Development of a Mathematical Model for 'Hayward' Kiwifruit Softening in the Supply Chain

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Technology at Massey University, New Zealand

Zhao Junyu, Matthew

2017

Abstract

Fruit loss is a major concern to the kiwifruit industry as it incurs a high cost to monitor and remove over soft or rotten fruit to meet export standards. Kiwifruit is exposed to various temperature scenarios due to different packhouse cooling practices, and temperature control is difficult to maintain throughout the supply chain. Fruit pallet temperatures are wirelessly monitored in the supply chain. This time temperature data provides valuable rich information which could be used to predict kiwifruit quality.

In the laboratory, green 'Hayward' kiwifruit were exposed to industry coolchain scenarios to investigate their influence on fruit firmness in subsequent storage. Cooling rate and storage temperature were identified to affect fruit firmness and chilling injury development significantly, where accelerated softening and increased chilling injury development was observed in late storage (> 100 d) when fruit were cooled directly to 0 °C. However, when fast cooled fruit were stored at 2 °C instead of 0 °C, low incidence of chilling injury was observed. The influence of cooling rate and storage temperature on kiwifruit quality suggests that industry should focus on the management practices adopted by packhouses in order to maintain acceptable quality after long term storage. A proportion of the firmness data results were used to develop a mechanistic style mathematical model of kiwifruit softening. Kiwifruit softening was mathematically described based on the correlation with starch degradation, breakdown of cell wall structure, and a description of the incidence of chilling injury development during storage. The model inputs consist of solely commonly collected at-harvest attributes: firmness, dry matter and soluble solids content and time-temperature data. Applying at-harvest attributes as model inputs enabled a capability to predict different softening curves as influenced by fruit maturity, and grower line differences. The developed model

demonstrated promising softening prediction with mean absolute errors (MAE) between 0.8 to 2.1 N when fruit were exposed to fluctuating temperatures and cooling profiles. A logistic model was used to estimate the proportion of chilling injured fruit. Based on the given time temperature information, the logistic model was able to predict the proportion of chilling injured fruit reasonably well ($R^2 = 0.735$). This chilling injury prediction was subsequently used to adjust the softening prediction during the late storage period (>100 d). Model validation was performed using the remaining data, identifying a lack of fit in both the rapid (MAE of 20.8 N) and gradual (MAE of 8.0 N) softening phase. The lack of fit in the rapid softening phase is proposed to be explained by the presence of an initial lag phase in softening which the developed model is unable to predict. The magnitude of firmness associated with starch content and cell wall integrity heavily influenced the lack of fit in the gradual softening phase. Fixing the initial amount of firmness associated to cell wall integrity to be constant for all maturities and grower lines improved the softening prediction.

Overall, this thesis contributes to the challenge of predictively modelling kiwifruit quality in the supply chain. However, there are still many opportunities for improvement including introducing the influence of: variation within the same batch; fruit maturity on chilling injury development; ethylene in the environment; pre-harvest management practices and extending the model to have more focus on high temperature conditions such as those experienced in the marketplace. Conducting studies on: the effect of curing on kiwifruit; using non-destructive techniques to provide information to help define model parameters for prediction; effect of high temperature exposure on kiwifruit softening are possible opportunities that may contribute to enable better prediction of kiwifruit quality in the supply chain in the future.

Acknowledgments

I wish to thank my supervisors, Dr. Andrew East and Prof. John Bronlund for the incredible amount of time and support given to me during my PhD. I greatly appreciate their positive feedback, continuous efforts and always encouraging me through tough times.

I would like to say many thanks to Peter Jeffery for his help when I had troubles in either my laboratory work or technical issue with computer and Sue Nicholson for her help and patience in assisting me in experimental planning and during my time in the laboratory. I am also thankful to Byron, Warwick and Gary for their technical support.

Heaps of thanks should go to Zespri International Ltd for the fellowship and supplying kiwifruit for this study. I am also thankful to Massey University for providing extra funding to relief my financial issues especially during my last year of study.

I am indebted to many of my friends and colleagues for their help and support throughout my studies in New Zealand. They are Tamarath (An) Pranamornkith, Gayani, Khairul, Pilirani, Jantana, Li Mo, Abdul and Srikanth. Special thanks to Ivana, Wensheng, Grace, Eileen, Seng Guan and Jordan for being an asset of good friends during my time in New Zealand.

Special thanks to my mother and father for their encouragement and appreciate my siblings (Roy, Vyennus and Dominic) for taking care of the family when I am away for my studies. Finally, I would like to thank Jenny, who is a very special person of my life. I am grateful for her continuous encouragement and support during all tough stages in my studies and always there to listen to my problems. I appreciated all the time you spent waiting for me to return to Singapore.

Table of contents

Abstract i

Acknowledgments iii

Table of contents iv

List of Figures x

List of Tables xv

Nomenclature xvii

1. Introduction 1

1.1. Thesis overview 3

2. Literature review 5

2.1. Kiwifruit 5

2.2. Kiwifruit ripening 5

2.2.1. Starch degradation 6

2.2.2. Breakdown of cell wall structure 7

2.2.2.1. Modification of cell wall polymeric network 8

2.2.2.2. Swelling of cell wall 10

2.2.3. Chilling injury development 11

2.3. Factors affecting fruit ripening 13

2.3.1. Temperature 13

2.3.2. Ethylene 14

2.3.3. Humidity 17

2.3.4. Fruit maturity 18

2.3.5. Rot development in kiwifruit 19

2.4. Kiwifruit supply chain 20

2.4.1. Pre-harvest treatments 20

2.4.2. Postharvest treatment 21

2.5. Softening models 25

2.5.1. Empirical approach 25

2.5.2. Mechanistic approach 26

2.5.2.1. Temperature dependency 26

2.5.2.2. Biological variability 27

2.6. Summary 28

3. Softening of 'Hayward' kiwifruit in different coolchain scenarios (*) 29

3.1. Introduction 29

3.2. Material and methods 31

3.2.1. Supply chain simulation 31

3.2.2. Fruit assessment 34

3.2.2.1. Fruit firmness 34

3.2.2.2. Soluble solids content 35

3.2.2.3. Decay incidence 37

3.2.2.4. Respiration rate 37

3.2.2.5. Statistical analysis 37

3.3. Result and discussions 38

3.3.1. Cooling profiles achieved 38

3.3.2. At-harvest attributes 41

3.3.3. Effect of cooling rate on kiwifruit quality during storage 42

3.3.4. Effect of storage temperature on kiwifruit quality during storage 48

3.3.5. Effect of break in temperature control on kiwifruit quality during storage 50

3.3.6. Effect of high temperature conditions on kiwifruit quality during storage 52

3.3.7. Effect of storage humidity on kiwifruit quality during storage 54

3.4. Conclusion 56

4. Factors influencing development of chilling injury in 'Hayward' kiwifruit during coolstorage (*) 57

4.1. Introduction 57

4.2. Materials and methods 59

4.2.1. Fruit source 59

4.2.2. Coolchain simulation 60

4.2.3. Fruit assessment 62

4.3. Results 65

4.3.1. Cooling profiles achieved 65

4.3.2. At-harvest fruit attributes 67

4.3.3. Chilling injury development during storage 69

4.3.4. Fruit firmness during storage 73

4.4. Discussion 76

4.4.1. Fruit maturity and grower line differences on kiwifruit quality 76

4.4.2. Influence of chilling injury on firmness measurement 80

4.4.3. Coolchain temperature effect on kiwifruit quality 82

4.5. Conclusion 87

5. Mathematical modelling of 'Hayward' kiwifruit softening 89

5.1. Introduction 89

5.2. Model conceptual framework 92

5.2.1. Breakdown of starch content 95

5.2.2. Breakdown of cell wall structure 97

5.2.3. Development of chilling injury 98

5.3. Model development 99

5.3.1. Model constraints 99

5.3.2. Mathematical formulation 100

5.3.3. Estimation of initial starch content (S_0) 102

5.3.4. Model of starch breakdown effect on firmness (F_A) 102

5.3.5. Model of the breakdown of cell wall structure on firmness (F_B) 107

5.3.6. Model for the development of chilling in jury effect on firmness 113

5.3.7. Prediction of kiwifruit firmness (F_{pred}) 116

5.4. Model summary 117

5.5. Conclusion 120

6. Developed model performance 121

6.1. Introduction 121

6.2. Materials and methods 122

6.2.1. Grower difference 123

6.2.2. Cooling rate effect on fruit firmness 125

6.2.3. Storage temperature effect on fruit firmness 127

6.2.4. Break in temperature control effect on fruit firmness 129

6.2.5. Effect of exposure to high temperature on fruit firmness 131

6.2.6. Prediction on the proportion of chilling injured fruit 132

6.3. Model error and sensitivity 133

6.3.1. Model inputs 134

6.3.2. Global model parameters 140

6.4. Overall discussion 142

6.5. Conclusion 144

7. Validation of 'Hayward' kiwifruit softening model in coolstorage 146

7.1. Introduction 146

7.2. Material and methods 147

7.2.1. Predictive modelling 149

7.3. Results and discussion 149

7.3.1. Fruit maturity difference 149

7.3.2. Fruit grower difference 156

7.3.3. Prediction of chilling injured fruit 163

7.4. Overall discussion 165

7.5. Conclusion 167

8. Overall discussion and recommendations 168

8.1. Introduction 168

8.2. Establishment of model 169

8.3. Model softening prediction 170

8.3.1. Prediction of lag phase 171

8.3.2. Prediction of gradual softening phase (F_{B0}) 178

8.3.3. Prediction of incidence of chilling injury (CI) 181

8.4. At-harvest attributes as model inputs 183

8.5. Application to industry 184

8.6. Possible future opportunities 185

8.6.1. Curing of kiwifruit 185

8.6.2. Pre-harvest effect on fruit storability 186

8.6.3. Predict fruit firmness with biological variation 187

8.6.4. High temperature exposure 189

8.7. Conclusion 190

9. References 191

Appendices 224

List of Figures

Figure 2.1: Schematic representation of key events 6

Figure 3.1: Example of time temperature profile of the air inside the box 29

Figure 3.2: Time temperature profile of direct (A) and gradual (B) cooling 33

Figure 3.3: Description of various temperature scenarios used for 'Hayward' kiwifruit from 2012 harvest 36

Figure 3.4: Fruit rots found at the side or stem end of 'Hayward' kiwifruit. Red arrow indicates the rotten area 37

Figure 3.5: Room and tray air time temperature for direct (A) and gradual (B) cooling to 0 or 2 °C. 39

Figure 3.6: Real time temperature profile of the different temperature scenarios simulated in the laboratory. 40

Figure 3.7: Effect of cooling profile on 'Hayward' kiwifruit softening at constant storage conditions of 0 (A) and 2 (B) °C 43

Figure 3.8: Effect of cooling profile on the accumulation of soluble solids in 'Hayward' kiwifruit at control storage conditions of 0 (A) and 2 (B) °C. 45

Figure 3.9: Effect of cooling rate on 'Hayward' kiwifruit respiration rate at 0 (A) and 2 (B) °C. 46

Figure 3.10: Effect of cooling rate on the incidence of rotten fruit in 'Hayward' kiwifruit subsequently stored at 0 or 2 °C. 48

Figure 3.11: Effect of storage temperature on 'Hayward' kiwifruit softening when direct cooled (A) or gradual cooled (B) to storage temperature at 0 or 2 °C. 49

Figure 3.12: Effect of break in temperature control on the kiwifruit firmness after 9 or 15 weeks of storage 51

Figure 3.13: Effect of high temperature exposure on kiwifruit firmness after 10 weeks of storage at 0 °C. 53

Figure 3.14: Effect of humidity at 30 °C on kiwifruit firmness after 14 weeks of storage at 0 °C..... 55

Figure 4.1: The proposed cooling profiles to cool fruit to storage temperature 61

Figure 4.2: Example of severity of chilling injury found along the outer pericarp of 'Hayward' kiwifruit. 63

Figure 4.3: The comparison between a fruit with chilling injury symptom (left) and a rotten fruit (right). 64

Figure 4.4: Cooling profiles designed and created for 6 different coolchain scenarios.. 66

Figure 4.5: Incidence of chilling injured fruit as influence by storage time, storage conditions and fruit maturity. 72

Figure 4.6: Softening of kiwifruit harvested at different maturity as influenced by supply chain temperature conditions. 75

Figure 4.7: Softening of kiwifruit harvested at different maturity during storage in six different supply chain conditions. 78

Figure 4.8: Softening of kiwifruit harvested at different maturity during storage in six different supply chain conditions 79

Figure 4.9: Histograms of fruit firmness as influenced by grower line (G1, G2 and G3) and maturity 81

Figure 4.10: Time temperature profile of control and various "switch" treatments. 0 refers to fruit stored at 0 °C. 85

Figure 4.11: (A & B) Softening of kiwifruit exposed to different temperature "switch" treatments. 86

Figure 4.12: Percentage of fruit with chilling injury after 125, 150, 175 and 200 d of storage 87

Figure 5.1: Average kiwifruit firmness and soluble solids content during storage at 0 °C. 94

Figure 5.2: The overall conceptual model development process. 102

Figure 5.3: (A) Modelled accumulation of soluble solids content for 2012 season, ... 105

Figure 5.4: Correlation between fruit firmness and starch content in the first 50 days of cool storage. 106

Figure 5.5: Estimation of average initial firmness contribution of cell wall breakdown based on at-harvest attributes. 109

Figure 5.6: The modelled change in firmness due to breakdown of cell wall structure (%) at various temperatures. 112

Figure 5.7: (A) Fitted model with the incidence of chilling injured fruit collected in the 2013 harvest season. 114

Figure 5.8: Logistic model used to describe the incidence of chilling injured fruit 116

Figure 5.9: Summary of the firmness prediction model developed 118

Figure 5.10: Simplified overall equations explaining the variables needed to predict fruit firmness in coolchain. 119

Figure 6.1: The model prediction (solid lines) against the experimental data 124

Figure 6.2: Experimental data points and modelled average fruit firmness (solid lines) during storage 126

Figure 6.3: Experimental data points and modelled average firmness (solid lines) of fruit that were cooled either directly or gradually to storage temperature 128

Figure 6.4: Experimental data points and modelled average firmness (solid lines) of fruit exposed to a break in temperature control 130

Figure 6.5: Experimental data and modelled average firmness of fruit exposed to high temperature conditions after 10 weeks of storage 132

Figure 6.6: Experimental data points and modelled average firmness of fruit with chilling injury development 133

Figure 6.7: Scatter plots of initial fruit firmness (F_0), soluble solids (B_0) and dry matter content (D_{m0}) 135

Figure 6.8: Sensitivity analysis of model inputs on predicting the softening curve. 136

Figure 6.9: Scatter plots of initial starch content (S_0), soluble solids (B_0) and dry matter content (D_{m0}) 137

Figure 6.10: Sensitivity analysis of model inputs on predicting the softening curve. .. 139

Figure 6.11: Sensitivity analysis of model inputs on predicting the softening curve. .. 140

Figure 6.12: Sensitivity analysis of model parameter (a) on predicting the softening curve 141

Figure 6.13: Sensitivity analysis of model parameter (k_w) on predicting the softening curve of the same grower line. 142

Figure 6.14: The developed model on predicting the softening of 'Hayward' kiwifruit 143

Figure 7.1: Correlation between fruit firmness and starch content during storage period. 150

Figure 7.2: The linear correlation of fruit firmness and starch content is invalid when starch content is above 8 %. 151

Figure 7.3: The predicted softening curve of fruit using different fruit maturity at-harvest attributes. 153

Figure 7.4: Model validation on the experimental data collected in 2013. 155

Figure 7.5: Difference between firmness prediction (F_{pred}) when adopting the modified correlation between starch and fruit firmness. 156

Figure 7.6: The predicted softening curve of early maturity fruit from different grower lines using at-harvest attributes 158

Figure 7.7: Model validation on softening pattern of late maturity fruit from three different grower lines collected in the 2013 season. 159

Figure 7.8: Comparison of softening prediction using at-harvest attributes of respective grower lines or an average across 3 grower lines 161

Figure 7.9: Model validation on softening pattern of late maturity fruit from three different grower lines 162

Figure 7.10: Comparison of the predicted and experimental data for the proportion of chilling injured fruit 164

Figure 7.11: Comparison on the predicted fruit softening curve of rapidly cooled ($R_{12h,0}$) against gradually cooled ($G_{2w,0}$) fruit to 0 °C. 165

Figure 8.1: (A) Correlation of fruit firmness and starch content with a plateau when starch content is above 8 %. 171

Figure 8.2: The accumulation of soluble solids content 174

Figure 8.3: The model prediction on the softening curve by modifying the rate of starch degradation (k_s) to be 3 times lower when starch content is above 8 %. 176

Figure 8.4: Model prediction of firmness of fruit in 2012 (A) and 2013 (C) harvest season. 179

Figure 8.5: The possible approach to relate ethylene to fruit firmness 181

List of Tables

Table 3.1: Average at-harvest attributes of kiwifruit from respective grower lines. 41

Table 3.2: Contingency table for the relationship of cooling rate on the incidence of rotten fruit in 'Hayward' kiwifruit 47

Table 3.3: Contingency table for the relationship of storage temperature on the incidence of rotten fruit in 'Hayward' kiwifruit.....50

Table 4.1: Average at-harvest attributes of kiwifruit from 3 grower lines harvested at 3 different maturity stages.....68

Table 4.2: Contingency table for the relationship of storage treatment on incidence of chilling injured fruit after 172 d of storage. 70

Table 4.3: Contingency table for the relationship of fruit maturity on incidence of chilling injured fruit after 172 d of storage. 71

Table 4.4: Contingency table for the relationship of grower line on incidence of chilling injured fruit after 172 d of storage. 72

Table 4.5: ANOVA table displaying the sum of square (SS), mean square (MS), F and P value of respective factors.....73

Table 5.1: Summary of different mechanisms hypothesized to occur during the observed phases of kiwifruit softening. 93

Table 5.2: The values of the model parameters with the standard deviation.....104

Table 5.3: Values of the model parameters with the standard deviation 111

Table 5.4: Estimated values of activation energies for different reactions.....112

Table 6.1: At-harvest attributes of kiwifruit from respective grower lines from 2012 season. 122

Table 7.1: The model inputs and parameters used to predict fruit firmness in coolchain. 148

Table 7.2: Average At-harvest attributes of kiwifruit from 3 growers harvested at 3 different maturity stages..... 148

Table 7.3: Average at-harvest attributes of kiwifruit from 3 different maturity stages. 153

Table 7.4: The different approach to define model parameter a and F_{B0}..... 161

Table 7.5: The comparison of the proportion of chilling injured fruit (%) between model prediction (Pred) and experimental data (Exp)..... 163

Nomenclature

Symbol	Definition	Units
a	Correlationship between starch and firmness	N %$^{-1}$
AHU_a	AHU when 50 % of chilling injured fruit is reached	°C d
AHU	Accumulated heat unit	°C d
B	Soluble solid contents	°Brix
B_0	Initial soluble solid content	°Brix
B_{final}	Final soluble solids content	°Brix
F_{CI}	Lowest firmness measured	N
CI	Proportion of chilling injured fruit	%
CI_{max}	Maximum proportion of chilling injured fruit	%
CI_{min}	Minimum proportion of chilling injured fruit	%
D_m	Dry matter content	%
$E_{a,s}$	Activation energy for starch breakdown	J mol^{-1}
$E_{a,p}$	Activation energy for breakdown of cell wall structure	J mol^{-1}
F	Fruit firmness	N
F_0	Initial fruit firmness	N
F_A	Firmness correlated with starch degradation	N
F_B	Firmness contributed by cell wall component	N
F_{Fix}	Underlying basal firmness	N
F_{pred}	Predicted fruit firmness	N
F_{soft}	Predicted soft fruit firmness	N
k_b	Rate constant of accumulation of soluble solids content	d^{-1}
k_s	Rate constant of starch breakdown	d^{-1}
$k_{s,ref}$	Rate constant of starch breakdown at T_{ref}	d^{-1}
k_w	Rate constant of the breakdown of cell wall structure	d^{-1}
$k_{w,ref}$	Rate constant of the breakdown of cell wall structure at T_{ref}	d^{-1}
R	Universal gas constant, 8.314	J mol^{-1}K^{-1}
S	Starch content	%
S_0	Initial starch content	%
t	Time	d
T	Temperature	°C
T_{abs}	Temperature in absolute	K
T_{ref}	Reference temperature at 20 °C	K
T_b	Base temperature	°C
μ	Rate constant of chilling injury development	d^{-1}

Symbol	Definition
	Storage conditions
TB 9	Break in temperature control after 9 weeks of storage
TB 15	Break in temperature control after 15 weeks of storage
HT	High temperature treatments
DH	Different humidity conditions at 30 °C
$R_{12h,0}$	Fruit rapidly cooled to 0 °C within 12 hours
$R_{12h,2}$	Fruit rapidly cooled to 2 °C within 12 hours
$D_{3d,0}$	Fruit directly cooled to 0 °C within 3 days
$G_{2w,0}$	Fruit gradually cooled to 0 °C within 2 weeks
$C_{1w,0}$	Fruit rapidly and gradually cooled to 0 °C within 1 week
$C_{1w,2}$	Fruit rapidly and gradually cooled to 2 °C within 1 week

1. Introduction

Kiwifruit is the major fresh produce export in New Zealand and was worth approximately $ 1.2 billion in 2015 (Aitken & Hewett, 2015). 'Hayward' kiwifruit is the most widespread cultivar among the different cultivars and has good postharvest performance and acceptable flavours (Burdon *et al.*, 2004; Burdon & Lallu, 2011). Fruit loss has been a major problem for the industry, costing approximately $ 120 million per year for monitoring and removal of over soft or rotten fruit to meet export standards (Tanner *et al.*, 2012). As a result, there is an emphasis on the reduction of fruit loss.

Fruit are exposed to different temperature scenarios throughout the supply chain. Wireless monitoring system was implemented in the industry to monitor the fruit pallet temperatures (Bollen *et al.*, 2013), identifying several possible temperature scenarios which compromise fruit quality. Kiwifruit is exposed to various temperature scenarios due to different packhouse cooling capacity and practices, and temperature control is difficult to maintain throughout the supply chain.

Cooling profiles, storage temperature and storage room ethylene concentration have an impact on fruit quality (East *et al.*, 2016). Variation in initial fruit quality and fluctuation in storage conditions throughout the supply chain, make prediction of fruit quality difficult (Feng *et al.*, 2003a; Tijskens *et al.*, 2003). The presence of bruise or rotten fruit also affect fruit quality and hence predicting fruit quality during storage becomes even more complex (Mitchell, 1990; Jeffery & Banks, 1996; Burdon & Lallu, 2011; Van Den Dungen *et al.*, 2011).

Kiwifruit softening consists of three softening phases; the initial lag phase, the rapid softening phase and a gradual softening phase (White *et al.*, 2005; Schroder & Atkinson, 2006). Fruit ripening can be explained by starch degradation resulting in increased sugar

content and loss of cell wall integrity which softens the fruit texture (Beever & Hopkirk, 1990; Redgwell *et al.*, 1991; Redgwell *et al.*, 1992; Bonghi *et al.*, 1996). Kiwifruit can also develop chilling injury during cooling and low temperature storage (Lallu, 1997). The fruit postharvest performance during storage may be anticipated by understanding the fruit physiology and factors that influence fruit ripening.

This research aims to develop a mathematical model to predict 'Hayward' kiwifruit softening in the supply chain. The model will be developed using time temperature information and common at-harvest attributes; fruit firmness, soluble solids content and dry matter content which are easily collected in the commercial setting throughout the supply chain. The model prediction should benefit the industry by providing improved information on kiwifruit softening and thus enable improved logistics with the goal to reduce fruit losses. The model focuses on predicting fruit firmness in subsequent storage as influenced by supply chain temperature scenarios, ignoring all ethylene effects. Therefore, ethylene concentration will be controlled to be below the industry standard during storage.

There are many mathematical approaches to describe the softening of kiwifruit during storage at different temperatures. Jabbar (2014) and Benge *et al*. (2000) demonstrated the use of empirical models such as the Complementary Gompertz (CG) model to describe fruit softening. Empirical models fit a curve to a large pool of data and contain several parameters that describe the curve but do not have any underlying biological basis. The advantage of using mechanistic approach is the flexibility to predict fruit softening under variable supply chain conditions. Exponential kinetics and Michaelis Menten are used to describe fruit softening in various storage temperatures (Schotsmans *et al.*, 2005; Schotsmans *et al.*, 2008) and modified atmosphere conditions (Hertog *et al.*, 2004c) and may be used as a starting point for model development. These models however have not

included softening by chilling injury development. A successful model will be able to describe kiwifruit softening with the influence of chilling injury development under supply chain conditions, ideally based on the initial fruit properties and storage conditions. This research aims to:

- include temperature dependency in the model to account for all the possible temperature scenarios occur in the supply chain
- study the effect of chilling injury development on fruit firmness in subsequent storage
- validate the developed model to predict softening of fruit population from various maturities, grower lines and coolchain scenarios

1.1. Thesis overview

The literature review (Chapter 2) provides information on the current knowledge on 'Hayward' kiwifruit ripening and its supply chain. The factors influencing (e.g. pre and postharvest treatments) fruit ripening will be reviewed. The mechanisms potentially explain kiwifruit ripening will be discussed in chapter 2, which subsequently aids in the model development. This chapter also includes the different types of model (empirical and mechanistic model) used to describe fruit softening. This review demonstrates the potential application of the developed model to kiwifruit industry.

The time temperature information provided by the industry has identified several possible conditions in the supply chain that will potentially affect kiwifruit quality (Figure 3.1). These potential temperature scenarios formed a basis for simulation in the laboratory to evaluate their effect on fruit firmness in subsequent storage. Chapter 3 demonstrates the screening and identification of supply chain scenarios that influence the fruit firmness during storage.

Chapter 4 focuses on the effect of chilling injury development on fruit firmness in subsequent storage. The symptoms of chilling injury in kiwifruit are defined in this chapter to distinguish chilling injured fruit from normal soft fruit or rotten fruit. A series of different supply chain scenarios that were identified in chapter 3 were applied to kiwifruit from different maturities and grower lines. The incidence of chilling injury across different coolchain scenarios was quantified and associated with fruit firmness.

Chapter 5 illustrates the possible mechanisms that explain kiwifruit softening and subsequently aid in the model development. The occurrence of chilling injury development during coolstorage is then mathematically described forming part of an overall final predictive model for kiwifruit quality change during storage.

In chapter 6, the model is developed to predict fruit softening based on time temperature information and at-harvest attributes that can be collected easily by the industry. Chapter 6 demonstrates the model performance to predict fruit softening as influenced by fruit grower lines and coolchain conditions based on the data collected in chapter 3.

The developed model is then validated against an independent set of experimental data in chapter 7. The model capability to predict kiwifruit firmness with given supply chain conditions and at-harvest attributes to account for the variability between fruit harvest seasons and fruit maturity is assessed.

Chapter 8 consists of the overall discussion and recommendations including the summarised outcomes and the limitations of the established model. This chapter also discusses the possible future opportunities to be explored to improve the model capability to predict kiwifruit softening.

2. Literature review

2.1. Kiwifruit

Kiwifruit (*Actinidia deliciosa* cv. Hayward) soften and develop flavour and sweetness during ripening, reaching a good eating quality that is acceptable to consumers (Crisosto & Crisosto, 2001; Jaeger *et al.*, 2003; Burdon *et al.*, 2004). The industry follows a standard that fruit fall below 10 N are not exported as the fruit will fail to meet the eating firmness window of 6 to 8 N upon reaching oversea markets. Huge cost is involved to repack and sort fruit to meet this industrial threshold. Therefore, developing a mathematical model to predict kiwifruit firmness during storage will potentially benefit the industry to anticipate the fruit storability. In order to develop a mathematical model to describe kiwifruit softening, a comprehensive understanding of the mechanisms behind fruit ripening and factors that influence ripening is required.

2.2. Kiwifruit ripening

During the ripening of kiwifruit, various biochemical reactions occur, resulting in an increase in soluble solids content and decrease in fruit firmness. The increase in soluble solids can be explained by the breakdown of starch and the change in firmness is related to the breakdown of cell wall structure. These biochemical reactions occur at different stages of softening. Schroder and Atkinson (2006) illustrated the respective mechanisms taking place during stages of kiwifruit ripening (Figure 2.1). Starch degradation is proposed to occur during the initial lag phase and transition to the rapid softening phase, while breakdown of cell wall structure such as solubilisation and depolymerisation of pectin and breakdown of the middle lamella contributes to the remaining softening phases (Figure 2.1). Overall, kiwifruit ripening can be categorised into 4 different phases, including an initial lag (initiation), rapid, and gradual softening, and the over-ripe phases (Beever & Hopkirk, 1990; White *et al.*, 2005; Schroder & Atkinson, 2006; Jabbar, 2014).

The following sections will discuss more on the respective mechanism on influencing fruit firmness.

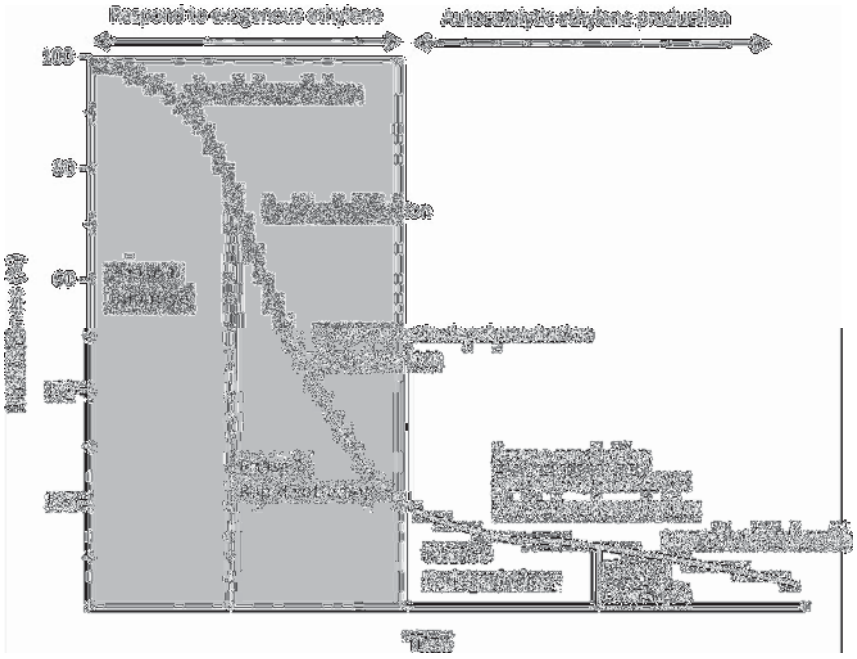


Figure 2.1: Schematic representation of key events during postharvest kiwifruit ripening. Phase 2 to 4 represent the different softening phases. (Source: Schroder and Atkinson, 2006).

2.2.1. Starch degradation

Degradation of starch content is an event that occurs during kiwifruit ripening. Starch accumulation occurs during fruit development and starts to breakdown when ripening (Beever & Hopkirk, 1990). Conversion of starch to sugar is an important metabolic aspect in ripening. Starch content decreases concomitant with an accumulation of soluble solids when kiwifruit ripens (Macrae *et al.*, 1992; Fonseca *et al.*, 2002). Mature kiwifruit have a starch content of 5 to 7 % fresh weight but after ripening the starch content decreases and sugar content increases up to 12 to 15 % (Beever & Hopkirk, 1990).

During kiwifruit ripening, there is a good correlation between starch degradation and the rapid loss of firmness in the early stage of softening (Macrae *et al.*, 1989). Although this correlation between starch breakdown and textural softening exist, the mechanism behind it remains to be elucidated. Bonghi *et al.* (1996) suggested starch

degradation may play an important role in the early stage of softening in kiwifruit. Unlike in kiwifruit, breakdown of starch content was found to contribute to textural softening in banana (Kojima *et al.*, 1994; Bhagyalakshmi *et al.*, 2002).

Starch degradation during ripening potentially affects cell turgor pressure. Turgor pressure contributes to the cell and tissue strength by providing the hydrostatic pressure within the cell and therefore influences textural properties (De Belie *et al.*, 2000). Having a low turgor pressure will cause the cell to collapse while too high in turgor pressure leads the cell to be brittle and likely to rupture (Lin & Pitt, 1986; Jackman & Stanley, 1992). An increase in soluble solids content during ripening will result in an increase in turgor pressure and thus an increase in firmness is expected. However, no distinct pattern of change in cell turgor has been observed during kiwifruit ripening (Harker & Hallett, 1994). Harker and Hallett (1994) proposed the cell wall of ripened kiwifruit cell became more plastic and elastic and thus resulted in a cell expansion rather than an increase in turgor pressure when placed in a hypotonic solution. Another possibility is solute accumulation may occur in both apoplast and symplast and thus maintaining a constant turgor pressure. Turgor pressure in beetroot tissue was found to remain unchanged even after an increase in osmotic pressure due to sucrose accumulation (Tomos *et al.*, 1992). Turgor pressure in tomato was found to be lower than expected from the osmotic potential, which may be due to the presence of solutes in the apoplast (Schackel *et al.*, 1991). Overall, these findings point out the possible reasons why no association between change in turgor pressure and starch breakdown was observed in kiwifruit ripening.

2.2.2. Breakdown of cell wall structure

Cell wall is made up of two distinct layers; mainly primary and secondary layers that provide the cell wall integrity. The primary layer is highly hydrated with approximately 65% water and consists of pectic polysaccharides, cellulose, hemicellulose

and soluble protein including enzymes (Brummell, 2006). Whereas the secondary layer contains large deposition of ligin, a complex structure made up of phenolic compounds (Lewis & Yamamoto, 1990).

Modifications to cell wall structure are commonly observed during fruit ripening (Van Buren, 1979; Brummell, 2006; Prasanna *et al.*, 2007). During kiwifruit ripening, breakdown of cell wall structure includes a change in pectin content (Soda *et al.*, 1987; Redgwell *et al.*, 1990; Bonghi *et al.*, 1996), swelling of the cell wall (Hallett *et al.*, 1992), and changes in the hemicellulose fraction (Percy *et al.*, 1996). The changes in cellular structure and composition could explain the difference in textural properties. Juiciness can be explained by the release of cell content when the cells are broken during chewing, while turgor pressure contributes to the crispness in the tissue. The breakdown of the pectic-rich middle lamella takes place during fruit ripening. This weakens the cell to cell adhesion and results in a mealiness textural property. Cellulose provides the cell rigidity while pectin contributes to cell elasticity.

2.2.2.1. Modification of cell wall polymeric network

Solubilisation and degradation of pectic polymers were found in tomato and kiwifruit ripening (Huber, 1992; Redgwell *et al.*, 1992). The pectin found in the cell walls changed from a relatively rigid state to a more mobile state during kiwifruit ripening (Newman & Redgwell, 2002). The 'softening' of pectin is a physical modification as no changes in the chemical composition is observed. Pectin starts to 'soften' in the early stages of ripening, preceding both pectin solubilisation and depolymerisation. Redgwell *et al.* (1992) demonstrated that degradation of pectic polymers in kiwifruit happened after solubilisation. Polygalacturonase (PG) and β-galactosidase are involved in the degradation of pectin (Wegrzyn & Macrae, 1992; Bonghi *et al.*, 1996). Wegrzyn *et al.* (1992) identified an increment in pectin methylesterase (PME) activity during ethylene

treatment followed by a rapid drop to a low level as kiwifruit softened. Although there was a detection of PME activity, little is established on the role of PME on kiwifruit cell wall *in vivo*. Many studies have shown a certain level of enzyme activities during ripening of kiwifruit. However, enzymes work interdependently and hence it is difficult to identify the key enzyme which triggers fruit softening.

Depolymerisation of hemicellulose occurs in fruit softening. Hemicellulose was extensively depolymerized during pepper (Harpster *et al.*, 2002) and papaya (Paull *et al.*, 1999) ripening. Similarly, a significant decrease in molecular weight of xyloglucan in tomato was observed during ripening (Maclachlan & Brady, 1994). The presence of long chain hemicellulose contributes to the rigidity of the cell wall. An increase in molecular weight of xyloglucans has been closely correlated to the increase in mechanical strength of cell walls in pea epicotyls (Miyamoto *et al.*, 1997). When long chain xyloglucans are depolymerised, a decreased in mechanical rigidity of cell wall is observed (Nishitani & Masuda, 1981). A decrease in the average molecular weight of xyloglucan from approximately 500 to 300 kDa (KOH - extracted) or 185 to 115 kDa (GTC – extracted) was observed during kiwifruit ripening (Redgwell *et al.*, 1991). The reduction in the molecular weight of xyloglucan weakens the cellulose – hemicellulose framework, promoting cell wall swelling. The nature of the cellulose crystallites or the polysaccharides adhering to crystallite surface remain unchanged when cell wall dissolution was extreme during kiwifruit ripening (Newman & Redgwell, 2002). Nevertheless, depolymerisation of hemicellulose may not be the key mechanism that results in loss of firmness. Other studies have shown that no traces of depolymerisation of hemicellulose were found during softening. For instance, no major variations or changes in xyloglucan during the ripening of grape berries (*Vitis vinifera L.*) were

observed (Nunan *et al.*, 1998). Similar findings were found in apple during ripening (Percy *et al.*, 1997).

Expansin is a protein found in fruit cell wall and plays a role in fruit softening. Expansin was found to bind between the interface of cellulose microfibrils and matrix polysaccharides, promoting the extension reversibly by breaking the non-covalent bonds within the polymeric network (McQueenmason & Cosgrove, 1995). Many studies failed to identify the presence of hydrolytic activity caused by expansin that explains the breakdown of cell wall structure. An established mechanism proposed is that expansin disrupts the non-covalent bonds between cell wall polysaccharides and thus promoting cell wall extension (Shcherban *et al.*, 1995). The cell wall extension allows the cell to maintain its structure during cell enlargement and expansion under high turgor pressure environment (Cosgrove, 2000). High levels of the expansin gene was found in tomato during ripening suggested that it promotes the breakdown of cell wall structure by exposing the inaccessible non-covalently bound polymers to hydrolysis by endogenous enzymes found in the cell wall and its expression was stimulated by ethylene (Rose *et al.*, 1997). Overexpression or suppression of expansin in transgenic tomato plants have shown evidence of softer or firmer fruit compared to the control (Brummell *et al.*, 1999). Expansin was discovered in strawberry during ripening implying that it is a common component of ripening (Civello *et al.*, 1999). Similarly, expansin was found in kiwifruit and plays a role in the release of pectin from unripe cell wall material (Schroder & Atkinson, 2006).

2.2.2.2. Swelling of cell wall

One of the modifications taking place during ripening is the swelling of cell wall. Swelling of cell wall is caused by pectin solubilisation. The absence of cell wall allows water to move into the void space between the cellulose-hemicellulose network

(Redgwell *et al.*, 1997). Cell wall swelling in kiwifruit during ripening is significant as the cell wall thickness in the outer pericarp was three to four times thicker than fruit at harvest (Hallett *et al.*, 1992). Redgwell *et al.* (1997) reported the presence of cell wall expansion in fruit such as avocado, blackberry, plum and persimmon. On the other hand, apples, pear, and watermelon display little increase in cell wall thickness during ripening, proposing that fruit with crisp texture do not display significant swelling of cell wall as compared to fruit with soft melting texture. Redgwell *et al.* (1997) interpreted that swelling of cell wall can be strongly correlated to pectin solubilisation but not pectin depolymerisation. The viscosity of the cell wall material increases during the swelling of cell wall, where the cell wall material of kiwifruit was found to be more viscous when the fruit is ripened (Redgwell *et al.*, 1992).

2.2.3. Chilling injury development

Chilling injury is a physiological disorder which develops when fruit are stored at low temperature, slightly above their freezing temperature. Chilling injury can be a reversible or irreversible process. Chilling injury can be classified as primary or secondary event (Marangoni *et al.*, 1996). During a primary event, phase change in membrane lipids is induced. This is a reversible process until a secondary event occurs. A secondary event causes a modification in the normal properties of the membrane lipids. Phase change in the cell membrane of avocados can be reversed by increasing the storage temperature, however once the cell membrane is damaged (i.e secondary phase), the process is found to be irreversible (Plattaloia & Thomson, 1987). Usually, the severe and permanent chilling injury symptoms found on fresh produce are the outcome of irreversible phase of the reaction.

The symptoms of chilling injury vary depending on the type of commodity. In 'Hayward' kiwifruit, the typical chilling injury symptoms are development of ring or

patch of granular, water soaked tissue in the outer pericarp and formation of diffuse pitting along with the development of a dark scald-like appearance in the skin (Lallu, 1997). Not all fruit display similar chilling injury symptoms as kiwifruit. Stone fruit such as apricots, plums and peaches display a mealy texture, woolliness, flesh browning, flesh translucency, loss of flavour, and fail to ripen (Vonmollendorff *et al.*, 1992). Tomato exhibits similar chilling injury symptoms as for stone when compared to the non-chilled tomatoes (Jackman *et al.*, 1992). Citrus fruit develop chilling injury symptoms such as cold pitting, brown staining, and increases in the susceptibility to decay and quality losses (Menesatti *et al.*, 2005). Development of browning at the core and flesh is the main chilling injury disorder for 'Yali' pear (Wu *et al.*, 1992). These chilling injury symptoms affect the overall quality and appearance of the commodity which results in consumer rejection and large volume of fruit wastage.

The loss of cell membrane integrity may be used to explain the mechanism behind the water soaked appearance in chilling injured kiwifruit. Excessive oxidative stress damages the cell membrane integrity and possibly leads to the water soaked appearance along the outer pericarp of kiwifruit. Oxidative stress is caused by excess reactive oxygen species (ROS), which the cell struggles to scavenge (Hodges *et al.*, 2004). ROS can be scavenged with the presence of antioxidant compounds, such as ascorbic acid and glutathione, and antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Mittler, 2002). Increasing the antioxidant enzymes helps to increase the chilling tolerance. An increase in antioxidant enzymes activities was found to reduce accumulation of ROS and thus lower chilling injury development in cucumber (Yang *et al.*, 2011) and kiwifruit (Yang *et al.*, 2012; Yang *et al.*, 2013).

Chilling injury affects the cell wall composition. Grainy texture is one of the chilling injury symptoms in kiwifruit and is explained by the reduction in cell to cell adhesion due to solubilisation of pectin in the middle lamella. This results in cells being able to remain intact without releasing their cellular contents when crushed (Brummell *et al.*, 2004). The grainy texture found in 'Braeburn' apple was found to be associated with the reduction of cell to cell adhesion between neighbouring cells (Harker & Hallett, 1992). The cell wall bulk porosity of chilling injured kiwifruit cell decreased with severity and the grainy appearance found in kiwifruit is proposed to be related with the presence of gas bubbles in cells (Bauchot *et al.*, 1999). Bauchot *et al.* (1999) also demonstrated that grainy appearance of kiwifruit tissue have 30% more cell wall material (CWM) and 70 % more galactosyl content in the CWM of outer pericarp tissue compared to the unaffected tissue.

2.3. Factors affecting fruit ripening

Previous section 2.2 has explained the possible biochemical reactions occurring during fruit ripening which result in an increase in soluble solids content and decrease in fruit firmness. Temperature, ethylene, humidity, fruit maturity and rot development are factors that can affect fruit ripening. It is important to understand their influence on fruit ripening and thus allowing the developed model to be responsive to these factors accordingly. The sections below discuss the effect of temperature, ethylene, humidity, fruit maturity and rot development on fruit ripening.

2.3.1. Temperature

Temperature is one of the major factors that influence the metabolism in kiwifruit and the rate of ripening (Hawkins, 1922; Beever & Hopkirk, 1990; Ritenour *et al.*, 1999; Prasanna *et al.*, 2007). A decrease in temperature reduces respiration rate (Wright & Heatherbell, 1967; Antunes & Sfakiotakis, 1997; Heyes *et al.*, 2010). Antunes and

Sfakiotakis (1997) reported an approximately tenfold increase in respiration rate when kiwifruit were exposed to increased temperature from 10 to 40 °C.

The rate of softening is also affected by temperature. Schotsmans *et al.* (2008) demonstrated this temperature dependence over a temperature range from 1.5 to 25 °C. This was also reported by Ritenour *et al.* 1999. However, when fruit are exposed to very high temperature conditions, the rate of softening was found to decrease. This phenomenon was observed in apples (Johnston *et al.*, 2001), avocados (Eaks, 1978), pears (Maxie *et al.*, 1974), plums (Tsuji *et al.*, 1984) and tomatoes (Biggs *et al.*, 1988) when exposed to high temperature conditions between 30 to 40 °C. Because rate of softening is sensitive to temperature, kiwifruit has a storage life of at least 20 to 24 weeks when stored at 0 °C (McDonald, 1990), but when stored at 20 °C the storage life decreases drastically to 3 weeks (White *et al.*, 2005; Jabbar, 2014). The industry stores kiwifruit at 0 °C to ensure a long storage life and this allows the export of large quantities of fruit to different countries by shipment.

2.3.2. Ethylene

Ethylene plays an important role in fruit softening evidence by exposure of fruit to ethylene triggering accelerated softening (Hewett *et al.*, 1999; Kim *et al.*, 1999; Jabbar & East, 2016). McAtee *et al.* (2015) demonstrated that kiwifruit follows a hybrid ethylene independent-dependent mechanism where the first phase of softening is independent to ethylene while the second phase of softening is dependent on ethylene. Besides ethylene, exposure to cold temperature triggers fruit softening (Snelgar *et al.*, 1993; Burdon *et al.*, 2007). The mechanism that triggers fruit ripening remains ambiguous, either being initiated by exogenous ethylene or chilling conditions. Therefore, it is challenging to describe the primary initiation of fruit softening.

Influence of ethylene on fruit softening has been studied in apples (Johnston *et al.*, 2002; Gwanpua *et al.*, 2012) and tomatoes (Biggs *et al.*, 1988; Alexander & Grierson, 2002). The presence of ethylene is known to influence softening rates in kiwifruit. There are many studies conducted to discuss the effect of ethylene on kiwifruit ripening during storage at 20 °C (Antunes & Sfakiotakis, 2000, 2002b; Antunes, 2007; Albert *et al.*, 2013) and 0 °C (Arpaia *et al.*, 1986; Jeffery & Banks, 1996; Wills *et al.*, 2001). Ethylene production is a complex biochemical reaction (Lieberman, 1979; Lelievre *et al.*, 1997), which can be inhibited with low activities of 1-aminocyclopropane-1-carboxylic acid content (ACC) and reduce ethylene-forming enzyme activity (Stavroulakis & Sfakiotakis, 1993; Antunes & Sfakiotakis, 1997). Fruit produces ethylene resulting in an accumulation of ethylene inside the fruit. Internal ethylene diffuses to the atmosphere when the ethylene concentration is lower in the atmosphere than fruit internal ethylene. The skin of the fruit is the principle barrier to ethylene loss from internal to the surrounding atmosphere. However, in situations where the concentration of ethylene in the atmosphere is greater than internal to the fruit, ethylene will diffuses into the fruit and affects softening.

Kiwifruit produces low levels of ethylene at harvest, less than 0.01 $nL.kg^{-1}h^{-1}$ (Burdon & Lallu, 2011). During storage at 0 °C, kiwifruit produces low level of ethylene in first 2 months of storage and production subsequently accelerates rapidly to the climacteric peak during the late storage period of 100 to 140 days (Chiaramonti & Barboni, 2010). Kiwifruit are found to produce high amount of ethylene when firmness falls below 10 N (Beever & Hopkirk, 1990; Mitchell, 1990; Lelievre *et al.*, 1997; Manolopoulou & Papadopoulou, 1998; Taglienti *et al.*, 2009; Burdon & Lallu, 2011). Therefore, accumulation of ethylene within a package is only likely to take place during the late storage period, provided there is an absence of rotten, chilling injured or damaged fruit. Rotten, chilling injured and damaged fruit have been found to produce ethylene at

higher rates (Hyodo & Fukasawa, 1985; Antunes & Sfakiotakis, 2002a; Feng *et al.*, 2003b; Burdon & Lallu, 2011) and thus their presence is likely to accumulate ethylene concentration within the atmosphere of the package, potentially affecting the neighbouring fruit in the same pack.

Chilling injured fruit were found to produce ethylene (Hyodo & Fukasawa, 1985; Antunes & Sfakiotakis, 2002a; Feng et al., 2003b), potentially increasing the amount of ethylene within the package. Since kiwifruit is found to be very sensitive to ethylene, the increase amount of ethylene produced by chilling injured fruit may potentially affect the neighbouring fruit within the same package, leading to a huge variation in fruit firmness across a single fruit pallet. Ethylene exposure was found to influence chilling injury development. Jabbar and East, (2016) showed that exposing fruit to ethylene concentration of 1 $\mu L\ L^{-1}$ resulted in a higher incidence (> 20 %) of chilling injured fruit.

In a real coolchain scenario, ethylene can accumulate within packages and therefore it is important to understand the effect of ethylene on fruit softening. However, the amount of ethylene in fruit or atmosphere is difficult to quantify accurately and thus making it a challenge for the industry to measure ethylene concentration throughout the coolchain. East *et al*. (2015) identified the difficulties to predict ethylene concentration within a pack as ethylene transmission across the packaging material is complex, where the packaging material is enclosed, but not perfectly sealed. Furthermore, the industry does not have the technology to measure ethylene concentration in the working range of 10 $nL\ L^{-1}$ to 10 $\mu L\ L^{-1}$ and thus quantifying ethylene concentration within a pack is impractical. Even though it is difficult to measure, the industry actively scrubs ethylene from the surrounding coolstore air during storage. Therefore, initially the effect of ethylene on fruit softening will be excluded in the model development.

2.3.3. Humidity

Kiwifruit stored in low humidity conditions result in moisture loss and skin shrinkage (Bautista-Banos *et al.*, 2000). Shrivel can occur in extreme cases of water loss, resulting in wrinkling or flaccidness in fruit. Approximate of 4 to 5 % weight loss during storage puts kiwifruit at risk of shrivelling (Burdon & Lallu, 2011). Besides weight loss, shrivelling is found to be influenced by softening and the water characteristics of the fruit's outer pericarp when the fruit soften (Burdon *et al.*, 2014a).

Weight loss in fruit is found to be related to water vapour pressure deficit (WVPD), where greater weight loss is observed at higher WVPD (Paull, 1999). Weight loss can be reduced by lowering WVPD through reducing air temperature, increasing humidity or providing a barrier to water loss (Grierson & Wardowski, 1978; Ben-Yehoshua, 1987). To avoid significant weight loss, kiwifruit are cooled promptly to storage temperature, stored at high humidity (95% RH), and a polyliner is wrapped around the tray of fruit to maintain a high humidity conditions within the pack.

Humidity has been found to influence rot development in kiwifruit. Bautista-Banos *et al.* (2000) found an increase in relative humidity coincides with a decrease in infection levels in kiwifruit. The incidence of rots in table grapes was found to decrease when relative humidity increased from 85 to 90 % and subsequently increased when relative humidity falls above 95 %, suggesting the optimal humidity to store table grapes is between 90 to 95 % RH (Pinto *et al.*, 2015). Storing fruit at optimal humidity conditions is a common practise to lower incidence of rot. Vegetables such as carrots, potatoes and cabbage have been reported to best store at a range of 90 to 95 % RH to reduce the incidence of rot (Ryall & Lipton, 1979). Kiwifruit is recommended to be stored at a relative humidity of 95 % at 0 °C (McDonald, 1990; Lallu *et al.*, 1992). Fruit are packed inside a polyliner, maintaining the humidity within the tray. However, when cooling fruit

to storage temperature, there is a likelihood that condensation will form on the polyliner, which may have consequences such as promoting rot development.

Overall, humidity indirectly influences fruit softening as it has an effect on rot development and rot influences fruit firmness. The model aims to predict fruit firmness under supply chain conditions and thus understanding the effect of humidity on fruit softening will aid in the model development, however it is a secondary factor and initially model parameters that explain the effect of humidity on firmness may be omitted.

2.3.4. Fruit maturity

Kiwifruit are harvested across different maturities. Fruit maturity has been found to influence subsequent fruit quality during long term storage. Late maturity fruit were found to be firmer in late storage than early maturity fruit (Mitchell *et al.*, 1992; Costa *et al.*, 1997). Kiwifruit are considered mature upon harvest but what determines their competence to ripen is still unclear. McAtee *et al.*, (2015) found the fruits competence to ripen was independent to ethylene exposure, whereas exposing fruit to cold temperatures triggers the fruit to ripen (Snelgar *et* al., 1993 and Burdon *et al.,* 2007). The changes required to initiate ripening may explain the presence of the lag phase in softening observed in early maturity fruit that is not observed in late maturity fruit (Beever & Hopkirk, 1990, Schroder & Atkinson, 2006 and Jabbar *et al.,* 2014). Since fruit maturity was found to affect softening, it is important that the developed model is able to account for maturity differences across the harvest season.

