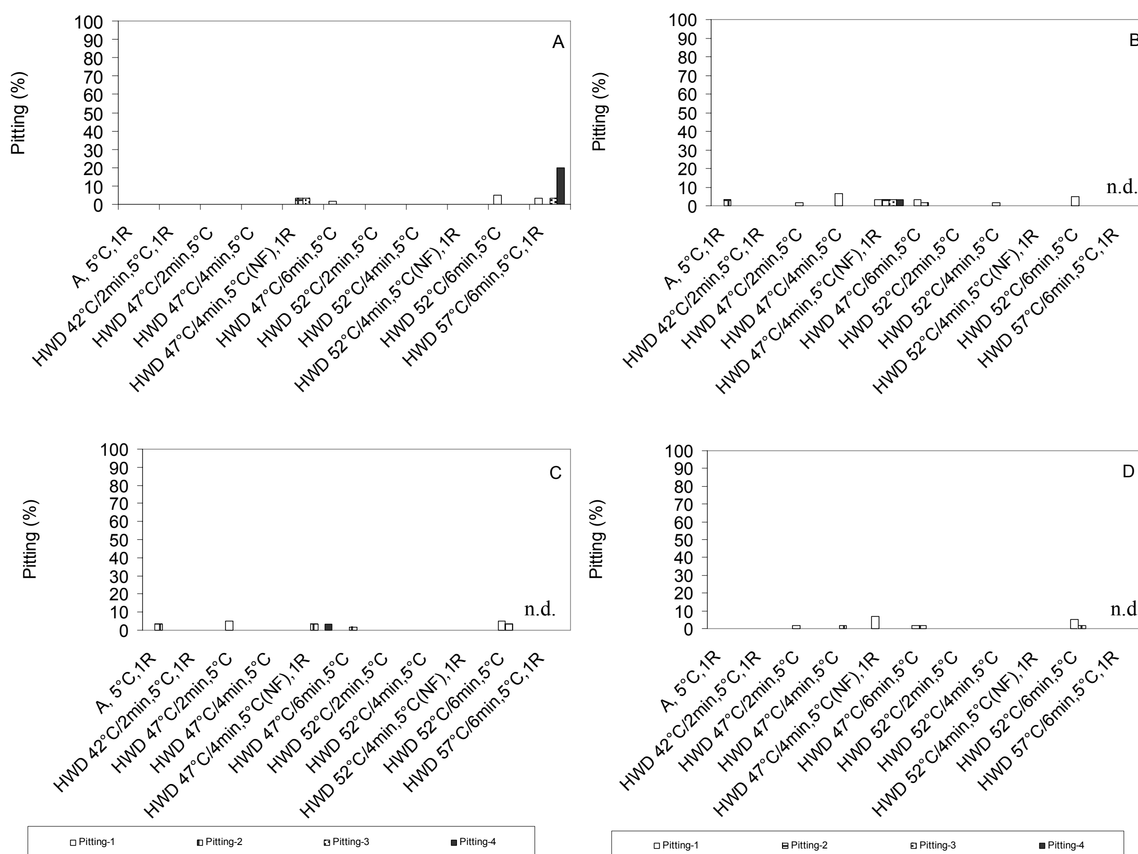

Figure A2 Incidence of pitting after 2(A), 4(B), 8(C) and 10 (D) weeks of storage of lime stored under RA at 5°C or pre-treated limes with different HWD conditions (47°C or 52°C for 2, 4 or 6 min, respectively) with fungicide or pre-treated limes with HWD at 42°C for 2 min, 47 and 52°C for 4 min without fungicide and 57°C for 6 min with fungicide then all treatments were stored under RA at 5°C, H5 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4). n.d. = no data.

The same fruit showed significant heat injury after 2 weeks (Fig A3-A). More generally, a small percentage of the fruit pre-treated with HWD at temperatures greater than 47°C showed heat injury symptoms at 4 weeks of storage (Fig A3-B). Dipping the fruit at 47°C for 2-6 min with fungicide (TBZ) is safe with respect to heat injury for the fruit. The HWD at 57°C for 6 min was clearly too hot for lime.