Fruit maturity has also been observed to influence chilling injury development. The lack of cold acclimatisation in field or advancement in ripening explains the incidence of chilling injury development in kiwifruit Koutsoflini *et al.* (2013) explained that the high incidence of chilling injury development in kiwifruit is caused by the lack of acclimatisation to low temperature. Susceptibility to chilling injury in kiwifruit has

also been observed to be lowered due to increase in cold acclimatisation in the field (Sfakiotakis *et al.*, 2005; Burdon *et al.*, 2007). Advancement in ripening due to delay storage at 20 °C or ethylene exposure was found to promote chilling injury development in kiwifruit (Koutsoflini *et al.*, 2013). Jabbar & East, (2016) also found that exposing kiwifruit to ethylene at harvest increases incidence of chilling injury.

Fruit maturity is estimated based on soluble solids content as it increases during ripening (Burdon & Lallu, 2011; Burdon *et al.*, 2013). In the New Zealand industry, a minimum soluble solids content of 6.2 °Brix is required before harvesting to obtain fruit with good quality and storage performance (Harman, 1981; Mitchell, 1990). Since soluble solids content is used to estimate fruit maturity and is easily collected by the industry, it can be used as a model input to differentiate fruit maturity across the harvest season.

2.3.5. Rot development in kiwifruit

Rot development occurs during or after postharvest storage depending on the survivability of the particular organism (Pennycook, 1985; Brook, 1990). The main postharvest pathogens that are responsible for fruit rots in New Zealand are *Botrytis*, *Cryptosporiopis*, *Phomopsis*, and *Cylindrocarpon* (Burdon & Lallu, 2011). *Botrytis* is able to survive at low temperatures and can be found at the stem end, body or stylar end of the fruit after 4 to 8 weeks of storage (Brook, 1992; Manning *et al.*, 2010; Burdon & Lallu, 2011). Pennycook (1985) explained that the spores of *Botrytis* are usually deposited at the picking wound during handling, packing and packhouse grading operations. When the uppermost cell layers of the picking wound are ruptured, the conidia germinate and grow rapidly into the fruit's vascular tissues.

Manning *et al.* (2010) discussed the possibility to reduce fruit rots caused by *Botrytis* by introducing curing. Curing is a process whereby kiwifruit are exposed to ambient temperature and humidity for a period of time after harvest and is usually applied

at the beginning of the supply chain before grading and packing. The influence of curing on rot development is possibly temperature and humidity dependent. Although the mechanism behind curing is not well defined, it was believed to be associated with a low degree of water loss that occurs before packing and/or cooling. Lallu and Webb (1997) found that fast cooling fruit to storage temperature increased the occurrence of *Botrytis* rot in kiwifruit due to the associated water loss or condensation at the picking scar.

The major physiological effect of rot development in kiwifruit is the resultant ethylene produced by the damaged fruit tissues. As discussed above that kiwifruit are sensitive to ethylene and therefore the amount of ethylene produced by rotten fruit potentially affects the neighbouring fruit within the same package. The presence of rotten fruit at harvest is likely to influence fruit softening. Burdon *et al.* (2011) discussed the occurrence of rot in kiwifruit would result in a significant difference in fruit firmness across different packs within a single pallet as the fruit in the same pack will soften rapidly compared to fruit in surrounding packs.

2.4. Kiwifruit supply chain

The kiwifruit supply chain includes harvesting from the orchard, grading, packing, cooling, storing, exporting and retailing before the products reach the end consumers. Kiwifruit are exposed to different pre and post-harvest treatments to extend fruit storability across the supply chain. In the development of a mathematical model to predict fruit firmness across different supply chain conditions, it is important to identify the possible factors that affect fruit softening across the supply chain.

2.4.1. Pre-harvest treatments

Pre-harvest treatments such as vine management (Boyd & Barnett, 2011; Patterson & Currie, 2011) or manipulation of light exposure (Tombesi *et al.*, 1993) are adopted by growers to improve fruit quality. Dry matter content is used as a quality index

for 'Hayward' kiwifruit. Generally fruit with high dry matter content is more acceptable to consumers (Crisosto *et al.*, 2011). Growers providing fruit with high dry matter content at harvest receive incentives, motivating them to adopt pre-harvest treatments. Boyd & Barnett (2011) demonstrated the application of girdling and low cropload to kiwifruit vines which resulted in an increase in fruit size and dry matter content. Similarly, Patterson & Currie (2011) showed that applying vine management techniques such as girdling, management of fruit bio stimulants, cane stringing and thinning on kiwifruit vines consistently deliver high productivity and taste potential fruit (i.e. high dry matter content).

Nutrient fortification is another pre-harvest treatment used to improve fruit quality. Calcium fortification on vines at full bloom stage potentially improves the storage life of kiwifruit (Xu *et al.*, 2015). Calcium ions form strong calcium bridges between pectin molecules (Brummell, 2006; Goulao & Oliveira, 2008) and thus achieves firmer fruit by altering the rate of cell wall degradation. Other nutrients including nitrogen, phosphorous, potassium and magnesium are likely to affect fruit firmness (Prasad & Spiers, 1992; Smith *et al.*, 1994; Feng *et al.*, 2003a). Since pre-harvest treatments were found to affect fruit quality, applying commonly collected at-harvest attributes (i.e. soluble solids and dry matter content) as initial model inputs allows quantification of pre-harvest treatments. However, this approach only describes the pre-harvest treatments that alter the soluble solids and dry matter content (i.e. vine management and manipulation of light exposure).

2.4.2. Postharvest treatment

Fruit quality is influenced by several factors throughout the supply chain. These coolchain scenarios include the exposure to different cooling rates, storage temperatures, breaks in temperature control, high temperature conditions and fluctuating humidity conditions. The New Zealand industry has been collecting time temperature information

of the supply chain to identify the possible temperature scenarios that compromise fruit quality. Since the model focus is to predict fruit firmness under supply chain conditions, it is important to investigate the effect of several temperature scenarios on fruit firmness.

In packhouses, fruit are graded, packed and cooled to storage temperature based on their cooling capacity and practises. Force air cooling, hydrocooling, room cooling and vacuum cooling are the several techniques used to cool fruit rapidly to storage temperature (Brosnan & Sun, 2001). Precooling is usually used to remove field heat from fruit after harvest to improve fruit quality (Findlay & Combrink, 1996). Lallu and Webb (1997) demonstrated that precooled kiwifruit are firmer during storage. A shorter cooling time is found to improve spear quality and less weight loss in asparagus (Lallu *et al.*, 2000). Applying precooling to strawberries after harvest has reported to improve the fruit quality (Pelletier *et al.*, 2011) and reduce fruit decay incidence (Nunes *et al.*, 2005).

Although precooling has been demonstrated to improve fruit quality, many studies have shown that precooling of fruit may cause an adverse impact on fruit quality. Lallu (1997) proposed that exposing kiwifruit to precooling promotes chilling injury development and thus affects fruit quality. Precooled kiwifruit is firmer compared to non-precooled fruit however, the incidence of Botrytis stem end rots has been shown to be higher in precooled fruit (Lallu & Webb, 1997). It is difficult for the industry to slow cool large amounts of fruit to storage temperature and thus precooling is usually adopted by packhouses.

Temperature conditioning is one of the several postharvest techniques used to lower the incidence of chilling injury by exposing commodity to temperatures that are slightly above the critical chilling range either in a single or multiple step conditioning. This may allow fruit to acclimatise to low temperature. By applying temperature

conditioning, the amount of phospholipids in membranes is maintained at high level, there is an increment in the degree of unsaturation in membrane fatty acids and suppression of the sterol to phospholipid ratio (Wang, 1994). Yang *et al*. (2013) demonstrated that chilling injury in 'Hayward' kiwifruit can be alleviated when exposed to temperature conditioning at 12 °C for 3 days due to an increase in antioxidant enzyme activities and maintaining higher levels of endogenous hormones. The incidence of chilling injury in 'Hongyang' kiwifruit was lowered when the fruit were gradually cooled from 15 to 0 °C (Yang *et al.*, 2012). Exposing banana (Pantastico *et al.*, 1968), cucumber (Nakamura *et al.*, 1985; Lafuente *et al.*, 1991), eggplants, tomatoes (Galvez *et al.*, 2010), pear (Lim *et al.*, 2005; Yan *et al.*, 2013), and mangoes (Rodeo & Esguerra, 2013) to temperature conditioning effectively reduces chilling injury.

Storage temperature was found to influence fruit quality. The occurrence and severity of chilling injury in kiwifruit are influenced by storage temperature. Over a storage temperature range of -0.5 to 2.5 °C, Lallu (1997) demonstrated that storing precooled fruit at storage temperature above 1 °C lowered the incidence of chilling injury development. In peaches, chilling injury occurs when stored at temperature between 2.2 and 7.6 °C, which is known as the killing temperature zone (Crisosto *et al.*, 1999). In addition, storing Lanes Late navel oranges at -1 °C had the highest incidence of chilling injury (Henriod *et al.*, 2005).

During storage, differences in temperature across several locations within industrial refrigerated coolrooms was observed due to oscillation around the set room temperature (East *et al.*, 2016). For instance, when the coolroom is set at 1 °C, the refrigeration system will be activated at 2 °C and switched off at 0 °C. Therefore, fruit that are located near the evaporator will be closer to 0 °C while fruit located away from the evaporator will be exposed to 2 °C. Since it was found that cooling rate and storage

temperature affect fruit quality by promoting chilling injury development, there may be a possibility to position pallets of fruit in coolroom according to the cooling profile. For instance, fruit that were cooled fast will be placed away from the evaporator (i.e. 2 °C) while fruit that were slow cooled could be located near the evaporator (i.e. 0 °C).

Due to logistic constraints, temperature can be difficult to maintain throughout the coolchain. Therefore, fluctuation in temperature is likely to occur across the supply chain. The New Zealand kiwifruit industry does not actively control temperature between on-shore storage facilities and the port, as a cost saving measure. A standard side curtain delivery truck is used to transfer kiwifruit between on-shore storage and the port which does not control temperature. Hence, a break in temperature control is likely to happen during the transportation from on-shore storage to the port prior to shipment. Temperature fluctuations and breaks have been shown to influence ripening of other fruits such as breaks in temperature for a day at 20 °C led to a significant softening in apples (East *et al.*, 2008). Similarly, the quality of strawberries (colour, weight loss, firmness, shrivelling, and decay and bruise incidence) was affected when exposed to temperature fluctuation during ground, in-flight and retail handing operations (Nunes *et al.*, 2003).

Kiwifruit are exported to countries in South East Asia and the Indian subcontinent. Bellavi Jayashiva (2012) demonstrated the change in fruit firmness when kiwifruit were exported to Indian markets, with an average ambient temperature of 35 °C. In addition, there is likelihood for fruit to be exposed to fluctuating humidity conditions, especially when exporting to countries with low humidity weather. Fruit are packed within a layer of polyliner, which maintains high humidity conditions during storage. However, exposing to high temperature and fluctuating humidity conditions may alter the temperature and humidity within the pack and thus compromising fruit quality.

2.5. Softening models

Using a mathematical approach to describe fruit softening pattern has been well established in many studies, ideally using mathematics to associate theory with experimental data. A mathematical model to characterise fruit softening behaviour can be represented by either an equation or a set of equations (Thornley & France, 2007). Mathematical approaches have been widely adopted to make quantitative softening predictions that can be compared with the real experimental data. The postharvest performance of apples under different storage conditions has been well described mathematically (Johnston *et al.*, 2001; Johnston *et al.*, 2002; Roth *et al.*, 2008; Van Pham *et al.*, 2008; Gwanpua *et al.*, 2012). Similarly, mathematical approaches have been used to describe the postharvest performance of banana (Chen & Ramaswamy, 2002; Quevedo *et al.*, 2009; Hashim *et al.*, 2012), cherry (Muskovics *et al.*, 2006), peach (Tijskens *et al.*, 1998; Tijskens *et al.*, 2012), and tomato (Van Dijk *et al.*, 2006a; Van Dijk *et al.*, 2006b; Pinheiro *et al.*, 2013). The following sections will focus on describing kiwifruit softening patterns using empirical or mechanistic approaches.

2.5.1. Empirical approach

Empirical models are developed by fitting curves to large sets of experimental data using mathematical or statistical equations such as complementary Michaelis – Menten (CMM), Complementary Gompertz (CG), or Weibull probabilistic models. Thornley *et al.* (2007) explained that empirical model contains several unknown parameters that do not have any scientific or biological explanations. A combination of two Weibull probabilistic models containing 4 different parameters were developed to describe kiwifruit softening based on fruit elasticity (Terasaki *et al.*, 2013). Benge *et al.* (2000) demonstrated use of a empirical approach to describe the kiwifruit softening pattern during storage but this approach failed to characterise softening with sufficient accuracy. Hence, more complicated models such as segmented Jointed Michaelis –

Menten (JMM) and Inverse Exponential Polynomial (IEP) were developed to better characterise the softening (Benge *et al.*, 2000). The application of Complementary Gompertz (CG) and Time Shift Complementary Gompertz (TSCG) were used to predict kiwifruit softening at 20 °C in air, introducing batch specific parameters to account for grower line variability (Jabbar, 2014). However, these models are too rigid and thus unable to describe softening behaviour when fruit are exposed to different conditions apart from the experimental data used to develop these models.

2.5.2. Mechanistic approach

A mechanistic approach can be used to mathematically describe fruit softening based on an extensive understanding or explanation of the phenomena being modelled (Thornley & France, 2007). Exponential kinetics, Michaelis Menten and linear kinetics are often used when developing mechanistic models. Adopting a mechanistic approach to describe fruit softening provides flexibility in modelling fruit softening behaviour under different storage conditions such as temperature or gas compositions. Kiwifruit softening under modified atmosphere conditions has been modelled mechanistically by relating the softening rate with gas exchange using the Michaelis Menten model (Hertog *et al.*, 2004c). Kinetics models have been used to describe kiwifruit softening when exposed to different storage temperatures (Schotsmans *et al.*, 2005; Schotsmans *et al.*, 2008). Similarly, a kinetic model was used to describe colour change in kiwifruit slices during hot air drying at different temperatures (Mohammadi *et al.*, 2008).

2.5.2.1. Temperature dependency

Fruit softening is dependent on temperature and thus the rate of softening is a function of temperature. Often, the Arrhenius equation is introduced to account for temperature dependency. Hertog *et al.* (2004) and Schotsmans *et al.* (2005 & 2008) applied the Arrhenius equation to describe the effect of temperature on kiwifruit softening.

Johnston *et al*. (2001) demonstrated that rate of softening in apple increased from 0 to 24 °C and subsequently decreased when exposed to temperature between 24 and 35 °C. The decrease in softening rate at high temperature is explained by reduced in ethylene biosynthesis (Klein & Lurie, 1990), and reduction in cell wall degradation (Klein *et al.*, 1990; Shalom *et al.*, 1993a). Therefore, Johnston *et al*. (2001) used Arrhenius equation to explain the increase in softening rate at temperature between 0 to 24 °C and introduced a Boltzman component to account for the decrease in softening rate at temperature between 24 and 35 °C.

2.5.2.2. Biological variability

Fruit are harvested across different orchards and maturities and thus biological variation exists between fruit. For instance, biological variation is distinguishable by the difference in at-harvest colour in tomato. Biological variability in postharvest behaviour has been interpreted using several different techniques (Lammertyn *et al.*, 2003; Scheerlinck *et al.*, 2004; Tijskens *et al.*, 2005; De Ketelaere *et al.*, 2006). Biological age is used to define the biological variability in tomato, assuming a ripening process from fruit setting to senescence is the same for all tomatoes (Tijskens & Evelo, 1994; Tijskens *et al.*, 2003; Hertog *et al.*, 2004b). Introducing variables such as biological age into kinetic models to describe the change in postharvest quality will allow to model fruit postharvest behaviour across a batch instead of individual fruit. Hertog *et al.* (2004a) applied a probabilistic kinetic approach to explain the postharvest variation in tomato colour in terms of variation in biological age. A similar approach was applied to describe the complex behaviour of a batch reflecting propagation of biological variation in the growth of Belgian endive (Hertog *et al.*, 2007b). The industry handles fruit in batches and thus integrating propagation of variation in mechanistic modelling will benefit the industry to predict the postharvest batch behaviour.

2.6. Summary

This chapter displayed the challenges faced by the kiwifruit industry to reduce fruit losses. There are several factors that affect kiwifruit ripening, including exposure to ethylene, temperature, fluctuating coolchain scenarios, and pre-harvest treatment. As a result, predicting kiwifruit ripening is a difficult task. Significant variation in fruit firmness occurs between batches (due to orchard management, harvest date, and production area) and within batches (due to presence of rotten or chilling injured fruit). These factors further complicate the prediction of fruit softening.

The use of mathematical modelling to describe fruit softening is not a new approach. The current models developed by Hertog *et al.* (2004) and Schotsmans *et al.* (2005 and 2008) demonstrated the ability to describe kiwifruit softening. However, these models did not include the effects of chilling injury development on fruit firmness or include at-harvest attributes as model inputs for different fruit maturities. This research aims to develop a mathematical model to describe 'Hayward' kiwifruit softening in supply chain conditions, including the effect of chilling injury development. Such a model will contribute towards improved information on fruit softening to the industry with the intention to reducing fruit losses.

3. Softening of 'Hayward' kiwifruit in different coolchain scenarios (*)

3.1. Introduction

Fruit quality is difficult to maintain throughout the supply chain as parameters such as temperature and humidity are likely to fluctuate over time. The industry has established a system to monitor temperature and humidity across the supply chain, from harvest to final consumer (Bollen *et al.*, 2013). After harvesting, fruit are transported to the packhouse for grading, sorting and packing. The fruit is then stored in a coolroom for up to 7 months before export to overseas markets in a refrigerated cargo ship or container. Excessive softening and rotten fruit result in an increase in fruit losses and additional labour cost for sorting and re-packing. Different cooling profiles, storage temperature, breaks in temperature control (Figure 3.1), high temperature environments, and fluctuating humidity at high temperature environments were identified from the time temperature profile of the coolchain as possible factors to cause potential risk on fruit quality.

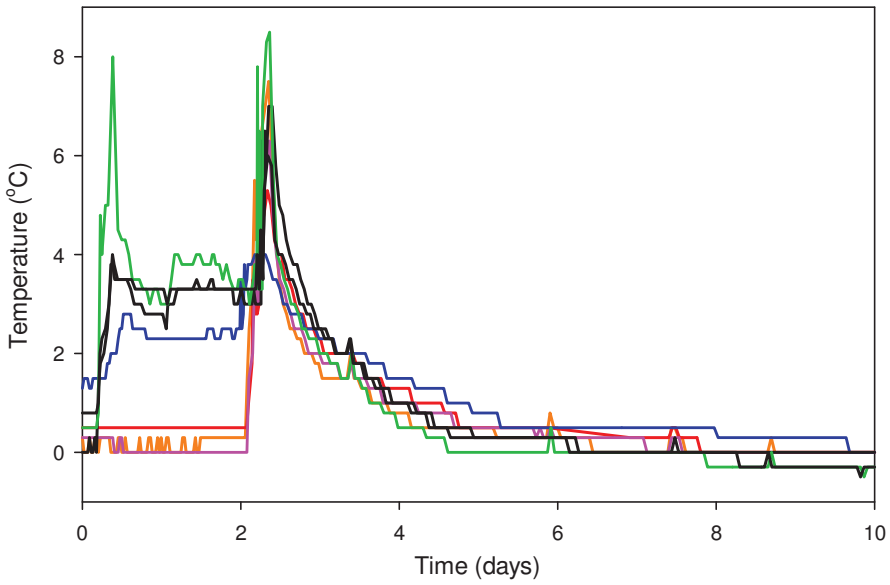


Figure 3.1: Example of time temperature profile of the air inside the box retrieved from commercial monitoring system.

(*) This chapter includes material published in the paper:
Zhao, J. M., Bronlund, J. E. and East, A. R. (2015). Effect of Cooling Rate on Kiwifruit Firmness and Rot Incidence in Subsequent Storage. *Acta Horticulturae*, 1079, 313-318

There are several occurrences throughout the supply chain that pose potential risk to kiwifruit quality. Ripening and senescence can be reduced by removing the field heat of harvested produce and thus maintaining good keeping quality of fruit and vegetables. Precooling is a common practice to effectly remove field heat before storage. However, it was found that kiwifruit are susceptible to chilling injury and rot development when precooled in 9 to 12 hours to storage temperature (Lallu & Webb, 1997).

The softening rate of kiwifruit is a function of storage temperature (Hertog *et al.*, 2004c; Schotsmans *et al.*, 2008). Exposing kiwifruit to high temperature will accelerate softening and thus decrease storability. Water or mass loss is the outcome of exposure to a lower humidity environment, which eventually leads to shrivel (Paull, 1999). Furthermore, water loss affects the fruit metabolism, accelerating fruit ripening (Burdon *et al.*, 1994). Due to logistical constraints, a break in temperature control can occur in supply chains, which may affect fruit quality. The likelihood for kiwifruit to be exposed to a break in temperature control is greater when using the standard curtain sider trucks as a tool for transportation. Studies have shown that the quality of apples (East *et al.*, 2008) and strawberries (Nunes *et al.*, 2003) were affected when exposed to breaks in temperature control. Exporting fruit to countries in the Indian subcontinent and South East Asia will potentially expose fruit to high temperature and fluctuating humidity environments.

Kiwifruit softening generally consists of three distinct softening phases. An initial lag phase is followed by a rapid decline and subsequently a gradual softening phase towards a lower asymptote (White *et al.*, 2005; Jabbar *et al.*, 2014). In addition, the rate of softening has been observed to increase during the gradual softening phase (Benge *et al.*, 2000; Schroder & Atkinson, 2006). White *et al.* (2005) suggested that the initial lag

phase is correlated to the time taken to become fully ripe and thus affected by the fruit maturity.

The industry has implemented a wireless monitoring system to monitor the fruit pallet temperatures (Bollen *et al.*, 2013), which can identify the several possible conditions in the supply chain that will potentially compromise the kiwifruit quality. The aim of this study is to investigate the storability of 'Hayward' kiwifruit as influenced by these conditions monitored in industry. Understanding the effect of these factors on storage performance will allow assessment of the importance of these deviations from optimal storage conditions and aid in the development of a predictive model for kiwifruit quality during storage with given time-temperature information.

3.2. Material and methods

3.2.1. Supply chain simulation

Approximately 108 trays of commercially produced 'Hayward' kiwifruit from the Bay of Plenty region were harvested in late May 2012 from 3 different grower lines. Fruit were commercially graded with count 36 size fruit delivered to Massey University, Palmerston North in modular bulk boxes and subsequently randomly packed into single layer trays with polyliners. A truck without temperature control was used to transport fruit from packhouse to Massey University. Care was taken to separate grower lines as each grower line was used as a replicate. Fruit were cooled using two different cooling methods, direct or gradual cooling and subsequent exposed to different temperature scenarios.

3.2.1.1. Cooling profiles

Trays of fruit were exposed to two different cooling methods, either direct or gradual cooling. Direct cooling (D) was achieved by placing the trays of fruit into a cool room set at 0 or 2 °C with 95 ± 5% RH (Figure 3.3). Gradual cooling (G) was achieved by placing the trays of fruit into a cool room with decreasing set point temperature from 16 to 0 or 2 °C over a period of 2 weeks (Figure 3.3). The fruit were subsequently stored in a cool room set at 0 ± 1 or 2 ± 1 °C with 95 ± 5% RH respectively for 25 weeks. The temperature and humidity of the cool room and air inside the fruit tray were monitored using data loggers, iButton (DS1923, Maxim Integrated, USA) throughout the cooling and storage period. The cool room temperature and humidity were monitored by placing the data loggers at different locations within the cool room (Figure 3.2). Fruit were cooled by stacking 20 - 24 trays in a column. The data logger was placed at the centre of the fruit tray, beneath the polyliner and thus measuring the air temperature inside the tray. The tray that contains the data logger was located at the centre of the stacked column during cooling. Temperature and humidity were recorded at intervals of 15 minutes.

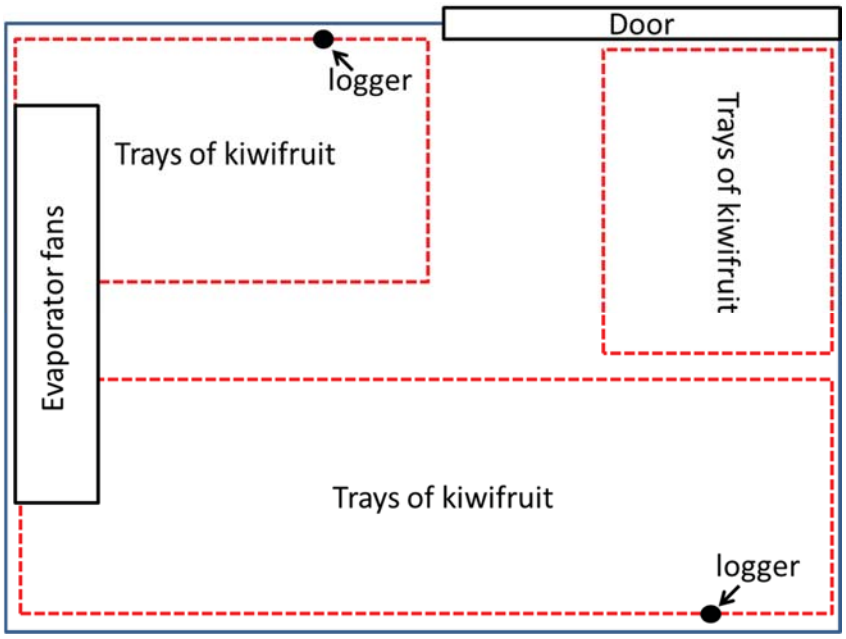


Figure 3.2: Configuration of the coolroom with the location of the trays and data logger to monitor the room temperature and humidity.

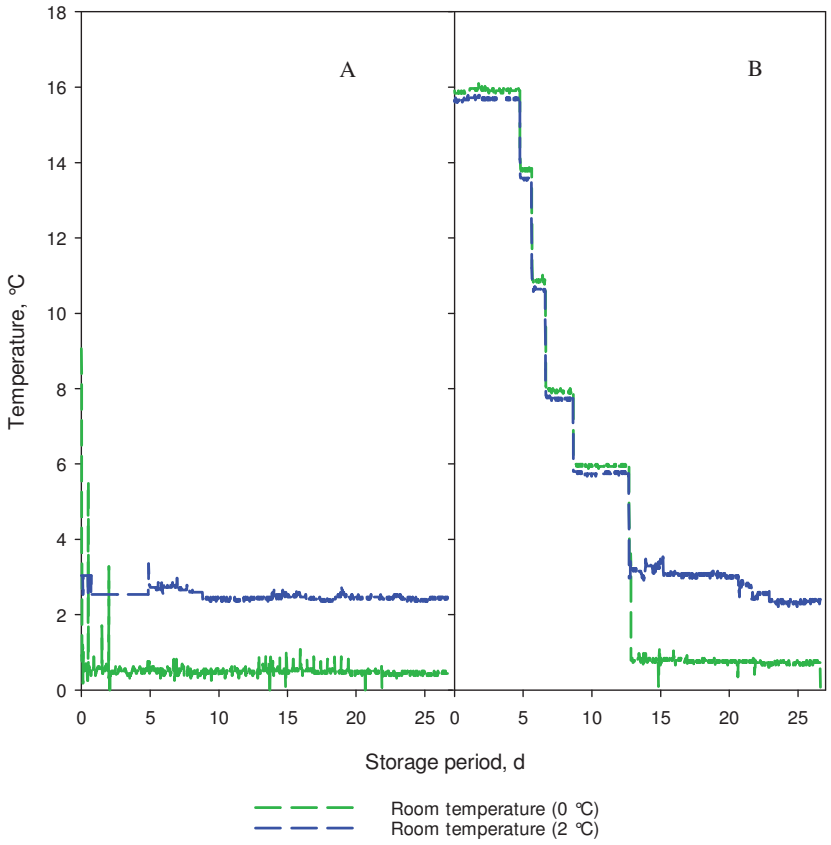


Figure 3.3: Time temperature profile of direct (A) and gradual (B) cooling to 0 or 2 °C.

3.2.1.2. Temperature scenarios

Kiwifruit were placed in a single layer tray with a layer of polyliner added to each tray. Temperature and humidity loggers (iButton) were placed inside the tray to monitor the temperature and humidity of the air inside the fruit tray before exposed to the different temperature scenarios. Three different temperature scenarios were designed to simulate possible coolchains for kiwifruit, whereby after a period of coolstore, fruit will be exposed to a break in temperature control, high temperature conditions or different humidity environments (Figure 3.4). To simulate a break in temperature control (TB), kiwifruit were exposed to 8 °C for 1 d after 9 or 15 weeks of storage at 0 or 2 °C respectively (Figure 3.4). Simulation was carried out to mimic the high temperature conditions (HT) by exposing fruit to 20, 25, 30 or 35 °C at 95% RH for a week after 10 weeks of storage at 0 °C (Figure 3.4). Fruit were left in respective temperature controlled rooms for a day before evaluating the fruit firmness. The exposure of fruit to different humidity conditions at high temperature (DH) was simulate by placing fruit in 35, 55, 75 or 95% RH at 30 °C for a week after 14 weeks of storage at 10 °C (Figure 3.7). The data loggers used for humidity measurement were calibrated using different salt solutions. Similarly, fruit were left at least a day for it to equilibrate to set humidity and temperature before conducting the measurement.

3.2.2. Fruit assessment

Measurements were conducted to assess the fruit quality (firmness, soluble solid content and incidence of rotten fruit) and physiological status (respiration rate) at intervals of 2 to 3 weeks across the storage period.

3.2.2.1. Fruit firmness

Kiwifruit were equilibrated to 20 °C overnight before measurement. Fruit exposed to high temperature conditions (25, 30 and 35 °C) were left at 20 °C for at least 3 hours before measurement. A penetrometer (QALink Willowbank Electronics Ltd.,

Napier, New Zealand), fitted with a standard 7.9 mm round Effegi probe and interfaced to a computer was used. A 2 mm slice of skin was removed from an equatorial region before measurement. The probe was set to penetrate the flesh to a depth of 8 mm at 20 mm s^{-1} with the minimum measurement being 1 N. Two measurement locations perpendicular to each other were used for each fruit. Fruit firmness readings were calculated based on an average of 36 fruit for each measurement. Rotten fruit were removed from the population prior to measuring fruit firmness.

3.2.2.2. Soluble solids content

Initial soluble solids content (SSC) was measured using a pocket refractometer (PAL-1, Atago, Tokyo, Japan). SSC was expressed as a percentage on the Brix scale. SSC readings were calculated based on an average of 36 fruit for every grower line for the first 5 measurements and 10 fruit for the remaining measurements.

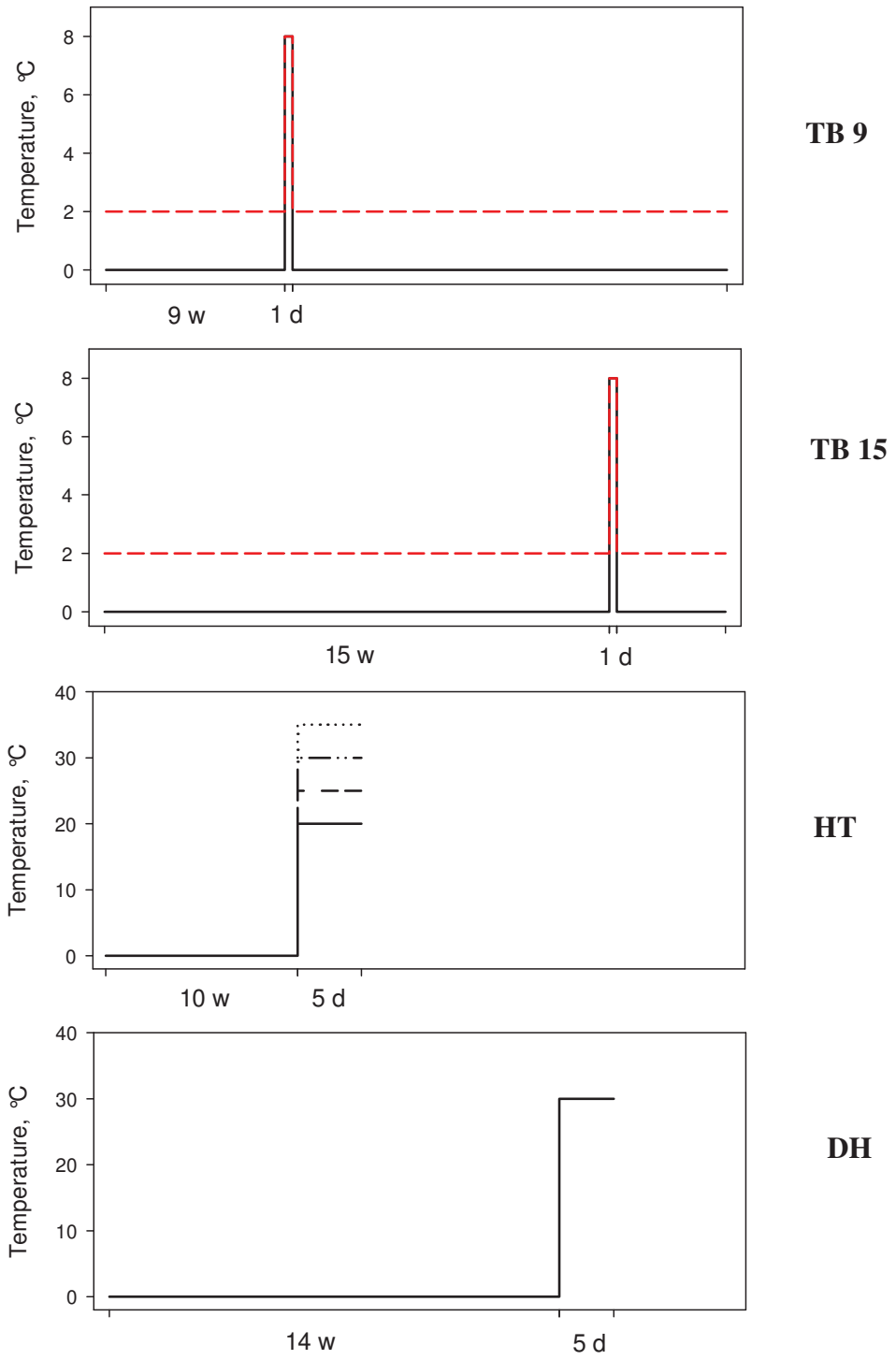


Figure 3.4: Description of various temperature scenarios used for 'Hayward' kiwifruit from 2012 harvest.d denotes days, w denotes weeks, TB denotes a break in temperature treatment, HT denotes high temperature treatment and DH denotes different humidity treatment.

3.2.2.3. Decay incidence

Decay incidence was assessed visually by inspecting for symptoms of rot which developed on the side or stem end (Figure 3.5). The incidence of decay was calculated as a percentage of the total fruit population (36 fruit per grower).

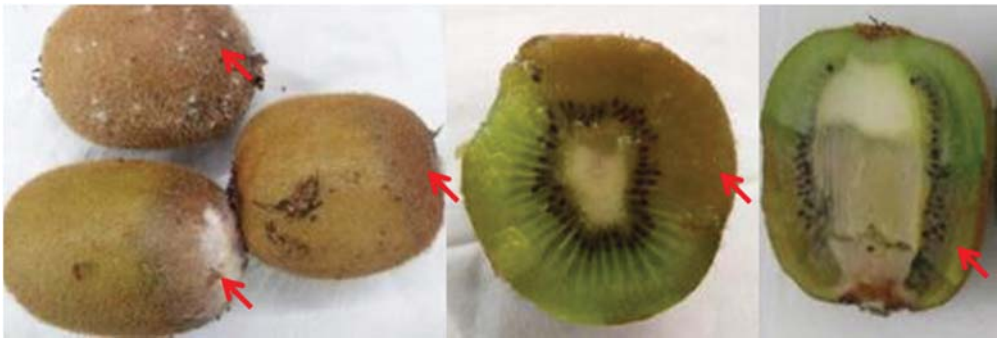


Figure 3.5: Fruit rots found at the side or stem end of 'Hayward' kiwifruit. Red arrow indicates the rotten area.

3.2.2.4. Respiration rate

Ten individual kiwifruit were each placed in a 500 mL glass jar sealed with a septum jar lid. Carbon dioxide concentrations were measured upon sealing and after 3 h at 0 or 2 °C. Headspace gas was sampled from the septum using a 1 mL syringe and injected to a carbon dioxide transducer (Analytical Development Company, Hoddesdon, UK) which was interfaced to an integrator (HP3396A, Hewlett Packard, USA). The respiration rate was calculated based on the accumulation of carbon dioxide concentration over time considering the fruit weight and remaining free volume of the jar. The fruit density was assumed to be 1037 kg m^{-3} (Jordan *et al*., 2000).

3.2.2.5. Statistical analysis

The experiments were conducted using a complete random design, with each grower line representing a replicate. Fruit firmness statistical analysis was performed using Minitab Version 15 (Minitab Inc, State College, PA, USA). Data were subjected to a General Linear Model, at each time with storage treatment and grower line as fixed factors. Comparison of means was undertaken using Tukey's test at $p \leq 0.05$. The

Anderson-Darling test was used to perform a normality test on sample populations. When required, Chi-square analysis was used to analyse the significance of the incidence count of rotten fruit.

3.3. Result and discussions

3.3.1. Cooling profiles achieved

Replicating the various coolchain scenarios in the laboratory for three different grower lines is a challenge. This was achieved by strict temperature control and monitoring. Figure 3.6 shows that the fruit were cooled differently to storage temperature (0 or 2 °C), where direct cooling took approximately 3 days to cool fruit to storage temperature while gradual cooling took approximately 2 weeks to cool fruit to storage temperature. Consistent cooling profiles and storage temperature across different grower lines enables to relate the subsequent fruit data to the cooling rate and storage temperature.

Different possible coolchain scenarios were simulated in the laboratory. A break in temperature control can occur in the supply chain during loading, transporting and the lack of coolstore facilities in the marketplace. Since a standard curtain sider truck is used to transport pallets of kiwifruit to the port for export and thus a break in temperature control is likely to take place. Figure 3.7 demonstrates that the tray took an approximately 1 day to reach 8 °C and approximately 3 days to return to storage temperature (TB). This replicates a break in temperature control in the coolchain as observed in industry (Figure 3.1). There is a possibility for kiwifruit to be shipped to countries in South East Asia or Indian subcontinents, exposing fruit to high temperature and fluctuating humidity environment. Without control of temperature and humidity, fruit can be easily exposed to fluctuating humidity and high temperature environment. Figure 3.7 shows that the tray of fruit took approximately 1 to 2 days to equilibrate to the desired temperature (HT) and humidity environment (DH).

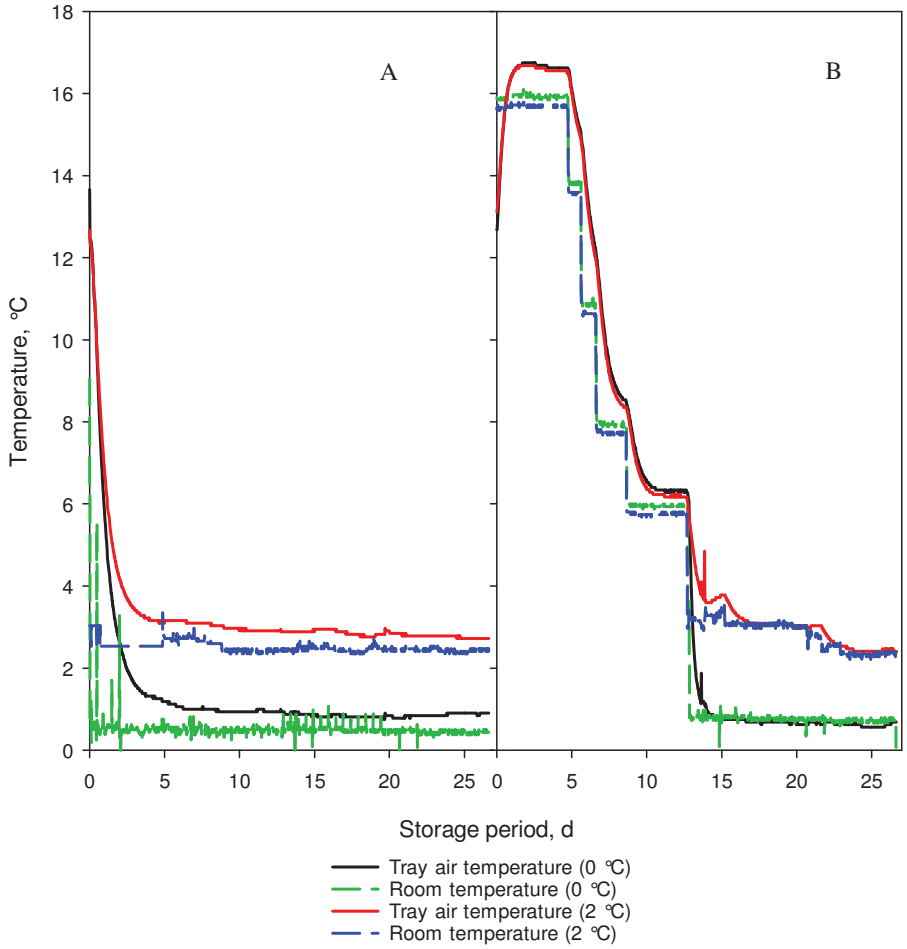
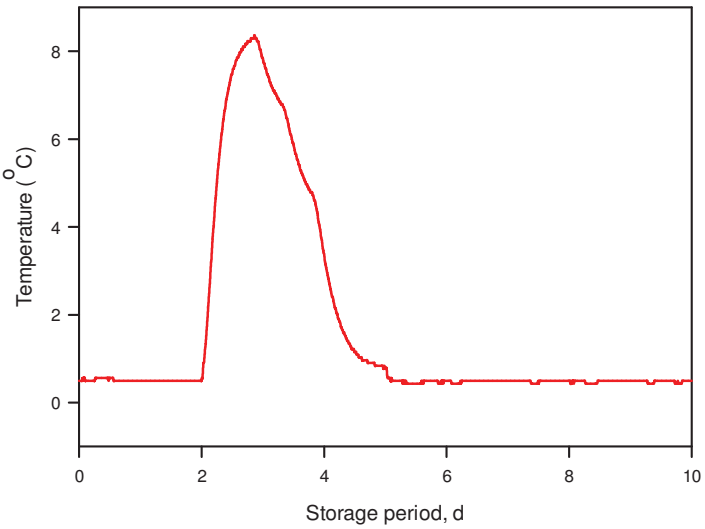
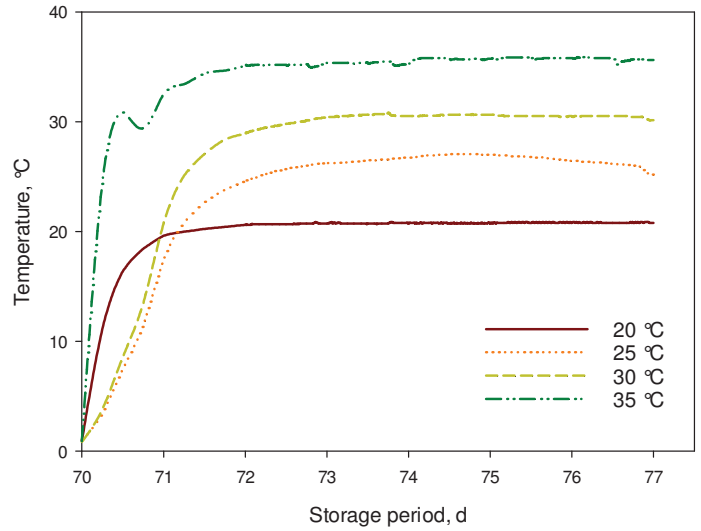


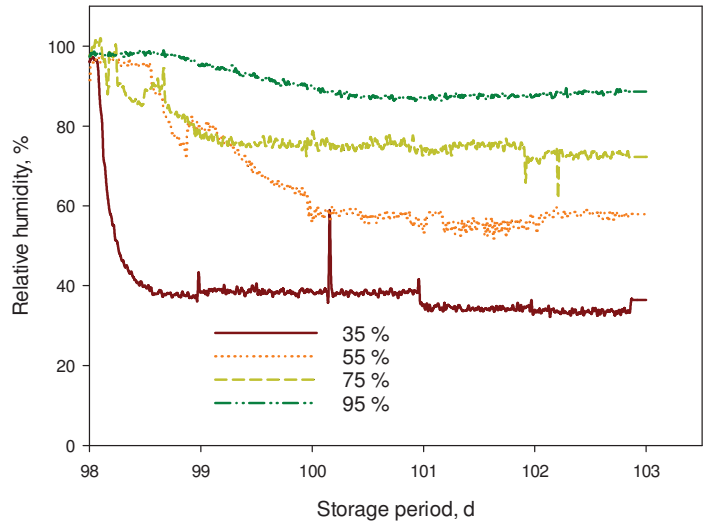
Figure 3.6: Room and tray air time temperature for direct (A) and gradual (B) cooling to 0 or 2 °C.



TB



HT



DH

Figure 3.7: Real time temperature profile of the different temperature scenarios simulated in the laboratory. TB denotes a break in temperature treatment, HT denotes high temperature treatment and DH denotes different humidity treatment.

3.3.2. At-harvest attributes

Fruit of different grower lines are exposed to different growing conditions and vine management procedures such as girdling, fruit crop load, light manipulation (Tombesi *et al.*, 1993; Boyd & Barnett, 2011; Patterson & Currie, 2011) and thus obtaining differences in at-harvest attributes. The at-harvest soluble solids content is used as an indicator to estimate the fruit maturity in kiwifruit industry, where a minimum of 6.2 % is required for commercial clearance (Mitchell, 1990; Costa *et al.*, 1997; Burdon *et al.*, 2013). Table 3.1 shows that all 3 grower lines were commercially mature, with a soluble solids content of more than 6.2 °Brix, which is above the commercial clearance. Although G3 has the lowest soluble solids content, the difference was large compared to G1 and G2. Similarly, G1 has the lowest initial firmness but the difference was not substantial compared to G2 and G3. No significant difference was observed in the dry matter content across all 3 grower lines (Table 3.1).

Table 3.1: Average at-harvest attributes of kiwifruit from respective grower lines. Each value represents the average initial firmness, soluble solids content and dry matter content of fruit from a single tray or respective grower line. p-value < 0.05 represents a significant difference between grower lines. Different letters in parentheses are statistically different at p = 0.05. NS denotes no significant difference.

Factors	Soluble solids content (B_0), °Brix		Firmness (F_0), N		Dry matter content (D_{m0}), %
G1	11.3	a	44.2	a	18.2
G2	11.6	a	51.6	b	18.4
G3	10.8	b	51.2	b	17.9
p - value	0.015		< 0.001		0.193
$LSD_{0.05}$	0.61		4.26		NS
n	36		36		15

3.3.3. Effect of cooling rate on kiwifruit quality during storage

A secondary rapid softening and a long gradual final phase are observed in the softening curves of stored kiwifruit (Figure 3.8). An initial lag phase can occur at the beginning of the softening curve during ripening. White *et al.* (2005) and Jabbar *et al.* (2014) both have demonstrated that 'Hayward' kiwifruit displayed an initial lag phase during the 6 to 9 days of ripening at 20 °C. However, the softening curves in this work did not show an initial lag phase, which may be due to the measurement interval between the first and second measurements being 21 days. Benge *et al.* (2000) also demonstrated the absence of initial lag phase in the softening of 'Hayward' kiwifruit when stored at 0 °C.

The cooling rate of kiwifruit to 0 °C had an influence on fruit firmness in the subsequent storage ($p < 0.05$, Figure 3.8). There was no effect of cooling rate on fruit firmness before 120 d of storage. However, an accelerated decrease in fruit firmness was observed in the long gradual softening phase, after 120 d of storage when 'Hayward' kiwifruit was directly cooled to 0 °C (Figure 3.8A). This accelerated decrease in fruit firmness was not found in fruit that were gradually cooled to 0 °C, maintaining a firmness of about 10 N after 120 d of storage. In addition, the accelerated softening in the long gradual softening phase of direct cooled fruit was not observed when subsequently stored at 2 °C (Figure 3.8B). This suggests that cooling rate affects the subsequent fruit firmness when fruit are stored at 0 °C only.

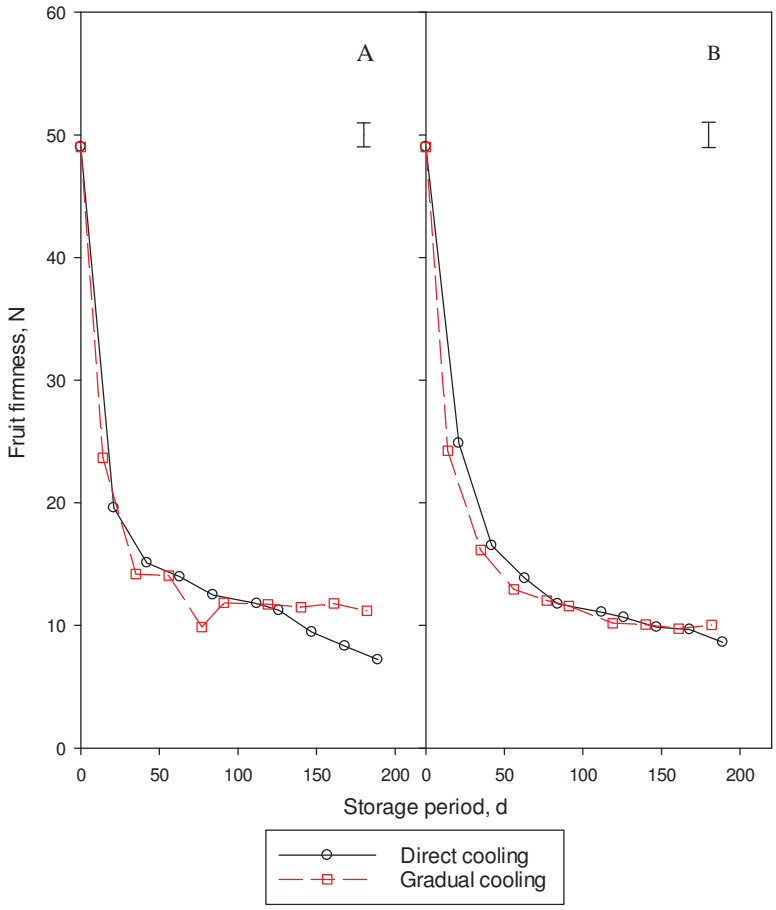


Figure 3.8: Effect of cooling profile on 'Hayward' kiwifruit softening at constant storage conditions of 0 (A) and 2 (B) °C. The data set points represent the average fruit firmness of 3 replicate growers of 36 fruit each (n = 108). Rotten fruit were removed from population prior to analysis. Error bars displayed represent the $LSD_{0.05}$.

A possible reason for firmness maintenance in gradually cooled fruit is that slow cooling may act as a period of acclimatisation, allowing fruit to build resistance to cold temperature, similar to temperature conditioning (Burdon and Lallu, 2011). It was found that applying temperature conditioning to kiwifruit (Yang *et al.*, 2012; Yang *et al.*, 2013), grapefruit (McDonald *et al.*, 1993) and avocados (Woolf *et al.*, 2003) induces resistance to low temperature and thus enhances fruit tolerance to chilling injury Consequently, the accelerated softening in the gradual softening phase of directly cooled fruit may be a result of the development of chilling injury. Previously, Lallu (1997) observed that shorter cooling time to storage temperature resulted in higher incidence of chilling injured fruit

while Yang *et al.* (2013) found that kiwifruit kept at 12 °C for 3 days before storage at 0 °C resulted in firmer fruit after 80 d of storage in comparison to directly cooled fruit.

Rapid increase in soluble solids content (SSC) was observed during the initial 50 d and reached a plateau for the remainder of the storage period at 0 and 2 °C (Figure 3.9). Similar increases in SSC have been observed during storage at 0 °C previously (Boquete *et al.*, 2004; Ma *et al.*, 2014). Cooling rates and storage temperature between 0 and 2 °C had no influence on 'Hayward' kiwifruit soluble solid accumulation ($p > 0.05$).

When kiwifruit ripen, they behave as climacteric fruit (Beever & Hopkirk, 1990; Mitchell, 1990). Often, a rise in respiration rate is observed for a short period, followed by a slow decrease as the fruit soften. Yang *et al.* (2013) demonstrated an initial decline in respiration rate within the first 10 d of storage at 0 °C, followed by a peak in respiration rate at 40 d, and a subsequent decrease from 40 to 120 d. The results from this work show that there was an initial decline in respiration rate during the first 50 d of storage with a decrease till end of storage at 0 and 2 °C (Figure 3.10). However, a rise in respiration rate was found after 120 d of storage when fruit were directly cooled and stored at 0 °C (Figure 3.10A).

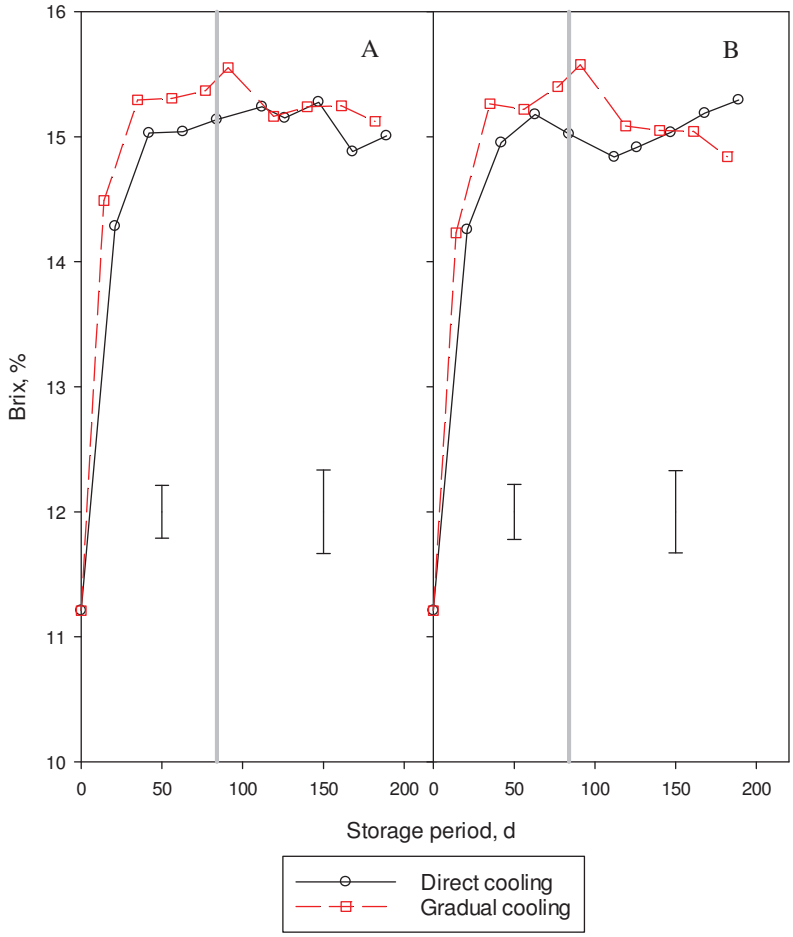


Figure 3.9: Effect of cooling profile on the accumulation of soluble solids in 'Hayward' kiwifruit at control storage conditions of 0 (A) and 2 (B) °C. The data set points represent 3 replicate growers of 36 fruit (n = 108) for the first 5 measurements and 10 fruit (n = 30) for the remaining storage period as indicated by the grey vertical line. Rotten fruit were removed from population prior to analysis. Error bars displayed represent the $LSD_{0.05}$.

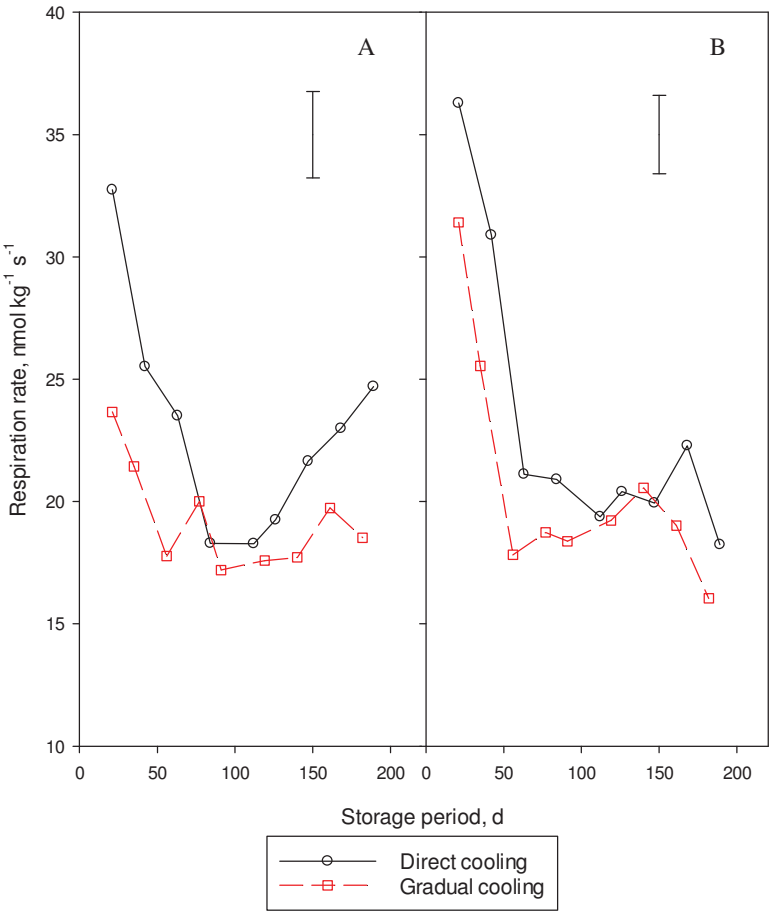


Figure 3.10: Effect of cooling rate on 'Hayward' kiwifruit respiration rate at 0 (A) and 2 (B) °C. The data set points represent 3 replicate growers of 10 fruit (n = 30). Error bars displayed represent the $LSD_{0.05}$.

Respiration rate can be used to indicate fruit metabolic activity. The respiration rate of directly cooled fruit was higher compared to gradually cooled fruit during storage (Figure 3.10A). The increase in respiration rate after 120 d of storage corresponds to the time when directly cooled fruit become softer than gradually cooled fruit. It is unknown if the increase in respiration rate observed after 120 d is a cause or effect of the accelerated softening observed towards the end of storage for the directly cooled fruit. This result may suggest that the fruit were undergoing chilling stress as fruit respiratory response can be altered when under chilling stress. Respiration rate of chilling injured persimmon has previously been found to be higher than non-chilling injured fruit (Macrae, 1987).

The incidence of decay in direct cooled fruit was lower compared to gradually cooled fruit (Table 3.2). Significant incidence of decay was only observed for directly cooled fruit at the end of storage (Figure 3.11). Similarly, the incidence of fruit decay in strawberry was found to be reduced with prompt cooling (Nunes *et al.*, 2005). The growth of pathogens that cause rot on fresh produce is temperature dependent, and thus it is recommended to store fresh produce at low temperature. It is possible that the higher incidence of rotten fruit in gradually cooled fruit is a result of the delay in cooling allowing the pathogen to advance in development during this time. However, the longer cooling period of gradually cooled fruit does not always lead to more rot development, and in fact may allow fruit to build resistance against decay. A delay in packing and cooling of kiwifruit for up to 7 days led in a lower incidence of subsequent rots (Pennycook & Manning, 1992).

Table 3.2: Contingency table for the relationship of cooling rate on the incidence of rotten fruit in 'Hayward' kiwifruit subsequently stored at 0 or 2 °C. Each count represents the sum of 3 replicate grower lines across the storage period. Chi-square = 34.57, df = 1, p < 0.001. Values in the parenthesis are contributions to chi square.

Cooling profiles	Sound	Rotten	Total
Direct cooling	2149 (0.249)	11 (15.56)	**2160**
Gradual cooling	2103 (0.249)	57 (15.56)	**2160**
Total	**4252**	**68**	**4320**

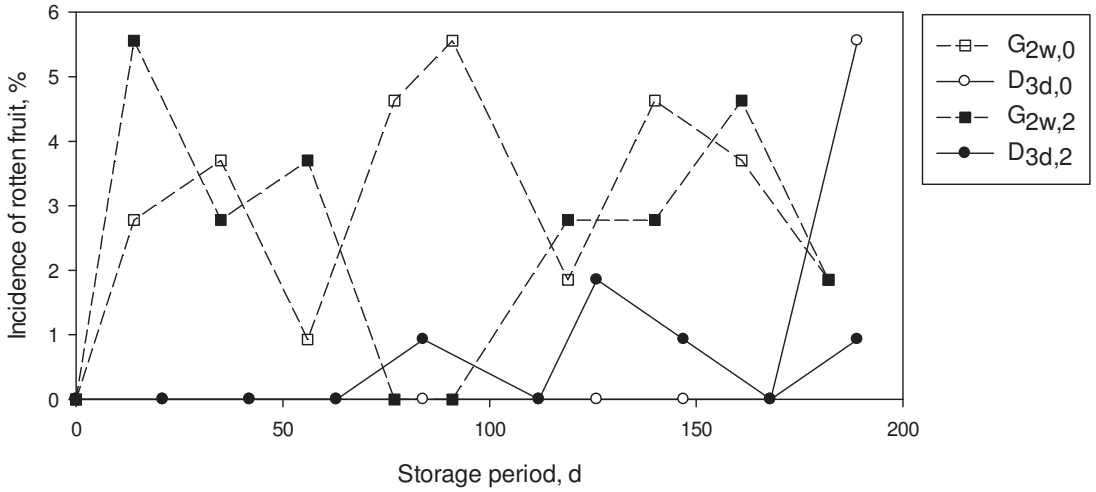


Figure 3.11: Effect of cooling rate on the incidence of rotten fruit in 'Hayward' kiwifruit subsequently stored at 0 or 2 °C. The data points represent incidence for 3 replicate growers of 36 fruit (n = 108). $G_{2w,0}$ denotes gradual cooling to 0 °C, $D_{3d,0}$ denotes direct cooling to 0 °C, $G_{2w,2}$ denotes gradual cooling to 2 °C and $D_{3d,2}$ denotes direct cooling to 2 °C.

3.3.4. Effect of storage temperature on kiwifruit quality during storage

Storage temperature affects kiwifruit softening, where fruit stored at higher temperature soften faster than in lower temperature (Hertog *et al.*, 2004c; Schotsmans *et al.*, 2005). Results showed that fruit stored at 2 °C were softer than fruit stored at 0 °C (Figure 3.12B). However, fruit that were direct cooled and stored at 0 °C became softer than fruit stored at 2 °C during late storage period, after 150 d of storage (Figure 3.12A). This result disagrees with the expectation that fruit stored at higher temperature will soften faster. As proposed earlier the more rapid decrease in firmness during the late storage period may be a result of chilling injury development. The risk of chilling injury development in kiwifruit decreases with increasing storage temperature. Lallu (1997) observed a significant reduction of chilling injured kiwifruit when stored at 2.5 °C compared to 0 °C. Overall, storing 'Hayward' kiwifruit at 2 °C leads to softer fruit but it may help to alleviate chilling injury development.

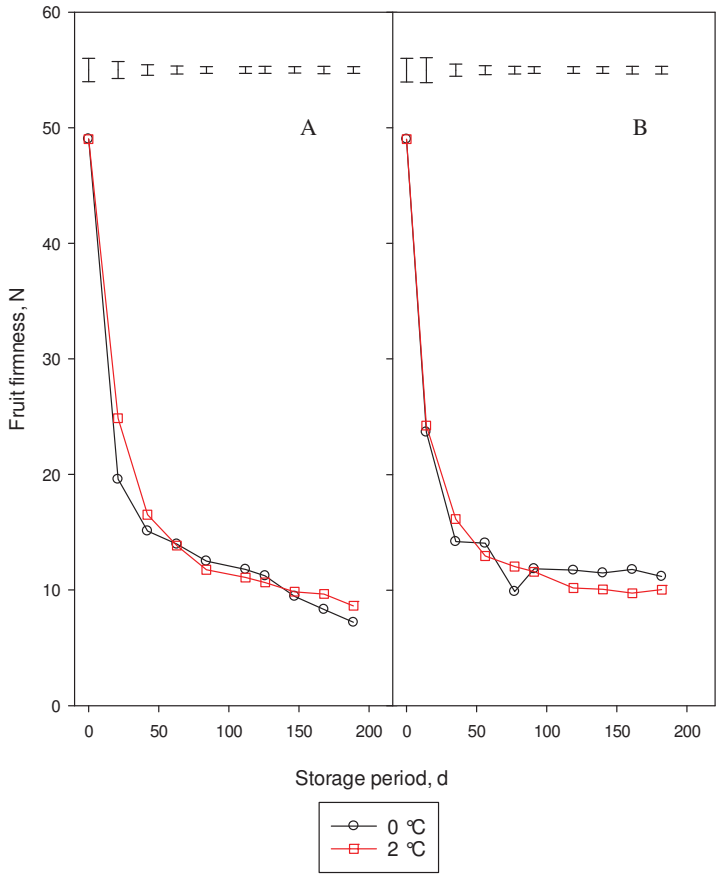


Figure 3.12: Effect of storage temperature on 'Hayward' kiwifruit softening when direct cooled (A) or gradual cooled (B) to storage temperature at 0 or 2 °C. The data set points represent the average fruit firmness of 3 replicate growers of 36 fruit each (n = 108). Rotten fruit were removed from population. Error bars displayed represent the $LSD_{0.05}$ at each time point.

Storage temperature did not influence the incidence of rotten fruit (Table 3.3). *Botrytis* rot happens during cool storage and can spread easily to surrounding fruit with contact (Brook, 1992). Fruit maturity, weather conditions in orchard and pre- and postharvest handling operations are factors that influence rots infection in kiwifruit (Brook, 1990; Hopkirk *et al.*, 1990b). Good postharvest management, for instance, curing of kiwifruit between temperature of 10 and 20 °C and relative humidity higher than 92% for less than 3 days helps to control rot in kiwifruit, without losing fruit quality during cool storage (Bautista-Baños *et al.*, 1997).

Table 3.3: Contingency table for the relationship of storage temperature on the incidence of rotten fruit in 'Hayward' kiwifruit subsequently stored at 0 or 2 °C. Each count represents the sum of 3 replicate grower lines across the storage period. Chi-square = 0.54, df=1, p = 0.463. Values in parenthesis are contributions to chi square.

Storage temperature	Sound	Rotten	Total
0 °C	2123 (0.004)	37 (0.265)	2160
2 °C	2129 (0.004)	31 (0.265)	2160
Total	4252	68	4320

3.3.5. Effect of break in temperature control on kiwifruit quality during storage

A break in temperature control of 8 °C for 1 day after 9 weeks of storage did not result in a significant difference on the fruit firmness ($p > 0.05$) (Figure 3.13). In apples, it was found that a break of 1 d at 20 °C resulted in significant softening (East *et al.*, 2008). The small break in temperature of 8 °C for a day is lower in comparison to exposure to the 20 °C for a day in apples studies and thus suggests that the exposed temperature is not high enough to cause a significant impact on kiwifruit softening. Strawberries (cv. Sweet Charlie) were considered unmarketable when exposed to fluctuating temperature regimes which were encountered during handling operations (Nunes *et al.*, 2003). Results obtained also show that a break in temperature after 15 weeks of coolstorage have no significant effect ($p > 0.05$) on the subsequent fruit firmness (Figure 3.13).

The current logistics chain between on-shore cool storage and port, which features no active temperature management, appears to be appropriate for the kiwifruit industry. However, kiwifruit is shipped over a range of maturities and after a range of lengths of time in storage and the results gathered only represent two fruit conditions and thus need to be treated with some caution.

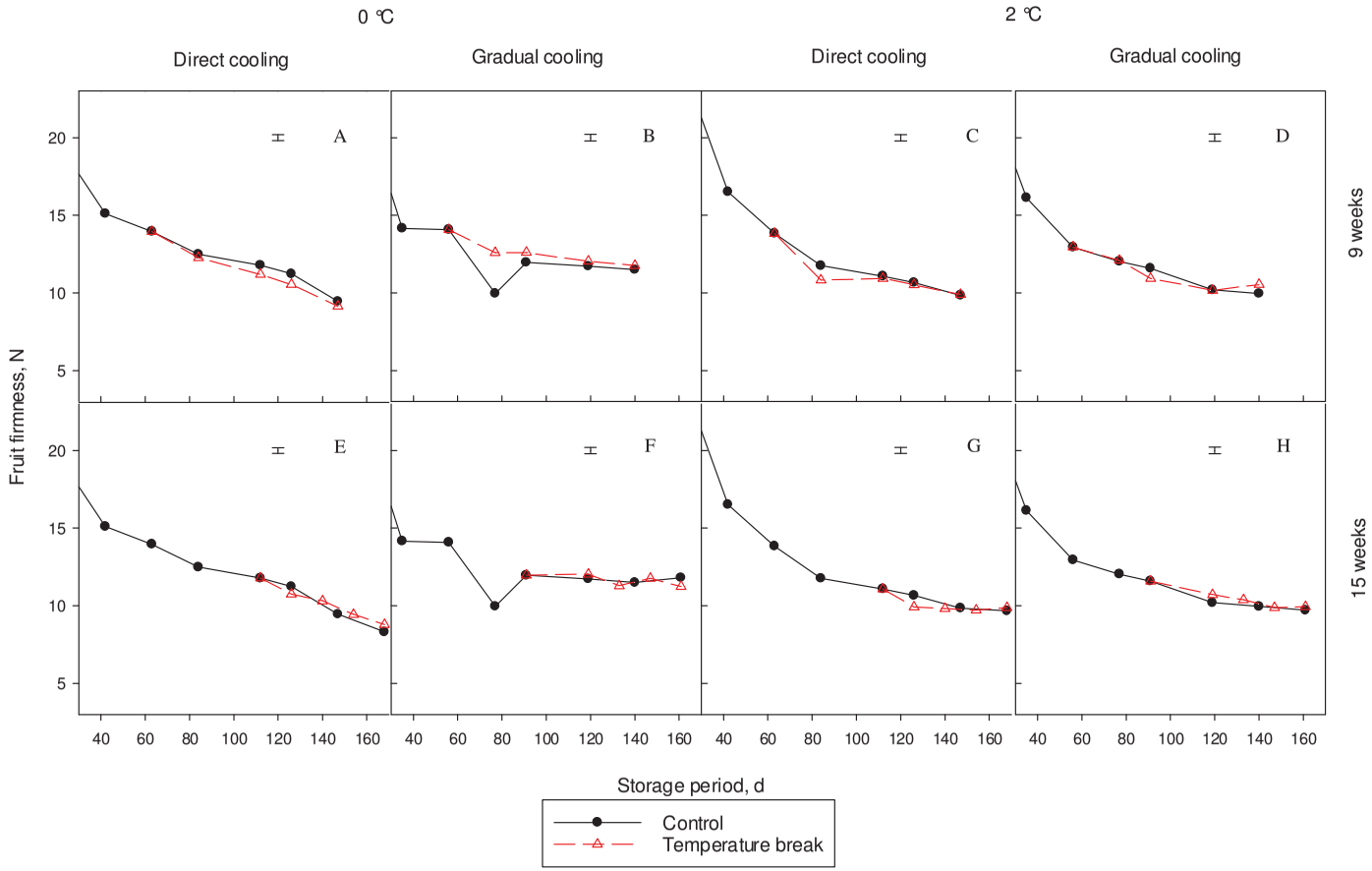


Figure 3.13: Effect of break in temperature control on the kiwifruit firmness after 9 or 15 weeks of storage at 0 (A, B, E and F) or 2 °C (C, D, G and H). Fruit were either direct cooled (A, E, C and G) or gradual cooled (B, F, D and H) respectively. Data points represent the average fruit firmness of 3 growers of 36 fruit (n = 108). Rotten fruit were removed from population prior to analysis. Error bars displayed represent the $LSD_{0.05}$.

3.3.6. Effect of high temperature conditions on kiwifruit quality during storage

Results demonstrated that exposing fruit to high temperature conditions after 10 weeks of storage has influenced the subsequent fruit firmness (Figure 3.14). Fruit exposed to different temperature display different softening. However, a lag phase prior to more rapid softening was observed when kiwifruit were exposed to 20 °C (Figure 3.14). This lag phase is not observed when fruit were exposed to 25, 30 or 35 °C. Previously, White *et al.* (2005) and Macrae *et al.* (1989) proposed that the presence of lag phase was correlated to the time taken for fruit to be fully ripened. However, in this case, fruit ripening has initated during the 10 weeks of storage at 0 °C before placing at elevated temperature conditions. Therefore, in this case early fruit maturity does not explain the lag phase that was reflected in the softening curve when exposed to 20 °C. The mechanisms behind the observed lag phase are unknown. Nevertheless, there is a possibility that the concentration of ethylene plays a part in regulating the transition between the lag phase and rapid softening phases. Jabbar and East (2016) demonstrated that exposing kiwifruit to exogenous ethylene concentration of 0.1 $\mu L\ L^{-1}$ and above after 10 weeks of storage at 0 °C is able to initiate rapid softening in kiwifruit. Kiwifruit was found to produce low level of ethylene when exposed to 20 °C after 60 days of storage at 0 °C for the first 3 days of exposure and subsequent producing high level of ethylene over the remaining storage period at 20 °C (Antunes & Sfakiotakis, 2002b). Therefore, there is a possibility that it took more than 3 days to accumulate more than 0.1 $\mu L\ L^{-1}$ of ethylene within the tray headspace in order to trigger rapid softening. This would possibly explain the reason for the observed lag phase when exposed to 20 °C.

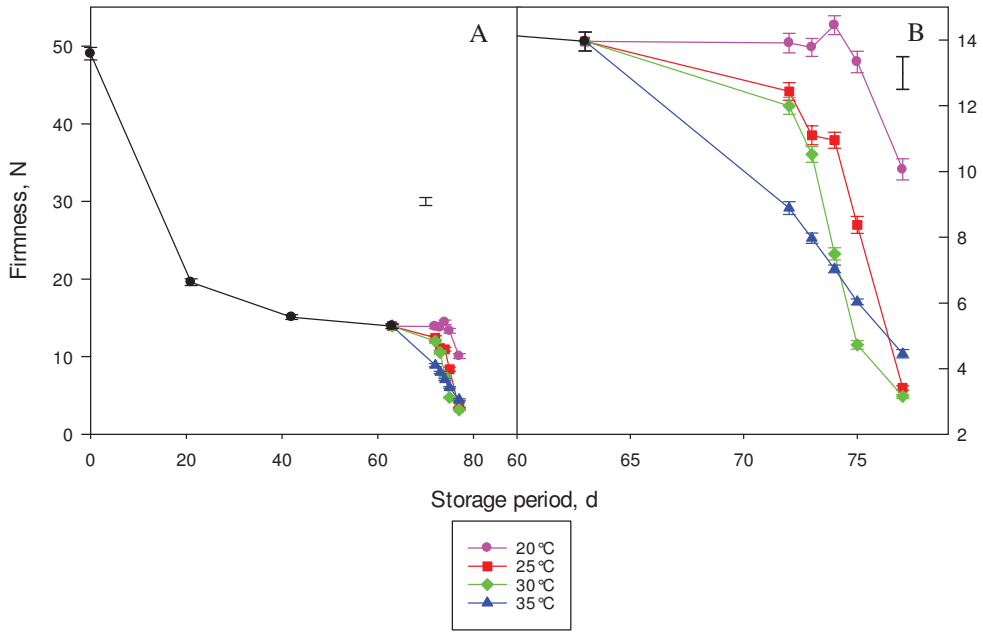


Figure 3.14: Effect of high temperature exposure on kiwifruit firmness after 10 weeks of storage at 0 °C. The data points represent the average fruit firmness of 3 growers of 36 fruit (n = 108). Rotten fruit were removed from population prior to analysis. Graph B represents the same data as graph A but focus on the late storage period. Error bars displayed represent the $LSD_{0.05}$.

Figure 3.14 shows that the softening rate of fruit stored at 35 °C was slower than fruit stored at 20 to 30 °C. Similarly, many studies have demonstrated a decrease in the softening rate of plums (Tsuji *et al.*, 1984), pears (Maxie *et al.*, 1974), avocados (Eaks, 1978), and tomatoes (Biggs *et al.*, 1988) when continuously held at temperatures between 30 to 40 °C. During kiwifruit ripening, several biochemical reactions take place, altering the fruit characteristic and physiology (Redgwell *et al.*, 1990; Redgwell *et al.*, 1997; Brummell, 2006). There is a possibility that these biochemical reactions were affected when exposed to 35 °C. The softening rate of 'Royal Gala' and 'Cox's Orange Pippin' apples was observed to decrease when exposed to temperature between 24 and 35 °C, which was suggested to be a result of the inactivation of enzymes that were directly or indirectly involved in cell wall disassembly (Johnston *et al.*, 2001). Many cell wall studies on apples have found a higher proportion of insoluble pectin when apple were exposed to

38 °C for 4 days, indicating that high temperature condition inhibits uronic acid degradation (Klein & Lurie, 1990; Shalom *et al.*, 1993b; Shalom *et al.*, 1996).

3.3.7. Effect of storage humidity on kiwifruit quality during storage

Humidity affects fruit quality in terms of mass or water loss which leads to shrivelling. Furthermore, humidity can affect fruit metabolism, accelerating fruit ripening (Burdon *et al.*, 1994; Wada *et al.*, 2008). At coolstorage conditions, kiwifruit were packed inside the polyliner and hence the humidity within the tray or pallet is usually at approximately 100 %. When humidity inside the tray is measured throughout the supply chain and it is consistently found to be maintained at more than 95 % (Bollen *et al.*, 2013). However, there is a possbility that the humidity will fluctuate when fruit are exposed to high climate temperatures as driving force for water loss can be expontentially larger at higher temperatures. Storage at different humidity conditions will affect the amount of water loss and thus altering the turgor pressure which may affect the fruit firmess. Figure 3.15 shows that fruit firmness was not affected greatly when at 30 °C and 35 to 95 % RH. Harker and Hallett (1994) found no obvious change in cell turgor pressure during fruit ripening, suggesting that during ripening the cell wall becomes more plastic and elastic and thus result in cell expansion rather than a change in turgor pressure. This may explain why little differences in firmness was observed between various humidity conditions (Figure 3.15). Furthermore, humidity was found to have little influence on fruit firmness but had great impact on weight loss and *Botrytis* infection level in kiwifruit when stored at three different humidity conditions (40-59%, 65-80% and 92-97%) for a week at 0 °C (Bautista-Banos *et al.*, 2000).

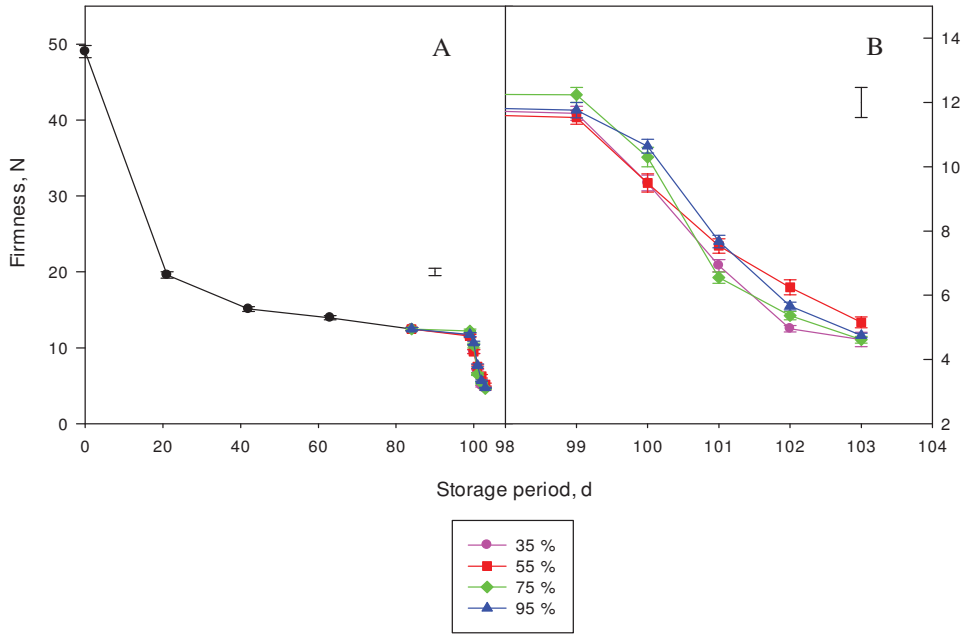


Figure 3.15: Effect of humidity at 30 °C on kiwifruit firmness after 14 weeks of storage at 0 °C. The data points represent the average fruit firmness of 3 growers of 36 fruit (n = 108). Rotten fruit were removed from population prior to analysis. Graph B represents the same data as graph A but focus on the late storage period. Error bars displayed represent the $LSD_{0.05}$.

It is critical to select the correct technique to evaulate the effect of water loss on fruit firmness as fruit firmness can be affected by changes in the structural cell wall components or cell turgor pressure. Fruit firmness was measured using a penetrometer and there is a possiblity that the penetrometer was unable to quantify the change in firmness at different humidity conditions due to water loss. It was found that using an invasive puncture test was unable to show the effect of humidity on 'Tradiro' tomato but an non-invasive technique was able to demonstrate the effect of humidity on the rate of softening (Hertog *et al.*, 2004a). Acoustic firmness measurement was used to show the increase in stiffness when apples were stored at high humidity conditions (De Belie *et al.*, 1999).

3.4. Conclusion

The rate of cooling to storage temperature (direct or gradual), storage temperature (0 or 2 °C) and exposure to high temperature conditions (20, 25, 30, 35 °C) are all factors that affect kiwifruit softening significantly. Development of chilling injury may be an outcome of cooling kiwifruit directly to storage temperature at 0 °C. However, breaks in temperature control and exposure to different humidity conditions at 30 °C did not influence subsequent fruit firmness. When developing the mathematical model to predict fruit softening, the model should be responsive to cooling rate, storage temperature and high temperature exposure.

Factors such as storage temperature and rate of cooling to storage temperature have been shown to affect the fruit firmness, possibly by inducing chilling injury during storage. However, chilling injury was not mentioned or quantified in this work. Therefore, in the next chapter more experiments are carried out to evaluate the incidence of chilling injured fruit when storing fruit as influenced by storage temperature (0 and 2 °C) and rate of cooling to storage temperature. The effect of maturity on chilling injury development and fruit firmness will be investigated in the next chapter. Understanding the effect of storage temperature and cooling rates on the incidence of chilling injury will benefit in the development of mathematical model to predict the softening curve.

4. Factors influencing development of chilling injury in 'Hayward' kiwifruit during coolstorage (*)

4.1. Introduction

Chilling injury can develop in fruit when stored at low temperature, above freezing temperature. Chilling injury occurs in peaches (Crisosto *et al.*, 1999), orange (Henriod *et al.*, 2005), banana (Pantastico *et al.*, 1968) and pears (Yan *et al.*, 2013). Previously, incidence of chilling injury development in kiwifruit was found to be lower when fruit were stored at higher storage temperature or took a longer time to cool fruit to 0 °C (Lallu, 1997; Lallu & Webb, 1997). Besides storage conditions, fruit maturity at harvest can influence development of chilling injury with incidence previously found to be higher in early harvest kiwifruit (Koutsoflini *et al.*, 2013). In order to predict storage quality, the effect of these factors on promoting chilling injury development is required.

The symptoms of chilling injury are dependent on the type of fruit. In 'Hayward' kiwifruit symptoms include the development of a ring or patch of granular, water soaked tissue along the outer pericarp, and formation of diffuse pitting along with the development of a dark scald-like appearance on the skin (Lallu, 1997; Bauchot *et al.*, 1999; Antunes & Sfakiotakis, 2002a; Burdon *et al.*, 2007). There is a similarity between symptoms of chilling injury and over ripening and thus make it difficult to identify whether excessive softening is caused by chilling injury or senescence. The development of chilling injury is usually caused by oxidative stress from excess reactive oxygen species (ROS) which subsequently damage the cell membrane (Hodges *et al.*, 2004; Antunes & Sfakiotakis, 2008). The loss of cell membrane structure may be the possible mechanism for the water soaked appearance found. Bauchot *et al.* (1999) suggested that grainy tissue found along the outer pericarp of a chilling injured kiwifruit is associated

(*) This chapter is summarised in the material published in the paper:
East, A., Zhao, M., Jabbar, A., Samarakoon, H., Bollen, F., Adkins, M.,Bronlund, J. Heyes, J. (2016). Why is predicting kiwifruit quality in the cool chain so difficult? Paper presented at the 4th IIR

with the presence of gas bubbles with a decreasing trend in bulk porosity of the cell wall with increased severity observed.

There are several postharvest techniques that are adopted to reduce the development of chilling injury in fruit. Commonly used techniques include chemical treatment such as ethoxyquin or squalene dipping (Wang & Baker, 1979; McDonald *et al.*, 1993), temperature conditioning (Nakamura *et al.*, 1985; Yang *et al.*, 2012) and intermittent warming (Biswas *et al.*, 2012; Biswas *et al.*, 2016). Exposing 'Hayward' kiwifruit to temperature conditioning of 3 days at 12 °C alleviated chilling injury development due to increased antioxidant enzyme activity and maintaining high levels of endogenous hormones (Yang *et al.*, 2013). Temperature conditioning was found to supress the increment in sterol to phospholipid ratio, increase the degree of unsaturation in membrane fatty acids, maintain high level of phospholipids content in membranes and enhance other factors that contribute to lower the development of chilling injury (Wang, 1994).

The industry inserts temperature loggers in pallets to monitor the temperature throughout the coolchain, identifying several different cooling profiles and storage temperatures (Bollen *et al.*, 2013). Harvested fruit are cooled to different storage temperatures based on the respective packhouse cooling capacity and practices. A difference in storage temperature may potentially be explained by positional and temporal temperature variation within industrial coolrooms (East *et al.*, 2016). Positional variations occur as fruit located near the evaporator air flow are exposed to cooler temperatures than fruit located at other locations.

Results from chapter 3 suggested the possibility of chilling injury development in 'Hayward' kiwifruit when cooled directly to storage temperature at 0 °C, observed as a

faster rate of softening during the later storage period (Figure 3.8). This chapter will focus on further investigating and quantifying chilling injury development in association with cooling rate in particular. A series of different coolchain scenarios were applied to fruit after harvest across different fruit maturities to further investigate the influence on fruit firmness and chilling injury development. Mathematically describing the occurrence of chilling injury development during coolstorage will aid in the development of a predictive model for kiwifruit quality during storage given time-temperature information, since chilling injury development affects fruit storability.

4.2. Materials and methods

4.2.1. Fruit source

Approximately 400 trays of commercially produced 'Hayward' kiwifruit from the Bay of Plenty region were harvested on three separate occasions. Fruit were harvested at 2 week intervals from the same 3 grower lines in order to represent early, mid and late maturity fruit. Fruit of approximately count size 30 were harvested from orchard, packed in single layer trays (without polyethylene polyliner) and delivered to Massey University, Palmerston North. A closed curtain truck without temperature control was used to transport fruit from orchard to Massey University. The temperature during transport was monitored with 2 iButton data loggers recording at 15 minutes intervals. The early maturity fruit were delivered on 29th April 2013, mid maturity fruit were delivered on 13th May 2013 and late maturity fruit were delivered on 27th May 2013 to Massey University.

Upon arrival, the pallet of fruit was kept in a room without temperature control (but with temperature monitoring) to continue curing overnight. On the following day, polyliners were added to each tray and each tray was randomly labelled to individual

treatment (coolchain simulation) and subsequent measurement timing. For each grower line, maturity and treatment condition combinations, 22 trays were used.

4.2.2. Coolchain simulation

Trays of fruit were exposed to different cooling profiles consisting of 4 different cooling methods; rapid (R), direct (D), gradual (G) or combination of rapid and gradual (C) cooling and 2 subsequent storage temperatures of 0 or 2 °C (Figure 4.1). Rapid cooling was accomplished by placing the trays of fruit in a pallet scale pre-cooler that was set up in a cool room at 0 °C (O'Sullivan *et al.*, 2016). The pre-cooler cooled the fruit rapidly to 0 °C within 12 h. Fruit were subsequently stored at either 0 ($R_{12h,0}$) or 2 °C ($R_{12h,2}$). Direct cooling was achieved by placing the trays of fruit into a cool room set at 0 °C and cooled using a room cooling technique ($D_{3d,0}$). Fruit were cooled in cool room by stacking 20 to 24 trays in a single vertical column. Gradual cooling was established by placing trays of fruit into a cool room initially at 16 °C with the set point temperature decreasing to 0 °C (at a rate of 1-2 °C per day) over a period of 2 weeks and subsequently stored at 0 °C ($G_{2w,0}$). A combination of rapid and gradual cooling was attained by placing the fruit in a pre-cooler to cool the fruit to 10 °C within 12 h, follow by a gradual cooling to 0 ($C_{1w,0}$) or 2 °C ($C_{1w,2}$) within 1 week. Once cooled, all the trays of fruit remained in a cool room set at 0 ± 1 or 2 ± 1 °C and 95 ± 5% RH respectively for up to 25 weeks.

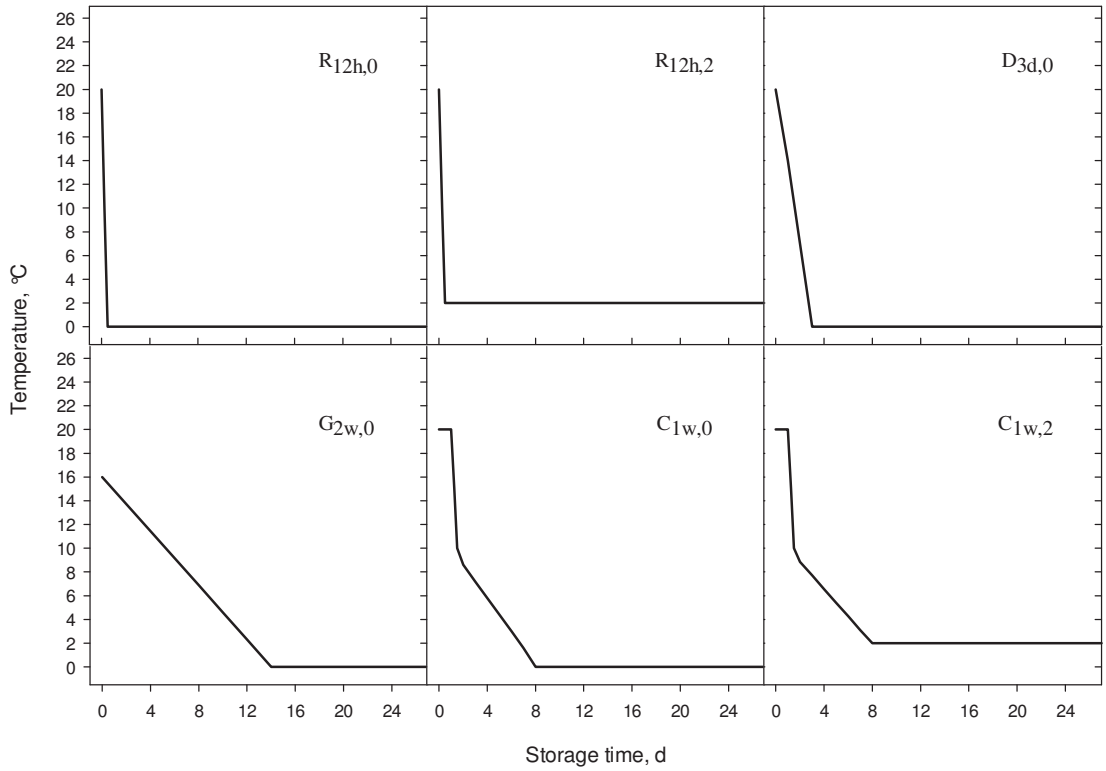


Figure 4.1: The proposed cooling profiles to cool fruit to storage temperature at 0 or 2 °C. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R1_{2h,2}$ refers to rapid cooling to 2 °C within 12 h, $D_{3d,0}$ refers to direct cooling to 0 °C within 3 d, $G_{2w,0}$ refers to gradual cooling to 0 °C within 2 w, $C_{1w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C_{1w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w.

The temperature and humidity of the cool room and air inside the fruit tray were monitored using iButton data loggers (DS1923, Maxim Integrated, USA) throughout the cooling and storage period. Two data loggers were used per grower, maturity and treatment combination. The data loggers were placed at the centre of the fruit tray, beneath the polyliner and thus measured the air temperature inside the tray. The tray that contained the data logger was located at the centre of the stacked column during cooling and storage. Temperature and humidity were recorded at 15 minutes intervals.

4.2.3. Fruit assessment

Measurements to assess fruit quality (firmness, occurrence and severity of chilling injured fruit, and incidence of rotten fruit) were conducted at fortnightly intervals for the first 60 days (0, 18, 32, 46 and 60 days), and later 3 weeks intervals until 25 weeks of storage (81, 109, 130, 151 and 172 days). The fruit measurements were conducted with a sample size of 30 fruit (a single layer tray) for the first 6 measurement days and 90 fruit (3 single layer trays) for the remaining 4 measurement days.

4.2.3.1. Initial dry matter and soluble solids content

Initial soluble solids content (SSC) was measured using a pocket refractometer (PAL-1, Atago, Tokyo, Japan). SSC was expressed as a percentage on the Brix scale. SSC readings were calculated based on an average of 30 fruit for early maturity fruit and 20 fruit for mid and late maturity fruit. Dry matter (DM) was measured by slicing a 2 to 3 mm thick equatorial slice from the fruit and placing it on a weighed petri dish. The slices were placed in food dehydrator (3000 series, Excalibur, California, USA) at 60 to 65 °C overnight. DM was calculated by the percentage of the dry weight over fresh weight. Petri dish and fruit slices were weighed using a weighing balance with 0.001 g accuracy (Mettler PG-503S, Toledo, Switzerland).

4.2.3.2. Occurrence and severity of chilling injury

Incidence of chilling injury was assessed visually by inspecting for symptom development on the outer pericarp after conducting a single equatorial slice. The symptoms include grainy tissue and a water soaked ring along the outer pericarp (Figure 4.2). The incidence of chilling injury was calculated as a percentage of the total fruit population (90 fruit per grower), with no distinction made for the severity of chilling injury symptoms.

Figure 4.2: Example of severity of chilling injury found along the outer pericarp of 'Hayward' kiwifruit. Grainy tissue and water soaked appearance were identified as symptoms of chilling injury. Severity increases from left to right across the figure. Red arrow indicates the chilling injury symptoms.

4.2.3.3. Decay incidence

Decay incidence was assessed visually by inspecting for symptoms of rots which developed on the side or stem end (Figure 3.5). The incidence of decay was calculated as a percentage of the total fruit population (30 or 90 fruit per grower). The water soaked appearance observed along the outer pericarp of a chilling injured fruit is different compared to a severely rotten fruit (Figure 4.3) and thus the ability to differentiate a rotten fruit from a chilling injury fruit is important and achievable.

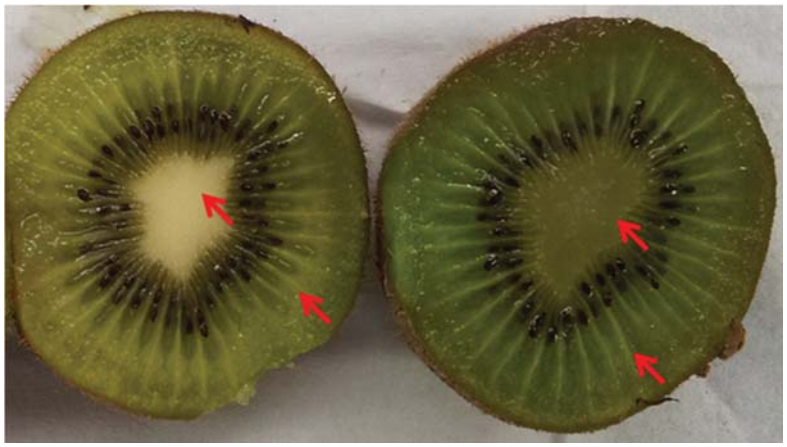


Figure 4.3: The comparison between a fruit with chilling injury symptom (left) and a rotten fruit (right).

4.2.3.4. Fruit firmness

Kiwifruit were equilibrated to 20 °C overnight before measurement. A penetrometer (QALink Willowbank Electronics Ltd., Napier, New Zealand), fitted with a standard 7.9 mm round Effegi probe and interfaced to a computer was used. A 2 mm slice of skin was removed from an equatorial region before measurement. The probe was set to penetrate the flesh to a depth of 8 mm at 20 mm s^{-1} with the minimum measurement being 1 N. The penetrometer was sent to Willowbank Electronics Ltd for calibration before the start of the experiment. Two measurement locations perpendicular to each other were used for each fruit. Fruit firmness readings were calculated based on an average of 30 fruit for the first 6 measurements and 90 fruit for the remaining 4 measurements. Rotten fruit were removed from the population when measuring the fruit firmness.

4.2.3.5. Statistical analysis

The experiments were conducted using a complete random design, with each grower line representing a replicate. Fruit firmness statistical analysis was performed using Minitab Version 15 (Minitab Inc, State College, PA, USA). Data were subjected to a General Linear Model, at each time with storage treatment, fruit maturity and grower as fixed factors. Comparison of means was undertaken using Tukey's test at $p \leq 0.05$. The

Anderson-Darling test was used to perform a normality test on sample populations. Chi-square analysis was used to analyse the significance of the incidence count of chilling injured fruit data.

4.3. Results

4.3.1. Cooling profiles achieved

Replicating coolchain scenarios in the laboratory and consistently for three different fruit timings is a challenge. Prior to arrival at the laboratory, the fruit were exposed to a differing fluctuating temperature environment during transportation and overnight curing. In particular, fruit of later maturity (M3) were harvested at significantly lower ambient temperature (6 to 10 °C) in comparison to the previous harvests (14 to 26 °C) during this phase (Figure 4.4).

The cooling process subsequent to this period was replicated with care to ensure that fruit across different maturities were exposed to similar cooling profiles. This was achieved by strict temperature control and monitoring. Figure 4.4 shows the different cooling profiles applied to the 3 different fruit maturities. Consistent cooling profiles and storage temperature across different fruit maturities allows relating the subsequent fruit quality data to the cooling profiles and storage temperatures.

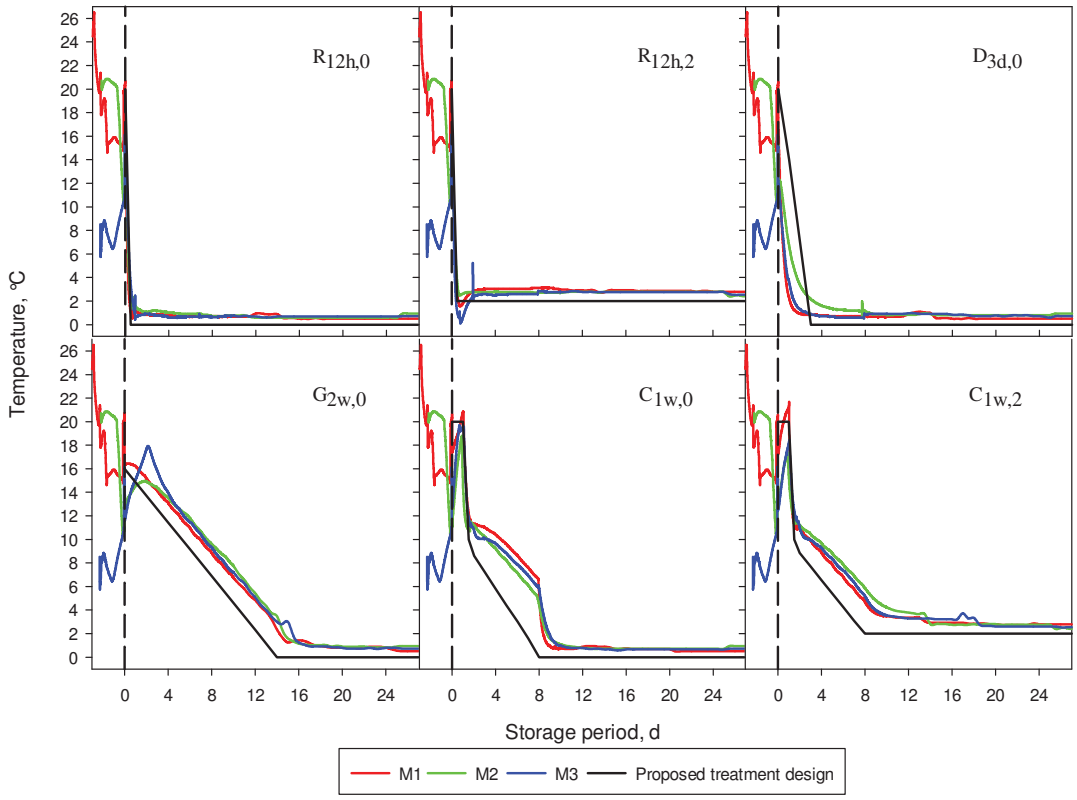


Figure 4.4: Cooling profiles designed and created for 6 different coolchain scenarios. M1, M2 and M3 denote the 3 respective fruit maturities (early, mid and late). The black solid line refers to the designed temperature profiles found in Figure 4.1. Temperature data prior to the vertical dashed line refers to temperature data collected during transport and curing process while the temperature data after the vertical dashed line refers to the cooling and storage temperature data. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R1_{2h,2}$ refers to rapid cooling to 2 °C within 12 h, $D_{3d,0}$ refers to direct cooling to 0 °C within 3 d, $G_{2w,0}$ refers to gradual cooling to 0 °C within 2 w, $C_{1w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C_{1w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w.

4.3.2. At-harvest fruit attributes

Initial dry matter content (D_{m0}) differed between grower lines ($p < 0.05$; Table 4.1). G1 had the highest dry matter content while G3 was the lowest. Fruit of different grower lines are exposed to different growing conditions and vine management procedures (e.g. girdling, fruit crop load, light manipulation) and thus result in differences in fruit dry matter content (Tombesi *et al.*, 1993; Boyd & Barnett, 2011; Patterson & Currie, 2011). Dry matter content also varied with fruit maturity, with the early harvest having less dry matter (Table 4.1). This result conflicts with Costa *et al*. (1997) who found no significant difference between dry matter content of fruit harvested on 3 different occasions. Variation in dry matter content is commonly observed between orchards and locations (Woodward & Clearwater, 2011).

Kiwifruit from three different growers were harvested every 2 weeks throughout the season and thus achieved fruit of very different maturities. This maturity change is clearly demonstrated in the initial soluble solids content (B_0) data (Table 4.1). Over the 4 weeks from early to late maturity, SSC increased from 5.5 to 11.1 °Brix. The at-harvest soluble solids content is used as a harvest index in the kiwifruit industry, where a minimum of 6.2 % is required for commercial clearance (Mitchell, 1990; Costa *et al.*, 1997; Burdon *et al.*, 2013). Fruit of early maturity for all grower lines had an average SSC of less than 6.2 %, indicating that they were not yet commercially mature. Grower line 3 (G3) consistently displayed the lowest initial soluble solids content (B_0). Variation in soluble solids content (B_0) across fruit maturity was observed to be greater than across grower line, suggesting that fruit maturity has greater influence on soluble solids content compared to grower line effect.

Table 4.1: Average at-harvest attributes of kiwifruit from 3 grower lines harvested at 3 different maturity stages. Different letters in parentheses are statistically different at p =0.05. NS denotes no significant difference.

Factors		Dry matter (D_m), %		Soluble solids content (B_0), °Brix		Fruit firmness (F_0), N	
Fruit maturity	Early	17.9	b	5.5	c	79.2	a
	Mid	18.5	a	7.5	b	75.8	b
	Late	18.4	a	11.1	a	56.0	c
p-value		0.002		< 0.001		< 0.001	
$LSD_{0.05}$		0.5		0.5		2.8	
n		60		60		90	
Grower line	G1	19.2	a	8.5	a	66.9	b
	G2	18.2	b	8.1	a	72.9	a
	G3	17.3	c	7.5	b	71.3	a
p-value		< 0.001		< 0.001		< 0.001	
$LSD_{0.05}$		0.5		0.5		2.8	
n		60		60		90	
Fruit maturity x Grower line	Early G1	18.4	b	6.1		76.2	b
	Early G2	18.2	b	5.4		82.8	a
	Early G3	17.1	c	5.1		78.7	a
	Mid G1	19.6	a	7.6		75.1	b
	Mid G2	18.3	b	7.9		75.3	b
	Mid G3	17.6	bc	7.1		77.1	ab
	Late G1	19.8	a	11.8		49.5	d
	Late G2	18.1	bc	11.0		60.6	c
	Late G3	17.3	bc	10.3		58.0	c
p-value		0.035		0.194		0.003	
$LSD_{0.05}$		1.1		NS		6.4	
n		20		20		30	

A maturity effect on fruit firmness was also evident, with more mature fruit being softer ($p < 0.05$, Table 4.1), agreeing with observations from previous studies (Macrae *et al.*, 1989; Costa *et al.*, 1997). Grower line also influenced initial firmness with G1 being generally less firm, although this was not the case for the mid maturity harvest (Table 4.1).

4.3.3. Chilling injury development during storage

Storage treatment (Table 4.2), fruit maturity (Table 4.3) and grower line (Table 4.4) were each assessed to determine the relationship with incidence of chilling injury. Chilling injury was first observed after 130 d of storage, with incidence increasing considerably to 172 d of storage (Figure 4.5). On an average incidence of chilling injury was 1.3 % at 130 days, 3.5 % at 151 days and 7.3 % at 172 days. Storage conditions had a dramatic influence on incidence of chilling injury (Table 4.2). Fruit exposed to rapid ($R_{12h,0}$) or fast ($D_{3d,0}$) cooling to 0 °C displayed higher incidence of chilling injured fruit compared to fruit exposed to slow ($G_{2w,0}$ and $C_{1w,0}$) cooling to 0 °C (Figure 4.5). After 172 d of storage, incidence of chilling injury in $R_{12h,0}$ and $D_{3d,0}$ averaged 16.8 % and 17.2 % respectively while $G_{2w,0}$ and $C_{1w,0}$ averaged just 2.6 % and 5.9 % respectively. This result clearly demonstrates that cooling rate to storage temperature at 0 °C plays a significant role in the subsequent incidence of chilling injured fruit.