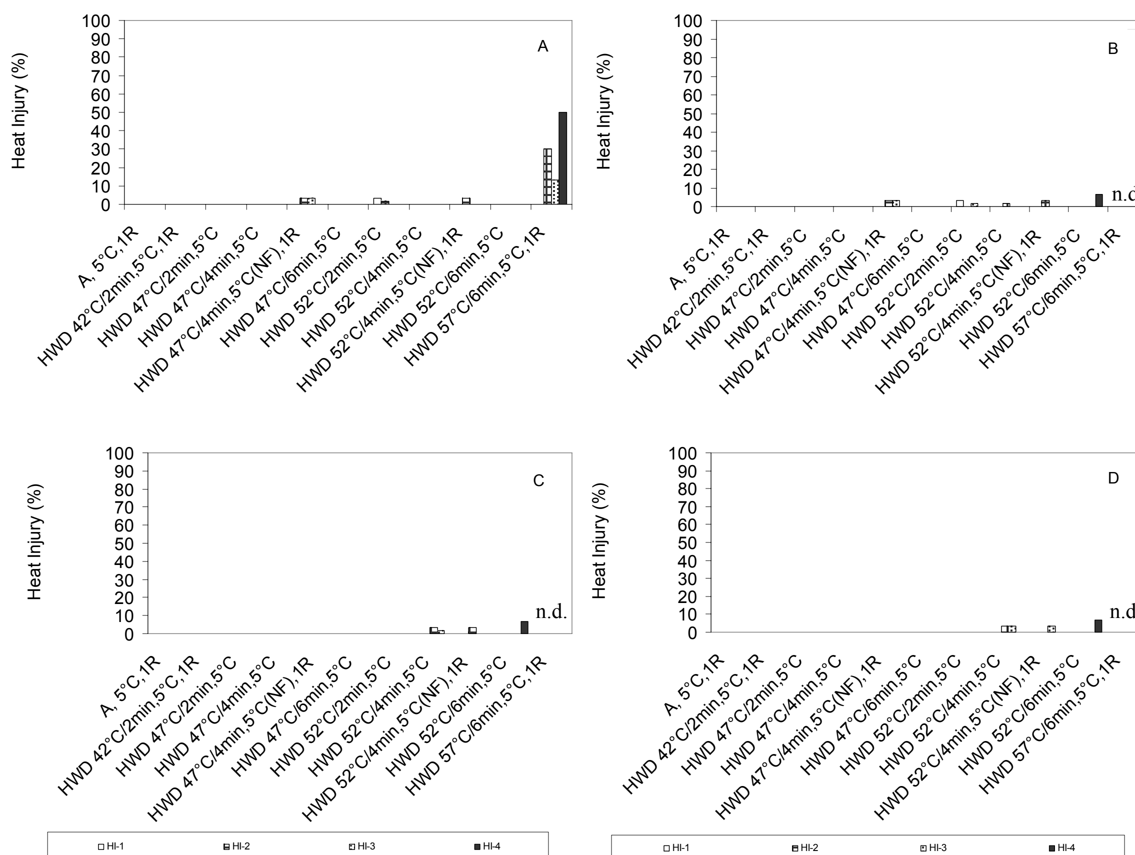

Figure A3 Incidence of heat injury after 2(A), 4(B), 8(C) and 10 (D) weeks of storage of lime stored under RA at 5°C or pre-treated limes with different HWD conditions (47°C or 52°C for 2, 4 or 6 min, respectively) with fungicide or pre-treated limes with HWD at 42°C for 2 min, 47 and 52°C for 4 min without fungicide and 57°C for 6 min with fungicide then all treatments were stored under RA at 5°C, H5 (severity: white bar = 1, cross-hatched = 2, grey = 3, black = 4). n.d. = no data.

Appendix III

Table A.1 Ranking of H° of the 24 green fruit at initial day 0 compared with the final H° of the same fruit at 12 weeks of storage.

Greenest fruit rank 0 day	Initial H°	Final H° at 12 weeks	*k* value	Final fruit rank 12 weeks
1	109.31	87.1	0.069293	2
2	108.89	90.53	0.079775	1
3	108.85	81.05	0.016867	21
4	108.71	82.63	0.01098	16
5	107.69	85	0.011698	7
6	106.95	80.49	0.084454	22
7	106.63	82.45	0.038555	17
8	105.97	84.69	0.033799	9
9	105.75	84.36	0.043549	12
10	105.63	84.61	0.07673	10
11	105.56	81.66	0.078293	19
12	104.77	86.01	0.032306	4
13	104.62	81.88	0.029628	18
14	104.61	82.88	0.026128	15
15	104.19	84.87	0.016542	8
16	103.86	84.41	0.102557	11
17	103.85	81.52	0.070545	20
18	102.72	83.19	0.106662	14
19	102.52	86.05	0.063294	3
20	102.46	85.64	0.121663	6
21	102.39	79.97	0.001282	23
22	101.61	85.74	0.080657	5
23	101.25	84.21	0.001956	13
24	100.48	79.94	0.050227	24
Means	104.97	83.79	0.051977	
SE	0.5255	0.5110	0.0071	

The ranks in Table A.1 indicate that there was not a strong pattern with respect to consistency of colour change. Some very green fruit (e.g. those ranked 3 and 6 initially) were quite yellow at the end whilst similar fruit (rank 1 and 2) remained quite green.

Appendix IV

Table A.2 Absorbance maxima and mass spectral data (negative mode) of lime juice flavonoids.

Absorbance maxima (nm)	m/z, $[M-H]^-$	MS^n	Putative Compound
-	387	341 179 164 161	Caffeic acid-O-glucoside(formic adduct)
270, 335	593	503 473 383 353	Apigenin-6,8-di-C-glucoside
257sh, 271, 348	623	533 503 413 383	Chrysoeriol-6,8-di-C-glucoside[1]
284, 330sh	595	287	Eriodictyol-7-O-rutinoside
255, 355	609	301 179 151	Quercetin-3-O-rutinoside
254, 268sh, 350	461	371 341 326 313 298	Chrysoeriol-6-C-glucoside (pos.Diosmetin)
252, 269sh, 343	579	271 177 151	Naringenin-7-O-rutinoside
256, 266sh, 353	623	315 300 271	Isorhamnetin-3-O-rutinoside
284, 328sh	609	301 286 283 257 242 199 125	Hesperetin-7-O-rutinoside
253, 267sh, 347	607	299 284 256	Diosmetin-7-O-rutinoside

m/z, $[M-H]^-$ represents molecular weight of the principal ion per unit charge.

MS^n is molecular weight of ion fragments.