Treatments stored at 2 °C had the lowest incidence of chilling injury, irrespective of the cooling profile used to reach this temperature. Fruit exposed to similar cooling profiles but stored at different temperature ($R_{12h,0}$ vs $R_{12h,2}$ and $C_{1w,0}$ vs $C_{1w,2}$) resulted in substantial chilling injury differences, demonstrating that storing fruit at 2 °C rather than 0 °C alleviates chilling injury development (Figure 4.5). Incidence of chilling injury in $R_{12h,0}$ (16.8 %) reduced to just 0.9 % when stored at 2 °C ($R_{12h,2}$). Similarly, incidence of chilling injury in $C_{1w,0}$ (5.9 %) was lowered to 0.5 % when fruit were stored at 2 °C ($C_{1w,2}$).

The results provide further evidence that the rate of cooling fruit to storage temperature and the subsequent storage temperature influence the development of chilling injury. These results coincide with the findings of Lallu (1997) solidifying the evidence that temperature conditions have an effect on chilling injury development in kiwifruit.

Therefore, the time temperature information available is a useful set of data that may aid in the estimation of the development of chilling injury in subsequent storage.

Table 4.2: Contingency table for the relationship of storage treatment on incidence of chilling injured fruit after 172 d of storage. Data is pooled from 3 grower lines across 3 maturities. Assessment of chilling injury was made after 172 d of storage. Chi-square = 357.8, df = 5, p < 0.001. Values in parenthesis are contributions to chi square. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R1_{2h,2}$ refers to rapid cooling to 2 °C within 12 h, $D_{3d,0}$ refers to direct cooling to 0 °C within 3 d, $G_{2w,0}$ refers to gradual cooling to 0 °C within 2 w, $C_{1w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C_{1w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w.

Storage treatments	Sound fruit	Chilling injured fruit	Total
$R_{12h,0}$	674 (7.86)	136 (99.78)	810
$R_{12h,2}$	803 (3.62)	7 (46.0)	810
$D_{3d,0}$	671 (8.49)	139 (107.72)	810
$G_{2w,0}$	789 (0.17)	21 (24.62)	810
$C_{1w,0}$	762 (0.17)	48 (2.11)	810
$C_{1w,2}$	806 (4.05)	4 (51.44)	810
Total	4505	355	4860

Fruit of late maturity had higher incidence of chilling injured fruit compared to fruit of early or mid-maturity after 172 d of storage (Figure 4.5; Table 4.3). At 172 d, early and mid-maturity fruit exposed to rapid cooling to 0 °C ($R_{12h,0}$) averaged 10.0 % and 14.1 % respectively while late maturity fruit incidence was 26.3 %. This increase in incidence of chilling injury for late maturity fruit was observed consistently in all storage treatments at 172 d of storage (Figure 4.5). Overall, the difference in chilling injury

incidence caused by maturity were significant with the high incidence for late maturity fruit contributing most to the Chi-squared statistic (Table 4.3). Koutsoflini *et al.* (2013) found that kiwifruit of early maturity increased the incidence of chilling injury development which conflicts with the result obtained.

Table 4.3: Contingency table for the relationship of fruit maturity on incidence of chilling injured fruit after 172 d of storage. Data is pooled from 3 grower lines across each maturity. Assessment of chilling injury was made after 172 d of storage. Chi-square = 68.4, df = 2, $p < 0.001$. Values in parenthesis are contributions to chi square.

Fruit maturity	Sound fruit	Chilling injured fruit	Total
Early	1547 (1.369)	73 (17.367)	1620
Mid	1526 (0.394)	94 (5.004)	1620
Late	1432 (3.232)	188 (41.015)	1620
Total	4505	355	4860

Fruit from G2 had the highest incidence of chilling injured fruit compared to fruit from G1 and G3 after 172 d of storage (Table 4.4). Based on the at-harvest soluble solids content, G3 was observed to be the least mature fruit (i.e. low SSC) followed by G2 and G1 (Table 4.1). Based on previous analysis, late maturity fruit had a significantly higher incidence of chilling injury while early maturity fruit had lower incidence of chilling injury. Hence it would seem that Table 4.3 and Table 4.4 provide a pair of contradictory results, in terms of maturity effect (at least defined by SSC) on chilling injury susceptibility. Other measures of maturity, dry matter and initial firmness (Table 4.1) offer no further explanation.

Table 4.4: Contingency table for the relationship of grower line on incidence of chilling injured fruit after 172 d of storage. Data is pooled from 3 fruit maturities. Assessment of chilling injury was made after 172 d of storage. Chi-square = 20.5, df = 2, $p < 0.001$. Values in parenthesis are contributions to chi square. G1, G2 and G3 refers to grower 1, 2 and 3 respectively.

Grower line	Sound fruit	Chilling injured fruit	Total
G1	1504 (0.004)	116 (0.046)	1620
G2	1467 (0.8)	153 (10.156)	1620
G3	1534 (0.696)	86 (8.835)	1620
Total	4505	355	4860

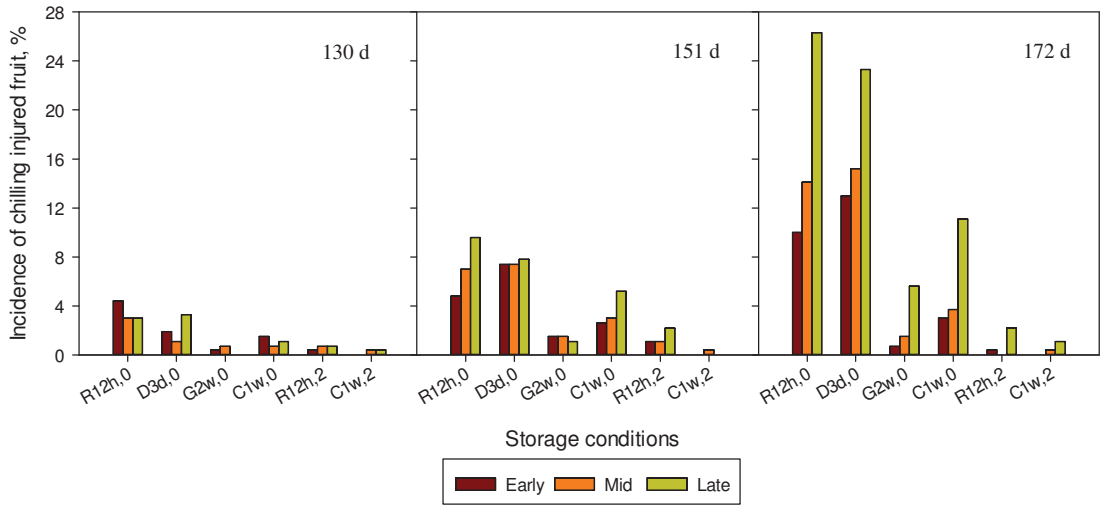


Figure 4.5: Incidence of chilling injured fruit as influence by storage time, storage conditions and fruit maturity. Storage condition code refers to the conditions described in Figure 4.4. $R12_{h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R12_{h,2}$ refers to rapid cooling to 2 °C within 12 h, $D3_{d,0}$ refers to direct cooling to 0 °C within 3 d, $G2_{w,0}$ refers to gradual cooling to 0 °C within 2 w, $C1_{w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C1_{w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w. The data represents the proportion of chilling injured fruit found across 3 grower lines (n = 270).

4.3.4. Fruit firmness during storage

In general, fruit maturity was found to have an effect on at-harvest fruit firmness and soluble solids content, while at-harvest dry matter was more influenced by grower line (Table 4.1; Appendix 1). Factors of grower line, maturity, storage treatment and storage period were analysed statistically to determine the effect on fruit firmness during storage (Table 4.5). Not surprisingly, storage period dominated the effect on firmness given this represents the softening expected during storage. Given the dominance of storage period on firmness, later analysis of treatments effect was conducted each storage time (Section 4.2.3.5). Beyond storage period, all other factors also had a significant effect on the fruit firmness in subsequent storage ($p < 0.05$). However, grower line differences explained the least of the variability (lowest SS) in the firmness data. Subsequently in order to simplify the data explanation and focus on the main effects, the results were presented with grower line data pooled.

Table 4.5: ANOVA table displaying the sum of square (SS), mean square (MS), F and P value of respective factors that were considered to have an effect on fruit firmness during storage. P < 0.001 represents a significant effect on fruit firmness.

Factors	DF	Seq SS	Adj SS	Adj MS	F	P
Grower line	2	3137	2758	1379	25	0.000
Fruit maturity	2	251248	252183	126091	2293	0.000
Storage treatment	5	116048	117881	23576	429	0.000
Storage period	9	7532167	7532167	836907	15216	0.000
Error	201	1588823	1588823	55.00		
Total	209	9491423				

Supply chain temperature profiles had a significant effect on fruit firmness ($p < 0.05$, Figure 4.6). Fruit exposed to rapid or fast cooling to storage temperature ($R_{12h,0}$ & $D_{3d,0}$) were firmer than fruit cooled slowly to storage temperature ($G_{2w,0}$ & $C_{1w,0}$) during the first 100 d of storage. However, fruit exposed to these cooling treatments ($R_{12h,0}$ & $D_{3d,0}$) were softer than slow cooled ($G_{2w,0}$ & $C_{1w,0}$) fruit after 150 d of storage (Figure 4.6).

This more rapid softening of fast cooled fruit mimics the results observed in previous season (Figure 3.8). Fruit exposed to similar cooling methods but stored at 2 °C were softer than fruit stored at 0 °C before 150 d of storage (Figure 4.6). However, Figure 4.6 shows that towards the late storage period (after 150 d) fruit stored at 0 °C and rapidly cooled ($R_{12h,0}$ & $C_{1w,0}$) had similar firmness as fruit stored at 2 °C ($R_{12h,2}$ & $C_{1w,2}$).

Storage temperature is expected to influence fruit softening, with fruit softening being faster when stored at higher temperature (Hertog *et al.*, 2004c; Schotsmans *et al.*, 2005). Results observed in the early storage period are in agreement with this expectation as the 2 °C increase in storage temperature accelerating softening, causing fruit to reach 10 N threshold faster. However, during the gradual softening phase (i.e. >75 d of storage) fruit being stored at 0 °C (and previously cooled rapidly, $R_{12h,0}$ and $D_{3d,0}$) are observed to soften faster than fruit stored at 2 °C (Figure 4.6). The result contradicts the expected influence of temperature on rate of softening as 0 °C stored fruit are softening at a faster rate than 2 °C fruit. Our hypothesis remains that this more rapid softening observed at 0 °C is a result of a proportion of these fruit developing chilling injury (Figure 4.5).

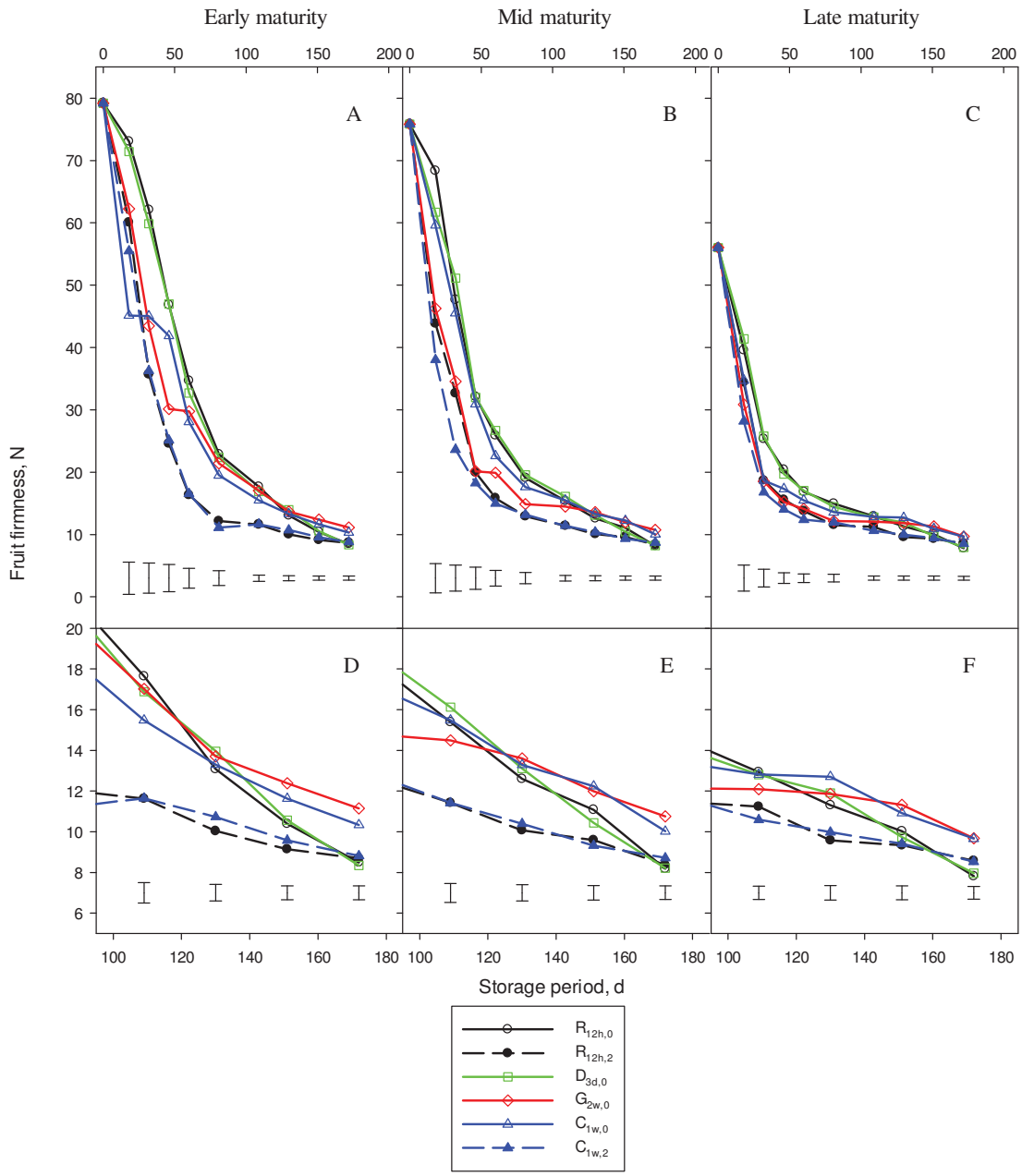


Figure 4.6: Softening of kiwifruit harvested at different maturity as influenced by supply chain temperature conditions. Solid lines represent fruit stored at 0 °C whereas long dashed lines represent fruit stored at 2 °C. Key refers to supply chain temperature conditions as provided in Figure 4.4. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R1_{2h,2}$ refers to rapid cooling to 2 °C within 12 h, $D_{3d,0}$ refers to direct cooling to 0 °C within 3 d, $G_{2w,0}$ refers to gradual cooling to 0 °C within 2 w, $C_{1w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C_{1w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w. Each data point represents the average firmness of fruit from 3 growers (n=90 for first 6 data points and n=270 for the remaining data points). Error bars displayed represent the $LSD_{0.05}$ at each time point. Graphs D to F represent the same data as A to C but focus on the late storage period (> 75 d of storage).

4.4. Discussion

4.4.1. Fruit maturity and grower line differences on kiwifruit quality

Kiwifruit were harvested from 3 different orchards at 3 different maturities (Table 4.1). Jabbar *et al.* (2014) demonstrated grower line differences on subsequent softening of 'Hayward' kiwifruit. At-harvest attributes are used to describe fruit maturity. Fruit of early maturity had higher firmness and lower soluble solids content in comparison to fruit of late maturity (Table 4.1). Fruit of early harvest were firmer in the first 100 d of storage (Figure 4.7) and subsequently become softer at the end of storage period (Figure 4.8), suggesting that early maturity fruit have poorer storability than late maturity fruit. The result observed a larger magnitude of firmness change in early maturity fruit than in late maturity fruit during the early storage period (< 100 d of storage; Figure 4.7). Previously, Costa *et al.* (1997) found that fruit harvested earlier were firmer at harvest and during early storage (< 60 d of storage) but became softer towards end of storage period (> 100 d of storage) compared to fruit harvested later. Similarly, Mitchell *et al.* (1992) found that fruit of late maturity were found to be the most firm after 6 months of storage.

Koutsoflini *et al*. (2013) proposed that early fruit maturity increases the incidence of chilling injury development in 'Hayward' kiwifruit due to a lack of acclimatisation to cool temperatures. Similarly, least advanced (i.e. early maturity fruit) 'Hort16A' kiwifruit were found to be more susceptible to chilling injury development but no clear minimum or maximum thresholds was identified across all orchards (Burdon *et al.*, 2014b). In this work, fruit of late maturity were found to have a higher proportion of chilling injured fruit (Figure 4.5; Table 4.3) in comparison to early maturity fruit, conflicting with the findings found by Koutsoflini *et al.* (2013) and Burdon *et al.* (2014). However, advancement of fruit ripening after harvest was also proposed to increase the susceptibility of fruit to chilling injury (Koutsoflini *et al.*, 2013). In this work, late maturity kiwifruit were more

advanced in ripening at harvest (i.e. lower firmness and higher soluble solids content) than early and mid-maturity fruit (Table 4.1) and hence this advancement in maturity may increase the susceptibility to chilling injury development. At-harvest soluble solids content can be used to estimate fruit maturity and thus G3 was observed to be the least mature followed by G2 and G1 (Table 4.1). However, the incidence of chilling injury was not found to increase between grower lines in this order (i.e. from G3 to G1; Table 4.4) demonstrating that using soluble solids content (°Brix) as a maturity indices alone is not indicative of chilling sensitivity. Additionally, Burdon *et al.* (2014) proposed that using at-harvest attributes such as flesh colour, soluble solids content, firmness and dry matter content were not convincible to predict the susceptibility of chilling injury in 'Hort16A' kiwifruit.

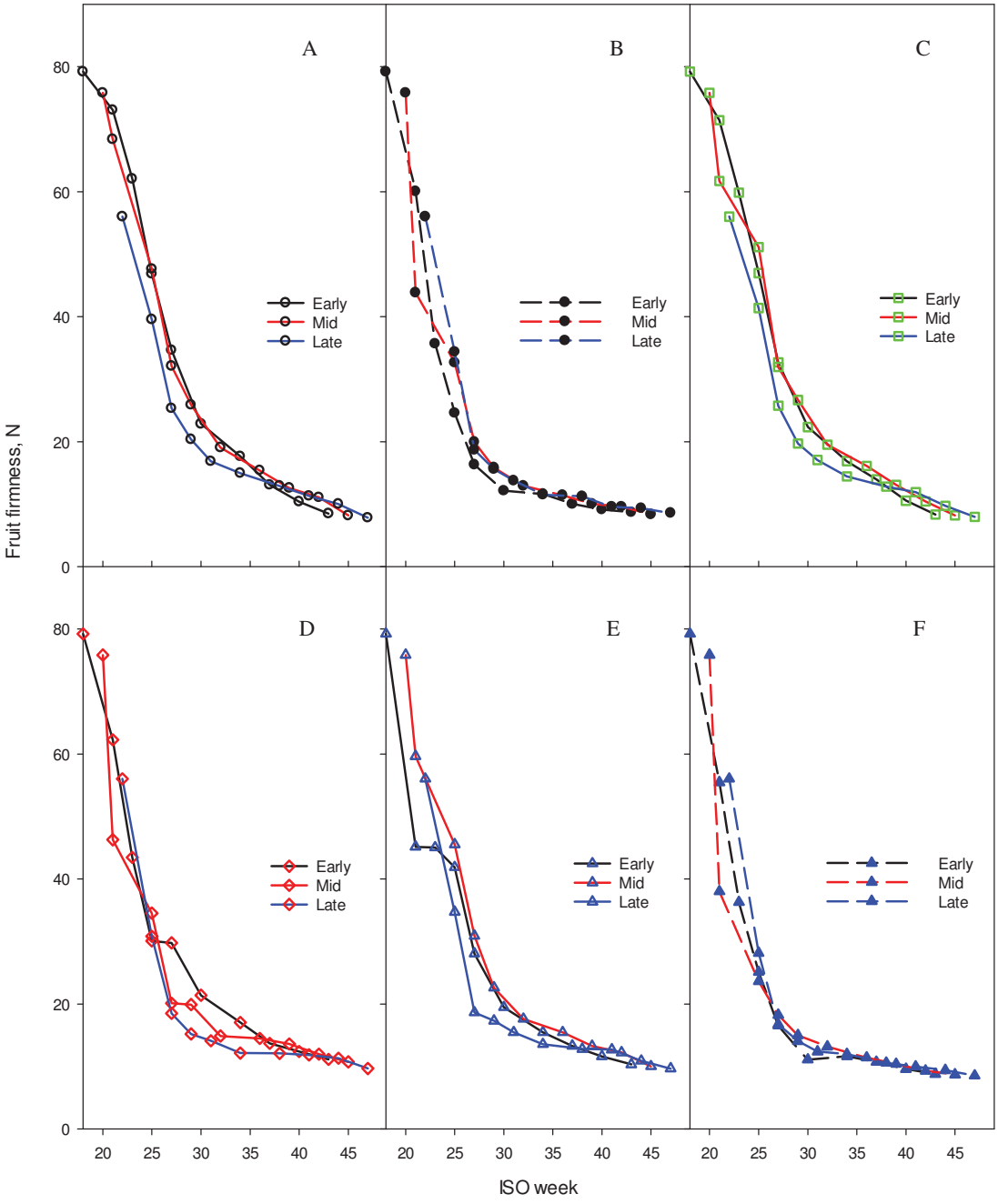


Figure 4.7: Softening of kiwifruit harvested at different maturity during storage in six different supply chain conditions. (A – F) represents treatments $R_{12h,0}$, $R_{12h,2}$, $D_{3d,0}$, $G_{2w,0}$, $C_{1w,0}$ or $C_{1w,2}$ respectively as described in Figure 4.4. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R1_{2h,2}$ refers to rapid cooling to 2 °C within 12 h, $D_{3d,0}$ refers to direct cooling to 0 °C within 3 d, $G_{2w,0}$ refers to gradual cooling to 0 °C within 2 w, $C_{1w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C_{1w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w. Each data point represents the average firmness of fruit from 3 growers (n=90 for first 6 data points and n=270 for the remaining data points). Rotten fruit were removed prior to analysis. Data is an alternative representation of the same data provided in Figure 4.6.

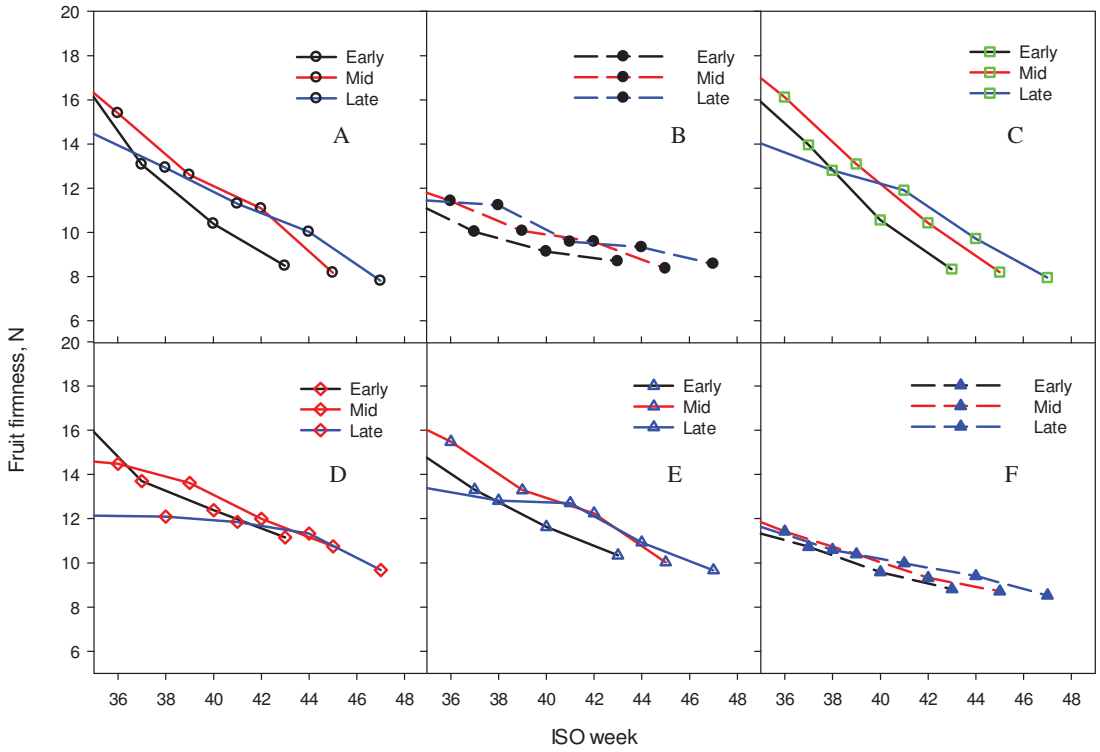


Figure 4.8: Softening of kiwifruit harvested at different maturity during storage in six different supply chain conditions, focusing on the storage period (> 36 ISO week). (A – F) represents treatments $R_{12h,0}$, $R_{12h,2}$, $D_{3d,0}$, $G_{2w,0}$, $C_{1w,0}$ or $C_{1w,2}$ respectively as described in Figure 4.4. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h, $R1_{2h,2}$ refers to rapid cooling to 2 °C within 12 h, $D_{3d,0}$ refers to direct cooling to 0 °C within 3 d, $G_{2w,0}$ refers to gradual cooling to 0 °C within 2 w, $C_{1w,0}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 0 °C within 1 w, $C_{1w,2}$ refers to rapid cooling to 10 °C within 12 h followed by slow cooling to 2 °C within 1 w. Each data point represents the average firmness of fruit from 3 growers (n=90 for first 6 data points and n=270 for the remaining data points). Rotten fruit were removed prior to analysis. Data is an alternative representation of the same data provided in Figure 4.6 and Figure 4.7.

4.4.2. Influence of chilling injury on firmness measurement

Should the development of chilling injury significantly influence firmness then this potentially could be observed through the development of a non-normal distribution of firmness within a single population, as the chilling injury development is speculated to result in simultaneously softening fruit more rapidly. In this experiment, the data collected at 172 d from the $R_{12h,0}$ treatment were used to plot a histogram and assess normality for each maturity and grower line combination. Figure 4.9 shows a single distribution fit, describing the distribution as normal ($p > 0.05$) in 8 of 9 occasions. Although 8 of the 9 populations were found to be normally distributed, the sub-population of chilling injured fruit were all observed to occupy the lower range of firmness in every population (< 10 N; Figure 4.9). A normal distribution fit suggests that chilling injured fruit may be soft but are not considerably distinguishable from a normal soft fruit by using penetrometer firmness measurement. Subsequently visual assessment on the cross section area of the fruit is required to identify chilling injury symptoms (Figure 4.2) and thus differentiate a chilling injured fruit from a normal soft fruit. Given that chilling injured fruit were unable to be differentiated from normal soft fruit by fruit firmness (Figure 4.9) repacking, sorting or even documenting chilling injury incidence of fruit in commercial coolchain is difficult.

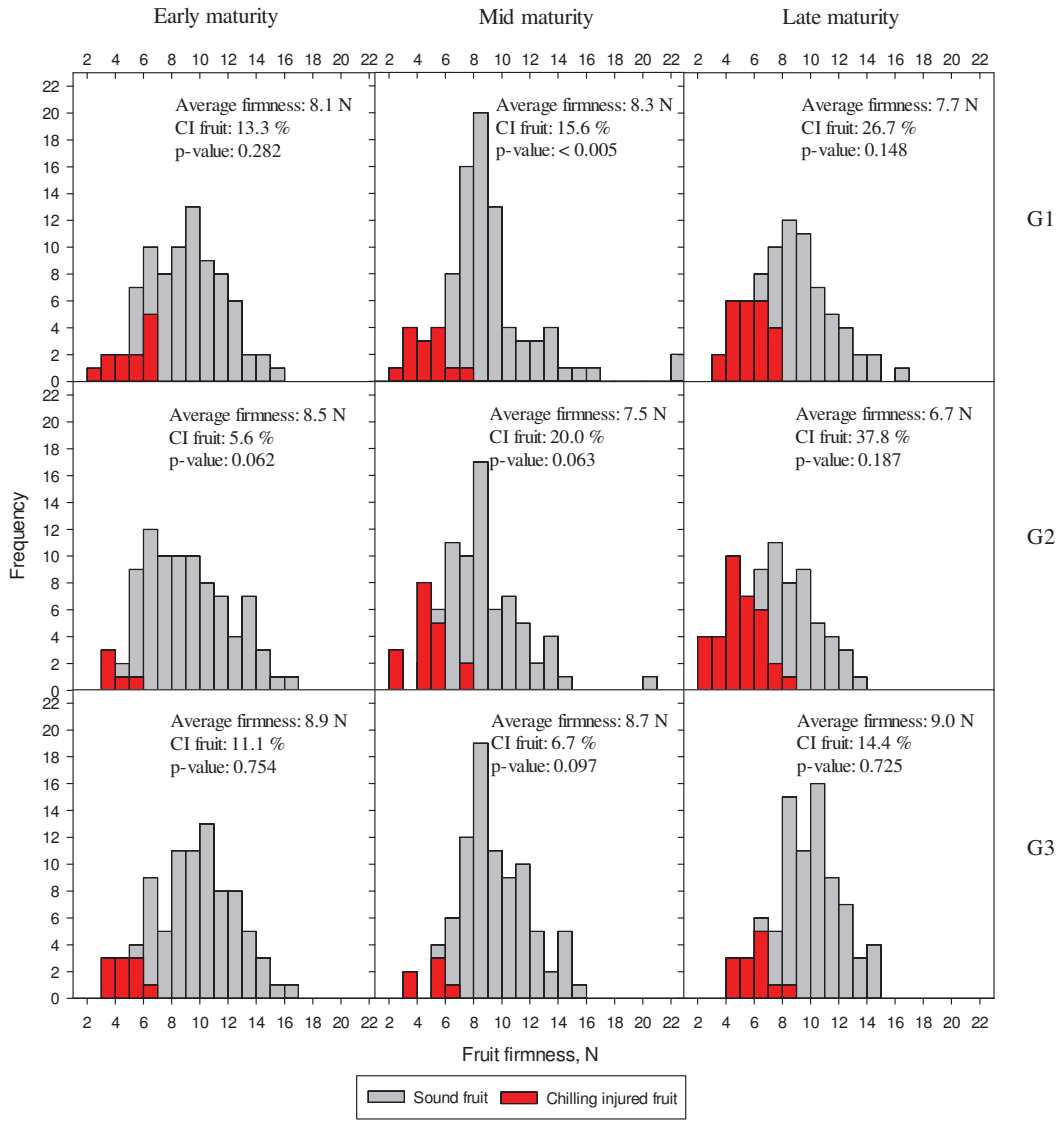


Figure 4.9: Histograms of fruit firmness as influenced by grower line (G1, G2 and G3) and maturity after 172 days of storage and exposure to the $R_{12h,0}$ cooling profile. $R_{12h,0}$ refers to rapidly cooled to 0 °C within 12 h. Each histogram contains 90 fruit from the respective grower line. Rotten fruit were removed from the population. An Anderson-Darling p-value < 0.05 refers to a population that is not normally distributed.

The percentage of chilling injured fruit found in those that were fast ($D_{3d,0}$) or rapid ($R_{12h,0}$) cooled to 0 °C were notably higher compared to fruit slowly cooled ($G_{2w,0}$ & $C_{1w,0}$) to 0 °C (Figure 4.5). At the same time when fruit were exposed to fast cooling to 0 °C ($R_{12h,0}$ & $D_{3d,0}$), during the late storage period (> 100 d) more rapid softening was observed (Figure 4.6). This suggests that chilling injury development in kiwifruit influences the average fruit firmness with chilling injury resulting in lowering the average fruit firmness. However, given that development of chilling injury does not cause fruit to result in substantially different firmness (Figure 4.9), this suggests that chilling injured fruit may potentially also influence average population firmness (i.e. the sound fruit within the same pack), due to an increase in ethylene in the immediate environment. Chilling injured fruit were found to produce ethylene (Hyodo & Fukasawa, 1985; Antunes & Sfakiotakis, 2002a; Feng *et al.*, 2003b), potentially increasing the concentration of ethylene within the package. Jabbar and East (2016) demonstrated that exposing kiwifruit to as low as 0.01 $\mu L\ L^{-1}$ at harvest induced rapid softening in kiwifruit. The higher concentration of ethylene accumulated within the package possibly results in a more rapid softening of the entire population. This principle is similar to the presence of rotten fruit affecting the neighbouring fruit within the same pack resulting in pre-mature softening due to ethylene produced by the rotten fruit (Burdon & Lallu, 2011).

4.4.3. Coolchain temperature effect on kiwifruit quality

4.4.3.1. The effect of cooling rate on kiwifruit quality

Precooling is applied to harvested fruit to remove field heat and delay the deteriorative and senescence processes and hence sustain a high level of quality (Brosnan & Sun, 2001). However, when kiwifruit was cooled to 0 °C within 3 days of harvest, the fruit were found to be firmer during the first 100 d of storage but soften faster towards the late storage period (Figure 4.6), with an increasing proportion of chilling injured fruit

(Figure 4.5). Chapter 3 also demonstrated that cooling rate to storage temperature affects the subsequent fruit firmness during the late storage period. Similarly, Lallu *et al.* (1997) proposed not to precool kiwifruit due to promotion of chilling injury development.

Longer cooling times (> 7 days) to storage temperature of 0 °C ($G_{2w,0}$ & $C_{1w,0}$) was found to significantly lower the risk of chilling injury development (Figure 4.5) and maintain firmer fruit during the late storage period (Figure 4.6). These cooling methods can be referred to as temperature conditioning, where it allows the fruit to build resistance against chilling injury during cool storage. Yang *et al.* (2013) demonstrated that exposing 'Hayward' kiwifruit to 12 °C for 3 days prior to storing at 0 °C alleviated chilling injury development by increasing the antioxidant enzymes activities and maintaining a high level of endogenous hormones. There is a possibility that by exposing kiwifruit to treatment $G_{2w,0}$ or $C_{1w,0}$ has increased the antioxidant activities and thus preventing chilling injury development.

In the industry, fruit are cooled and kept in a coolroom for storage prior to export. The industry follows a standard that fruit less than 10 N are not to be exported from New Zealand as the remaining storage life is not sufficient to guarantee the fruit reaching the marketplace. Therefore, the importance of this work was always to focus on predicting fruit firmness during the late storage period (i.e. > 100 days of storage), where the fruit firmness is possibly below the 10 N threshold. This study has shown that fast or rapid cooling fruit to 0 °C ($R_{12h,0}$ or $D_{3d,0}$) causes kiwifruit to soften in an accelerated rate during the late storage period and thus suggests that precooling kiwifruit leads to poor long term storability. Fruit quality of rapidly cooled fruit is also compromised due to the development of chilling injury. Different packhouses cool fruit to storage temperature based on their cooling capacity and management practices. Since this work identified the effect of cooling rate on chilling injury development, the cooling practices in packhouses

can be used as a reference to estimate the risk of chilling injury development and thus evaluate the potential for long term storability.

4.4.3.2. The effect of storage temperature (0 or 2 °C) on kiwifruit quality

This work, Lallu (1997), and Yang *et al.* (2012 & 2013) have demonstrated that either temperature acclimatisation or removal of temperature stress alleviate chilling injury development during storage. Fruit exposed to rapid cooling but stored at 2 °C ($R_{12h,2}$) lowers the incidence of chilling injury development (Figure 4.5) but with the consequence of softer fruit (Figure 4.6). This suggests that removal of the temperature stress (i.e. from 0 to 2 °C) alleviates chilling injury development. Positional and temporal temperature variations occur in industrial coolrooms and thus fruit softening is expected to be different across the coolroom. Based on the coolroom temperature distribution information, fruit that reaches the 10 N threshold can be potentially identified. The result shows that fast cooled fruit can be stored at 2 °C to alleviate chilling injury development (Figure 4.6) and thus suggesting that storing fast cooled fruit at locations away from the evaporate air flow (i.e. at slightly higher temperature), may assist chilling injury alleviation.

This work demonstrates that storage temperature influences fruit storability, where fruit exposed to rapid cooling to 0 °C ($R_{12h,0}$) were firmer with high incidence of chilling injury development and fruit were softer with lower incidence of chilling injury development when stored at 2 °C ($R_{12h,2}$). Therefore, there is a possibility that manipulating storage temperature during storage may achieve firmer fruit and reduce chilling injury development in subsequent storage. A later unpublished experiment conducted by author was conducted in 2014 at Massey University in which kiwifruit were placed at 0 or 2 °C and subsequently switched from 0 to 2 °C or 2 to 0 °C at 25 days

intervals during storage of 200 days (Figure 4.10). Results identified that fruit exposed to longer storage at 0 °C were firmer (Figure 4.11A & B), with a higher proportion of fruit above 10 N (Figure 4.11C & D). Although fruit were firmer when stored at 0 °C, a higher incidence of chilling injury was found after long term storage (> 125 day) (Figure 4.12). Figure 4.12 suggest that interchanging fruit from 2 to 0 °C promotes chilling injury development, whereas the opposite (0 °C to 2 °C) alleviates chilling injury development. Overall, the result suggests that subtle temperature differences near the target storage temperature of 0 °C during storage may have substantial influence on the firmness and chilling injury outcomes after long term storage.

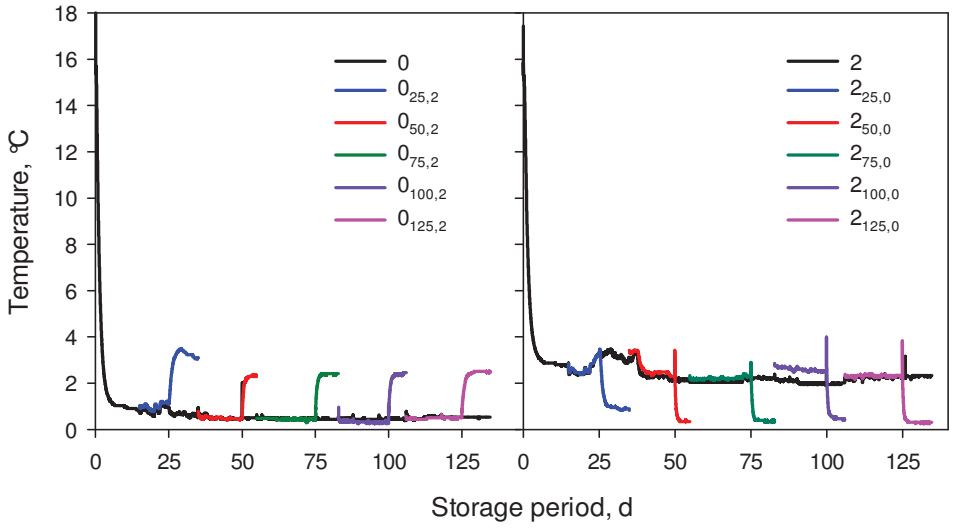


Figure 4.10: Time temperature profile of control and various "switch" treatments. 0 refers to fruit stored at 0 °C. Temperature switch from 0 to 2 °C was applied at 25 d ($0_{25,2}$), 50 d ($0_{50,2}$), 75 d ($0_{75,2}$), 100 d ($0_{100,2}$) and 125 d ($0_{125,2}$) of storage. 2 refers to fruit stored at 2 °C. Temperature switch from 2 to 0 °C was applied at 25 d ($2_{25,2}$), 50 d ($2_{50,2}$), 75 d ($2_{75,2}$), 100 d ($2_{100,2}$) and 125 d ($2_{125,2}$) of storage.

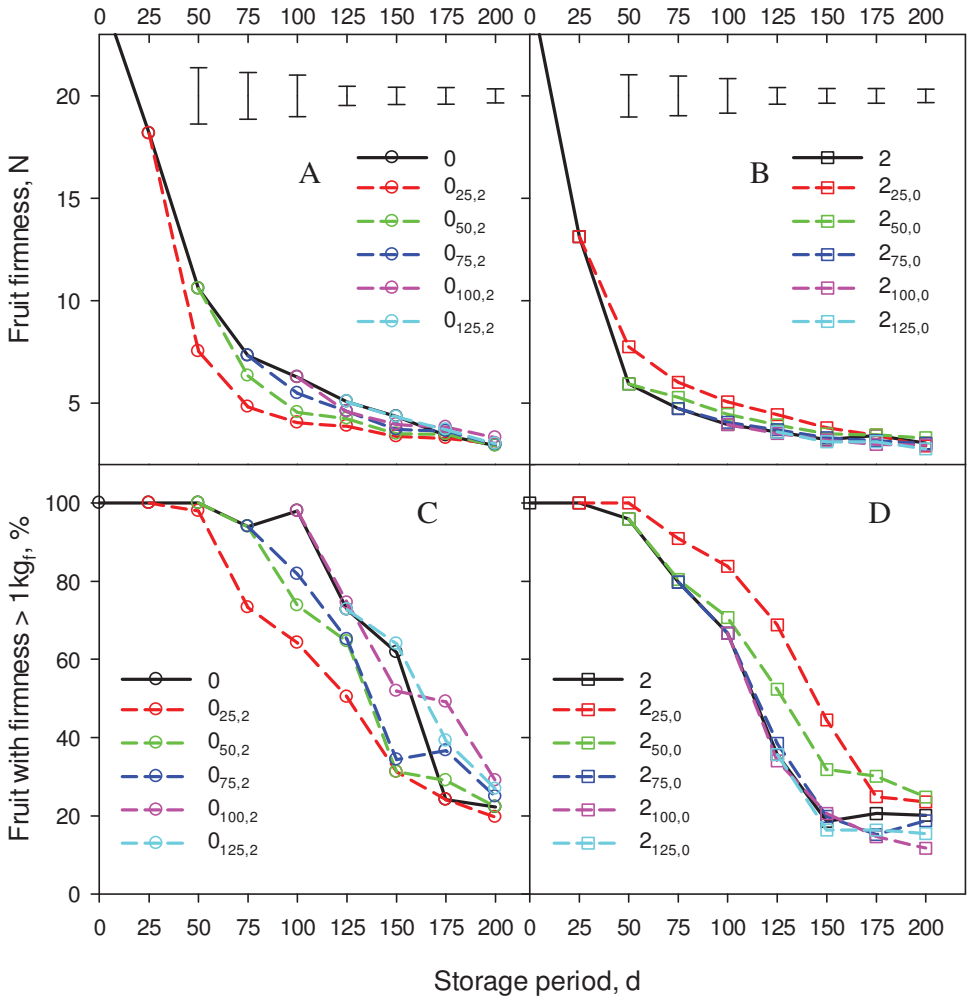


Figure 4.11: (A & B) Softening of kiwifruit exposed to different temperature "switch" treatments. Fruit were exposed to a temperature switch from 0 to 2 °C or 2 to 0 °C at every 25 d intervals. Treatment code represents initial temperature (0 or 2 °C), days of storage at that temperature (25 to 125 days) and final storage temperature (0 or 2 °C). 0 refers to fruit stored at 0 °C. Temperature switch from 0 to 2 °C was applied at 25 d ($0_{25,2}$), 50 d ($0_{50,2}$), 75 d ($0_{75,2}$), 100 d ($0_{100,2}$) and 125 d ($0_{125,2}$) of storage. 2 refers to fruit stored at 2 °C. Temperature switch from 2 to 0 °C was applied at 25 d ($2_{25,2}$), 50 d ($2_{50,2}$), 75 d ($2_{75,2}$), 100 d ($2_{100,2}$) and 125 d ($2_{125,2}$) of storage. Each data points represent an average firmness of 3 grower lines (n = 99 for first 3 data points and n = 297 for remaining data points). (C & D) Percentage of fruit with more than 10 N firmness during storage for various temperatures "switch" treatments.

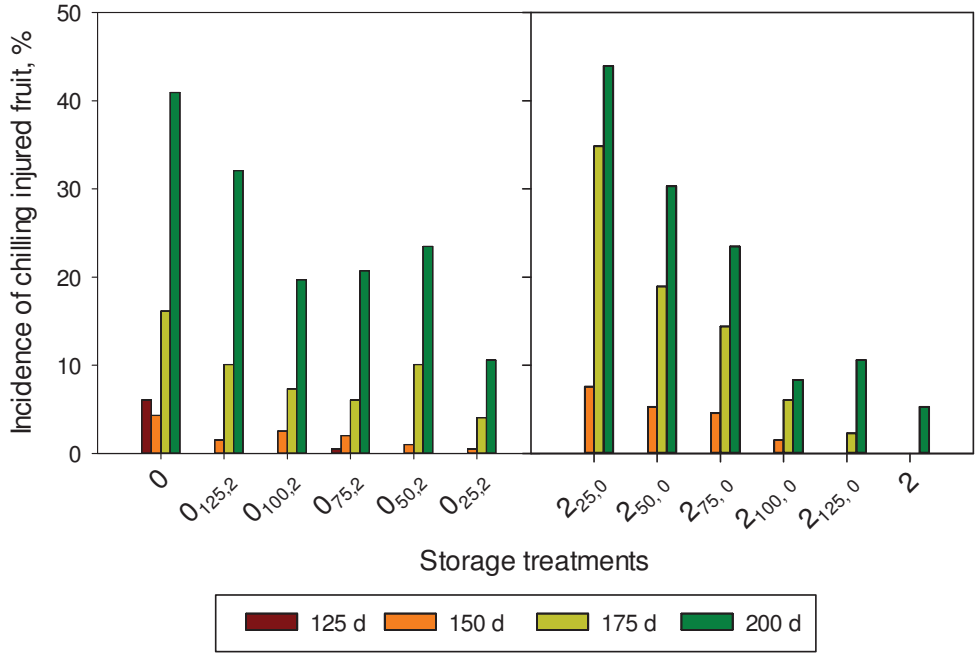


Figure 4.12: Percentage of fruit with chilling injury after 125, 150, 175 and 200 d of storage for various temperatures "switch" storage treatments (Figure 4.10). Fruit were exposed to a temperature switch from 0 to 2 °C or 2 to 0 °C. 0 refers to fruit stored at 0 °C. Temperature switch from 0 to 2 °C was applied at 25 d ($0_{25,2}$), 50 d ($0_{50,2}$), 75 d ($0_{75,2}$), 100 d ($0_{100,2}$) and 125 d ($0_{125,2}$) of storage. 2 refers to fruit stored at 2 °C. Temperature switch from 2 to 0 °C was applied at 25 d ($2_{25,2}$), 50 d ($2_{50,2}$), 75 d ($2_{75,2}$), 100 d ($2_{100,2}$) and 125 d ($2_{125,2}$) of storage. Each bar represents an average incidence of chilling injured fruit from 3 grower lines (n = 297).

4.5. Conclusion

Chilling injury development affected fruit quality during the late storage period (> 100 days), displaying symptoms such as grainy tissue and water soaked appearance along the outer pericarp. Furthermore, development of chilling injury in kiwifruit is likely to influence fruit firmness during storage, as an increase in the proportion of chilling injured fruit coincided with accelerated softening, during the late storage period (> 100 d). The storage conditions (cooling rate and storage temperature) influence the susceptibility of kiwifruit to chilling injury development, where rapid or fast cooling to 0 °C promotes chilling injury development whereas storing fruit at 2 instead of 0 °C alleviates chilling injury development. Kiwifruit are cooled to storage temperature differently depending on the management practices adopted at each packhouse. Since the results suggest that

cooling time to storage temperature has an impact on fruit firmness and quality, the cooling methods adopted by each packhouse should at focus for batches of fruit which are destined for long term storage. Fruit maturity was found to have an effect on the chilling injury development in kiwifruit, but still questionable whether fruit of late or early maturity are more susceptible to chilling injury development. Therefore, it suggests that more studies are needed to be done in order to understand the influence of fruit maturity on incidence of chilling injury in kiwifruit.

In the following chapter, an empirical modelling approach is used to estimate the proportion of chilling injured fruit by fitting. Accumulated heat units are used to quantify the difference between various storage temperature treatments. Making a reasonable estimation of the amount of chilling injured fruit in the population is critical as it contributes to lowering the population average fruit firmness. Having a mathematical prediction of the incidence of chilling injury will assist the development of a mathematical model to predict the softening curve of kiwifruit. These models of kiwifruit softening and chilling injury development are created in the next chapter.

5. Mathematical modelling of 'Hayward' kiwifruit softening

5.1. Introduction

Prediction of 'Hayward' kiwifruit quality in the coolchain is a challenge as there are many factors that potentially influence the quality in subsequent storage. Differences in grower lines, cooling profiles, storage temperatures, and ethylene exposure were identified to contribute to the uncertainty in fruit softening prediction (East *et al.*, 2016). Fruit maturity influences fruit firmness during storage, where fruit harvested later in season have better storability than fruit harvested early in the season (Harman, 1981; Mitchell *et al.*, 1992; Costa *et al.*, 1997). Jabbar (2014) investigated the differences in grower line on kiwifruit softening, where a wide time variation was discovered for 3% of the fruit to fall below the export threshold of 10 N. Previously chapters 3 and 4 demonstrated the effect of cooling rate and storage temperature on fruit firmness in subsequent storage, and by promoting or alleviating chilling injury development. The impact of temperature on kiwifruit firmness has been well documented previously in chapters 3 and 4 and other work (Mitchell, 1990; Schotsmans *et al.*, 2005; Schotsmans *et al.*, 2008; Bollen *et al.*, 2013; Jabbar, 2014). These factors that influence fruit softening should be included in the development of a predictive model to ensure that the model should respond accordingly.

There are several mathematical models that have previously been used to model kiwifruit softening during storage. Benge *et al.* (2000) used empirical complementary Michaelis – Menten type (CMM), Exponential (EXP), and Complementary Gompertz (CG) models. However, these models failed to characterise fruit softening sufficiently. A segmented Jointed Michaelis – Menten type (JMM) and Inverse Exponential Polynomial (IEP) better characterised softening (Benge *et al.*, 2000). Jabbar *et al.* (2014) demonstrated the use of Complementary Gompertz (CG) and Time Shift Complementary

Gompertz (TSCG) to predict kiwifruit softening at 20 °C in air. Grower line variability in softening was described using at-harvest measurements (initial soluble solid content and firmness) as predictors to determine batch dependent parameters. These empirical modelling approaches fit a curve to a large set of data and contain several parameters describing the curve that do not have any biological explanation. The rigidness of empirical models makes them difficult to characterise softening behaviour of kiwifruit when exposed to conditions apart from the experimental data used to create the model. This becomes problematic if requiring application to variable time temperature data to predict kiwifruit softening.

Another approach that is commonly used is mechanistic modelling, which uses understanding of the mechanisms to develop representative equations. Hertog *et al.* (2004) developed a complex model which related gas exchange and effect of modified atmosphere to predict kiwifruit softening. However, the model was not built to predict the development of chilling injury, which was found to affect fruit firmness in subsequent storage. Alternatively, Schotsmans *et al.* (2005, 2008) developed a kinetic model to describe the stiffness and textual changes in 'Hort16A' kiwifruit when stored at different storage temperature, where Arrhenius law was included to model temperature dependence. However, the developed models did not attempt to characterise accelerated softening due to chilling injury development. These models used at-harvest firmness to predict fruit softening in storage without considering the influence of fruit maturity and grower line, which affect fruit softening (Jabbar, 2014).

There are other mechanistic model examples for fruit quality that have been developed for peaches (Tijskens *et al.*, 1998), apples (Johnston *et al.*, 2001; Gwanpua *et al.*, 2012), tomatoes (Van Dijk *et al.*, 2006a; Van Dijk *et al.*, 2006b) and cucumber (Schouten *et al.*, 2002). The benefit of using the mechanistic model approach is being

able to predict the softening behaviour under variable conditions across the range of conditions used for development. However, the challenge of developing a good mechanistic model is acquiring a detailed understanding of the mechanisms behind the process being modelled (i.e. softening of kiwifruit in this case).

The aim of this chapter is to develop a mechanistic type model that characterises softening behaviour observed in 'Hayward' kiwifruit and thus enable prediction of softening in the coolchain. For ease of application, the prediction model was constrained to consist of inputs that are easily collected in the commercial setting throughout the supply chain. Factors considered in varying the firmness during storage are initial fruit firmness and maturity, and storage temperature conditions; while ethylene effects were ignored. Ethylene is known to affect kiwifruit softening and chilling injury development, even at coolstorage temperatures (Jabbar & East, 2016). However, as measurement methods limit detailed ethylene condition data collection in the current supply chain, it would be inappropriate to develop a model that requires ethylene conditions as input data. Should firmness prediction be achieved, industry will benefit in providing improved information on kiwifruit softening and hence enable improved logistics with the aim of reducing fruit losses.

Fruit softening is a complex biological process, involving several enzymes and reactions. Therefore, several assumptions were made in order to develop a mathematical model to describe fruit softening. The first assumption is the breakdown of starch results in fruit softening, hence describing the rapid softening phase. The second assumption is when there is no starch left, the breakdown of cell wall is the only mechanism that leads to the gradual phase of softening. The third assumption is that the observed rapid softening that occurs during the last stage of softening (> 120 d) is a result of chilling injury development. Fruit softening curve can be predicted based on these established

assumptions. However, these assumptions may over simplified the softening process, resulting in an over or under softening predictions.

5.2. Model conceptual framework

Starch degradation, cell wall swelling, modification of cell wall structure, loss of turgor pressure, and chilling injury development are all major events that occur during kiwifruit ripening that affect fruit texture. Schroder and Atkinson (2006) described the various key events that occurring during kiwifruit ripening in four distinct phases, including an initial lag phase (phase 1), rapid softening (phase 2), a long gradual softening (phase 3) and followed by over-ripe softening (phase 4). In this work, the initial lag phase was not observed when fruit were stored at 0 or 2 °C (Figure 3.8, Figure 4.6). Similarly, Benge *et al.* (2000) also observed no initial lag phase in softening when kiwifruit were stored at 0 °C. This initial lag phase is however observed when kiwifruit are stored at 20 °C (Macrae *et al.*, 1989; White *et al.*, 2005; Jabbar *et al.*, 2014). A contributing factor to the differences observed at each temperature may be the difference in measurement rate. Commonly, when fruit are monitored at 20 °C, fruit quality measurement is conducted within days while the measurement intervals at 0 or 2 °C are commonly at least 2 weeks. This slower rate of measurement at 0 or 2 °C may result in missing measurement of a short lag phase component of softening which may occur at this temperature. Given that no lag phase was observed in the data (Figure 5.1), the model developed will not attempt to describe an initial lag phase.

Numerous biochemical modifications occur during kiwifruit ripening (Burdon & Lallu, 2011). Developing an exact mathematical model to describe the contribution of each modification to softening would not only be challenging but also result in a highly parameterised model, requiring a large set of input information to describe each of the processes. Hertog *et al.* (2004) developed a kiwifruit softening model that considered

storage conditions, including changes in oxygen and carbon dioxide concentration. Although the model is able to describe the fruit softening curve to an extent, the influence of fruit maturity, grower line, and chilling injury development on fruit softening is not well defined. The industry handles fruit across different maturity and grower lines sourced from wide ranging geographical locations and thus an ability to incorporate the effect of fruit maturity and grower lines on the softening prediction will be advantageous. A balance between being mechanistically representative yet simplistic enough to enable industrial application is required. As a result the mechanistic model formulation will focus on describing three major biochemical modifications occurring during 3 phases of the softening curves (Table 5.1).

Table 5.1: Summary of different mechanisms hypothesized to occur during the observed phases of kiwifruit softening.

Phase	Proposed mechanism	Section
A	Correlates to the breakdown of starch	5.2.1
B	Breakdown of cell wall structure	5.2.2
C	Chilling injury development	5.2.3

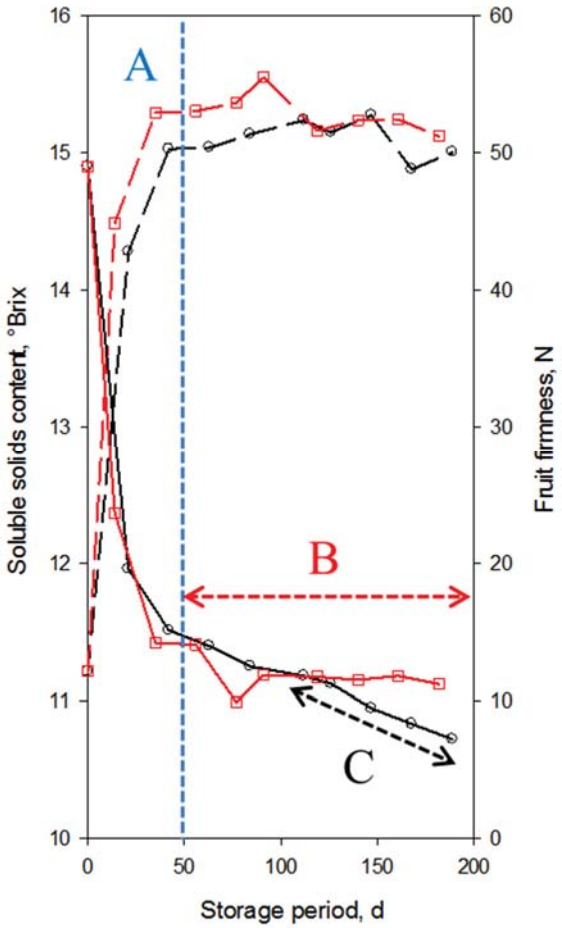


Figure 5.1: Average kiwifruit firmness and soluble solids content during storage at 0 °C. A, B and C represent the three different phases of kiwifruit softening reflected in Table 5.1. The data represents the average fruit firmness and soluble solids of fruit stored at 0 °C after direct (black line) or gradual (red line) cooling to 0 °C as collected in the 2012 season and previously presented in Figure 3.8 and Figure 3.9. The long dash line refers to the soluble solids data while the solid line refers to the fruit firmness data. Fruit firmness data consist of an average of 3 replicate growers of 36 fruit each (n = 108), whereas soluble solids content data consist of an average of 3 replicate growers of 36 fruit (n = 108) for the first 5 days and 10 fruit (n = 30) for the remaining storage period.

The first phase (A) consists of the rapid initial softening, the second phase (B) consists of the gradual softening and the last phase (C) refers to the accelerated softening rate towards the end of the storage period as a result of chilling injury development (Figure 5.1). In phase A, the rapid softening coincides with the breakdown of starch to sugars observed as the increase in fruit soluble solids content (Figure 5.1; Section 5.2.1). When there is little or no starch remaining, the mechanism causing the gradual softening

is dominated by the breakdown of the cell wall structure (Section 5.2.2). Development of chilling injury affects the fruit firmness during long storage, where a high proportion of chilling injured fruit is observed. Therefore, softening accelerates towards the late storage period (Section 5.2.3). The remaining parts of this section discuss the concepts that underpin the development of the mathematical model that represents these 3 different phases described in Figure 5.1 and Table 5.1.

5.2.1. Breakdown of starch content

Starch degradation has been observed to coincide with the rapid softening phase in kiwifruit (Arpaia *et al.*, 1987; Macrae *et al.*, 1989). Bonghi *et al.* (1996) suggested that starch degradation may play an important role in the early stages of kiwifruit softening. However, the mechanism that relates degradation of starch to fruit softening in kiwifruit is not known. Starch content decreases concomitant with an accumulation of soluble solids when kiwifruit ripen (Macrae *et al.*, 1989; Macrae *et al.*, 1992). The breakdown of starch in kiwifruit during ripening is caused by amylase activity. Bonghi *et al.* (1996) demonstrated a high level of amylase activity at harvest which subsequently declined when kiwifruit softened. The decrease in amylase activity corresponds with the decrease in starch content from high level (5 to 7 %) at harvest to negligible level after ripening.

'Hayward' kiwifruit contains high starch content of 5 - 7 % fresh weight or 40 - 50 % dry weight at harvest (Bowen *et al.*, 1988; Macrae *et al.*, 1989; Matsui & Kitagawa, 1990; Walton & Dejong, 1990). During fruit development, the average starch granule size increases from 3 - 4 µm to 10 – 12 µm and once the fruit matures is 6 – 8 µm (Sugimoto *et al.*, 1988). The starch is found in the outer pericarp, the locule wall area of the inner pericarp and the core of kiwifruit (Hallett *et al.*, 1992; Harker & Hallett, 1994; Hallett *et al.*, 1995). Structural changes occur to starch granules during postharvest ripening. Starch granules found in the outer pericarp of a firm fruit (75 N) are no longer visible when the

fruit soften to 5 N (Hallett *et al.*, 1995). This demonstrates that starch dramatically reduces in physical size during kiwifruit ripening.

While dramatic reduction in starch granule size occurs during initial softening, the mechanism of how this starch breakdown translates to a lower penetrometer firmness measurement has not been explained. In banana, breakdown of starch content was found to contribute to textural softening, where a significant decrease in starch content was detected during ripening (Kojima *et al.*, 1994; Bhagyalakshmi *et al.*, 2002). A decrease in banana starch content from 22.4 % to 6.9 % of the pulp during ripening led to a decrease in elastic modulus (Finney *et al.*, 1967). Breakdown of starch increases osmotic pressure in banana pulp (Von Lesecke, 1950). This increase in osmotic pressure is often associated with a decrease in turgor pressure (Meyer *et al.*, 1965) which may potentially contribute to softening during banana ripening (Falk *et al.*, 1958).

Breakdown of starch during ripening affects the turgor pressure which eventually influences the textural properties. Macrae *et al.* (1989) found that during kiwifruit ripening at 0 °C, starch content was found to decrease from 54.1 to 13.1 mg g^{-1} FW within the first 4 weeks and subsequently to non-detectable levels after 12 weeks. There is a possibility that the initial rapid softening is partly due to the decrease in the cell turgor. However, Harker and Hallett (1994) demonstrated that no distinct pattern of change in cell turgor was observed during kiwifruit ripening. It was proposed that during ripening, the cell wall of kiwifruit becomes more plastic and elastic and hence leads to cell expansion rather than an increase in turgor pressure.

Despite the currently undefined link between starch degradation and softening in kiwifruit, the coincidental nature of these processes and the fact that both dry matter and soluble solids content are standard industry fruit maturity measurements may provide

opportunity to model the initial rapid softening phase including the influence of fruit grower line and maturity. Dry matter and soluble solid content are used to estimate fruit starch content, which potentially assesses the fruit maturity.

5.2.2. Breakdown of cell wall structure

Many events occur within the cell wall matrix during ripening, including solubilisation and degradation of the pectic polymers, and destabilisation of the cellulose-hemicellulose network (Percy *et al.*, 1996; Redgwell *et al.*, 1997; Brummell, 2006). These events will lead to a reduction in cell to cell adhesion, weakening of cell wall strength, and swelling of the cell wall, resulting in fruit softening. Cell wall modification occurs during kiwifruit ripening. A loss in staining intensity of cell wall material suggests modification of the cell wall structure and composition occurs during kiwifruit ripening (Hallett *et al.*, 1992). Solubilisation and degradation of pectic polymer was found during kiwifruit ripening, with degradation of pectic polymers in kiwifruit occurring after solubilisation (Redgwell *et al.*, 1992). Harker *et al.* (1994) found that in the early stage of softening, cell to cell adhesion was strong causing the cell to rupture upon applying tensile stress to the fruit, exposing the cell interior. However, during the later stage of softening, cell to cell adhesion weakens and hence neighbouring cells separated from each other without rupturing, when the same amount of tensile stress was applied to the fruit.

There are several enzymes that are responsible for the modification of cell wall structure. Polygalacturonase (PG) and β-galactosidase (β-GAL) are involved in the degradation and solubilisation of pectin (Wegrzyn & Macrae, 1992; Bonghi *et al.*, 1996). Pectin methylesterase (PME) activity of kiwifruit was found to increase during ethylene treatment followed by a rapid drop to low level as fruit softened, proposing that PME activity might initiate the rapid softening during ripening in response to ethylene treatment (Wegrzyn & Macrae, 1992). Although there was detection of PME activity,

little is established on the role of PME on kiwifruit cell wall *in vivo*. Another enzyme that contributes to cell wall modification is xyloglucanase, which reduces the molecular weight of xyloglucan and thus weakens the cellulose–hemicellulose framework and promotes cell wall swelling. Redgwell *et al.* (1997) suggested swelling of the cell wall is a complex process which involves both mechanisms of pectin solubilisation and weakening of cellulose-hemicellulose framework. Swelling of the cell wall occurs in ripe kiwifruit, where cell wall thickness in the outer pericarp is 3 to 4 times greater than fruit at harvest (Hallett *et al.*, 1992).

Fruit softening is a complex process where many enzymatic processes are occurring either simultaneously or consecutively. The role of each enzyme affecting the cell wall structure during ripening has been well studied. However, it is difficult to identify one key enzyme that initiates softening, since every enzyme plays a distinct role in modifying the cell wall structure. Therefore, instead of identifying one key enzyme, all the enzymes that are responsible for the breakdown of cell wall structure are modelled as a simplified single enzyme system. This enables description and prediction of the softening mechanism caused by enzymatic activity.

5.2.3. Development of chilling injury

Chilling injury is a physiological disorder that occurs when fruit or vegetables are stored at low temperature, but above freezing temperature. The development of chilling injury is found in 'Hayward' kiwifruit when stored at cool storage for several months (Lallu, 1997; Bauchot *et al.*, 1999). In this work, the symptoms of chilling injury on kiwifruit were identified as a grainy appearance and water soaked area along the outer pericarp (Figure 4.2). Bauchot *et al.* (1999) showed that the grainy appearance was associated with the presence of gas bubbles in the outer pericarp, where an increase in bulk porosity of the cell wall was observed in chilling injured fruit. The mechanism

behind the development of water soaked area in the outer pericarp is still unclear. One suggestion is that the water soaked area may be due to the loss of cell membrane integrity. Yang *et al.* (2013) demonstrated that applying temperature conditioning to kiwifruit will alleviate chilling injury development by reducing the reactive oxygen species (ROS) level which potentially damages the cell membrane.

Chilling injury development in kiwifruit affects fruit quality and storability. This work has suggested that chilling injury also influences fruit firmness and softening rate at later period of storage (Figure 4.6). Cooling rate to storage temperature and subsequent storage temperature were found to play an important role in the incidence of chilling injury development. Lallu (1997) and this work (Figure 4.5) have shown that a higher proportion of fruit develop chilling injury when exposed to a faster cooling rate to storage temperature and stored at 0 °C for several months. Therefore in this model, chilling injury development and its influence on softening will be modelled with emphasize on the effect of cooling rate and storage temperature on subsequent development.

5.3. Model development

5.3.1. Model constraints

The purpose of the mathematical model is for potential widespread industrial application. The use of grower line dependent parameters is required to describe the well-known grower line difference observed by Jabbar *et al.* (2014). Each grower dependent parameters will require a prior estimation from data collected in industry. Using data that is already collected and relatively easy and cheap to measure is also beneficial for industrial application. Commonly available data that is currently collected is at-harvest soluble solid content and dry matter while some suppliers also collect firmness. The mathematical model was developed to rely on the time temperature data collected in a RFID temperature monitoring system (Bollen *et al.*, 2013). As previously stated, all

ethylene effects were ignored as technologies to conduct widespread measurements of ethylene concentration in the working range (10 nL L^{-1} to 10 µL L^{-1}) in the industrial setting do not currently exist, even though low concentration of ethylene (10 nL L^{-1}) was found to influence fruit softening significantly (Jabbar & East, 2016). McAtee *et al.* (2015) found that in the first phase of softening, kiwifruit do not produce ethylene but it is highly sensitive to ethylene. Therefore, during the experimental work, ethylene concentrations were monitored and controlled to be below the industry standard of less than 30 nL L^{-1} to minimise any ethylene effect on the data collected.

5.3.2. Mathematical formulation

The model will be developed by establishing equations to describe the respective softening phases described in section 5.2 (Figure 5.1). A general softening curve that does not include chilling injury is explained based on the correlation of the breakdown of starch content and breakdown of cell wall structure (Table 5.1). These processes are initially modelled without accounting for the occurrence of chilling injured fruit (eq. 5.1). Hence, initially fruit firmness (F_{soft}, N) was modelled as an addition of three components:

1. Change in firmness due to correlation with starch degradation (F_A)
2. Change in firmness due to breakdown of cell wall structure (F_B)
3. An underlying basal firmness (F_{Fix})

$$F_{soft} = F_A + F_B + F_{Fix} \qquad \text{[eq 5.1]}$$

The underlying basal firmness (F_{Fix}) is set at 1 N, based on the lowest firmness value the penetrometer can detect. Firmness of badly rotten kiwifruit achieved a value of approximately 1 N using the penetrometer and thus it was assumed that given infinite time all fruit will soften to a limit of 1 N. Hertog *et al.* (2004) estimated F_{Fix} for two different harvest seasons. However, this requires *a priori* knowledge of final firmness,

which is restrictive of predictive use of the model. Therefore, F_{Fix} was fixed as 1 N irrespective of the harvest season in order to enable predictive use of the model.

An additional mechanism will later be added to account for chilling injury related softening in the population (Section 5.3.6). This mechanism works by predicting the incidence of chilling injury (*CI*) and the apportioning a low firmness value to that proportion of the fruit in the population. The remainder of the population without chilling injury (1-*CI*) are expected to have the firmness caused by normal softening (F_{soft}). Overall, fruit firmness (F_{pred}) is predicted based on the proposed mechanism in Table 5.1 (eq 5.2).

$$F_{pred} = CI(F_{CI}) + (1 - CI)F_{soft} \qquad \text{[eq 5.2]}$$

An outline of the data flow used is provided in Figure 5.2. Initial dry matter and soluble solids content are used to predict initial starch content. Starch degradation influence on firmness (F_A) is related to initial starch content and temperature. Cell wall breakdown influence on firmness (F_B) is related to initial firmness, starch content and temperature, while chilling injury estimation requires temperature data only. The following sections explain how each of the model components are mathematically described and estimated.

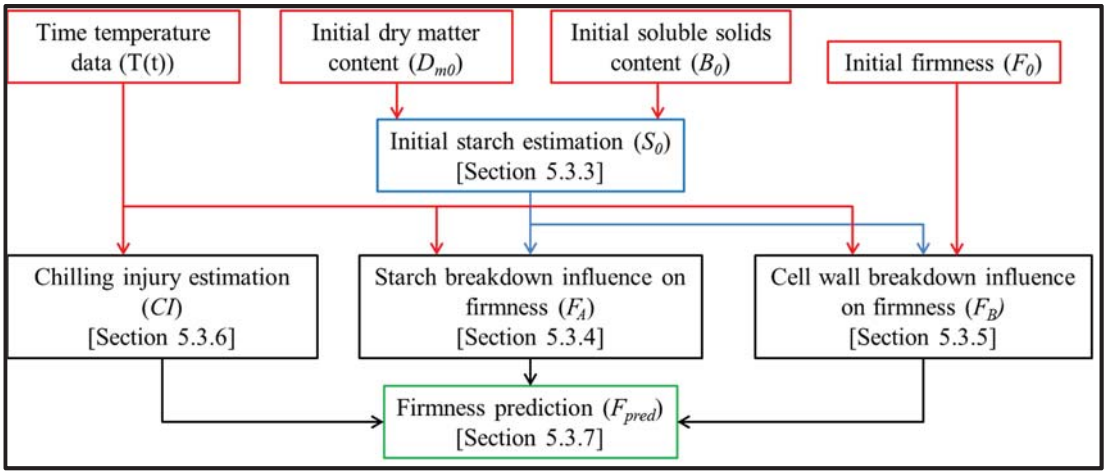


Figure 5.2: The overall conceptual model development process. The units of respective parameters can be found on the nomenclature.

5.3.3. Estimation of initial starch content (S_0)

The initial amount of starch at harvest (S_0, %) in a grower line of fruit can be estimated based on the difference of the initial soluble solid content (B_0, %) and final soluble solid content (B_{final}, %), assuming that all starch present initially in fruit will eventually convert to soluble solid content (eq. 5.3). The final soluble solid content (B_{final}) can be estimated based on an established correlation from the initial dry matter content (D_{m0}, %; eq. 5.4; Burdon *et al.*, 2004). Initial starch content (S_0) of individual fruit can therefore be estimated using the initial dry matter (D_{m0}) and initial soluble solid content of respective fruit.

$$S_0 = B_{final} - B_0 \qquad \text{[eq. 5.3]}$$

$$B_{final} = -3.755 + 1.057 D_{m0} \qquad \text{[eq. 5.4]}$$

5.3.4. Model of starch breakdown effect on firmness (F_A)

Section 5.2.1 discussed the evidence of the correlation between the breakdown of starch and the rapid softening phase. While there is no mechanistic explanation for the breakdown of starch causing the initial rapid softening, many researchers have shown the strong correlation of starch breakdown and rapid softening (Macrae *et al.*, 1989; Bonghi

et al., 1996). Hence in this work the rapid softening phase was modelled by relating the process to the starch breakdown. This assumption is developed based on data such as Figure 5.1, where rapid soluble solid content accumulation and rapid softening coincide in the first 50 days of storage. Starch breaks down to simple sugars causing a dramatic increase in measured soluble solids content (Burdon & Lallu, 2011; Burdon *et al.*, 2013). Therefore, it was assumed that the rate of accumulation of soluble solids (k_b, d^{-1}) is inversely equal to the rate of breakdown of starch during ripening (k_s, d^{-1}).

$$k_s = -k_b \qquad \text{[eq 5.5]}$$

5.3.4.1. Kinetics of accumulation of soluble solids content (k_b)

The accumulation of soluble solids during storage was modelled as a first order reaction from the time of harvest (eq. 5.6).

$$\frac{\mathrm{d}[B]}{\mathrm{dt}} = k_b(B_{final} - B) \qquad \text{[eq. 5.6]}$$

Where $B = B_0$ at t= 0

The conversion of starch to soluble solids during kiwifruit ripening is dependent on temperature (Macrae *et al.*, 1989). Therefore, to account for the temperature dependence, the Arrhenius equation is introduced (eq. 5.7).

$$k_b = k_{b,ref} \mathrm{e}^{\frac{E_{a,b}}{\mathrm{R}}\left(\frac{1}{T_{ref}} - \frac{1}{T_{abs}}\right)} \qquad \text{[eq. 5.7]}$$

The rate constant ($k_{b,ref}$, d^{-1} at 293 K, T_{ref}) and activation energy ($E_{a,b}$, J mol^{-1}) parameters were estimated using the experimental data of the accumulation of soluble solids content (Figure 3.9), the initial soluble solids content (B_0) data from 3 different grower lines, different cooling treatments (direct or gradual cooled) to 0 or 2 °C and storage at 20 °C in the 2012 harvest season. Optipa v6.0 (Hertog *et al.*, 2007c) was used

to estimate the model parameters ($E_{a,b}$, $k_{b,ref}$; Table 5.2). Single step simulation followed by optimisation was used to determine the value of the model parameters. Appendix 2 shows the various fitted curves generated by Optipa v6.0 to estimate the model parameters using experimental data. Figure 5.3A demonstrates the model (k_b & $E_{a,b}$) prediction of the rate of soluble solids accumulation.

Table 5.2: The values of the model parameters with the standard deviation are estimated based on the accumulation of soluble solids, initial soluble solids content, dry mater and the estimated final soluble solids content. The reference temperature was set at 20 °C.

Model parameter	Estimated value	Standard deviation
$k_{b,ref}$ (d^{-1})	0.14	2.92 x 10^{-3}
$E_{a,b}$ (J mol^{-1})	20,105	1,029

Assuming the inverse equality for the rate of starch breakdown to the rate of accumulation of soluble solids content (eq. 5.5), the rate of starch breakdown was modelled as a first order decay influence by temperature (eq. 5.8 and 5.9). Given the inverse equality assumption, the rate constant and activation energy for soluble solids content accumulation (Table 5.2) were also applied to the starch breakdown ($k_{s,ref}$ & $E_{a,s}$).

$$\frac{d[S]}{dt} = k_s[S] \qquad \text{[eq. 5.8]}$$

Where $S = S_0$ at t = 0

$$k_s = k_{s,ref} e^{\frac{E_{a,S}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T_{abs}}\right)} \qquad \text{[eq. 5.9]}$$

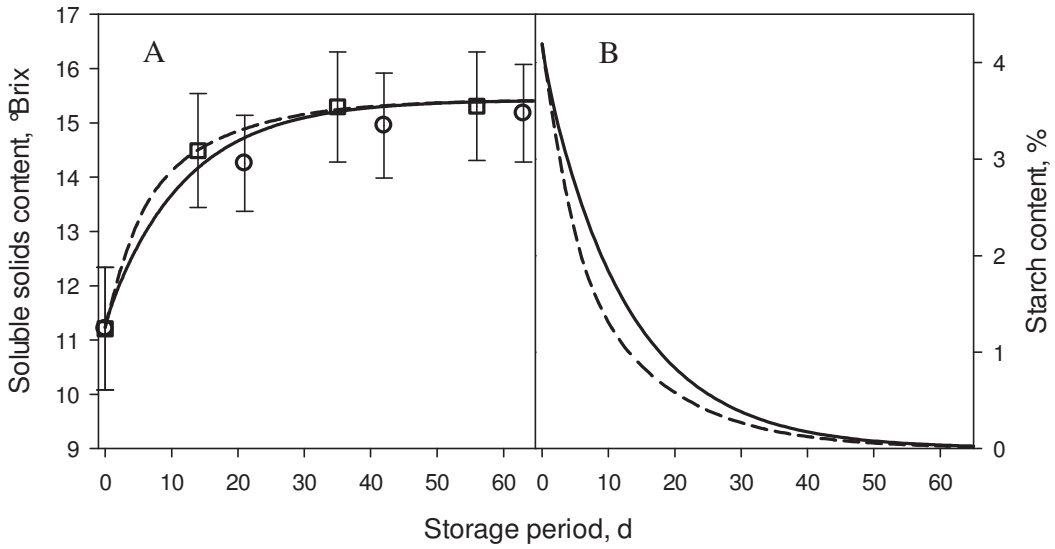


Figure 5.3: (A) Modelled accumulation of soluble solids content for 2012 season, where fruit of different grower lines were either direct (DC, solid line) or gradual (GC, broken line) cooled to 0 °C. Refer to Figure 3.6 for the time temperature information on the cooling profiles of DC or GC to 0 °C. Each data point represents the average of 108 individual fruit soluble solids content collected from 3 different grower lines, containing 36 fruit from each grower line. Error bar represents the standard deviation. (B) The subsequent modelled rate of starch degradation based on direct (DC) or gradual (GC) cooling profiles.

The activation energy of a typical enzymatic reaction ranges between 0 to 33,600 J mol^{-1} (Ricardo & James, 2007). Previously, the rate constant for starch breakdown in potato at 20 °C was found to be 0.02 d^{-1} with an activation energy of 34,200 J mol^{-1} (Nourian *et al.*, 2003). Although the activation energy is lower compared to the breakdown of starch in potatoes, the estimated model parameters (Table 5.2) are not unreasonable to describe the temperature dependence of starch breakdown during kiwifruit ripening. Figure 5.3B demonstrates the predicted starch degradation as influenced by various independent cooling profiles, where rate of starch degradation is affected by cooling rates.

5.3.4.2. Relationship of starch content to firmness (*a*)

Given the ability to model accumulation of soluble solids content and provide a prediction of starch content and the consistent and strong coincidence between fruit

firmness and starch breakdown (Macrae *et al.*, 1989), an empirical constant (a) was developed to relate starch content to firmness (eq. 5.10).

$$F = a[\mathrm{S}] + \mathrm{b} \qquad \text{[eq 5.10]}$$

This parameter (a) was estimated as the slope of a straight line fitted through starch content and fruit firmness data collected in 2012 harvest season (Figure 5.4). Therefore, the constant (a) was established at 7.49 N $\%^{-1}$. The intercept of the fitted line (b = 17.36 N) represents the average firmness at harvest which is a contribution of both the cell wall structure (F_B) and underlying basal firmness (F_{Fix}). Given that F_{Fix} is fixed at 1 N on average, an additional 16.36 N of softening is available to describe the effects of cell wall structure breakdown (Phase B).

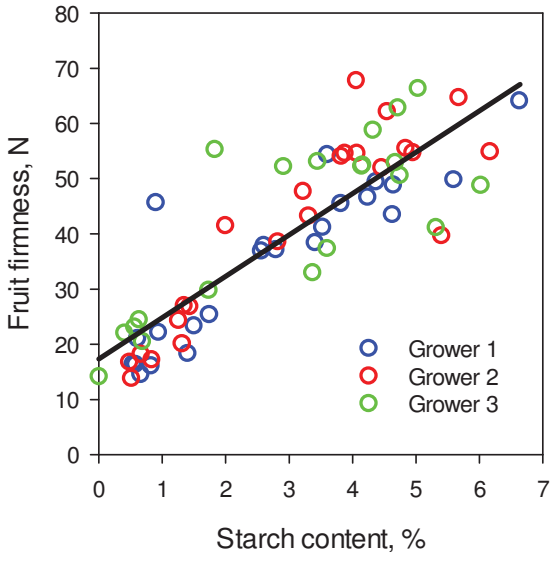


Figure 5.4: Correlation between fruit firmness and starch content in the first 50 days of cool storage. The gradient of the fitted line refers to the model parameter (a). Each point (open circle) represents an individual fruit one of 3 grower lines.

Given the assumption of the inverse equality for the rate of starch breakdown to rate of accumulation of soluble solids (eq. 5.5) and a description of the relationship between fruit firmness and starch content (a = 7.49 N $\%^{-1}$), the firmness change due to starch degradation (F_A) was modelled as a first order decay (eq. 5.11), where the initial firmness as a result of the starch content (F_{A0}) was directly related to the estimated initial starch content (S_0) through the previously estimated average relationship established (eq. 5.12).

$$\frac{\mathrm{d}[F_A]}{\mathrm{dt}} = k_s a[S] \qquad \text{[eq. 5.11]}$$

Where $F_{A0} = a[S_0]$ at t = 0

$$F_{A0} = 7.49[S_0] \qquad \text{[eq. 5.12]}$$

5.3.5. Model of the breakdown of cell wall structure on firmness (F_B)

The breakdown of cell wall structure is a complex process where many different enzymatic reactions take place. During ripening, the loss of cell to cell adhesion and weakening of cell wall strength result in a drop in fruit firmness (Section 5.2.2). Rapid softening coincides well with the breakdown of starch (Section 5.2.1). When starch becomes diminished, the subsequent softening can be attributed to the breakdown of the cell wall structure. Thus, conceptually the breakdown of cell wall structure was largely related to the gradual softening period. The degree of cell wall breakdown during the gradual softening phase is not quantified in this study. Therefore, the model parameters that explain the change in fruit firmness affected by cell wall breakdown (F_B) in the gradual softening period are estimated by fitting a curve to the experiment data collected in 2012 season (Figure 3.8).

5.3.5.1. Estimating the contribution of cell wall to initial firmness (F_{B0})

Assuming that the relationship between starch content and subsequent contribution to firmness (eq. 5.10) holds on every occasion (i.e. a is a constant), the remaining firmness (b) which is attributable to cell wall properties can vary depending on grower line differences at harvest. Assuming the average correlation (eq. 5.10) established between fruit firmness and starch content holds and substituting the average remaining firmness (b) for the model expression of firmness relating to cell wall breakdown (F_B) and the underlying basal firmness (F_{Fix}) will result in eq. 5.13. In effect making this assumption results in applying the same gradient to grower lines of different initial starch and firmness properties (Figure 5.5), resulting in different estimation of the remaining firmness attributable to the cell wall properties at harvest (F_{B0}). Assuming that the relationship holds at harvest and rearranging to make F_B the subject enables estimation of initial fruit firmness contribution of cell wall structure breakdown (F_{B0}) for each grower line (eq. 5.14).

$$F = 7.49[S] + F_B + F_{Fix} \qquad \text{[eq. 5.13]}$$

$$F_{B0} = F_0 - 7.49[S_0] - F_{Fix} \qquad \text{[eq. 5.14]}$$

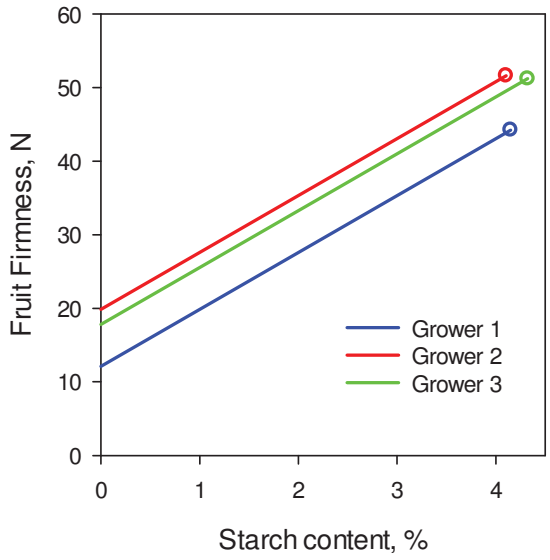


Figure 5.5: Estimation of average initial firmness contribution of cell wall breakdown based on at-harvest attributes. Each data points represents the average initial measurement of fruit firmness (n = 36), soluble solids content (n = 36), and dry matter content (n= 15) from respective grower lines.

As a result, soluble solids content (B_0), dry matter content ($D_{m,0}$) (through the calculation of S_0), at-harvest firmness (F_0), and the assumption of the underlying basal firmness (F_{Fix}) are all used to calculate $F_{B,0}$ in equation 5.13. The inputs used to estimate F_{B0} were at-harvest attributes which are grower line and maturity dependent and thus F_{B0} will be influenced by fruit grower line (Figure 5.5) and maturity. For the data sets used in model creation, since grower 2 has a relatively high initial firmness, but slightly lower initial starch content, a greater magnitude of firmness is associated to cell wall breakdown (F_{B0} = 19.6 N, F_{A0} = 31.0 N). Alternatively, grower 3 has the highest starch content and hence the degree of softening of this grower line is more associated with the rapid softening phase (F_{B0} = 17.9 N, F_{A0} = 32.3 N).

5.3.5.2. Kinetics of the breakdown of cell wall structure (k_w)

There are many enzymes involved in cell wall breakdown working interdependently and thus it is a challenge to identify a key enzyme to explain the breakdown of cell wall structure. For simplification, a general rate constant is used to account for the complex enzymatic reactions (k_w, d^{-1}). The change in firmness explained by the breakdown of cell wall structure (F_B, N) is assumed to follow a first order decay (eq. 5.15). F_{B0} is estimated using equation 5.14 based on at-harvest attributes.

$$\frac{d[F_B]}{dt} = -k_w[F_B] \qquad \text{[eq. 5.15]}$$

Where $F_B = F_{B0}$ at t = 0

The breakdown of cell wall structure is dependent on temperature (Tijskens *et al.*, 1998; Van Dijk *et al.*, 2006b). Therefore, the Arrhenius equation is applied to account for the temperature dependence (eq. 5.16). The model parameters, rate constant at reference temperature ($k_{w,ref}$, d^{-1} at 293 K, T_{ref}) and activation energy ($E_{a,w}$, J mol^{-1}) were estimated based on the experimental data collected in 2012 harvest season (Figure 3.8). Fruit firmness was predicted by the combination of starch degradation and breakdown of cell wall structure, and a minimum measurable firmness of 1 N (eq. 5.1). By providing time temperature information, experimental data of firmness with time (Figure 3.8 to Figure 3.15) and the previously estimated model parameters ($k_{s,ref}$; $E_{a,s}$ and a), Optipa v6.0 (Hertog *et al.*, 2007c) was used to estimate the model parameters ($k_{w,ref}$; $E_{a,w}$, Table 5.3). A single step simulation followed by optimisation was again used to determine the value of the model parameters. Appendix 3 demonstrates the various fitted curves generated by Optipa v6.0 to estimate the model parameters using firmness data collected.

$$k_w = k_{w,ref} e^{\frac{E_{a,w}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T_{abs}}\right)} \qquad \text{[eq. 5.16]}$$

Table 5.3: Values of the model parameters with the standard deviation estimated on softening curves represented 3 grower lines, 16 temperature profiles and known model parameters ($k_{s,ref}$, $E_{a,s}$ and a). The reference temperature was set at 293 K.

Model parameter	Estimated value	Standard deviation
$k_{w,ref}$, d^{-1}	6.70×10^{-2}	1.18×10^{-3}
$E_{a,w}$, J mol^{-1}	106,850	885

Enzyme such as PME, PG and β-GAL are often attributed to the breakdown of cell wall structure. Van Dijk *et al.* (2006) and others have explained the change in activity of PME, PG and β-GAL using kinetic models by estimating the activation energy for respective enzymes. The estimated activation energy describing the temperature dependence of the breakdown of cell wall structure during ripening ($E_{a,w}$ = 106,850 J mol^{-1}) was within the range reported in these previous findings (Table 5.4) and thus is not unreasonable. Schotsmans *et al.* (2008) estimated an average rate constant for 'Hort16A' kiwifruit of 0.20 d^{-1} at 293 K from 3 harvest dates which is greater in comparison to the estimated $k_{w,ref}$ (0.07 d^{-1}). The reference rate constant ($k_{w,ref}$) is used to describe the gradual softening whereas the rate constant estimated by Schotsmans *et al.* (2008) describes the overall softening curve and hence obtaining a value lower than 0.2 d^{-1} is not irrational. Applying the estimated model parameters ($k_{w,ref}$ and $E_{a,w}$), the rate of change in firmness due to cell wall breakdown (F_B) can be modelled accordingly to various exposed temperature (Figure 5.6), where the rate increases with temperature.

Table 5.4: Estimated values of activation energies for different reactions that break down the cell wall structure described in the model.

Type of model	Fruit	Estimated activation energy, E_a	Reference
PME activity	Tomatoes	78,700	Van Dijk *et al.* (2006)
β-GAL activity	Tomatoes	129,000	Van Dijk et al. (2006)
PG activity	Tomatoes	99,800	Van Dijk *et al.* (2006)
	Peaches	113,450	Tijskens *et al.* (1998)
Breakdown of pectin by pectin degrading enzymes	'Braeburn' apples	82,000	Gwanpua *et al.* (2011)
Firmness breakdown	Cox' Orange Pippin' apples	82,699*	Johnston *et al.* (2001)
	Royal Gala' apples	71,766*	Johnston *et al.* (2001)
	Peaches	123,072	Tijskens *et al.* (1998)
	Tomatoes	82,500	Van Dijk *et al.* (2006)

* refers to the value was calculated based on the rate constants at different temperatures and by applying the Arrhenius equation provided.

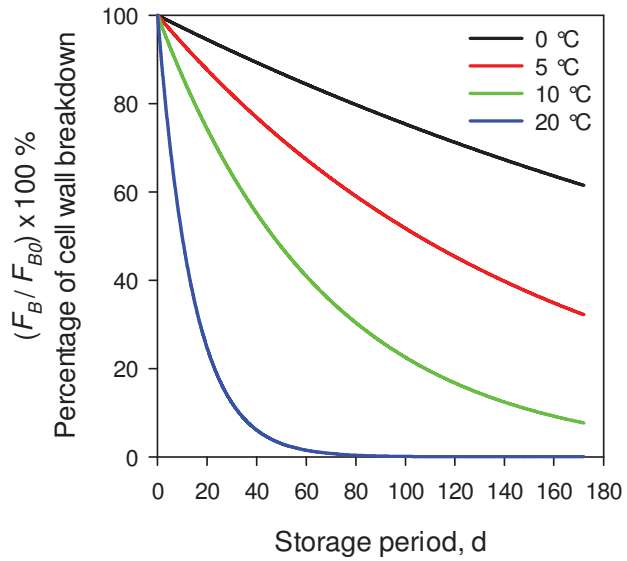


Figure 5.6: The modelled change in firmness due to breakdown of cell wall structure (%) at various temperatures.

5.3.6. Model for the development of chilling in jury effect on firmness

The rate of cooling fruit to storage temperature and the subsequent storage temperature have an effect on the incidence of chilling injury, where faster cooling rate and low storage temperature both promote chilling injury development (Figure 4.5). The complexity of chilling injury development during long term storage (> 100 days) as influenced by the cooling profiles applied at the beginning of storage (< 21 days) makes it difficult to mathematically describe. An empirical model was chosen to predict the incidence of chilling injured fruit, using the data (Figure 4.5) and the associated time temperature information (Figure 4.4). The objective for the 2012 harvest season was to identify the possible temperature scenarios that affect fruit softening and thus the proportion of chilling injured fruit across different temperature scenarios was unfortunately not collected. This lack of data (i.e. incidence of chilling injured fruit) for the 2012 harvest season resulted in this portion of the model being fitted to the 2013 data, where incidence of chilling injury was collected.

Given that cooling rate, storage temperature and storage time all affect the development of chilling injury, calculation of accumulated heat units (*AHU*, °C d) was used to summarise the temperature profiles during the entirety of storage. Accumulated heat units have been previously used to predict the harvest date for cucumber (Perry & Wehner, 1996) and shelf-life of asparagus (King *et al.*, 1988). AHU was determined by estimating the area under the time temperature data (Figure 4.4), greater than the base temperature, T_b (eq. 5.17). A key finding from the experimental work was that rapid cooling in combination with 2 °C storage resulted in low incidence of chilling injury development, yet at 0 °C storage chilling injury incidence was high. In order to differentiate between these coolchain scenarios and assuming that temperatures are never < 0 °C, a base temperature (T_b) of 0 °C was chosen. Fruit that were exposed to rapid

cooling and storage at 0 °C will obtain relatively low AHU values whereas fruit exposed to slow cooling or storage at 2 °C will obtain relatively high AHU values (Figure 5.7).

$$AHU(t) = \sum_{t=0}^{t}(T(t) - \mathrm{T_b})\,\Delta t \qquad \text{[eq. 5.17]}$$

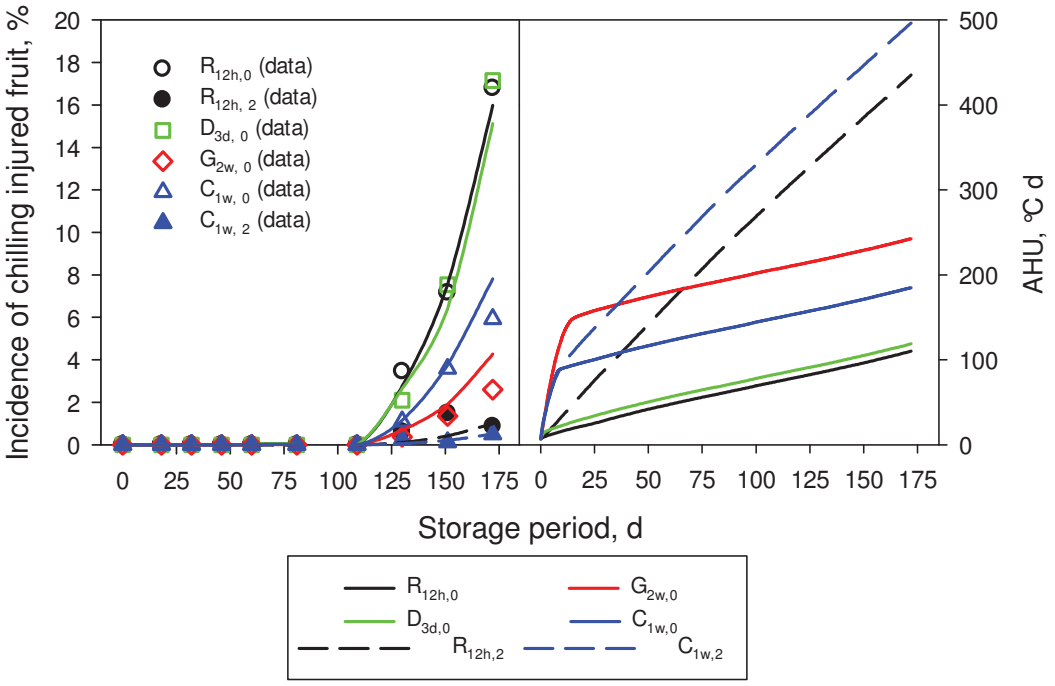


Figure 5.7: (A) Fitted model with the incidence of chilling injured fruit collected in the 2013 harvest season. Each data points represents average incidence of chilling injured fruit from 3 grower lines across different fruit maturity found in Figure 4.5 (n = 270). (B) Average calculated accumulated heat units (AHU, °C d^{-1}) for respective coolchain scenarios (Figure 4.4) during storage for up to 172 days.

The calculation of *AHU* summary of various storage conditions is used as an input to predict the incidence of chilling injured fruit (*CI*) with an empirical logistic model (eq. 5.18). A logistic model is useful for describing a system in which a change occurs between 2 limits (in this case between 0 and 100 % CI incidence). Logistic models have been used previously to predict colour development during ripening of banana (Chen & Ramaswamy, 2002), cherries (Muskovics *et al.*, 2006) and apples (Tijskens *et al.*, 2009). The empirical parameters μ (d^{-1}) and AHU_α (°C d^{-1}) can be estimated by fitting the experimental data collected (Figure 4.5), given that the minimum and maximum proportion of chilling injured fruit is naturally 0 and 100 % respectively.

$$CI = 100 - \frac{100}{1+e^{-\mu(AHU-AHU_{\alpha})}} \qquad \text{[eq. 5.18]}$$

5.3.6.1. Fitted model parameters (μ and AHU_{α})

The unknown model parameters (μ *and* AHU_{α}) were estimated using solver analysis (Microsoft Excel v14.0.6112.5, Microsoft Corporation, Redmond, WA, USA). The parameter, μ was kept constant while AHU_{α} was described as a linear function with storage time (eq. 5.19). Experimental time temperature information (Figure 4.4) and the incidence of chilling injured fruit (Figure 4.5) were used to estimate these parameters. After fitting the model to the experimental data (Figure 5.7A), values of the unknown parameters were found to be μ = 0.0108 d^{-1}, c = 4.44 °C, and d = 817.29 °C d

$$AHU_{\alpha}(t) = c(t) - d \qquad \text{[eq. 5.19]}$$

Figure 5.8 demonstrates the flexibility of the logistic model to describe the proportion of chilling injured fruit in different coolchain scenario (i.e. difference in AHU) during storage. When fruit were cooled rapidly to 0 °C (i.e. AHU values are low), the amount of chilling injured fruit is estimated to be higher, with the proportion of chilling injured fruit increasing with longer storage period.

Incidence of chilling injured fruit was found to be influenced by fruit maturity (Figure 4.5). The limitation of adopting the current logistic model is that it is unable to take fruit maturity into account, and hence could be improved by adding parameters that describe fruit maturity to the logistic model. Although the current logistic model (eq. 5.18) is unable to account for fruit maturity, it is still able to describe the incidence of chilling injured fruit reasonably well (R^2 = 0.735; Figure 5.8), which aids in the final model development to predict fruit softening pattern.

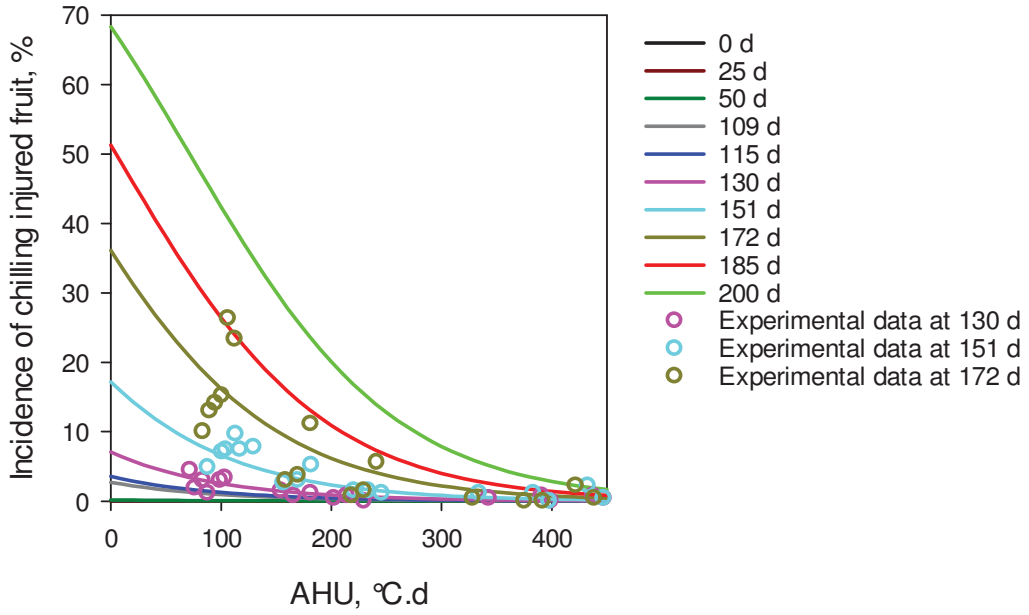


Figure 5.8: Logistic model used to describe the incidence of chilling injured fruit by fitting the curve to the experimental data. AHU is calculated based on the time temperature data collected during storage (Figure 4.4). Each data point (open circle) represents the incidence of chilling injured fruit from different fruit maturity across 6 different cooling profiles (Figure 4.5).

5.3.7. Prediction of kiwifruit firmness (F_{pred})

For modelling, chilling injury is assumed to affect fruit firmness in subsequent storage. The prediction of the incidence of chilling injured fruit during storage is used to assign a proportion of the fruit in the population to a firmness of 1 N (F_{CI}), equation 5.20. The remaining population firmness is predicted by the effects of starch degradation and cell wall breakdown (F_{soft}; eq. 5.1). Using this ratio allocation equation means that initially when chilling injury development is not triggered, firmness prediction is solely driven by the two aforementioned physiological processes (F_{soft}; eq. 5.1).

$$F_{pred} = CI(F_{CI}) + (1 - CI)(F_A + F_B + F_{Fix}) \quad \text{[eq. 5.20]}$$

5.4. Model summary

A fruit firmness prediction model was developed based on the 20 equations described in the above sections (Figure 5.9). After parameter fitting, easy adaptation and usage can be achieved. The model can be simplified substantially as summarised in (Figure 5.10). This simplification has occurred by:

- Substituting eq. 5.4 into eq. 5.3
- Substituting eq. 5.5 into eq. 5.8 and replacing $k_{b,ref}$ = 0.14 d^{-1}, $E_{a,b}$ = 20105 J mol^{-1}, R = 8.314 J $mol^{-1}K^{-1}$ and T_{ref} = 293 K into eq. 5.7
- Substituting eq. 5.5 into eq. 5.11 and replacing a = 7.49 N $\%^{-1}$
- Replacing a = 7.49 N $\%^{-1}$ and F_{Fix} = 1 N into eq. 5.14
- Replacing $k_{w,ref}$ = 6.70 x 10^{-2} d^{-1}, $E_{a,w}$ = 106850 J mol^{-1}, R = 8.314 J $mol^{-1}K^{-1}$ and T_{ref} = 293 K into eq. 5.16
- Substituting T_b = 0 °C in eq. 5.17
- Substituting eq. 5.19 into eq. 5.18 and replacing c = 4.44 °C, d = 817.29 °C d and μ = 1.08 x 10^{-2} d^{-1} into eq. 5.18
- Replacing F_{CI} = 1 N and F_{Fix} = 1 N into eq. 5.20

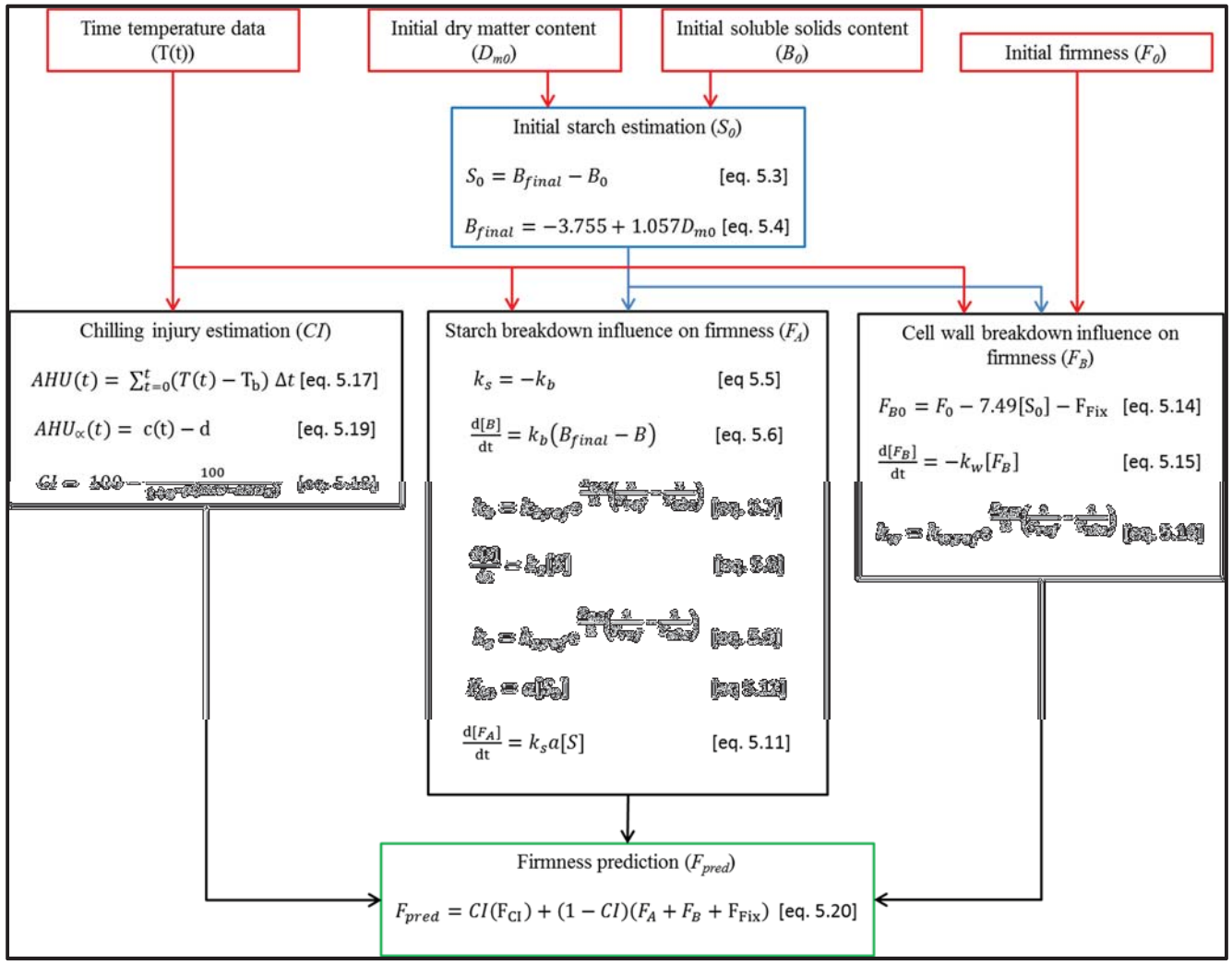


Figure 5.9: Summary of the firmness prediction model developed to describe the softening curve of a particular fruit grower line.

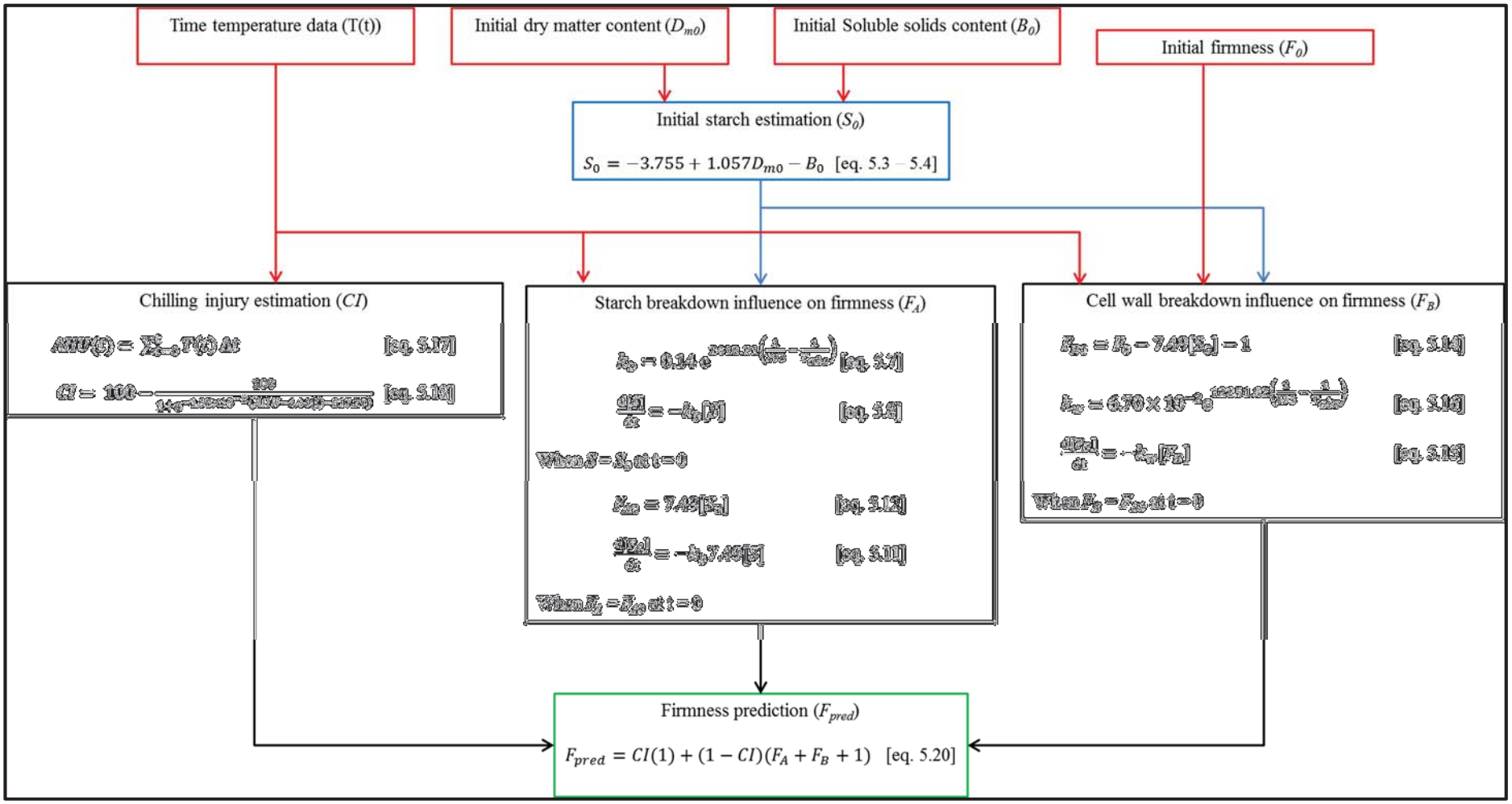


Figure 5.10: Simplified overall equations explaining the variables needed to predict fruit firmness in coolchain.

5.5. Conclusion

The softening model is developed based on 3 processes (i.e. the correlation to starch degradation (F_A), cell wall breakdown influence on firmness (F_B), chilling injury development (F_{CI}) and an underlying basal firmness of 1 N (F_{Fix}).The resulting model consists of 11 equations, for of which are factor of time (eq. 5.11, 5.15, 5.18 and 5.20). Inputs required for the model are initial dry matter content (D_{m0}), soluble solids content (B_0), fruit firmness (F_0) and supply chain temperature conditions (T(t)). In order to develop a predictive model to describe fruit softening, the mechanism behind fruit softening may have been simplified into 3 main processes, neglecting other processes such as ethylene effect on softening and transition of maturation to ripening. The model weakness will be identified in the subsequent chapters.

The following chapter (Chapter 6) will demonstrate how this model performs against the data used to create the model, while the subsequent chapter (Chapter 7) will provide a true validation in which the predictions from this model are compared to data not used in constructing the model.

6. Developed model performance

6.1. Introduction

In the previous chapter, a predictive model was developed to describe kiwifruit softening based on 3 fundamental processes; correlation of starch degradation to fruit firmness (F_A), breakdown of cell wall component influencing firmness (F_B) and development of chilling injury affecting subsequent firmness (F_{CI}). In chapter 3 several factors were observed to affect fruit firmness during coolstorage. Chapter 5 used this data and the chilling injury data from chapter 4 to develop the mathematical model. The subsequent developed model is expected to be flexible and able to predict the softening as influenced by the following conditions:

1. Fruit grower line differences
2. Coolchain conditions including:
    a. Direct or gradual cooling
    b. Different storage temperature (i.e. 0 or 2 °C)
    c. A break in temperature control (i.e. 1 d at 8 °C)
    d. Exposure to high temperatures environment (i.e. 20, 25, 30 or 35 °C)

The aim of this chapter is to evaluate the performance of the developed model to describe the softening curve based on different at-harvest attributes and time temperature information. Results collected in 2012 harvest season (Chapter 3) were used to compare with the predicted softening curves. The model is expected to make reasonable firmness prediction based on the model inputs and coolchain scenarios.

6.2. Materials and methods

This chapter investigates how well the created model described those results used to develop the model, and the sensitivity of the model to data input (D_{m0}, B_0, F_0 and T(t)). The at-harvest attributes from each respective grower lines were used as model inputs (Table 6.1). Time temperature information of respective cooling and storage conditions was used (Figure 3.6 and Figure 3.7). Firmness prediction simulation was performed using Matlab R2011b (MathWorks Inc, Natick, Massachusetts, USA) (Appendix 4). The model parameters (i.e. a, k_{ref} and E_a) were fixed as described in Figure 5.10.

Table 6.1: At-harvest attributes of kiwifruit from respective grower lines from 2012 season.

Grower	Firmness (F_0), N	Soluble solids content (B_0), °Brix	Dry matter content (D_{m0}), %
G1	44.2	11.3	18.2
G2	51.6	11.6	18.4
G3	51.2	10.8	17.9

The model predicts the average firmness of respective grower lines (i.e. G1, G2 and G3) using the average at-harvest attributes (Table 6.1) and supplied coolchain temperature conditions. The average predicted firmness is thereafter compared against the average of the experimental data. The error of fits between the predicted (P) and experimental data (O) were determined using Mean Absolute Error, MAE (eq. 6.1.).

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}|O_i - P_i| \qquad \text{[eq. 6.1]}$$

6.2.1. Grower difference

The difference between growers is expressed by the initial firmness, soluble solid content and dry matter content (Table 6.1). Hence, using these parameters as model inputs helps to differentiate the softening curve of grower lines. Generally, fruit with high initial firmness will have low soluble solids content since the available starch has not broken down. This section investigates how the developed model describes grower line differences as a result of the different initial model inputs.

Since the model inputs for each grower line are different, different softening curves are expected to result. Figure 6.1 shows the softening curves for each grower lines for a range of coolchain scenarios. The initial firmness (F_0) for G1 is lower than G2 and G3, which were relatively similar between each other (Table 6.1). According to the correlation established in Figure 5.4, a lower F_0 will result in lesser magnitude change in firmness associated with cell wall breakdown (F_B) and more associated with rapid softening phase (F_A). Hence, the softening curve of G1 is expected to be faster (Figure 6.1).

The predicted softening curves based on different time temperature information fitted reasonably well with the experimental data, displaying a difference between grower lines. The MAE ranged falls 0.7 to 4.0 N. The difference between grower lines was observed to be larger for model than for data (Figure 6.1E, F, G and H). When fruit were exposed to direct cooling and subsequently stored at 0 °C (Figure 6.1A & E), chilling injury is anticipated resulting in prediction of more rapid softening after 120 d of storage. The chilling injury component integrated into the model has enable predicting the subsequent accelerated softening in the late storage period (Figure 6.1E). The rate of softening predicted well but the spread of grower lines is larger than observed in the experimental data (Figure 6.1E).

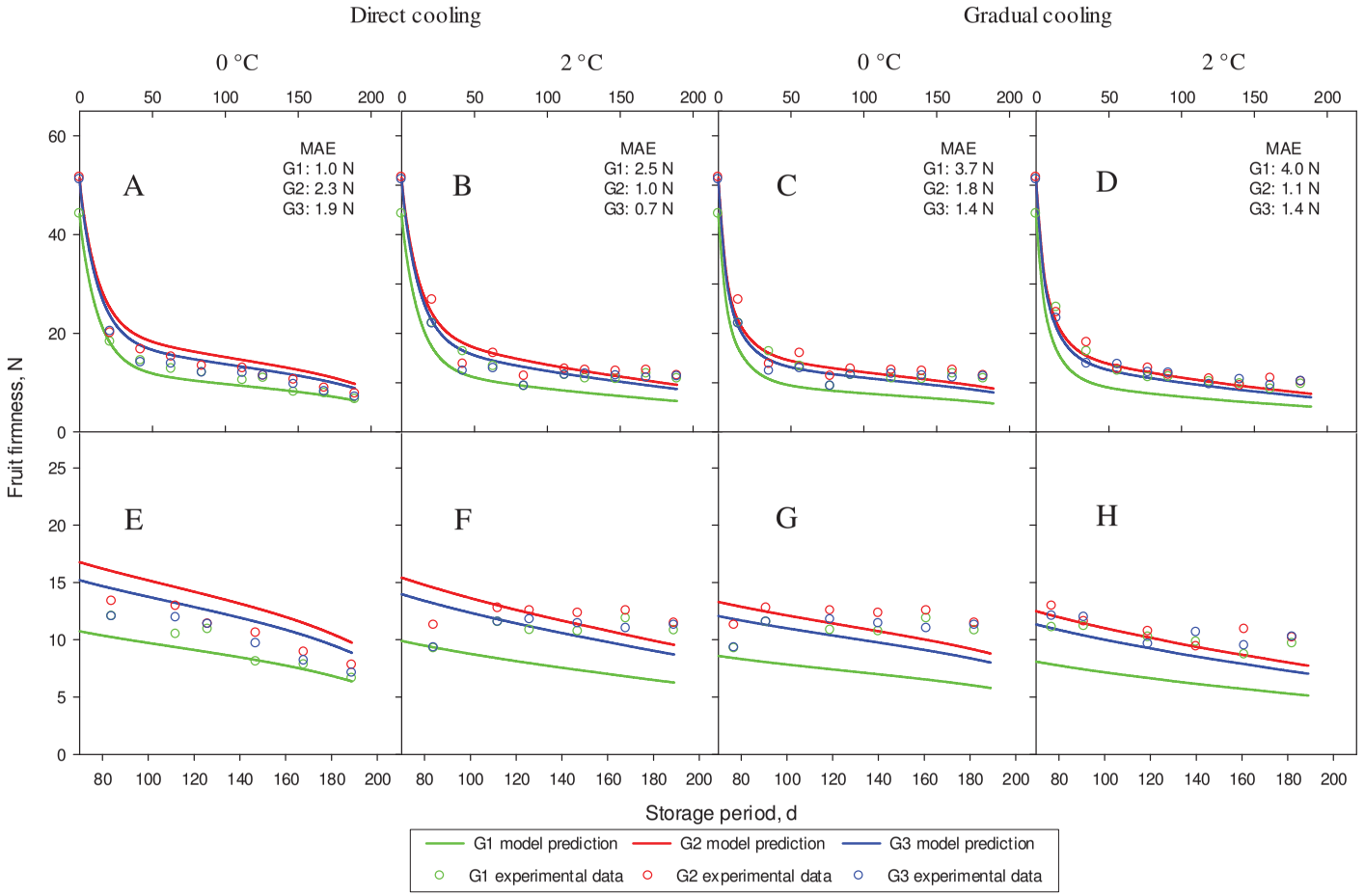


Figure 6.1: The model prediction (solid lines) against the experimental data using the average initial firmness, soluble solids content and dry matter for 3 different grower lines of 36 fruit each. G1, G2 and G3 represent the different grower lines. Each experimental data points refers to the average fruit firmness (n = 108). Figure A to D represent different cooling methods while Figure E to H represent the same data as A to D but focus on firmness less than 20 N.

The average firmness after 120 d of storage for gradual cooled fruit to 0 or 2 °C was underestimated by the model (Figure 6.1G and H). The rate of softening in the late storage period (> 120 d) becomes pseudo-parallel to each other, maintaining consistent gradient but displaying the difference between grower lines (Figure 6.1F, G & H).

6.2.2. Cooling rate effect on fruit firmness

The rate of cooling kiwifruit to storage temperature was shown to influence fruit firmness in subsequent storage (Chapter 3). Initially, the rate of cooling influences softening in that fruit are firmer when cooled faster. Later in storage, faster cooling to storage temperature at 0 °C resulted in faster softening during the gradual softening phase (Figure 3.8). This was suspected to be caused by the development of chilling injury and thus resulting in reduced fruit firmness. The developed model describes the trends in fruit firmness as influenced by the cooling rate to storage temperature at 0 and 2 °C, where fruit were firmer when cooled faster to storage temperature (Figure 6.2) but accelerate softening in the late storage period at 0 °C (i.e. after 120 d of storage). The model displayed consistent trends that were found in the experimental data and literature findings. However, the predicted softening curves were not fitted closely with the experimental data, with a MAE range between 0.8 to 2.1 N (Figure 6.2). The modelled softening curve of gradual cooling was observed to be lower compared to the experimental data. Furthermore, the modelled softening curve of direct cooling did not intersect with the modelled softening curve of gradual cooling, whereas the experimental data showed an intersection after 120 d of storage (Figure 6.2C).

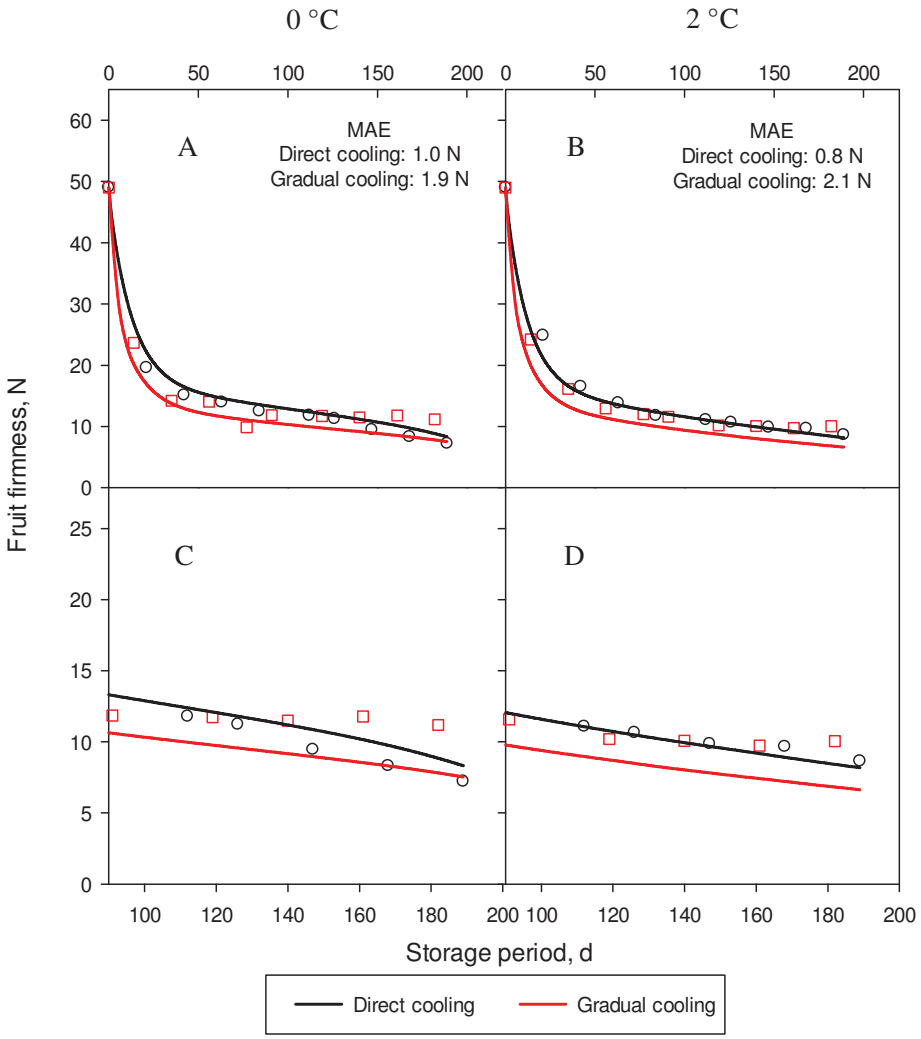


Figure 6.2: Experimental data points and modelled average fruit firmness (solid lines) during storage at 0 (A & C) or 2 °C (B & D) as influenced by rate of cooling. Cooling profiles are provided in Figure 3.6 Each data point represents the average firmness of 3 replicate growers of 36 fruit each (n = 108), refer to Figure 3.8. Rotten fruit were removed from population prior to analysis. Graphs C & D represent the same data as A & B but focus on the late storage period (> 100 d of storage).

6.2.3. Storage temperature effect on fruit firmness

Chapter 3 demonstrated the effect of storage temperature on fruit firmness during coolstorage (Figure 3.12). Exposing fruit to 2 °C resulted in a faster softening. Fruit with a similar cooling profile but stored at 2 °C softened faster than fruit stored at 0 °C in subsequent storage, provided that there was no chilling injury developed during storage (Figure 3.12B). Figure 6.3 shows that the developed model is able to characterise the softening behaviour of fruit stored at 0 or 2 °C, where fruit were predicted to be softer in subsequent storage when stored at 2 °C. The rapid softening phase is influenced by the initial cooling rate and thus fruit that were exposed to similar cooling profiles are expected to have similar softening rate in the rapid softening phase. The model exhibits its capability to predict similar softening rate in the rapid softening phase due to the initial cooling methods and subsequently a lower firmness when fruit were stored at 2 °C (Figure 6.3). This provides evidence that the developed model is able to describe the change in fruit firmness in subsequent storage when fruit were stored at different storage temperatures.

When fruit were cooled directly to 0 °C, accelerated softening was observed after 120 d of storage and thus the firmness of fruit stored at 0 were similar to fruit stored at 2 °C. Figure 6.3A and C show that the modelled average firmness of fruit stored at 0 and 2 °C (direct cooling) were similar at the end of storage period (after 180 d of storage) but the intersection of 0 °C with 2 °C happens 50 days later than observed in the experimental data.

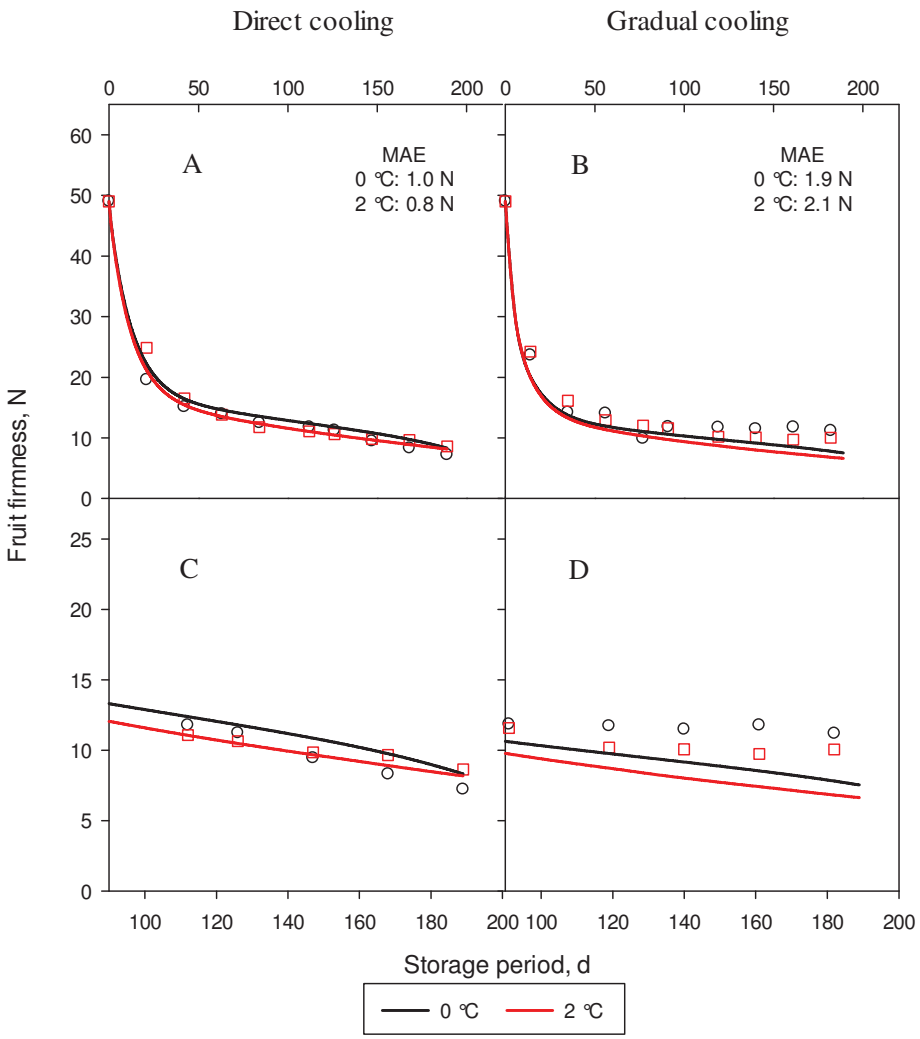


Figure 6.3: Experimental data points and modelled average firmness (solid lines) of fruit that were cooled either directly or gradually to storage temperature as influence by storage temperature. Cooling profiles are provided in Figure 3.6. Each data point represents the average fruit firmness of 3 replicate growers of 36 fruit each (n = 108) as previously presented in Figure 3.12. Rotten fruit were removed from population prior to analysis. Graphs C & D represent the same data as A & B but focus on the late storage period (> 100 d of storage).

6.2.4. Break in temperature control effect on fruit firmness

Chapter 3 showed that a break in temperature control to 8 °C for a day did not have a significant effect on the fruit firmness in subsequent storage (Figure 3.13). Due to the temperature dependence of the model, a decrease in fruit firmness will be expected to be predicted when exposed to a break in temperature control for a day. Figure 6.4 demonstrates that the model predicted a slight drop in fruit firmness after exposing fruit to a break in temperature control of 8 °C for a day. This small drop resulting in a 0.5 to 2 N which corresponds to the experimental finding that a break in temperature control of 8 °C for a day does not influence the fruit firmness significantly in subsequent storage (Figure 3.13). The least square difference between fruit population after 9 weeks and 15 weeks of storage was also between 0.5 N and 2 N (n = 108). Therefore, the model agrees with the experimental data in that a break of temperature control to 8 °C for a day is unlikely to result in a measurable decrease in firmness.

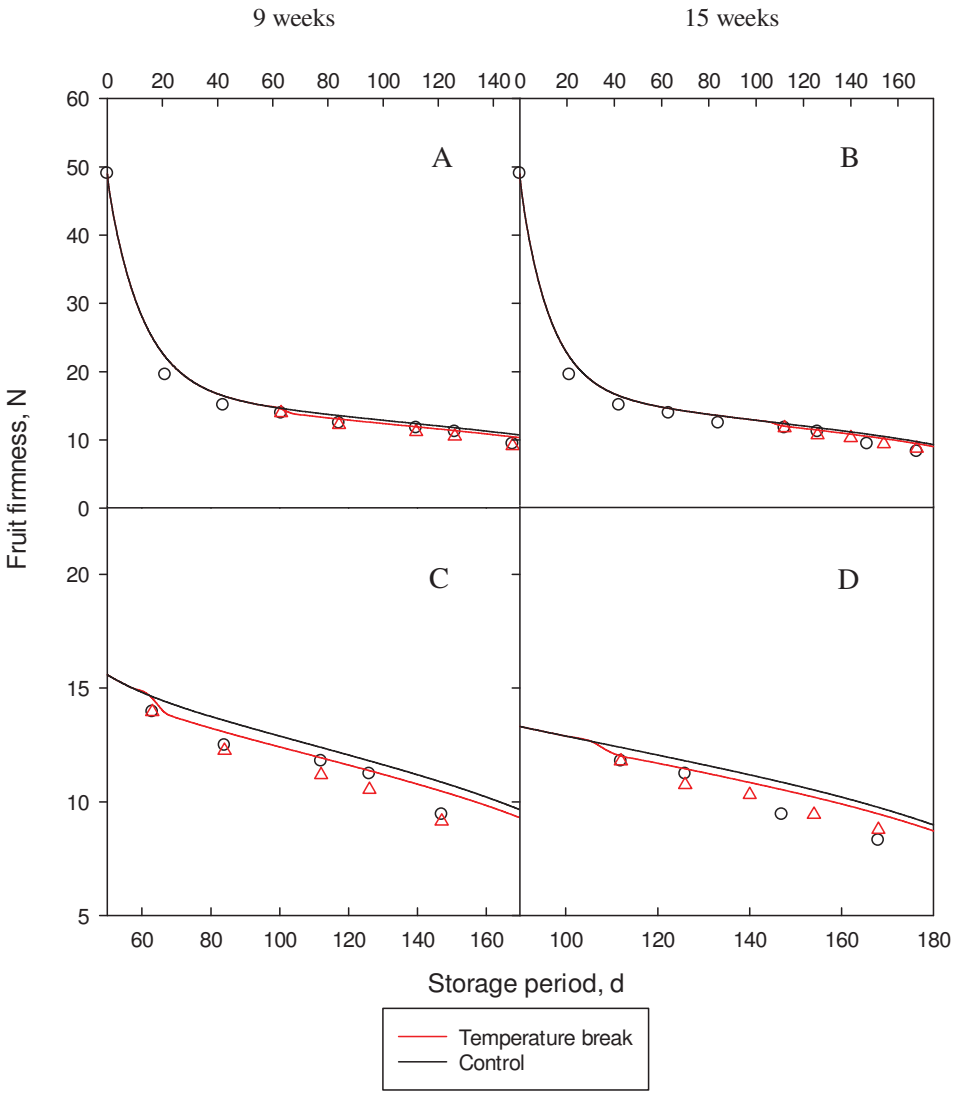


Figure 6.4: Experimental data points and modelled average firmness (solid lines) of fruit exposed to a break in temperature control at 8 °C for 1 d (red solid lines) after 9 or 15 weeks of storage Control (black solid line) refers to fruit that were not exposed to a break in temperature control. Each data points refers to the average fruit firmness of 3 replicate growers of 36 fruit each (n = 108). Data presented is the same as that presented in Figure 3.13. Graphs C & D represent the same data as A & B but focus on the late storage period (> 60 d of storage).

6.2.5. Effect of exposure to high temperature on fruit firmness

The effect of high temperature condition on kiwifruit firmness during storage is well discussed in chapter 3. Fruit softening rate increased when exposed to temperatures ranging from 20 to 30 °C and decreased when exposed to 35 °C (Figure 3.14). Figure 6.5 shows that the rate of softening increases when exposed to high temperature conditions. Due to the temperature dependency of the model, the softening rate was predicted to increase with increasing temperature conditions, from 20 to 35 °C, where modelled firmness fitted well with the experimental data of fruit exposed to 25 and 30 °C (Figure 6.5). However, the experimental data shows a decrease in softening rate at 35 °C. Moreover, when fruit were exposed to 20 °C, a lag phase of approximately 3 days was observed. This suggests that the model lacks the capability to predict the fruit firmness accurately when fruit are exposed to 20 and 35 °C, where the MAE was 1.4 and 2.5 N respectively (Figure 6.5). The model is developed based on temperature dependency using Arrhenius equation but there may be other factors that result in the lag phase at 20 °C and the decline softening rate at 35 °C. One of the possible factors that explains the lag phase at 20 °C after 10 weeks of storage is the influence of internal ethylene concentration on the transition from lag to rapid softening phase (Jeffery & Banks, 1996).

A decrease in softening when continuously exposed to temperature above 30 °C has been demonstrated previously in apple. Johnston *et al.* (2001) used a modified Arrhenius equation which consists of the Arrhenius and Boltzman components to determine the rate constant for firmness change in apples during storage at high temperature conditions. The Arrhenius component helps to describe the increase in rate constant when apples were exposed from 0 to 24 °C while the Boltzman component describes the decrease in rate constant when exposed between 24 and 35 °C. This approach is not adopted during the model development because it adds more unknown

parameters in the model which are difficult to explain and complicates the overall model. The likelihood for kiwifruit to be continuously exposed to above 30 °C for a long period is likely when fruit were to export to countries with high temperature environment in South East Asia and Indian subcontinent.

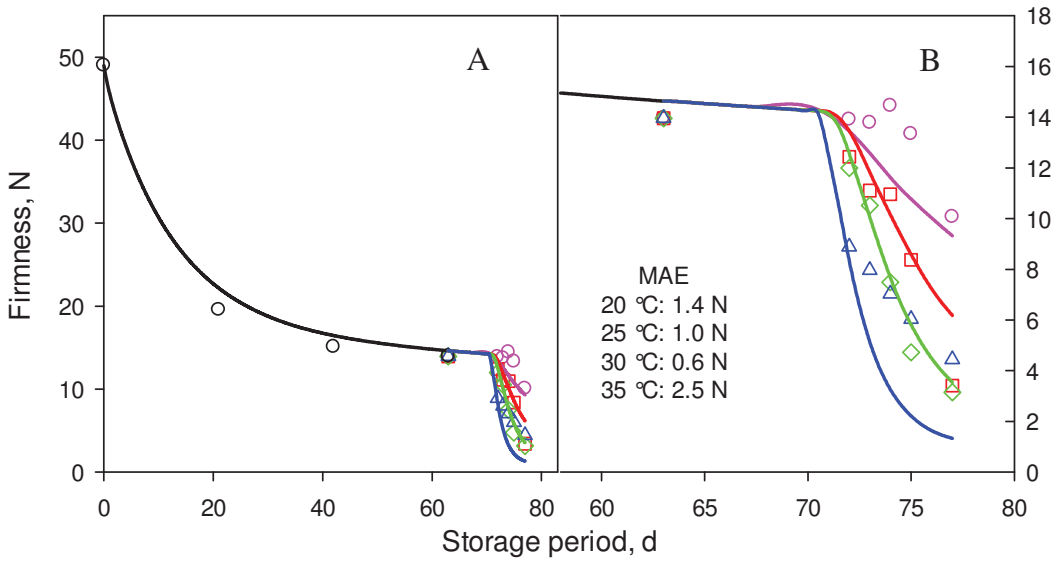


Figure 6.5: Experimental data and modelled average firmness of fruit exposed to high temperature conditions after 10 weeks of storage at 0 °C. Control refers to fruit stored at 0 °C (black) throughout the storage period. Each data point refers to the average fruit firmness of 3 replicate growers of 36 fruit each (n = 108). The data presented are identical to the presented in Figure 3.14. Rotten fruit were removed from population prior to analysis. Graph B represents the same data as A but focus on the late storage period (> 60 d of storage). Fruit were exposed to 20 (pink), 25 (red), 30 (green) and 35 (blue) °C.

6.2.6. Prediction on the proportion of chilling injured fruit

Results from chapter 3 suggested an effect of chilling injury on the fruit firmness. Higher incidence of chilling injury development correlated with softer fruit towards the late storage period (Figure 4.5 & Figure 4.6). Equation 5.1 explained the physiological softening of kiwifruit. However, this does not include more rapid softening caused by the development of chilling injury. An empirical model was used to predict the proportion of chilling injured fruit during storage (eq. 5.17). An assumption was then made that fruit with chilling injury development at late storage period had a firmness value of 10 N (eq.

5.19). Figure 6.6 illustrates the difference in the prediction of fruit firmness during the late storage period between a model with (eq. 5.19) and without (eq. 5.1) this chilling injury component. The addition of the chilling injury model (eq. 5.17) assisted description of the lower fruit firmness during the late storage period.

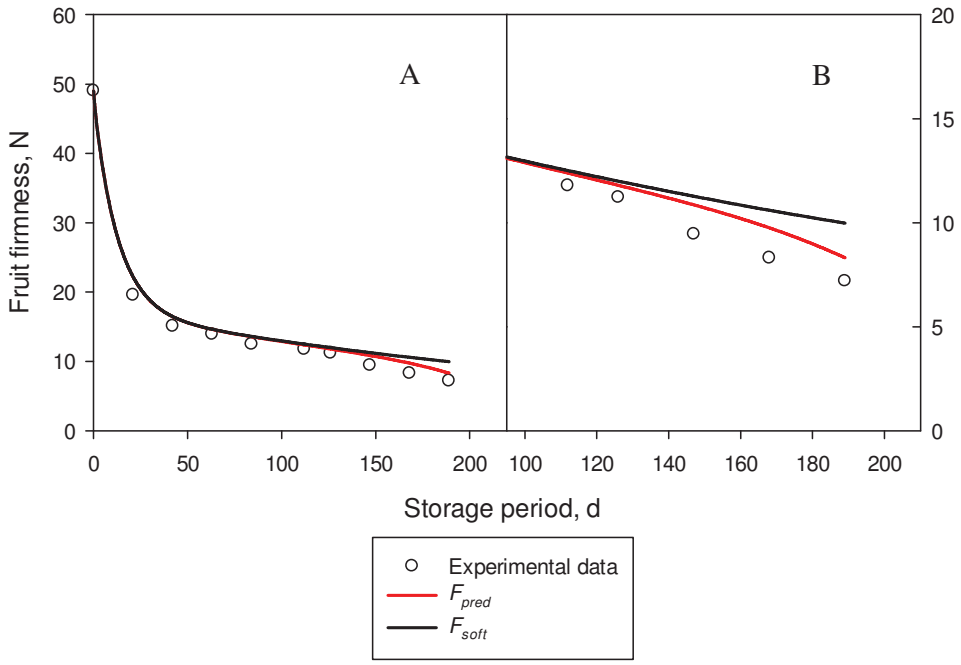


Figure 6.6: Experimental data points and modelled average firmness of fruit with chilling injury development. Fruit were directly cooled to 0 °C. The black solid line represents the model without including the chilling injury component (eq. 5.1) while the red solid line represents the model with chilling injury component (eq. 5.19). Each data point represents an average firmness of 3 replicate growers (n = 108). The data presented are identical to the presented in Figure 3.8. Rotten fruit were removed prior to analysis. Graph B represents the same data as A but focus on the late storage period (> 100 d of storage).

6.3. Model error and sensitivity

The previous sections have demonstrated the flexibility and limitations of the developed model to kiwifruit firmness during softening. The next section will focus on the sensitivity analysis of the model with respect to the model parameters and model inputs.

6.3.1. Model inputs

A sensitivity analysis was conducted to evaluate how responsive the modelled fruit softening curves are to the model inputs (initial firmness, soluble solids or dry matter content). When conducting the sensitivity analysis on the model inputs, the model parameters (k_{ref} and E_a) and time temperature information (T(t)) remained constant. Sensitivity analysis was conducted by using the extreme values of model inputs (D_{m0}, F_0, B_0 or S_0) from the individual from the 3 different grower lines.

Initially correlations between the input data were explored (Figure 6.7). No correlation was found between initial dry matter content and initial firmness or initial soluble solids (Figure 6.7B & C). However, a negative correlation between initial firmness and soluble solids content was found, where a high initial firmness will correspond with a low initial soluble content (Figure 6.7A).

In order to relatively test sensitivity of the model, the input values for the initial firmness and soluble solids content should follow the correlation. The alternative is to have these inputs tested independently. However, this can result in the model making predictions for scenarios that cannot occur in reality (i.e. high soluble solids content, high firmness) and hence over-demonstrate the sensitivity of the model.

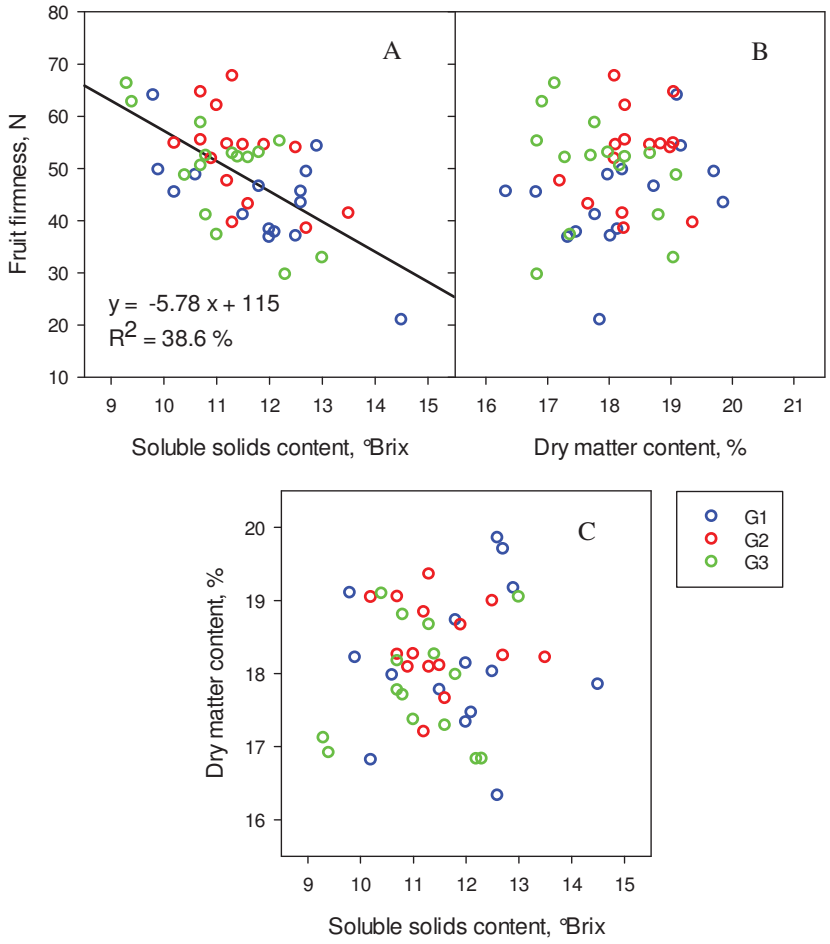


Figure 6.7: Scatter plots of initial fruit firmness (F_0), soluble solids (B_0) and dry matter content (D_{m0}) for individual fruit of the 2012 season represented as 15 fruit from each of 3 grower lines. (A) Correlation between initial fruit firmness and soluble solids content (r = -0.621, p-value < 0.05), (B) and (C) show no correlation between initial fruit firmness and dry matter content (r = 0.105, p-value > 0.05) and initial soluble solids and dry matter content (r = 0.107, p-value > 0.05).

Figure 6.8 shows the influence of initial firmness and soluble solids content on the resulting firmness prediction. Cases when the inputs do not follow the correlation (Figure 6.7), the prediction curve will be illogical, as identified in Figure 6.8A & I. Figure 6.8 shows that the modelled softening curve was found to be similar across different model inputs (F_0 and B_0). However, the change in F_0 or B_0 results in different location for slow softening phase, where higher F_0 or B_0 leads to higher firmness during slow softening. Figure 6.8 also demonstrates that higher B_0 contribute lesser to the fast softening phase to overall softening.

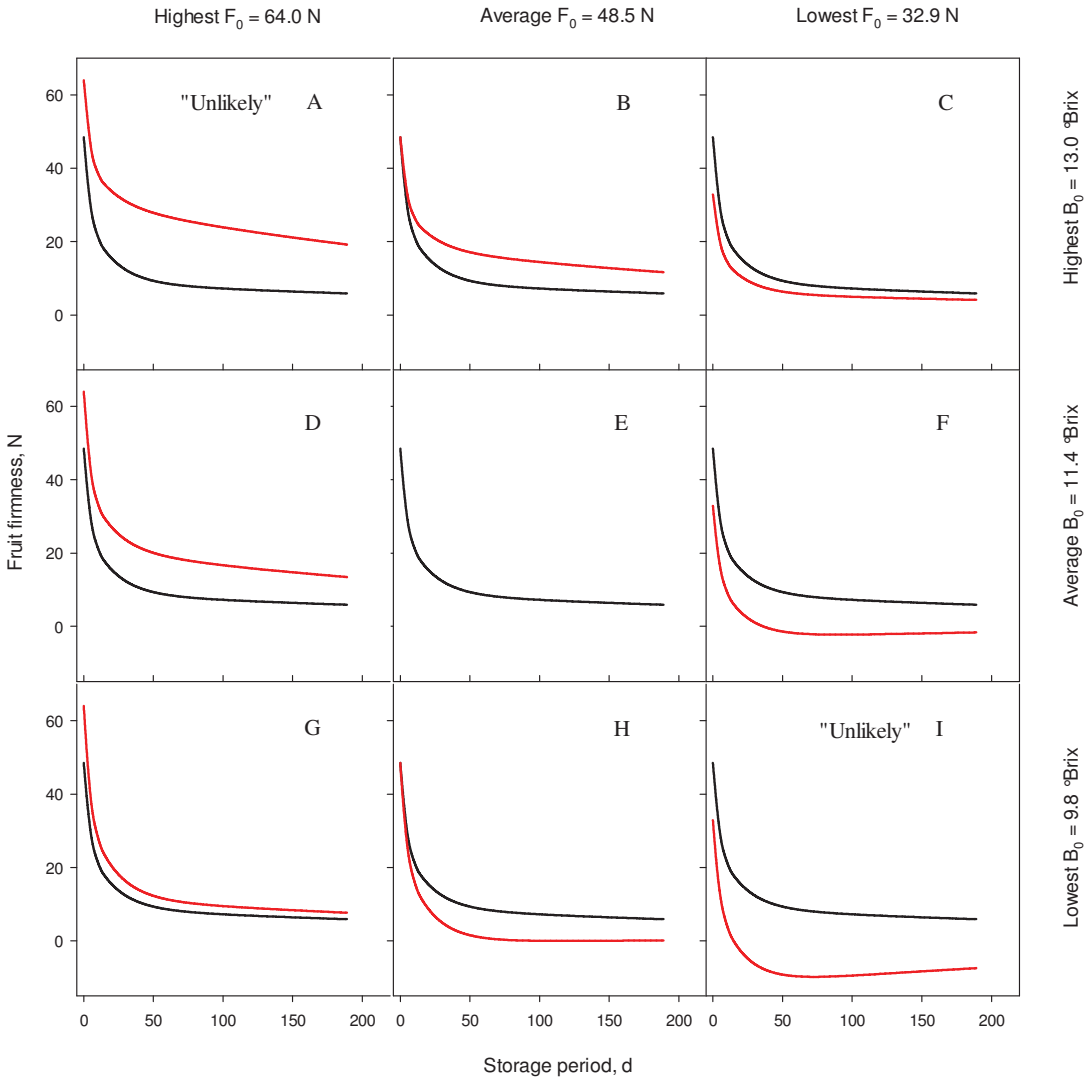


Figure 6.8: Sensitivity analysis of model inputs on predicting the softening curve. The black line represents the model with average model inputs while the red line represents model with different extremes of the model inputs (initial firmness and soluble solids content, SSC). For these curves, global model parameters and time temperature data ($R_{12h,0}$) were kept constant. "Unlikely" refers to the unlikelihood for the softening curve (red solid line) to occur.

Figure 6.7C shows no correlation was found between initial soluble solid content (B_0) and dry matter content (D_{m0}). However, these parameters are used to estimate the fruit starch content, which was found to correlate with fruit firmness (Figure 5.4). Since B_0 and D_{m0} will influence the estimation of initial starch content, it will also affect the softening prediction. Figure 6.9 shows that D_m has a positive correlation with the fruit starch content while the B_0 has a negative correlation with the fruit starch content. These figures indicate that the input values for B_0 and D_m should follow the correlation in order to perform a logical softening prediction.

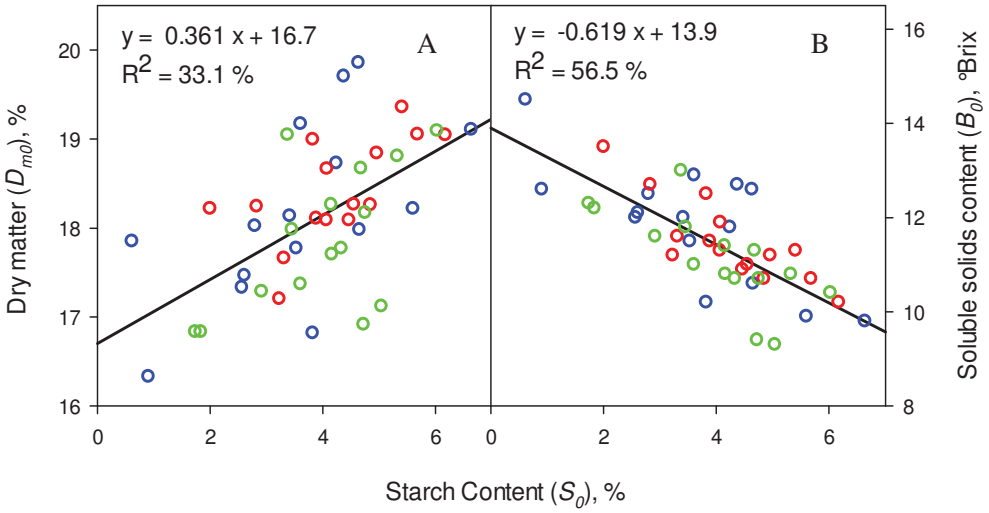


Figure 6.9: Scatter plots of initial starch content (S_0), soluble solids (B_0) and dry matter content (D_{m0}) for individual fruit of the 2012 season represented as 15 fruit from each of 3 grower lines (A) Correlation between dry matter content, D_{m0} ($r = 0.575$, $p < 0.05$) and (B) initial soluble solid content, B_0 ($r = -0.752$, $p < 0.05$) on fruit starch content, S_0.

Dry matter content is used to estimate the final soluble solids content (eq. 5.4) which subsequently determine the amount of starch present in the fruit (eq. 5.3). The first phase of softening is correlated to the degradation of starch content (F_A) and thus upon depletion of starch, the second phase of softening is depending on the rate of the loss of cell wall composition (F_B). Based on equation 5.1, the firmness change is dependent on the starch degradation, loss of cell wall composition and a minimum measured firmness

(F_{Fix}). When global model parameters and F_{Fix} are kept constant, the amount of starch estimated based on the initial model inputs (B_0 and D_{m0}) will determine the amount of F_{B0} which influences the second phase of softening. Figure 6.10 shows that a high estimation of S_0 will predict a lower value of F_{B0} and thus affect the predicted softening curves.

Figure 6.10 shows the possible softening prediction based on different initial model inputs to estimate the starch content (B_0 and D_{m0}). When the estimated S_0 is high and F_{B0} is low (Figure 6.10A, B & C), the softening will be largely dictated by starch degradation, with the firmness plateauing after 50 days of storage to a very low firmness. On the other hand, when S_0 is estimated to be lower than F_{B0}, the model predicts a softening curve that is highly influenced by the rate of the breakdown of cell wall composition (Figure 6.10G, H & I) with little initial rapid softening. Dry matter content is used to estimate the starch content which subsequently affects the magnitude of firmness change due to F_A or F_B. Figure 6.10 demonstrates the influence of initial dry matter content (D_{m0}) in predicting a sensible softening pattern. Figure 6.10 shows that higher initial dry matter content (D_{m0}) had greater magnitude of firmness change in the rapid softening while lower D_{m0} had a greater magnitude of firmness change in the gradual softening phase. Therefore, it is important to collect reliable at-harvest attributes (F_0, B_0 and D_{m0}) in order for the model to make logical softening prediction.

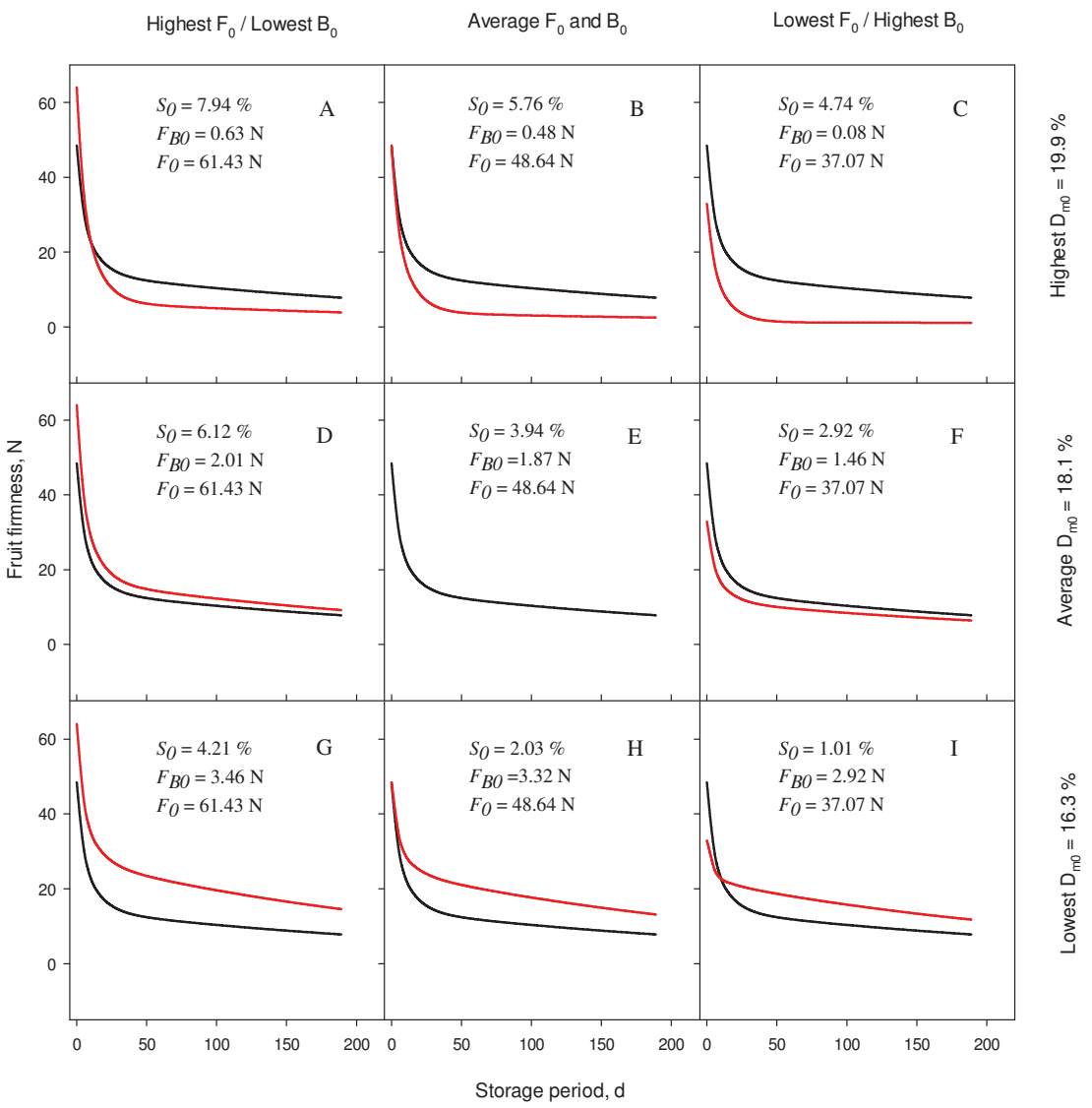


Figure 6.10: Sensitivity analysis of model inputs on predicting the softening curve. The black line represents the model with average model inputs while the red line represents model with different extremes of initial firmness (F_0), soluble solids content (B_0) and dry matter content (D_m). For these curves, model parameters and time temperature data were kept constant (details as for Figure 6.8).

Famiani *et al.* (2012) and Figure 6.9A demonstrated a positive relationship between dry matter content and amount of starch. This may indicate that fruit with high starch content take longer to ripen and thus retain high flesh firmness during storage. The softening prediction coincides with these findings as fruit with high estimated starch content has better storability compared to fruit with low estimated starch (Figure 6.11). The at-harvest firmness and soluble solid content vary over the harvest season, with a distinctive trend of reduction in fruit firmness and increase in soluble solids content across

the season (Mitchell *et al.*, 1992; Jabbar, 2014). Including fruit firmness and soluble solids content as model inputs will aid differentiation of fruit harvest timing.

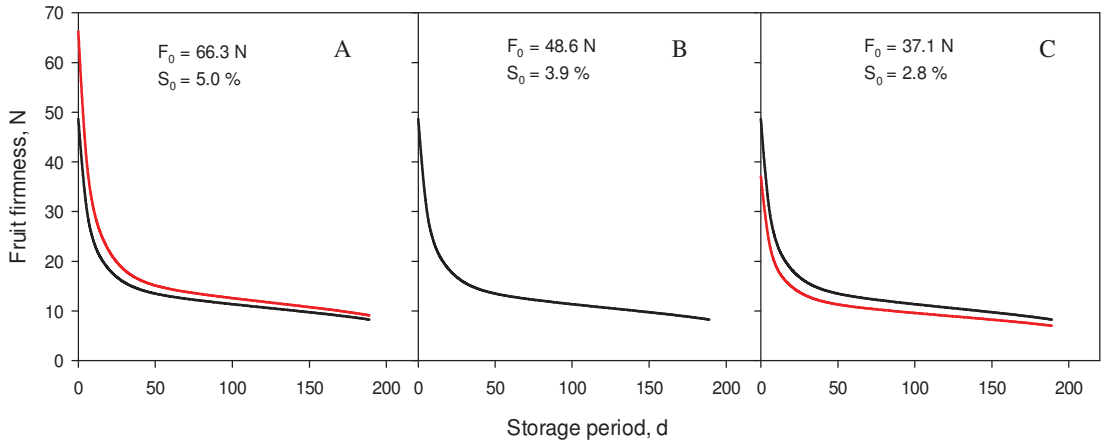


Figure 6.11: Sensitivity analysis of model inputs on predicting the softening curve. The black line represents the model with average model inputs (F_0 = 48.6 N and S_0 = 3.9 %) while the red line represents model with different extremes of initial firmness (F_0) and starch content (S_0) as displayed on respective graphs. The model inputs of the red solid line in graph A were (F_0 = 66.3 N and S_0 = 5.0 %) while in graph C the model inputs were (F_0 = 37.1 N and S_0 = 2.8 %). For these curves, model parameters and time temperature data were kept constant.

6.3.2. Global model parameters

Currently, the model parameter (a) was set at 7.49 N $\%^{-1}$ (Figure 5.4), which was estimated from the fruit firmness and starch correlation. This value (a) dictates the amount of firmness loss as a result of starch degradation. A sensitivity analysis is performed to examine the impact of the model parameter (a) on the overall softening curve, knowing that the value was obtained based on the correlation between fruit firmness and starch content. The initial model inputs (F_0, B_0 and D_m) and time temperature data were remained constant while the model parameter (a) will be varied by more than 20 % of the original value. With an increase of 20 % of the model parameter (a) a lower value of $F_{B,0}$ is predicted, resulting in a lower magnitude of firmness change during the second phase of softening and thus softening mainly being caused by the rate of starch degradation (Figure 6.12A). On the other hand, decreasing 20 or 40 % of the model parameter (a) a higher value of $F_{B,0}$ is predicted, allowing a greater magnitude of firmness change during the

second phase of softening (Figure 6.12B & C). Figure 6.12 demonstrates a 20% increase in *a* will under predict the fruit firmness in the late storage period. In contrary, a decrease of 20 or 40% resulted in an over estimation of fruit firmness in the late storage period. The prediction of fruit firmness during the late storage period is critical as it allows the industry to evaluate the fruit storability.

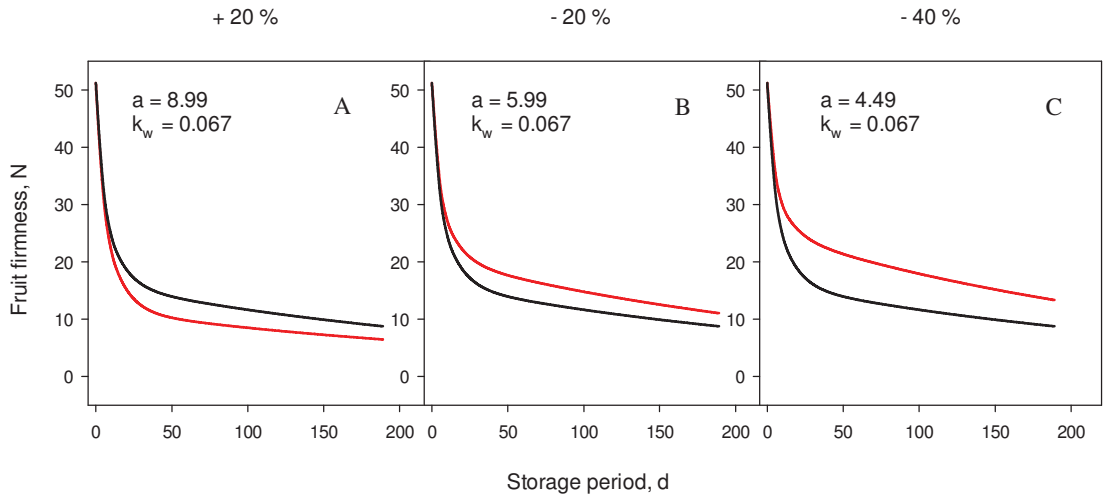


Figure 6.12: Sensitivity analysis of model parameter (*a*) on predicting the softening curve of the same grower line. The black line represents the model with values of an ordinary softening curve (a=7.49, k_w=0.067) while the red line refers to the model with *a* changed by 20% or more. The model inputs and time temperature data were kept constant.

For the first phase of softening which is correlated to starch degradation, experimental data on the accumulation of soluble solids content was collected during storage period and thus able to be used to describe the model parameters (k_s and $E_{a,s}$). However, the global model parameters (k_w and $E_{a,w}$) describing the second phase of softening were obtained by enabling optimisation software to estimate these values based on the a large pool of experimental data collected. Consequently, sensitivity analysis of model parameter, k_w is conducted to investigate its impact on predicting the softening curve. Figure 6.13 demonstrates the influence of k_w on the gradual softening phase, where an increase in the rate will under predict the fruit firmness (Figure 6.13A) while decreasing the rate (20 or 40%) will over predict the fruit firmness (Figure 6.13B & C)

during the late storage period. The prediction of fruit firmness in the late storage period is critical as it influences the industry's decision to retain fruit in coolstore or alternatively export fruit.

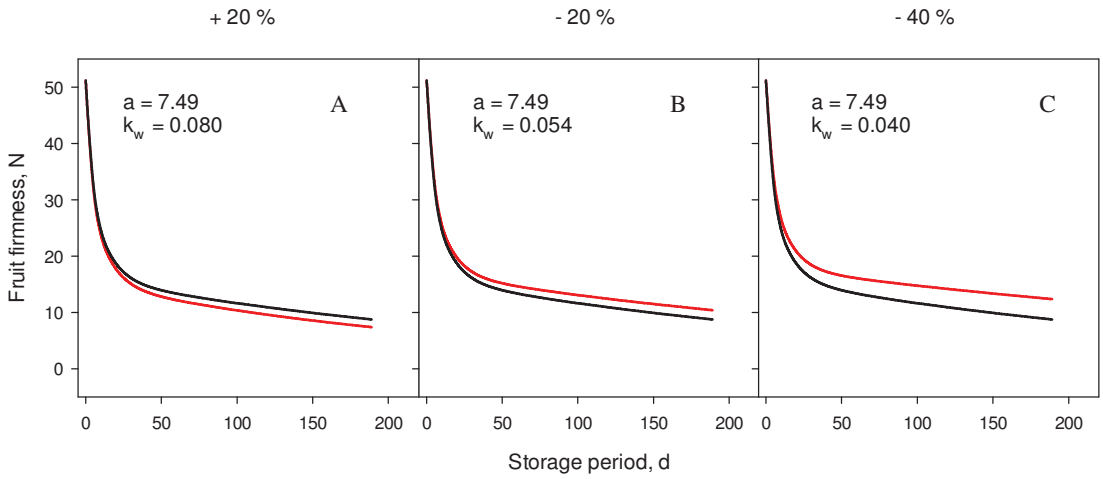


Figure 6.13: Sensitivity analysis of model parameter (k_w) on predicting the softening curve of the same grower line. The black line represents the model with values of an ordinary softening curve (a=7.49, k_w=0.067) while the red line refers to the model with k_w changed by 20% or more. The model inputs and time temperature data were kept constant.

6.4. Overall discussion

The developed mathematical model characterised the softening based on two exponential decay reactions that are occurring during ripening. These reactions are used to explain the two distinct softening phases, rapid and gradual softening. During the rapid softening, different events of enzymatic reactions are happening, including starch breakdown, pectin solubilisation and depolymerisation (Schroder & Atkinson, 2006). These reactions are assumed to correlate well with the rate of starch degradation (F_A) and thus it is explained by the starch component as illustrated in Figure 6.14. Schroder & Atkinson (2006) also reported that loss of cell to cell adhesion and pectin depolymerisation occurred during the second gradual softening. Gradual softening is explained by the cell wall structure component, F_B (Figure 6.14). Since fruit firmness is predicted according to decreasing rate of F_A and F_B, the estimation of S_0 and F_{B0} are

critical. Model inputs such as initial firmness (F_0), soluble solids content (B_0), dry matter content (D_m) are used to estimate the S_0.

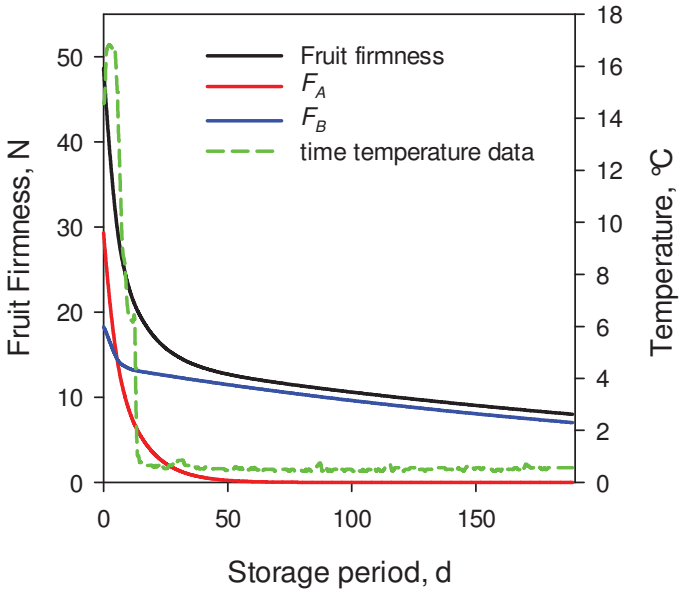


Figure 6.14: The developed model on predicting the softening of 'Hayward' kiwifruit that were gradually cooled and stored at 0 °C. F_A and F_B are parameters found in equation 5.1.

The model is unable to predict the softening curve of fruit exposed to similar time temperature profile but stored in a controlled atmosphere (CA) environment. Studies have demonstrated that storing kiwifruit at CA conditions helps to prolong the storage life and retain flesh firmness (Harman & McDonald, 1989; Oz & Eris, 2010). Furthermore, Harman & McDonald (1989) found that CA storage alters the degradation of pectin in kiwifruit by decreasing the rate of pectin hydrolysis and hence retaining flesh firmness. On the other hand, it was suggested that CA storage decreases the rate of starch degradation and thus maintaining the firmness during storage (Arpaia *et al.*, 1985). Based on these understandings, CA storage affects either cell wall degradation or starch degradation or both, altering the rate constant (k_w and k_s). Hertog *et al.* (2004b) previously developed a model based on the approach of associating rate of gas exchange to the

softening of 'Hayward' kiwifruit. However, this model is unable to predict fruit softening with the possibility of chilling injury development.

Currently, the developed model is used to predict the average firmness across the storage period under different storage and cooling conditions. This was made possible by inputting the average value of F_0, B_0 and D_{m0}. The industry categorises kiwifruit based on the grower line. Therefore, allowing the industry to predict the softening of individual grower lines is more beneficial. The grower line that is predicted to reach 10 N earlier will be exported before the firmness falls below this threshold. Figure 6.1 demonstrated that fruit softening is dependent on grower line where different grower lines exhibit different softening pattern. Jabbar (2014) also demonstrated variation in softening behaviour between different growers. The variation in softening is attributed to the differences in growing location or orchard management practices (Mitchell *et al.*, 1992; Arpaia *et al.*, 1994).

6.5. Conclusion

The softening of kiwifruit in the coolchain is modelled using a mechanistic approach describing the softening based on starch degradation, loss of cell wall structure and chilling injury development. The initial inputs for the model are fruit firmness (F_0),

soluble solids content (B_0) and dry matter content (D_{m0}) which can be easily collected throughout the supply chain. The model demonstrated its robustness in predicting the softening curve when fruit were exposed to fluctuating temperature environment throughout the coolchain. This includes the different cooling methods, storage temperatures, exposure to high temperature conditions and a break in temperature control of 8 °C for a day. Model sensitivity was conducted on the model inputs and global parameters, identifying the model shortcomings in predicting the softening curve. The model demonstrates promising predictions on fruit softening of 3 different grower lines when fruit were stored at different storage conditions, based on the at-harvest attributes as model inputs. The mathematical model is developed based on the experimental result found in chapter 3. A validation with an independent set of experimental result is required and will be discussed in the next chapter.

7. Validation of 'Hayward' kiwifruit softening model in coolstorage

7.1. Introduction

The ripening of kiwifruit follows a softening pattern described by three different phases; an initial lag phase, a rapid softening phase and a final gradual softening phase (White *et al.*, 2005; Schroder & Atkinson, 2006; Jabbar *et al.*, 2014). In season variability resulting from pre and postharvest factors contributes to the variation in fruit storability (Burdon *et al.*, 2013; Jabbar *et al.*, 2014; East *et al.*, 2016). In order to develop an easily applicable mathematical model to predict kiwifruit storability in the coolchain, an assumption was made that softening was described by three processes; softening correlated with starch degradation, breakdown of cell wall structure and development of chilling injury. The rates of softening by the first two mechanisms were assumed to follow first order kinetics (Chapter 5). The mathematical model was developed and fitted adequately, describing the trends in the data (2012 harvest season; Chapter 3) observed experimentally.

This study has identified that cooling rates and storage temperature affect both fruit softening rate and chilling injury development (Chapter 3 and 4). The Arrhenius equation was introduced to describe the temperature dependence on fruit softening rates. Furthermore, accumulated heat units (*AHU*) were used to empirically model the development of chilling injury influenced by the cooling rates and storage temperature (Chapter 5). Kiwifruit are exposed to fluctuating temperatures throughout the coolchain (Bollen *et al.*, 2013). Time temperature information (T(t)) of the coolchain is necessary to predict the fruit softening pattern and the incidence of chilling injured fruit. The difference in softening rate between grower lines was explained by fruit at-harvest attributes (F_0, B_0 and D_{m0}), whereas the fitted model parameters were assumed to be independent to grower line differences (Figure 6.1). The first phase rate constant ($k_{s,ref}$)

and activation energy ($E_{a,s}$) were fitted based on the accumulation of soluble solids content whereas the second phase rate constant ($k_{w,ref}$) and activation energy ($E_{a,w}$) were fitted to the softening data collected in 2012 harvest season.

Independent validation is required to further test whether the developed model is able to predict kiwifruit firmness within the supply chain, given time temperature information (T(t)) and initial fruit quality (firmness (F_0), soluble solids content (B_0) and dry matter content (D_{m0})) data. Fruit at-harvest attributes are collected destructively and thus average inputs are used. As a result, the model predicts mean fruit firmness. In chapter 5, the mathematical model was developed based on data from three kiwifruit grower lines from the 2012 harvest season. The objective of this chapter was to validate the developed model with an independent data set obtained from a different harvest season (2013, chapter 4) in order to further explore the ability of the model to predict the softening of kiwifruit in the coolchain. There is variability in fruit softening with harvest season. The model should be able to account for this variability from the initial fruit quality (F_0, B_0 and D_{m0}). The at-harvest attributes and time temperature information varied for the 2013 harvest season, while the global model parameters remained unchanged (Table 7.1).

7.2. Material and methods

As described in above, the validation data was reported in section 4.3, 9 batches of 'Hayward' kiwifruit representing 3 grower lines harvested over 3 maturities were collected in 2013 and cooled using several methods to reach a storage temperature of 0 or 2 °C (Figure 4.4). Fruit firmness and chilling injury incidence were measured regularly through to 172 days of storage. At-harvest firmness, soluble solids content and dry matter were measured from single trays of 30 fruit randomly selected from each grower and maturity (Table 7.2).

Table 7.1: The model inputs and parameters used to predict fruit firmness in coolchain.

Inputs	Assumption	Parameters
Fruit at-harvest attributes	Initial fruit properties explain difference in grower line and fruit maturity	F_0, B_0, D_{m0}, S_0
Model parameters were estimated from 2012 harvest season data (Chapter 5)	Parameters are independent to grower line or seasonal variability	a, F_{Fix}, $k_{s,ref}$, $E_{a,s}$, $k_{w,ref}$, $E_{a,w}$, AHU_α, μ
Time temperature data	Time temperature data explains the cooling rate and storage temperature across coolchain	T(t)

Table 7.2: Average at-harvest attributes of kiwifruit from 3 growers harvested at 3 different maturity stages (Table 4.1). Each value represents the average firmness, soluble solids content and dry matter content of fruit from a single tray for a respective grower line and fruit maturity. p-value < 0.05 represents a significant difference between factors. Different letters in parentheses are statistically different at p =0.05.

			At-harvest attributes										
Factors			**Dry matter (D_{m0}), %**		**p - value**	**n**	**Soluble solids content (B_0), %**		**p - value**	**n**	**Fruit firmness (F_0), N**		**p - value**	**n**
Fruit maturity x Grower line	Early	G1	18.4	b			6.07	de			76.2	b		
		G2	18.2	b			5.42	e		30	82.8	a		
		G3	17.1	c			5.13	e			78.7	a		
	Mid	G1	19.6	a			7.64	c			75.1	b		
		G2	18.3	b	0.04	20	7.87	c	0.19	20	75.3	b	0.003	30
		G3	17.6	bc			7.08	c			77.1	ab		
	Late	G1	19.8	a			11.8	a			49.5	d		
		G2	18.1	bc			11	ab		20	60.6	c		
		G3	17.3	bc			10.3	b			58	c		

7.2.1. Predictive modelling

Fruit firmness readings collected across the storage period were used to validate with the developed model (Section 4.3). Firmness prediction simulations were performed using the model developed using Matlab R2011b (MathWorks Inc, Natick, Massachusetts, USA). The simulation was setup using model inputs (F_0, B_0 and D_{m0}) that were obtained from each at-harvest measurements (Table 7.2) and model parameters (k_{ref} and E_a) that were discussed in chapter 5. The error in the fits between the predicted softening curve and experimental data collected in 2013 was quantified using the Mean Absolute Error, MAE (eq. 6.1).

7.3. Results and discussion

7.3.1. Fruit maturity difference

The differences in at-harvest attribute across grower lines and maturity assists validation. The resulting data set means that the developed model was tested across a wide range of initial conditions throughout the storage period. Fruit of different maturity can be differentiated by at-harvest soluble solids content. Early harvested fruit tend to have lower soluble solids content compared to fruit that are harvested later. When kiwifruit transits from maturing to ripening, a physiological shift from starch accumulation to breakdown occurs and hence an accumulation of soluble solids content occurs (Burdon & Lallu, 2011). The transition from accumulation to breakdown of starch is also influenced by ambient temperature, usually at low temperature of below 7 or 10 °C (Snelgar *et al.*, 1993; Burdon *et al.*, 2007). Starch content was found to decrease as fruit mature. In chapter 5 a relationship was established between fruit firmness and starch content during storage period using the data collected in 2012 (Figure 5.4 and Figure 7.1). This was used to determine the model parameter a, which affect the estimation of F_{B0}.

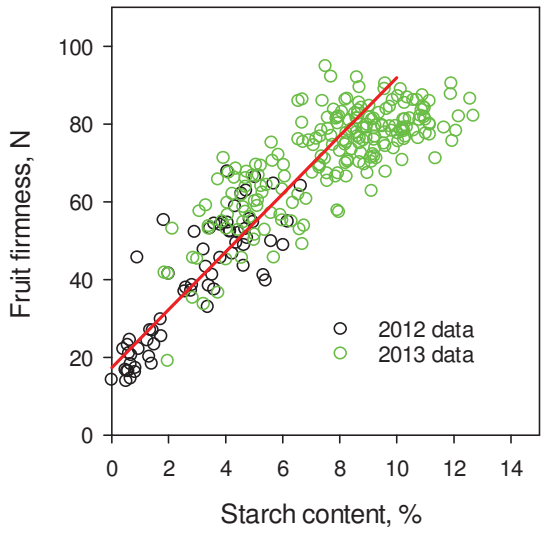


Figure 7.1: Correlation between fruit firmness and starch content during storage period. The red line refers to the fitted line of F = 7.49[S] + 17.36 using the 2012 data. Black circles represent the data collected in 2012 whereas the green circles represent the data collected in 2013.

The firmness and starch content correlation found in chapter 5 shows a positive linear relationship between fruit firmness and starch content, where firmness increases with starch content. Fruit firmness can be estimated by extrapolating the fitted line with a given starch content, assuming the correlation is still valid with the different seasons fruit. The fruit firmness and starch content of fruit obtained in 2013 were mostly higher compared to fruit collected in 2012 (Figure 7.1). The data collected in 2013 were plotted against the established correlation based on data obtained in 2012 to analyse if the correlation is still valid when the starch content and firmness fall at the higher end of the correlation. Figure 7.1 shows that the increasing linear relationship between fruit firmness and starch content is not valid for firmness above 80 N and starch contents above 8 %. This suggests that the fruit may have a maximum firmness and it does not increase with increasing starch content (Figure 7.2). Fruit harvested in the early and mid-season had high initial firmness and starch content. In this scenario, the correlation is found to be untrue therefore limiting the model's ability to predict the softening accurately.

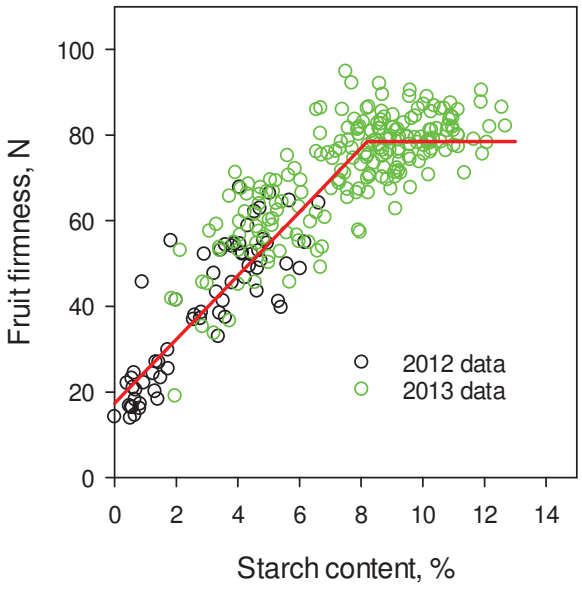


Figure 7.2: The linear correlation of fruit firmness and starch content is invalid when starch content is above 8 %. Black circles represent the individual at-harvest fruit data collected in 2012 and green circles represent individual at-harvest fruit data collected in 2013. The slope of the fitted line below 8 % starch remains the same at 7.49 N $\%^{-1}$.

At-harvest attributes of fruit from different maturity are applied to the developed model to evaluate the model flexibility in predicting the softening pattern of fruit with various maturities when exposed to different storage conditions (Table 7.3). Fruit maturity was found to have a greater effect on the at-harvest attributes in comparison to grower line (Appendix 5) and thus the at-harvest attributes used are an average of the 3 different grower lines (G1, 2 and 3) across early, mid and late maturity (Table 7.3). The benefit of using the average at-harvest attributes of 3 different grower lines as the model inputs instead of averaging the firmness prediction of respective 3 different grower lines is that this allows the industry to predict the average fruit firmness of a single pallet with similar fruit maturity without the uncertainty of grower line variability in the prediction. The use of grower line specific at-harvest properties will be investigated in subsequent analysis.

Figure 7.3 shows that the model is able to predict three distinct softening patterns based on fruit maturity across 3 different storage treatments ($R_{12h,0}$, $R_{12h,2}$ and $G_{2w,0}$). Soluble solids content at harvest is usually used as an indicator to predict the fruit storability and maturity. Fruit with low soluble solids content tend to have poor storability whereas fruit with high soluble solid content at harvest have good storability and provides acceptable flavour when at eating ripe firmness (Snelgar *et al.*, 1993; Burdon & Lallu, 2011). Soluble solids content was found to be related to fruit maturity, where soluble solids content increased across the harvest season (Table 7.3). Similarly, fruit harvested later in the season were found to be firmer at the end of the storage compared to fruit harvested early in the season (Costa *et al.*, 1997). The model predicts late maturity fruit are firmer during storage compared to early maturity fruit, even though the at-harvest firmness is lower than early maturity fruit. The magnitude of firmness change is dependent on the extent of the correlation with starch degradation (F_A) and cell wall breakdown influence on firmness (F_B). Therefore, a greater magnitude of firmness change in fruit with high initial starch content, contributed to the rapid softening phase. Alternatively, a greater magnitude of firmness change in fruit with low initial starch content is attributed to the gradual softening phase. This explains the model prediction on fruit of late maturity to have better storability compared to early maturity fruit (Figure 7.3), which coincides with the findings in literature and chapter 4 (Figure 4.8).

Table 7.3: Average at-harvest attributes of kiwifruit from 3 different maturity stages. Each value represents the average firmness (n = 90), soluble solids content (n = 90 for early maturity fruit and n = 60 for mid and late maturity fruit) and dry matter content (n = 90 for early maturity fruit and n = 60 for mid and late maturity fruit) of fruit from 3 replicated growers (G1, 2 and 3). Different letters in parentheses are statistically different at p =0.05.

	At-harvest attributes								
Fruit maturity	**Dry matter, %**	**p-value**	**n**	**Soluble solids content, °Brix**	**p-value**	**n**	**Fruit firmness, N**	**p-value**	**n**
Early	17.8 b		90	5.5 c		90	79.2 a		
Mid	18.5 a	< 0.05	60	7.5 b	< 0.05	60	75.8 b	< 0.05	90
Late	18.4 a		60	11.0 a		60	56.0 c		

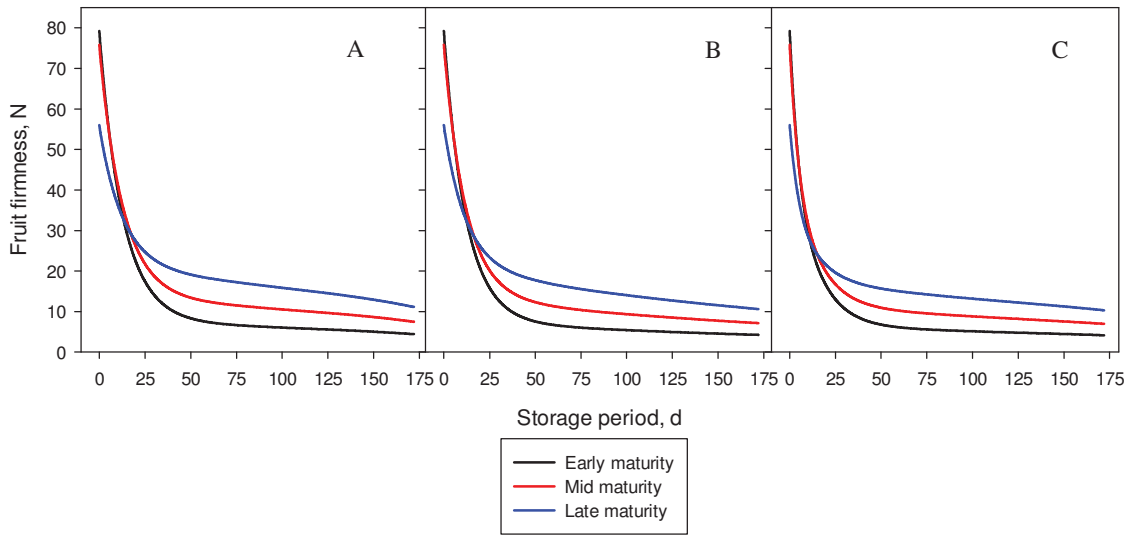


Figure 7.3: The predicted softening curve of fruit using different fruit maturity at-harvest attributes. The simulation was set up using the time temperature information of (A) rapid cooling to 0 °C ($R_{12h,0}$), (B) rapid cooling to 2 °C ($R_{12h,2}$) , and (C) gradual cooling to 0 °C ($G_{2w,0}$).

The model discussed in chapter 5 was used to simulate the softening profiles by applying the at-harvest attributes (Table 7.3) and time temperature information (Figure 4.4) of 3 different storage treatments; rapid cooling to 0 °C ($R_{12h,0}$), rapid cooling to 2 °C ($R_{12h,2}$) or gradual cooling to 0 °C ($G_{2w,0}$). The model follows the assumption established

in chapter 5 on the linear relationship between fruit firmness and starch content (Figure 7.1). The predicted softening pattern of fruit from different maturities and different cooling curves demonstrated some large differences for early and mid-maturity fruit (Figure 7.4). In particular the rapid softening phase was predicted to be much more rapid than the observed results, significantly under predicting the fruit firmness in the first 100 days of storage and subsequently under predicting the gradual softening phase.

The model was developed based on fruit harvested in 2012, where the at-harvest attributes (Table 6.1) were similar to the at-harvest attributes of the late maturity fruit in 2013 (Table 7.2). No significant difference was found between the at-harvest soluble solids content (B_0) of the 2012 season and late harvested fruit from the 2013 season ($p = 0.382$). This may explain why the model can predict the softening curves of late maturity fruit close to the experimental data collected in 2013, with MAE ranging between 1.4 to 3 N (Figure 7.4).

The rates of softening in the rapid softening phase were found to be more distinctive between fruit exposed to various cooling methods for fruit harvested early in the season and became less distinctive when harvested later in the season (Figure 4.6). The difference in softening rate between fruit maturities suggests that fruit maturity influences the rate of softening during the rapid softening phase. A transition from the accumulation of starch to breakdown occurs when matured kiwifruit starts to ripen (Beever & Hopkirk, 1990). Fruit harvested early in the season may not be fully matured and thus affecting the rate of starch breakdown. Since the rate of softening during the rapid softening phase is correlated to the rate of starch breakdown, the transition from accumulation to breakdown of starch will influence the softening rate in the rapid softening phase. Therefore, under prediction of firmness before 100 days of storage

strongly suggests that the rate of softening during the rapid softening phase is influenced by the fruit maturity.

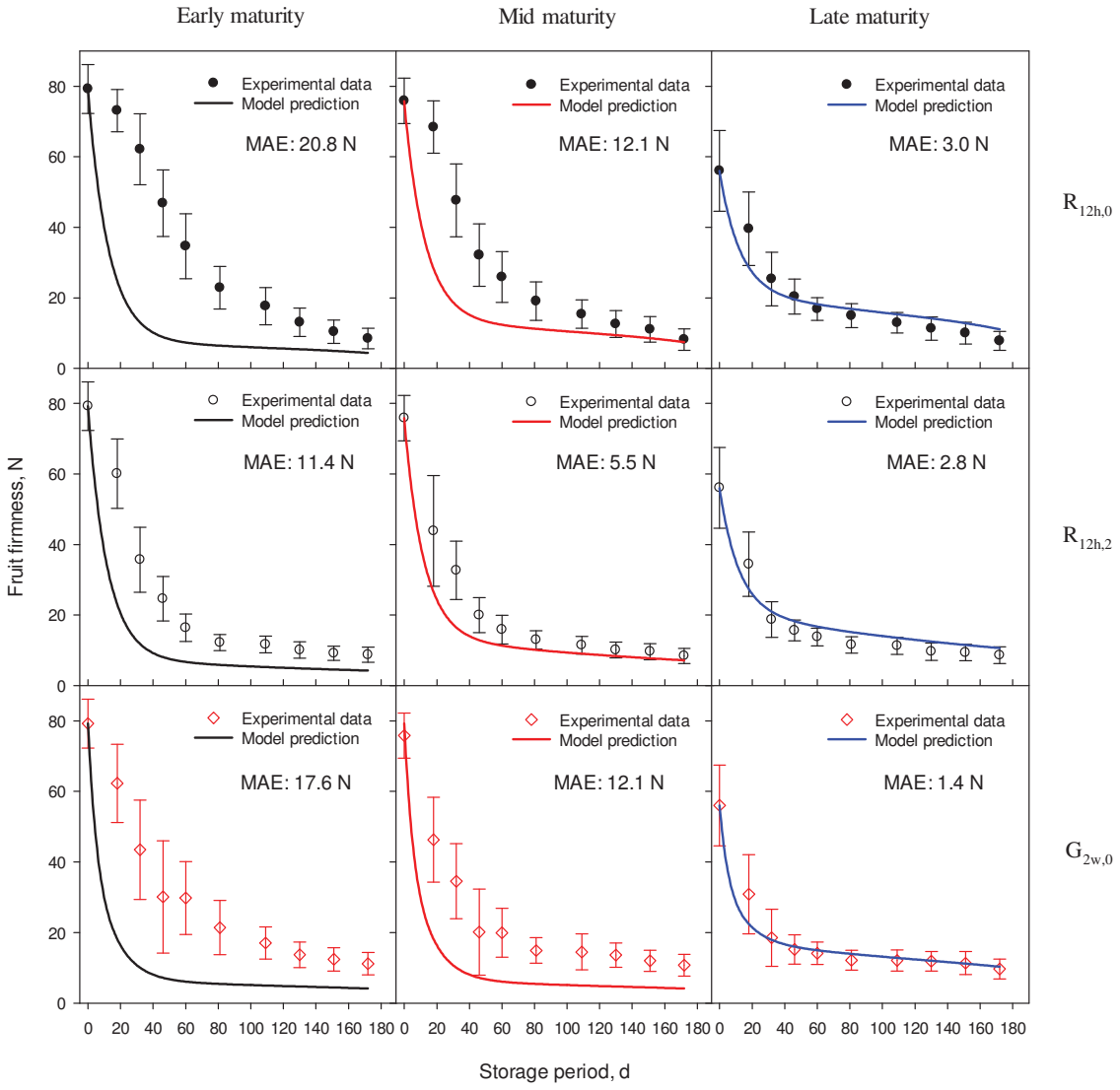


Figure 7.4: Model validation on the experimental data collected in 2013. The fruit were harvested at different periods across the season, exposed to 3 different cooling methods and subsequently stored at 0 or 2 °C. Each data point represents the average fruit firmness (n=30 for first 6 data points and n=90 for the remaining data points). The error bars represent the standard deviation. Rotten fruit were removed from the population prior to analysis. MAE represents mean absolute error.

The model was developed using fruit of late maturity and thus predicting the softening pattern of early and mid-maturity fruit was an extrapolation. When the model was used to make predictions outside of the assumptions and correlations used to formulate it, the probability to make an accurate prediction will be lowered. Variation in

fruit maturity is one of the possible reasons of achieving poorer predictions of the softening curve during the rapid softening phase in early harvest fruit.

Another possible reason for this lack of fit was that the linear correlation between starch and fruit firmness was found to break down when starch content was above 8 %. Therefore, the increasing linear relationship between starch and fruit firmness (Figure 7.1) was modified to be non-existent when starch content is above 8 % (Figure 7.2). Adopting this modification in the correlation will delay softening to occur until the starch content is less than 8 % and thus will predict a lag phase (Figure 7.5). The following chapter provides more discussion regarding the potential modification of this correlation.

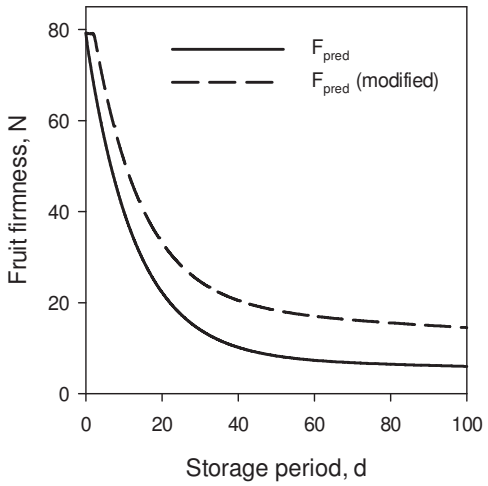


Figure 7.5: Difference between firmness prediction (F_{pred}) when adopting the modified correlation between starch and fruit firmness. The dashed line refers to the firmness prediction using the modified correlation. The solid line refers to the firmness prediction using the increasing linear relationship between starch and fruit firmness. The simulation was set up using the time temperature information of rapid cooling to 0 °C ($R_{12h,0}$).

7.3.2. Fruit grower difference

The industry harvests fruit from different growers for export to different countries. Differences in at-harvest properties of fruit from different grower lines have been found to have an effect on the prediction of softening pattern (Jabbar, 2014). Therefore, it would be useful if the model could predict fruit softening based on grower difference by using

the at-harvest attributes. The rapid softening phase was assumed to be correlated to starch degradation with subsequent phases caused by breakdown of cell wall component and chilling injury development during postharvest storage. However, other pre-harvest conditions have been found to affect kiwifruit softening, such as calcium content (Hopkirk *et al.*, 1990a; Antunes *et al.*, 2005; Gerasopoulos & Drogoudi, 2005), exposure to cold weather (Snelgar *et al.*, 2005), girdling (Seager *et al.*, 1995; Boyd & Barnett, 2011), fruit crop load (Famiani *et al.*, 2012) and exposure to sunlight (Tombesi *et al.*, 1993; Tavarini *et al.*, 2009). Fruit harvested from different grower lines are exposed to different pre-harvest conditions and thus affect the at harvest measurements such as soluble solids content, initial fruit firmness and dry matter content. Consequently, at-harvest attributes of each grower line (Table 7.2) were used as model inputs to predict the softening pattern.

Chapter 6 demonstrated the model capability to predict the softening pattern of fruit harvested from different growers (Figure 6.1). Three different sets of at-harvest measurement were collected from 3 independent growers (G1, 2 and 3) of late maturity (M3) fruit and were used as model inputs to predict the softening pattern (Table 7.2). Based on the at-harvest attributes of the 3 different grower lines of late maturity fruit, the model is expected to predict a faster softening rate for G1 due to a greater magnitude of firmness change associated with the rapid softening phase (i.e. higher starch content) and the softening pattern of G1 to be different compared to G2 and G3 as the initial firmness of G1 was significantly different ($p < 0.05$) from fruit from growers G2 and G3, whereas the initial firmness of G2 and G3 were not different. Figure 7.6 demonstrates the model predictions of these different softening curves based on the at-harvest attributes of respective grower lines when exposed to different storage treatments. Based on the at-harvest attributes, the model was able to predict a difference in softening curve between

G1, G2 and G3 whereas the softening curve of G2 and G3 were similar (Figure 7.6). Furthermore, the model predicts a faster softening rate for G1, which is expected.

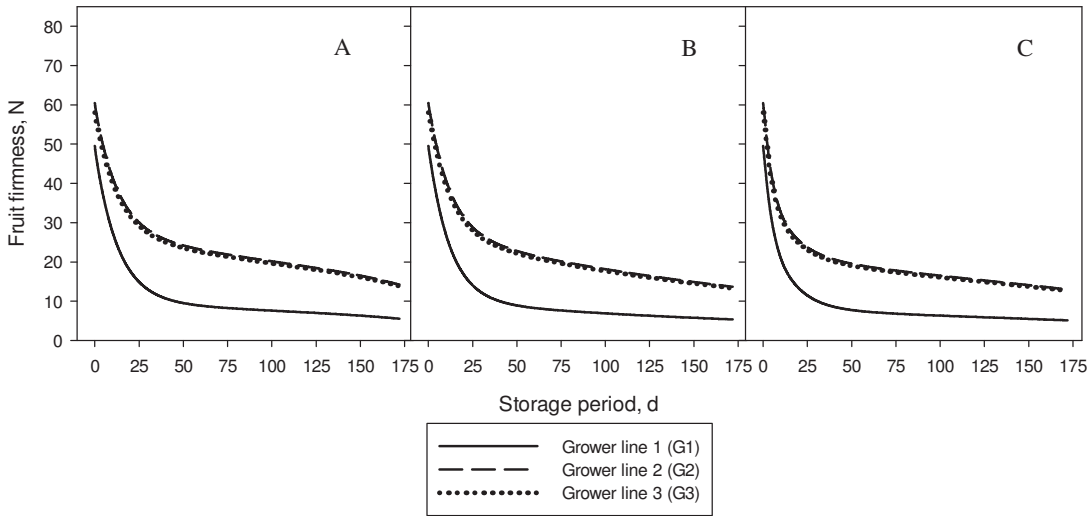


Figure 7.6: The predicted softening curve of early maturity fruit from different grower lines using at-harvest attributes found in Table 7.2. The simulation was set up using the time temperature information of (A) rapid cooling to 0 °C ($R_{12h,0}$), (B) rapid cooling to 2 °C ($R_{12h,2}$) , and (C) gradual cooling to 0 °C ($G_{2w,0}$).

The predicted softening curves were compared with the experimental data to validate the model prediction across various grower lines. The previous section demonstrated the poor softening prediction of early and mid-maturity fruit. Therefore, experimental data collected from fruit of late maturity across 3 different grower lines were used to compare with the model predictions. Figure 7.7 demonstrates the comparison of the model predictions using at-harvest attributes across different grower lines against the experimental data, with MAE ranging between 2 to 8 N. The variance of the predicted softening curve across respective grower lines of late maturity fruit (Figure 7.7) were observed to be greater in comparison to the softening prediction based on the average at-harvest attributes of the different grower lines from late maturity fruit (Figure 7.4). This suggests that the average at-harvest attributes from a large set of data (i.e. all 3 grower lines) improves the softening prediction, where the average at-harvest attributes based on

individual grower lines incurred greater variance in softening prediction. The variance was reduced when averaging the at-harvest attributes of all grower lines to predict the average softening curve of late maturity fruit.

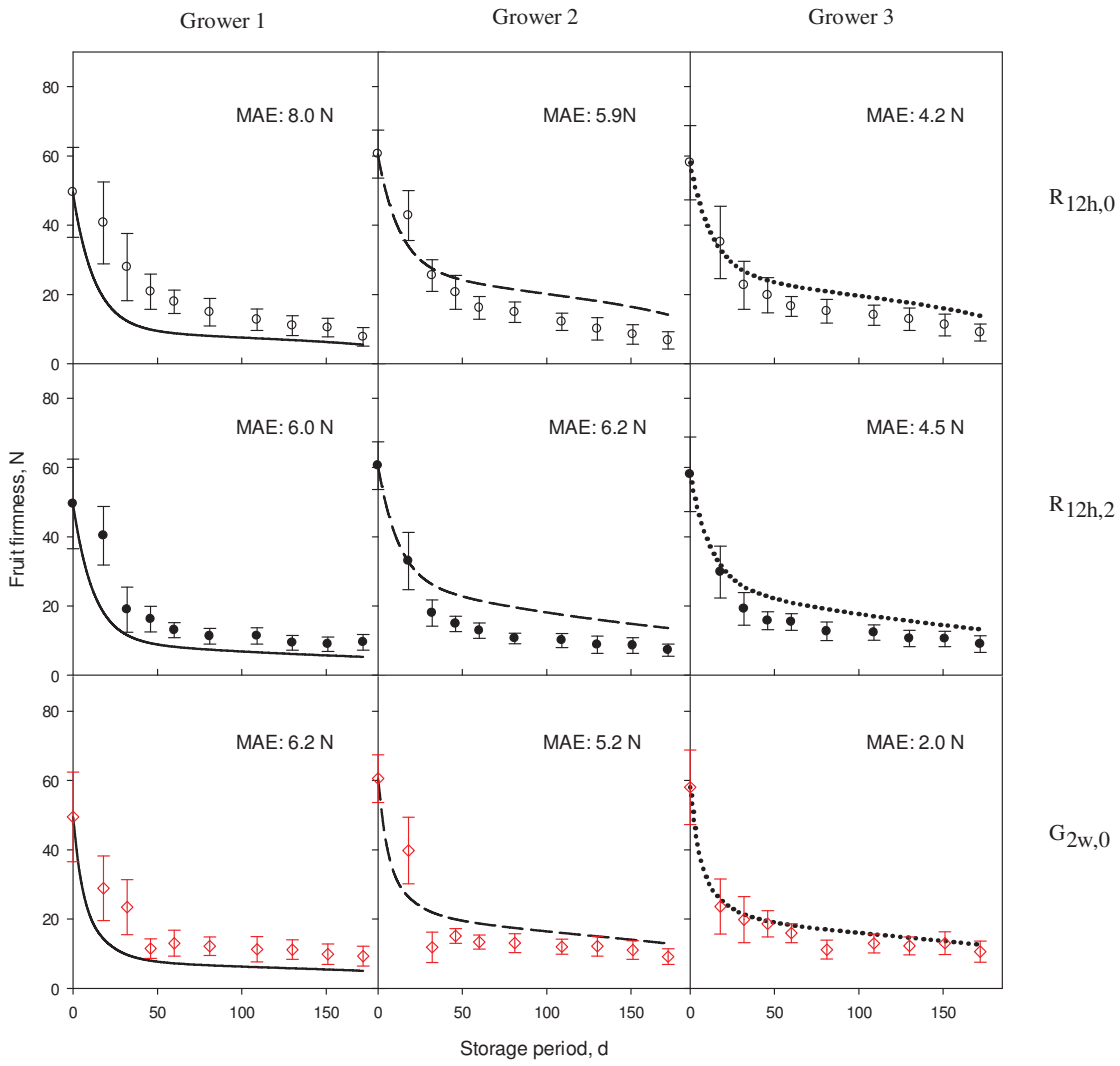


Figure 7.7: Model validation on softening pattern of late maturity fruit from three different grower lines collected in the 2013 season. Solid lines, dashed lines and dotted lines represent the predicted softening curves of different grower lines of late maturity fruit. The simulation was set up using the time temperature information of (A) rapid cooling to 0 °C ($R_{12h,0}$), (B) rapid cooling to 2 °C ($R_{12h,2}$) , and (C) gradual cooling to 0 °C ($G_{2w,0}$). Each data point represents the average fruit firmness of respective grower lines (n=30 for first 6 data points and n=90 for the remaining data points). The error bars represent the standard deviation. Rotten fruit were removed from the population prior to analysis. MAE represents mean absolute error.

Hypothetically, using the average at-harvest attributes of a specific grower line (i.e. Grower 1) should give better predictions of the average softening pattern of that

particular grower line compared to using the average at-harvest attributes from all 3 grower lines. However, this was found to be untrue when a comparison was made between prediction of individual grower lines and average of the 3 grower lines (Figure 7.8), where the prediction based on the average at-harvest attributes across 3 grower lines better described the softening of respective grower lines. The experimental results reflected in Figure 7.8 show that during the late storage period (> 50 d), the difference in firmness across the 3 grower lines is smaller compared to during the early storage period (< 50 d) and thus suggesting less variation in the change of firmness during the gradual softening phase between grower lines.

Changes in cell wall components are assumed to occur (Chapter 5) during the gradual softening phase, which involves several enzymatic activities. Currently, it is difficult to identify the specific enzymatic activity that is responsible for the softening during the gradual softening phase. PG activity was found to be associated with the softening in the later softening phase; its activity detectable when fruit reaches a firmness of below 10 N (Bonghi *et al.*, 1996; Schroder & Atkinson, 2006). Ethylene is found to influence kiwifruit softening. Kiwifruit produces ethylene when the firmness reaches below 15 N (Burdon & Lallu, 2011; Samarakoon, 2013) and accumulation of ethylene within the package is likely to occur when fruit firmness reaches less than 20 N (Chiaramonti & Barboni, 2010; Jabbar & East, 2016). These factors suggest that gradual softening initiates when kiwifruit reaches a certain firmness (F_{B0}). Table 7.4 shows that this can be mathematically implemented by fixing F_{B0} across all grower lines with varying F_{A0} (i.e varying a), instead of previously adopted approach (Chapter 5) by fixing a at 7.49 N $\%^{-1}$ with vary F_{B0}. Applying this concept into the model will allow better prediction of the gradual softening phase, with MAE ranging between 1.1 to 3.8 N (Figure 7.9).

Table 7.4: The different approach to define model parameter *a* and F_{B0}.

Model parameter	Model in Chapter 5	Revision
F_{B0}	$F_0 - aS_0 - F_{fix}$	constant = 16.4 N
a	constant = 7.49 N $\%^{-1}$	$F_0 - (F_{B0} + F_{fix}) / S_0$

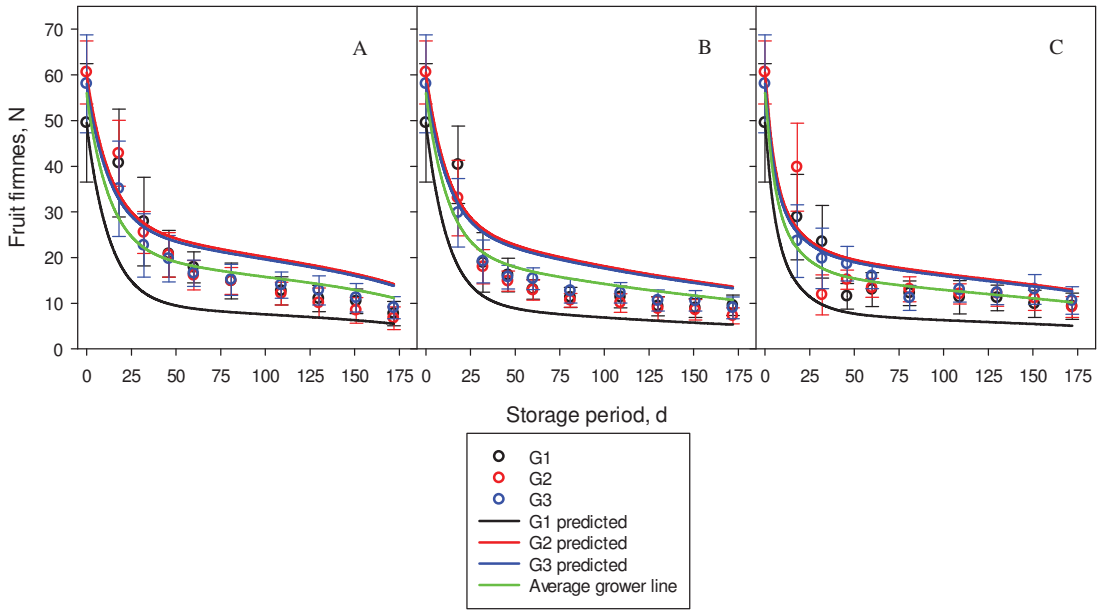


Figure 7.8: Comparison of softening prediction using at-harvest attributes of respective grower lines or an average across 3 grower lines (G1, 2 and 3) with experimental data of late maturity fruit. The simulation was set up using the time temperature information of (A) rapid cooling to 0 °C ($R_{12h,0}$), (B) rapid cooling to 2 °C ($R_{12h,2}$) , and (C) gradual cooling to 0 °C ($G_{2w,0}$). Each data point represents the average fruit firmness of respective grower lines (n=30 for first 6 data points and n=90 for the remaining data points). The error bars represent the standard deviation. Rotten fruit were removed from the population prior to analysis.

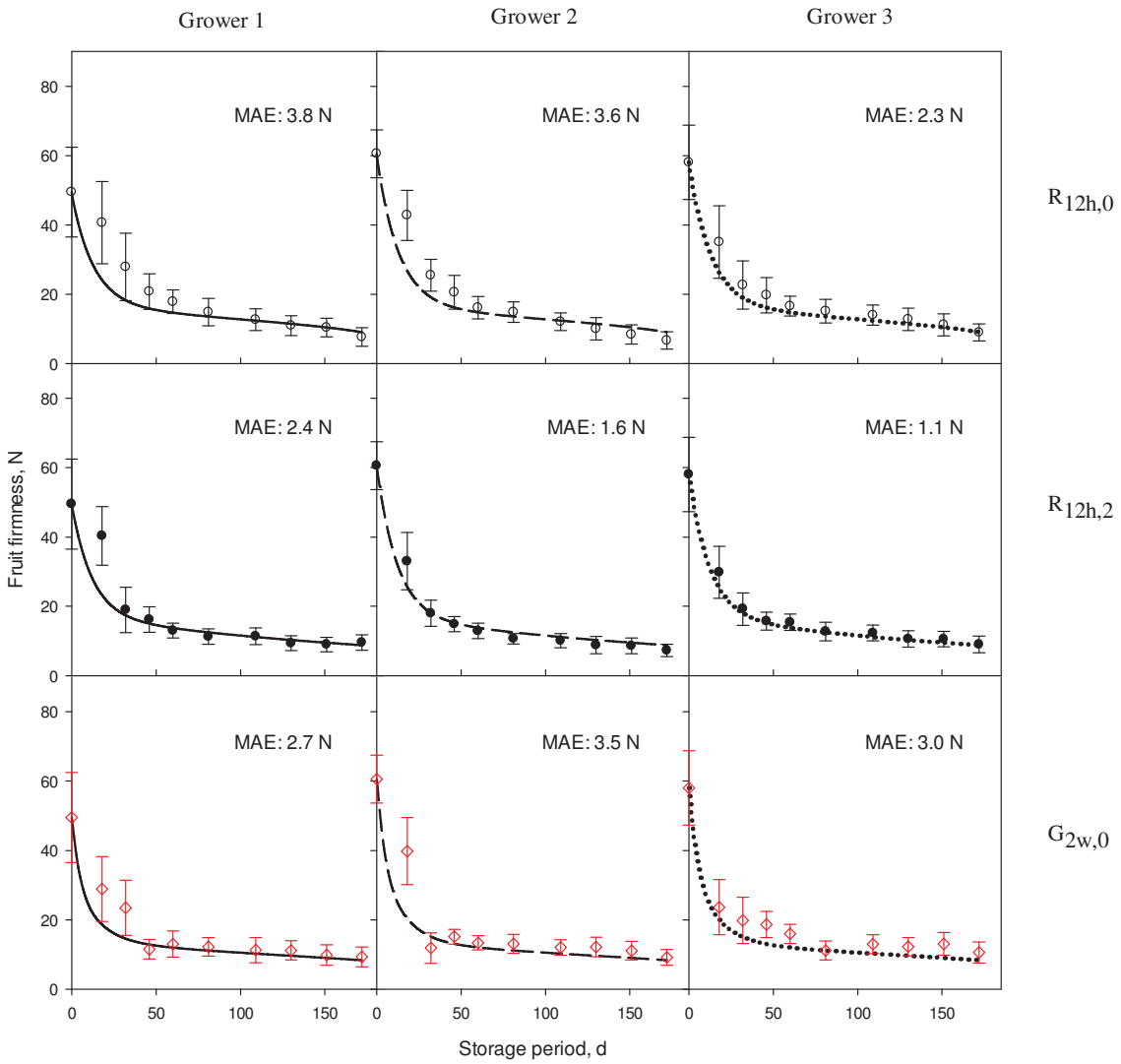


Figure 7.9: Model validation on softening pattern of late maturity fruit from three different grower lines collected in the 2013 season (F_{B0} = 16.4 N and vary a). Solid lines, dashed lines and dotted lines represent the predicted softening curves of different grower lines of late maturity fruit. The simulation was set up using the time temperature information of (A) rapid cooling to 0 °C ($R_{12h,0}$), (B) rapid cooling to 2 °C ($R_{12h,2}$) , and (C) gradual cooling to 0 °C ($G_{2w,0}$). Each data point represents the average fruit firmness of respective grower lines (n=30 for first 6 data points and n=90 for the remaining data points). The error bars represent the standard deviation. Rotten fruit were removed from the population prior to analysis. MAE represents mean absolute error.

7.3.3. Prediction of chilling injured fruit

As reported in chapter 4, fruit exposed to rapid cooling to 0 °C ($R_{12h,0}$) promotes chilling injury, resulting in a higher proportion of chilling injured fruit (Figure 4.5) and thus contributes to the accelerated decrease in average fruit firmness in the gradual softening phase (Figure 4.6). On the other hand, storing fruit at 2 °C ($R_{12h,2}$) or gradual cooling to 0 °C ($G_{2w,0}$) alleviates chilling injury development. The logistic model (eq. 5.17) is able to predict the proportion of chilling injured fruit when fruit were rapid cooled to 0 °C, leading to a higher proportion of chilling injured fruit (Table 7.5). The incidence of chilling injury were predicted to be lower when fruit were exposed to slow cooling ($G_{2w,0}$) or storage at 2 °C ($R_{12h,2}$).

Table 7.5: The comparison of the proportion of chilling injured fruit (%) between model prediction (Pred) and experimental data (Exp) collected in 2013. The fruit were rapid cooled to 0 °C ($R_{12h,0}$) or 2 °C ($R_{12h,2}$) or gradually cooled to 0 °C ($G_{2w,0}$). Chilling injured fruit were observed after 130, 151 and 172 d of storage.

Fruit maturity	Storage period	Cooling profiles					
		$R_{12h,0}$		$R_{12h,2}$		$G_{2w,0}$	
		Pred	Exp	Pred	Exp	Pred	Exp
Early	130 d	3.2	4.4	0.2	0.4	0.8	0.4
	151 d	8.5	4.8	0.7	1.1	2.2	1.5
	172 d	17.7	10	1.5	0.4	4.8	0.7
Mid	130 d	2.8	3	0.1	0.7	0.7	0.7
	151 d	7.5	7	0.4	1.1	1.9	1.5
	172 d	16	14.1	0.9	0	4.3	1.5
Late	130 d	2.4	3	0.1	0.7	0.6	0
	151 d	6.6	9.6	0.2	2.2	1.7	1.1
	172 d	14.4	26.3	0.6	2.2	3.8	5.6

The results reported in chapter 4 suggested that the incidence of chilling injured fruit was influenced by fruit maturity, where more chilling injured fruit were found in late maturity fruit (Figure 4.5). The current empirical model does not account for the effect of fruit maturity on predicting the proportion of chilling injured fruit. Figure 7.10 compares the predicted and experimental incidence of chilling injury showing an over prediction in

the proportion of chilling injured fruit for early maturity fruit and an under prediction for late maturity fruit.

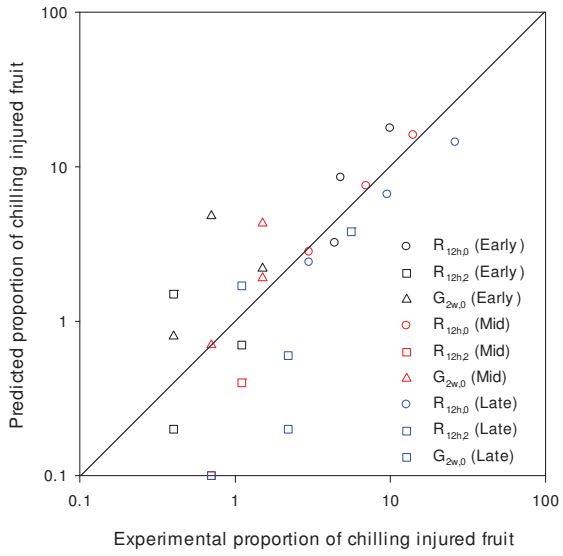


Figure 7.10: Comparison of the predicted and experimental data for the proportion of chilling injured fruit reflected in Table 7.5. Both axes are in log scale. Early, Mid and Late refer to the fruit maturity.

Chapter 5 discussed the prediction of the accelerated softening in the gradual softening phase by using an empirical approach to predict the proportion of chilling injured fruit. The chilling injury component added to the model helps to predict the proportion of chilling injured fruit which affects the subsequent firmness during storage. By adopting this approach, fruit that are exposed to storage conditions which promote chilling injury development will result in softer fruit during late storage period. Figure 7.11 shows that the model is able to predict an accelerated softening in gradual softening phase (> 140 d) of rapidly cooled fruit ($R_{12h,0}$) but not on gradually cooled fruit ($G_{2w,0}$). The experimental result (Figure 4.6C & F) demonstrated that the softening curve of rapidly cooled fruit intercepts with gradually cooled fruit after 120 d of storage. Although the developed model is able to predict the accelerated softening in the gradual softening phase, it failed to intercept with the softening curve for gradually cooled ($G_{2w,0}$) fruit that

was observed in the experimental data after 120 d of storage (Figure 7.11). This suggests that the predicted proportion of chilling injured fruit (Table 7.5) is not sufficient to predict an accelerated softening rate to intercept with the softening curve of gradually cooled fruit after 120 d of storage. The following chapter will discuss the possible reasons of the poor prediction on the firmness during late storage period and suggestions to improve the chilling injury component on softening prediction.

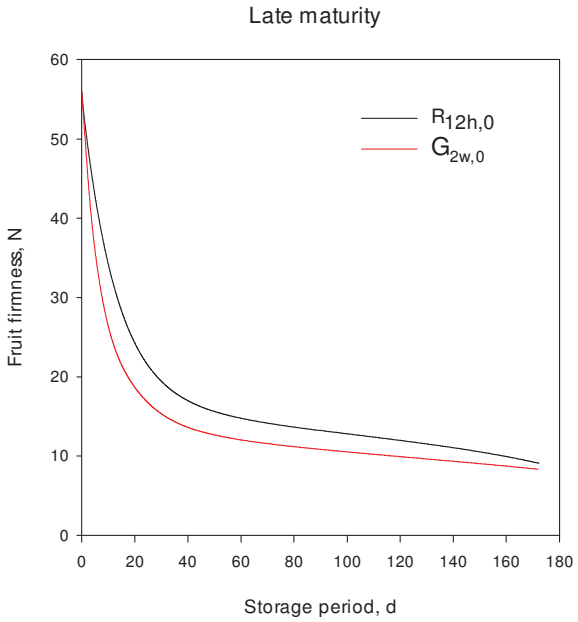


Figure 7.11: Comparison on the predicted fruit softening curve of rapidly cooled ($R_{12h,0}$) against gradually cooled ($G_{2w,0}$) fruit to 0 °C.

7.4. Overall discussion

Fruit harvested in different seasons and from various grower lines have different at-harvest attributes. The at-harvest attributes consist of initial fruit firmness (F_0), dry matter content (D_m) and soluble solid content (S_0). Previous sections have discussed the development of model to predict the fruit softening curve based on at-harvest attributes and time temperature information. Although the predicted softening curves did not fit closely with the experimental results especially in the rapid softening phase, the predicted softening trends suggest that at-harvest attributes can be used as model inputs to differentiate fruit maturity and grower lines. The benefits of using at-harvest attributes

are that the values are easy to collect when fruit are harvested and they do not require additional parameters to the final model to account for the fruit maturity and growers variability on the softening prediction. Jabbar *et al*. (2014) demonstrated the use of batch-dependent softening description parameters (B, κ and τ) that were associated with the at-harvest attributes to account for the batch variability in the softening of 'Hayward' kiwifruit.

The model validation has demonstrated the advantages of incorporating two different kinetic reactions and an empirical model to explain the softening pattern of kiwifruit during coolchain instead of using a single kinetic reaction as suggested by Schotsmans *et al*. (2008). A single kinetic reaction was used to explain the temperature dependence of textural changes in 'Hort16A' kiwifruit (Schotsmans *et al.*, 2008) reasonably well, displaying a biphasic pattern. The softening pattern of 'Hayward' kiwifruit is explained by four different softening phases which cannot be described by applying a single kinetic reaction. Adopting the method established by Schotsmans *et al*. (2008) may able to explain the temperature dependence of softening in the rapid softening phase but not during the gradual softening phase, due to the presence of chilling injured fruit which affects the subsequent fruit firmness. Another approach adopted earlier is using the rate of gas exchange to predict fruit softening (Hertog *et al.*, 2004c). Similarly, this approach does not include the effect of chilling injury development on fruit firmness. Furthermore, both models by Schotsmans *et al*. (2008) and Hertog *et al*. (2004) did not account for maturity and grower variability on the softening prediction. The model developed in this study uses information that are easily collected throughout the coolchain (i.e. time temperature information and at-harvest attributes) to estimate the fruit softening, allowing easy application to the industry.

7.5. Conclusion

Model validation has revealed the model capability in predicting the softening curve of fruit from different maturity and grower by including model input using the at-harvest attributes that are easily to collect throughout the supply chain and thus making it applicable to the industry. The model was developed based on fruit of late maturity. When applying parameters of early and mid-maturity fruit, the model is extrapolated well beyond the established correlations made earlier. The extrapolation is not valid and thus results in poor predictions of the softening curve of early and mid-maturity fruit, especially in the rapid softening phase. The under prediction of the fruit firmness in the rapid softening phase was possibly due to the influence by fruit maturity.

This chapter also address the prediction on the gradual softening phase and incidence of chilling injury. The prediction of gradual softening across different grower lines was improved by fixing the model parameter F_{B0} as a constant (16.4 N) and varying a. This modification suggests that gradual softening phase is initiated when fruit reach a certain firmness (F_{B0}). The empirical model (eq. 5.17) is capable to predict the incidence of chilling injury based on the given storage conditions. However, the estimated proportion of chilling injured fruit is unable to predict an accelerated softening that was observed in the experimental data. In the next chapter, the possible reasons to explain the poor prediction on the softening pattern will be discussed.

8. Overall discussion and recommendations

8.1. Introduction

One of the major concerns in the kiwifruit industry is postharvest fruit loss. Variation between grower lines and maturities make prediction of fruit quality throughout the coolchain a challenge (Jabbar, 2014). The cost to the industry is approximately $120 million per year when considering the costs to monitor, and re-pack to remove over soft fruit or rotten fruit to meet export standards (Tanner *et al.*, 2012). This work uses a mechanistic modelling approach to describe fruit softening and applies an additional empirical approach to estimate the incidence of chilling injured fruit. The outcome of this research is a mathematical model that predicts 'Hayward' kiwifruit firmness in the coolchain and hence has the potential to assist industry to reduce fruit wastage and associated costs.

Initially, supply chain features that had the potential to influence fruit quality were identified from industrially collected time temperature information (Figure 3.1). A simulation of these supply chains was conducted in the laboratory to identify the factors that affect fruit quality in coolchain (Chapter 3). Fruit firmness was postulated to be additionally affected by chilling injury development, as accelerated softening was observed in the late storage period that coincided with the increase in proportion of chilling injured fruit (Chapter 4). Three different softening phases were defined to describe the kiwifruit softening pattern. These softening patterns were then mathematically described using a mechanistic approach through the attribution of each softening phase to: correlation to starch degradation, breakdown of cell wall structure, and chilling injury development respectively (Chapter 5).

The developed model performance was evaluated after fitting the predicted curve with the 2012 experimental data (Chapter 6). The predictive capability of the developed mathematical model was validated with various independent data sets of kiwifruit softening in different coolchain scenarios simulated in the laboratory (Chapter 7). At-harvest attributes were used as model inputs to account for the differences in fruit grower line and maturity. Chapter 7 discussed the model capability in predicting the softening curve and in particular discussed the under prediction of fruit firmness during the rapid softening phase. This chapter aims to summarise the outcome of the model development and validation process and at the same time discussing the limitations of this research, application to the industry, and possible research opportunities.

8.2. Establishment of model

Identifying the factors that affect fruit quality in the coolchain is important as the developed model should be responsive to these factors in order to predict the softening pattern. The cooling methods and storage temperatures were monitored closely to ensure that the fruit were cooled and stored to simulate various coolchain scenarios. The presence of rotten fruit within the fruit population affects the average fruit firmness data and thus requires selecting the correct data. Finding rotten fruit within a fruit population is inevitable. Generally, pathogens that are responsible for decay can tolerate and grow at low temperatures and hence rots can occur during or after storage (Pennycook, 1985; Brook, 1992; Manning *et al.*, 2003). Rotten fruit was removed from the population before firmness measurement. Hence the softening curve obtained in this work is assumed to be not influenced by the effect of rots. However, rotten fruit produce high amounts of ethylene which potentially affects the neighbouring fruit in the same package (Burdon & Lallu, 2011). Since kiwifruit are highly sensitive to ethylene, premature softening may occur. During early storage, the impact of rotten fruit on fruit firmness will be significant

as the fruit is still firm. However, the impact was observed to be less significant in late storage when the fruit becomes soft (closer to 10 N). Jabbar & East (2016) found that exposing kiwifruit to ethylene concentrations of between 0.01 to 1 µL L^{-1} after 10 weeks of optimal storage resulted in an accelerated softening during storage. Therefore, despite the fact that rotten fruit have been removed before measurement, there is potential for this rotten fruit to affect the entire tray average. As a solution, Jabbar (2014) proposed to reject any trays of fruit which contained rotten fruit by implementing an experimental practice where replacement trays are available. This method was attempted in this research but failed to work due to the unpredictable occurrence of rotten fruit as these fruit can also be found in the replacement trays. In both 2012 and 2013 season, rotten fruit was observed to make up approximately 1.8 % of the total population. Rotten fruit were also found in all storage treatments.

8.3. Model softening prediction

Model validation identified the lack of fit in both the rapid and gradual softening phase. The lack of fit in the rapid softening phase of early and mid-maturity fruit may be explained by the inadequacy to predict the initial lag phase. The poor prediction of the gradual softening phase may be interpreted by the magnitude of firmness associated with starch content (F_A) and cell wall integrity (F_B). Chapter 7 also identified the models limitation to predict the incidence of chilling injury in fruit of early and late maturity. This section will discuss further on the possible reason to explain the lack of fit and potential model adjustments that may provide a solution.

8.3.1. Prediction of lag phase

Chapter 7 demonstrated the model limitation of over predicting the softening rate in the rapid softening phase and thus this phase needs further refinement in order to improve predictive accuracy. The firmness and starch content correlation found in Figure 5.4 demonstrates a positive linear relationship between fruit firmness and starch content. However, the fruit firmness and calculated starch content of fruit obtained in 2013 season were higher than those of the 2012 season (Figure 8.1A). The data collected in 2013 were added to the established correlation from the 2012 season to ascertain if the correlation remained. Figure 8.1A demonstrates that the linear relationship between firmness and starch content becomes invalid above 8 % starch content as the firmness maximum limits at approximately 80 N. Fruit harvested in the early and mid-season had high initial firmness and starch content resulting invalidating the use of the established correlation and thus limiting the model's ability to predict the softening accurately in the rapid softening phase, for these fruit maturities.

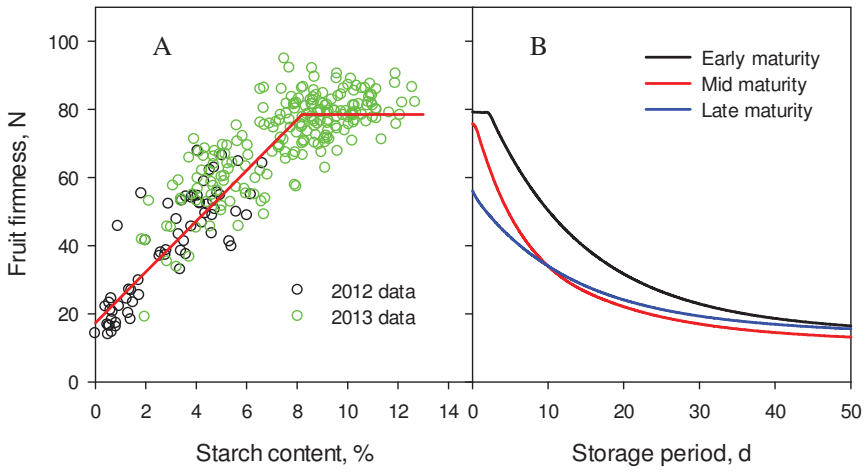


Figure 8.1: (A) Correlation of fruit firmness and starch content with a plateau when starch content is above 8 %. (B)Fruit softening curve of fruit harvested at different period in the season and rapidly cooled to 0 °C ($R_{12h,0}$). The model initial conditions are 5.5 °Brix (B_0), 17.9 % (D_{m0}) for early maturity fruit, 7.5 °Brix (B_0), 18.5 % (D_{m0}) for mid maturity fruit, and 11.1 °Brix (B_0), 18.4 % (D_{m0}) for late maturity fruit.

Instead of assuming a linear correlation between fruit firmness and starch content, it is possible to define the relationship as two stage, being proportionally linear to 8 % starch and with all fruit being 80 N above 8 % starch (Figure 8.1A). This assumption allows the degradation of starch content to influence the softening when starch content is below 8 %. In the model, the rate of starch breakdown would remain as a first order degradation (eq. 5.8). By adopting this approach, the rapid softening phase of fruit with high starch content (i.e. >8 %) only commences once starch content falls below 8 % and thus allows the presence of lag phase in the model (early maturity, Figure 8.1B), when starch is degrading but not influencing firmness.

The model was first created without a lag phase as the experimental work did not demonstrate the presence of an initial lag phase in the softening curve (Figure 3.8 and Figure 4.6). However, Jabbar *et al*. (2014), Schroder and Atkinson (2006) and White *et al.* (2005) have all demonstrated an initial lag phase when kiwifruit were stored at 20 °C. Potentially, the initial lag phase is not found on the softening curve when fruit are stored at 0 or 2 °C because of the time intervals used between each measurement (usually 2 weeks at least). By adopting the two stages correlation shown in Figure 8.1A, the model was able to predict an initial lag phase in early mature fruit, with an average initial starch content of 9.6 % (Figure 8.1B). White *et al.* (2005) suggested that the presence of an initial lag phase is influenced by fruit maturity as it is correlated with the time taken to become fully ripe.

Figure 7.4 demonstrated the under prediction on the rate of rapid softening for early and mid-maturity fruit. The rate of softening during the rapid softening phase is correlated to the rate of starch breakdown and thus the transition from accumulation to breakdown of starch on vine could potentially influence pre-harvest softening. Beever and Hopkirk (1990) interpreted that the transition from accumulation of starch to

breakdown occurs when matured kiwifruit start to ripen. Fruit harvested early in the season may not be fully mature and thus the rate of starch breakdown is reduced. The under prediction of firmness before 100 days of storage strongly suggests that the rapid softening phase is influenced by fruit maturity (Figure 7.4).

While the mechanisms which trigger the initiation of ripening in kiwifruit are still to be elucidated, a better understanding on the transition from fruit maturation to ripening will provide insight on the prediction of the lag phase in the softening curve. The transition from fruit maturation to ripening is a complex metabolic process that is regulated by both developmental and hormonal factors. McAtee *et al.* (2015) suggested that the "competence to ripen" in kiwifruit is independent to ethylene but exposing kiwifruit to ethylene will trigger fruit to ripen. An up-regulation of several genes (regulators of ripening) was observed when kiwifruit were stored under ethylene condition, suggests that exogenous ethylene plays a part in ripening (Zhang *et al.*, 2016). Besides exogenous ethylene, Snelgar *et al.* (1993) and Burdon *et al.* (2007) suggested that the "readiness to ripen" in kiwifruit is dependent on the exposure to cold environmental temperatures. A proteomic study demonstrated a change in protein abundance level, indicating that both exogenous ethylene and chilling treatment elicit kiwifruit ripening (Minas *et al.*, 2016). The following sections will propose some ideas to predict and model a lag phase in the softening curve of early maturity fruit.

The current developed model assumes that the rate of starch breakdown follows a first order rate that is dependent on the amount of starch content when under fixed storage temperature conditions and is set to be temperature dependent due to the fluctuating coolchain conditions. The pattern of soluble solids accumulation was described as a simple sigmoidal curve, displaying a gradual increase and subsequent a period of fast increase and eventually a slow period (Burdon *et al.*, 2013; Burdon, 2015). Burdon *et al.*

(2003) collected data on the accumulation of soluble solids content during the early stage of ripening (i.e. between 5 to 11 °Brix; Figure 8.2) while the collected experimental data (2012 harvest season) falls toward the late storage period (i.e. >11 °Brix). The rate of soluble solids accumulation in 'Hayward' kiwifruit demonstrated a gradual increase when the soluble solids content was between 5 to 7 %, followed by a rapid increase when it reaches above 7 %, and finally reached a plateau at around 15 °Brix (Figure 8.2). This suggests that the rate of starch breakdown and subsequent accumulation of soluble solids follows a simple sigmoidal curve which coincides with Burdon (2015).

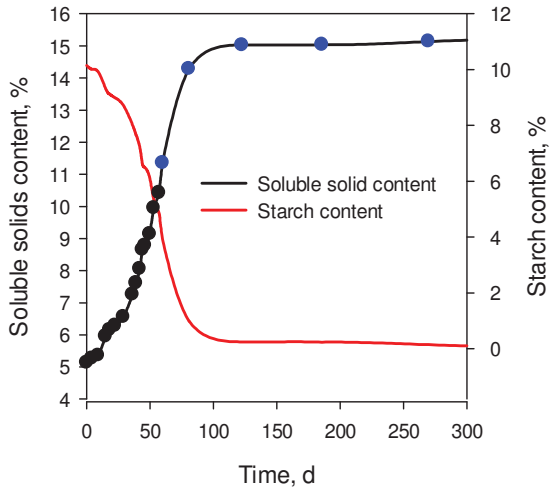


Figure 8.2: The accumulation of soluble solids content (black solid line) from Burdon *et al.* (2013) (black circle) and data obtained in this study (blue circles) from Figure 3.9 and hence the subsequent proposed rate of starch degradation (red solid line)

The predictive model was developed based on the results obtained in 2012 season, where the initial soluble solids content (B_0) was above 11 % for all three grower lines. Burdon *et al.* (2013) fitted the accumulation of soluble solid using two different approaches, a logistic fit and fitting using two linear components, exhibiting a 3 times difference between the upper and lower linear fit on the accumulation of SSC with different break-points across grower lines. This study did not research the accumulation

of SSC of fruit with low initial soluble solids content (< 5 °Brix) and hence unfortunately to estimating the rate of starch degradation of fruit with initial low B_0 is difficult. Exploring the effect of fruit maturity on the starch degradation based on the accumulation of soluble solids content may help to explain the initial lag phase and thus improve the softening prediction in early storage period (before 100 d), since the prediction of the early softening phase will affect the prediction in the subsequent slow softening phase.

The under prediction of firmness during the rapid softening phase may be remedied by utilising a logistic function to describe the rate of accumulation of soluble solids content as provided by Burdon *et al.* (2013) and experimental data collected in 2012 (Figure 8.2). Since the current study did not have sufficient results to justify the logistic function in the rate of accumulation of soluble solids content and the break-point, a simplified approach was adopted by adjusting the rate of starch degradation to be three times lower (0.047 d^{-1}) of the proposed rate when the starch content falls above 8 % and returns to the original value (0.14 d^{-1}) when starch content reaches below 8 %. The break-point is set at 8 % due to the correlation established between fruit firmness and starch content (Figure 8.1A). There may be a possibility that the break-point varies across different grower lines as observed by Burdon *et al.* (2013). The model estimates the starch content from the initial SSC (B_0) and dry matter content (D_{m0}), and based on the rate of starch degradation to predict the change in fruit firmness in the rapid softening phase.

The proposed approach allows the prediction of the initial lag phase based on fruit maturity, where fruit of early maturity will predict a longer lag phase (Figure 8.1B). With the presence of a lag phase, more firm fruit are predicted and thus an improvement towards the prediction of the rapid softening phase is observed. Although the rapid softening phase was not predicted accurately, the subsequent fruit firmness in gradual softening phase was better estimated (Figure 8.3). The disadvantage of this simple

approach is that it is somewhat speculated based on reported data. The next section will discuss a potential mechanistic approach to better explain the softening in the rapid softening phase.

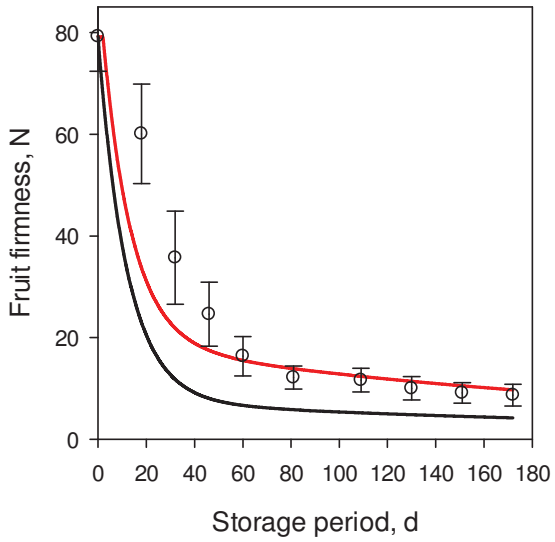


Figure 8.3: The model prediction on the softening curve by modifying the rate of starch degradation (k_s) to be 3 times lower when starch content is above 8 %. The model initial conditions are 79.2 N (F_0), 5.5 °Brix (B_0), 17.9 % (D_{m0}) for early maturity fruit. The open circles represents the average fruit firmness of early maturity fruit from 3 different grower lines that were rapidly ($R_{12h,2}$) cooled to 2 °C (n=90 for first 6 data points and n=270 for the remaining data points). The error bars represent the standard deviation. Black line represents the prediction without lag phase whereas the red line represents the modified prediction with lag phase.

8.3.1.1. Proposed mechanisms

Starch degradation and accumulation of soluble solids content are complex enzymatic reactions that take place during kiwifruit ripening (Weibel & Gomez, 1962; Macrae *et al.*, 1992; Hallett *et al.*, 1995). Although the process of starch degradation is not well defined in kiwifruit, a hypothesis is made to explain starch degradation mechanistically. Starch degradation follows a typical enzymatic reaction (eq. 8.1) which involves enzymes (E) binding to the substrate starch (S) to form a complex (ES) which subsequently breaks down starch to soluble solids (P).

$$E + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES_1 \overset{k_2}{\rightarrow} P \qquad \text{[eq. 8.1]}$$

Burdon (2015) and Figure 8.2 demonstrate that the change of soluble solids content follows a simple sigmodal curve; slow at the beginning and increasing exponentially with storage until reaching an asymptote. This suggests enzyme inhibition occurs during the process of starch degradation. During banana ripening, enzyme activities were found to be affected by the effect of indole-3-actic acid (IAA) and thus result in a delay of the starch degradation process.(Purgatto *et al.*, 2001). IAA content was observed in kiwifruit to decline rapidly during ripening (Chen *et al.*, 1997; Chen *et al.*, 1999). The presence of IAA during ripening may potentially initially delay starch degradation in kiwifruit before reaching negligible content, similar to findings by Purgatto *et al.* (2001) and thus conceivably affect fruit softening.

Starch degradation can also be influenced by the availability of bound enzyme and free enzyme. A similar classic example would be the degradation of cell wall structure during ripening of tomato fruit, which leads to release of wall-bound enzymes and thus promote further pectin hydrolysis (Rushing & Huber, 1984, 1990). Kinetic equations can be used to describe the assumption of a proportion of bound enzymes and free enzymes which affects the rate of starch degradation in the early storage period. Total enzymes (E_T) consist of the amount of free enzyme (E) and bound enzymes (E_b). Adopting this method may enable explanation of the initial lag phase based on the amount of free enzyme available initially, speculating that the enzyme responsible to breakdown starch are mostly bound in early maturity fruit. Therefore the initial low amount of free enzymes will predict a long initial lag phase. Alternatively, when large amounts of free enzymes are presented initially (i.e. for late maturity fruit) the predicted initial lag phase will be short or negligible. This approach will evolve 2 different rates of reaction and thus

explaining the 2 different rates observed in accumulation of soluble solid content or starch degradation (Figure 8.2). The drawback of this method is introducing more unknown variables (e.g. enzyme concentrations and rate constants) that are difficult for the industry to quantify or associate with at-harvest parameters and may compromise on overall softening prediction.

8.3.2. Prediction of gradual softening phase (F_{B0})

Chapter 7 demonstrates a poor prediction of the gradual softening phase (Figure 7.7) due to the magnitude of firmness change by the estimated model parameters, F_{B0} and a (Section 7.3.2). The prediction was improved (Figure 7.9) by fixing F_{B0} as a constant (16.4 N) and allow a to vary according to initial starch content (S_0) and firmness (F_0). This modification suggests that there might be a fixed amount of firmness change in the gradual softening phase and thus suggests that F_{B0} should be set as a constant. Figure 8.4 demonstrates a more promising softening prediction when F_{B0} is fixed across 2 harvest seasons (2012 and 2013). Adopting this modification results in a better prediction (MAE reduced from 8.0 N to 3.8 N) of the gradual softening phase which allows good estimation of fruit firmness during the later storage period, before reaching the industrial threshold standard of 10 N.

The model was developed without including an effect of ethylene on fruit softening while understanding the fact that kiwifruit is highly sensitive to ethylene. Undamaged kiwifruit was found to produce ethylene when firmness is 10 N or less (Burdon & Lallu, 2011; Samarakoon, 2013) while Chiaramonti and Barboni (2010) demonstrated that ethylene is produced between 100 and 140 d of storage. The fruit firmness was observed to be less than 20 N across different fruit maturity between 100 to 140 d of storage (Figure 4.6). Therefore, accumulation of ethylene within the pack is likely to occur when fruit firmness reaches less than 20 N. There is a possibility that

ethylene influences fruit firmness in the gradual softening phase (< 20 N) and thus it may be required to relate ethylene with firmness change during the slow softening phase.

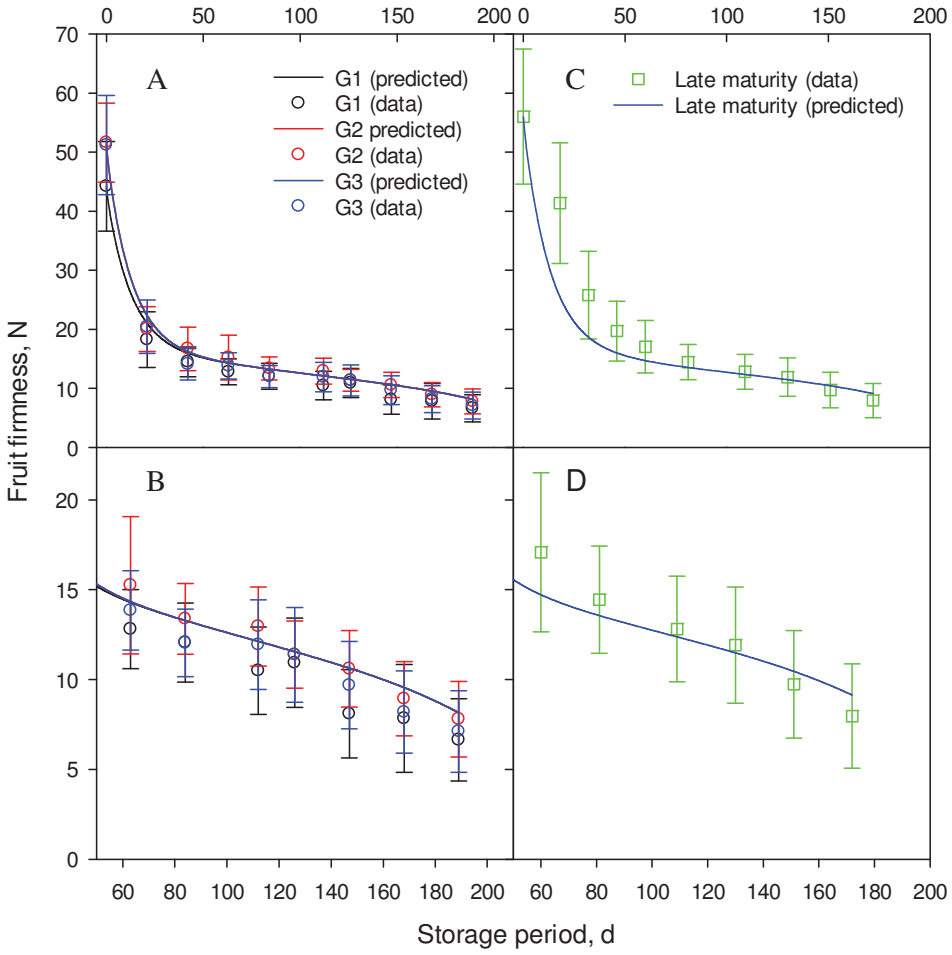


Figure 8.4: Model prediction of firmness of fruit in 2012 (A) and 2013 (C) harvest season. Fruit were fast cooled to 0 °C and subsequent stored at 0 °C. (A and B) Each data point represents the average firmness of fruit from 3 individual grower lines; G1, G2 and G3 (n = 36). (C and D) each data points represents the average firmness of late maturity fruit from 3 grower lines (n = 90 for first 6 data points and n=270 for the remaining data points). The model initial inputs are 44.2, 51.6 and 51.2 N (F_0), 11.3, 11.6 and 10.8 °Brix (B_0), 18.2, 18.4 and 17.9 % (D_{m0}) for respective grower lines in graphs A and B, whereas the initial inputs are 79.2 N (F_0), 5.5 °Brix (B_0), 17.9 % (D_{m0}) for graphs C and D. Graphs B and D represent the same data as A to C but focus on the late storage period (> 60 d of storage). The error bars represent the standard deviation.

The lack of ethylene sensing technology to measure concentrations in the coolchain easily and cheaply results in a paucity of information. Additionally, the complexity of ethylene transmission across packaging materials which are enclosed, but

not perfectly sealed, makes it difficult to predict ethylene concentration within the package interacting with the fruit (East *et al.*, 2015). Figure 8.5 illustrates the possible approach to estimating ethylene conditions is to relate fruit firmness to ethylene production, ethylene production to ethylene concentration and subsequently estimate accelerated softening as a result of ethylene. Samarakoon (2013) established the rate of ethylene production in kiwifruit as a function of firmness and temperature. This established relationship aids in estimating the amount of ethylene produced based on fruit firmness. Jabbar and East (2016) quantified the effect of exogenous ethylene on fruit softening in subsequent storage, demonstrating a significant loss in firmness when exposed to ethylene concentration of 0.01 $\mu L\ L^{-1}$ at harvest but observed no substantial differences in softening when applied after 10 weeks of storage. Overall, the findings from Samarakoon (2013) and Jabbar and East (2016) show the possibility to integrate the effect of ethylene onto the softening prediction, should ethylene production and internal box ethylene concentration be related (Figure 8.5). Another factor to consider is quantifying the ethylene produced by fruit that are damaged physically or physiologically or by rots. Fruit with chilling injury were found to produce ethylene (Hyodo & Fukasawa, 1985; Antunes & Sfakiotakis, 2002a; Feng *et al.*, 2003b). Quantifying the amount of damaged fruit due to physical impact or rots is practically impossible without direct measurement, given the low incidence of these random events. Applying the empirical prediction on chilling injured fruit may possibly enable an estimate of the amount of ethylene produced by chilling injured fruit. The next section will discuss on the prediction of chilling injured fruit.

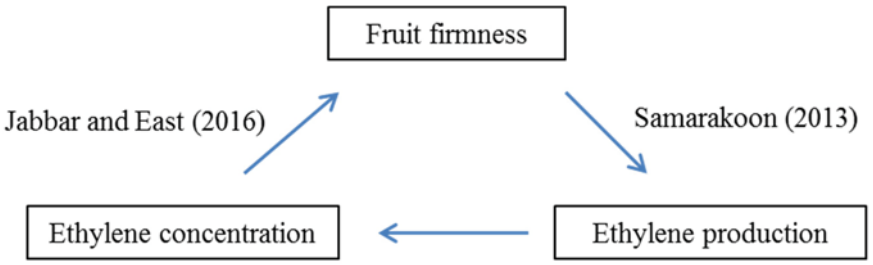


Figure 8.5: The possible approach to relate ethylene to fruit firmness

8.3.3. Prediction of incidence of chilling injury (*CI*)

The incidence of chilling injury development was predicted based on the time temperature information (via estimating AHU). Previously, chilling injury development in kiwifruit was found to be influenced by cold temperature acclimatisation and advancement in ripening (Koutsoflini *et al.*, 2013). Exposure to slow cooling to storage temperature allows the fruit to acclimatise to cold temperature, whereas exposing fruit to high storage temperature allows fruit to advance in ripening. Therefore, time temperature information can be used to calculate AHU and thus quantify fruit exposure to cold temperature acclimatisation. Koutsoflini *et al.* (2013) demonstrated that fruit of early maturity had highest incidence of chilling injury development and decreased from early to late maturity. However, in this work an increase in incidence of chilling injury development in late maturity fruit was observed. Therefore, there is a lack of consistent evidence for fruit maturities influence on the incidence of chilling injury development in kiwifruit. More studies need to be conducted to relate fruit maturity and chilling injury development. Consequently, model parameters that account for fruit maturity influence on chilling injury were not included in the model development (eq. 5.17). A complicating factors to this problem is that the indices for maturity are also used as indicators of fruit ripening status and thus could be associated with explaining the advancement in ripening (during slow cooling) which affects chilling injury development.

Fruit with high incidence of chilling injury corresponded with lower average firmness. However, these populations with high incidence of chilling injured fruit were

observed to be normally distributed (Figure 4.9), without 2 distinct populations (i.e. chilling injury and non-chilling injury). Additionally, the firmness of chilling injured fruit was all below 10 N. While the average chilling injured fruit is softer compared to the average normal soft fruit, the ability to differentiate individual fruit by firmness is not possible (Figure 4.9). In modelling the reduced firmness caused by chilling injury development, the proportion of chilling injured fruit within the fruit population was assigned a firmness of 1 N (F_{CI}) while the remaining population firmness is predicted by normal softening (eq. 5.19). Adopting this approach allows predicting a lower average firmness when the population consists of a high incidence of chilling injured fruit. However, the predicted incidence of chilling injury was unable to estimate the accelerated softening in the gradual softening phase well (Figure 7.11).

Another possible approach to describe the accelerated softening due to chilling injury is to use cell membrane permeability as a mechanism to predict fruit firmness as a function of time. The loss of cell membrane integrity may be used to explain the mechanism behind chilling jury development as Yang *et al.* (2012 and 2013) found an increase in cell membrane permeability coincided with a decrease in fruit firmness and increase in incidence of chilling injured fruit in storage. The setback of using this approach is the difficulties to quantify cell membrane permeability accurately and there are other factors that may explain the loss of cell membrane integrity.

The development of chilling injury has adverse impact on the fruit quality, resulting in poor consumer experiences. Therefore, anticipating fruit quality based on the coolchain conditions will be advantageous to the industry. The proportion of chilling injured fruit can be estimated using an empirical approach (eq. 5.17). However, this approach may not be able to estimate the proportion of chilling injured fruit accurately, possibly due to the influence of fruit maturity. Instead, the empirical approach can be

proposed as a risk assessment for chilling injury development in a particular pallet of kiwifruit based on the time temperature information. This risk assessment will be useful for the industry to identify the pallets of fruit that have a higher probability of developing chilling injury and hence require possible management procedures such as repacking and sorting. By making good judgements on the fruit quality, the industry can reduce cost associated with fruit wastage and labour.

8.4. At-harvest attributes as model inputs

At-harvest fruit firmness was found to decrease as the harvest season progressed (Mitchell *et al.*, 1992; Costa *et al.*, 1997). Solely relying on at-harvest fruit firmness as model input to predict the fruit quality during storage is not justifiable. Variation between fruit to fruit is large and thus introduces further uncertainty to estimate fruit maturity (Smith *et al.*, 1994; Pyke *et al.*, 1996; Feng *et al.*, 2003a; Woodward & Clearwater, 2011). Including other parameters such as soluble solids content and dry matter allows better description of the fruit maturity and quality. Using these at-harvest attributes to estimate the fruit maturity is a fairly common approach. The Strif Index coefficient consisting of fruit firmness, soluble solid content, and starch content is used to estimate apple physiological maturity at harvest in some parts of the world (DeLong *et al.*, 1999).

Fruit maturity was proposed to influence the softening pattern in particular due to the presence of an initial lag phase and rate of gradual softening phase (i.e. the absence of initial lag phase and slower softening in gradual softening phase of late mature fruit). Therefore, it is important to estimate fruit maturity to make a reasonable prediction on the fruit quality during storage. At-harvest attributes such as soluble solids content and dry matter were suggested to be the best indicators of fruit maturity. This work suggests that the amount of starch reserves in kiwifruit determines the length of the initial lag phase and define the magnitude of rapid softening phase. The starch content can be estimated

from the initial soluble solids and dry matter content. Using dry matter content to predict fruit quality (in terms of taste) has been well established as the starch available is correlated to the final soluble solids content after ripening (Beever & Hopkirk, 1990; Jordan *et al.*, 2000; Burdon *et al.*, 2004; Harker *et al.*, 2009; Crisosto *et al.*, 2011). Feng *et al.* (2003) proposed the use of percentage of dry matter that has been solubilised (SSFDM) at harvest as a better indicator to estimate kiwifruit physiological maturity. SSFDM was defined as a percentage of the calculated soluble solids content of a whole fruit (SSF, %; (Jordan *et al.*, 2000)) over dry matter content. This approach relates the amount of solubilised dry matter to quantify fruit maturity, where low amount of SSFDM refers to fruit of early maturity. The developed model correlates the initial lag phase and rapid softening phase with starch degradation and thus requires good estimation of the starch content at-harvest. MacRae *et al*. (1989 and 1992) used chemical methods to quantify the starch content in kiwifruit. In this work, the starch content is determined based on the difference between the measured initial (B_0) and the final soluble solids content (B_{final}), which is estimated using the correlation established by Burdon *et al.* (2004). It is possible to better estimate starch content by using the soluble solids of the whole fruit (SSF) instead of the measured soluble solids content (B_0) as SSF represents the soluble solids content to a whole fruit basis which allows better comparison with other fruit constituent measurements (Jordan *et al.*, 2000). A better estimation of the final soluble solids content (B_{final}) will also benefit in determining the amount of starch at harvest.

8.5. Application to industry

Storage conditions (i.e. cooling rate and storage temperature) influence fruit firmness change during storage. The first 2 weeks which include cooling fruit to storage temperature were found to influence chilling injury development which affects the fruit

quality and storability during the late storage period. This finding highlights the importance of managing fruit cooling to storage temperature (Bollen *et al.*, 2013). Currently, cooling capacity and practices vary across packhouses. The industry has collected a plethora of data on temperatures throughout the coolchain. This set of information is very valuable and potentially can be applied to the developed model to assess its capability to predict fruit softening over a large range of real temperature scenarios. The model prediction shortcomings especially in the rapid softening phase which subsequently affects the firmness prediction in the gradual softening phase have been well documented (Chapter 7). Applying the time temperature information and at-harvest attributes data from the industry to the developed model will able to identify more components that cause the rigidness on softening prediction.

8.6. Possible future opportunities

This work developed a model (with shortcomings) to predict kiwifruit softening in coolchain conditions. There are more opportunities to explore which may improve the model capability to predict kiwifruit softening. Currently, the model is developed focusing on the influence of cooling rates and storage temperature on fruit firmness and incidence of chilling injury. However, there are many more areas to explore which can influence kiwifruit quality in storage.

8.6.1. Curing of kiwifruit

Result collected in 2012 harvest season indicated that cooling rate had a significant effect on the incidence of rotten fruit (Section 3.3.3). However, results obtained from the 2013 harvest season did not replicate the outcome. The inconsistency in the findings may suggest that there are several factors that influence the development of rots in kiwifruit. Burdon *et al.* (2011) proposed that water loss or condensation at the picking scar, or creating opportunity for pathogens to invade the fruit tissues are possible

factors that will influence the likelihood for rot development. An ability to reduce rot development in kiwifruit will benefit the industry.

The process of curing is exposing kiwifruit to ambient temperature and humidity for a period of time after harvest. Curing is applied at the start of the supply chain, before grading and packing. Curing kiwifruit lowers the incidence of rot development in kiwifruit (Beever, 1992; Bautista-Banos *et al.*, 1995; Lallu *et al.*, 1997; Manning *et al.*, 2010). The effectiveness of the curing process is likely to be dependent on temperature and humidity. Much research has been conducted to study the effect of temperature and humidity during curing on incidence of infection by *Botrytis cinerea* (Pennycook & Manning, 1992; Bautista-Baños *et al.*, 1997). Pennycook and Manning (1992) documented the existence of curing phenomenon in kiwifruit by demonstrating that the ability of *Botrytis* infections on picking wounds was influenced by the temperature, humidity and duration of postharvest conditions. In this study, the effect of curing on 'Hayward' kiwifruit firmness in subsequent storage was not explored. Since curing is implemented immediately prior to cooling and coolstorage, there is a possibility that manipulation of temperature and humidity at the start of the supply chain will influence chilling injury development and thus affect fruit firmness in subsequent storage. Therefore, the mechanisms behind curing which improves kiwifruit quality (firmness and chilling injury development) during storage may potentially be included in the developed model.

8.6.2. Pre-harvest effect on fruit storability

Pre-harvest treatment such as vine management (Boyd & Barnett, 2011; Patterson & Currie, 2011) or manipulation of light exposure (Tombesi *et al.*, 1993) influences the dry matter content of kiwifruit. The model uses dry matter content as a model input and has the potential to account for these possible effects of vine management. However, pre-

harvest treatments involving nutrient fortification on kiwifruit such as calcium also affect fruit firmness (Feng *et al.*, 2003a; Xu *et al.*, 2015). Calcium ions form strong ionic bonded calcium bridges between pectin molecules (Brummell, 2006; Goulao & Oliveira, 2008), potentially altering the rate of cell wall degradation. The model does not include any effect of calcium on fruit softening and thus it would be useful to estimate the fruit nutrient content to quantify the preceding pre-harvest history. Besides calcium, minerals such as nitrogen, phosphorous, potassium and magnesium concentrations have previously been found to affect fruit firmness (Prasad & Spiers, 1992; Smith *et al.*, 1994; Feng *et al.*, 2003a). The gradual softening phase is explained by the loss of cell wall integrity and thus the presence of nutrients that affects the breakdown of cell wall structure will potentially alter the rate of softening.

Currently, at-harvest attributes are collected using destructive techniques such as refractometer and penetrometer. Recently, many studies have investigated the use of non-destructive techniques to estimate fruit quality and storability. Non-destructive techniques include Near Infrared Spectroscopy (NIR) (McGlone & Kawano, 1998; McGlone *et al.*, 2002; Costa *et al.*, 2003; Clark *et al.*, 2004; Liu *et al.*, 2011), X-ray (Feng *et al.*, 2010; Trejo Araya *et al.*, 2013; Cantre *et al.*, 2014) and optical coherence tomography (Li *et al.*, 2015). NIR was found to be capable to estimate kiwifruit at-harvest attributes such as soluble solids, dry matter content and fruit firmness (Feng, 2003). This has demonstrated the potential to apply NIR to determine the model inputs and thus allowing the developed model to predict fruit firmness during storage.

8.6.3. Predict fruit firmness with biological variation

Introducing biological variation into the model will improve prediction of fruit firmness within a batch. Biological variation can be expressed by either fruit maturity indices or biological age. The biological age concept has previously been used to quantify

the variation in colour change in tomato (Hertog *et al.*, 2004b; Hertog *et al.*, 2007a) and growth of Belgian endive (Hertog *et al.*, 2007b). The propagation of biological variation within a batch can be predicted by using a probabilistic kinetic approach. Hertog *et al.* (2007a and 2007b) demonstrated using such approach to define the distribution of colour change in tomato and growth in stem length of Belgian endive as a function of time and temperature during postharvest storage. The batch variability and keeping quality of cucumber is modelled based on stochastic and kinetic approach; the stochastic part describe the biological variability whereas kinetics part depends on the processes that estimates keeping quality (Schouten *et al.*, 2004). In this work, at-harvest attributes including initial soluble solids and dry matter content are used to describe fruit maturity and thus biological variation. A single batch consists of variation of fruit with different maturity indices. Variation of dry matter content was observed between orchards (Woodward & Clearwater, 2011). The current model is developed to estimate the average fruit firmness in subsequent storage, without the capability to predict the batch relative frequency distribution of firmness over time. The probabilistic kinetic approach can be implemented to fruit firmness as a function of maturity (soluble solids and dry matter content), which is liable to biological variation. Assuming that the kinetic model parameters are independent to biological variation, adopting the probabilistic kinetic approach to the developed model may describe the distribution of kiwifruit firmness in a batch as a function of time and temperature during storage, based on the initial firmness distribution measured at harvest. An ability to predict the distribution of firmness within a batch during postharvest storage will allows the industry to better optimise their logistics, as it is more likely to identify poor storing batches of fruit as commercial batch failure is usually representative of only a small (i.e. 2 – 4%) proportion of failure within a batch.

8.6.4. High temperature exposure

Temperature has a major effect on fruit quality during postharvest storage. When exposing kiwifruit to temperatures above 30 °C, the softening rate was found to decrease (Figure 3.14 and Figure 6.5). Therefore, applying the Arrhenius equation to account for the temperature dependence of softening may not be applicable above 30 °C. Alternatively, a modified Arrhenius equation could be implemented to ensure that the softening rate decreases at temperature above 30 °C. A simple modified Arrhenius equation consisting of Arrhenius and Boltzman components was applied to estimate firmness loss in apple at different non-optimal temperatures, where the softening rate was found to increase from 0 to 22 °C and decreased subsequently to 35 °C (Johnston *et al.*, 2001). Simple exponential, Boltzman and the Inverse exponential polynomial were used to predict kiwifruit softening along the supply chain in India, with ambient temperature of 35 ± 2 °C (Bellavi Jayashiva, 2012). When kiwifruit is exported to countries such as the Indian subcontinent or South East Asia, adopting this approach will be useful to make a reasonable prediction on the fruit firmness when exposed to high temperature conditions.

8.7. Conclusion

Postharvest fruit loss has been a major concern to the kiwifruit industry and thus reducing fruit loss will be beneficial to the industry. This thesis attempted to describe kiwifruit softening pattern using a mechanistic modelling approach based on at-harvest attributes and time temperature information collected from the coolchain, ignoring other pre-harvest factors and ethylene. Cooling rate and storage temperature were found to affect fruit firmness in subsequent storage by promoting chilling injury development. Kiwifruit softening was modelled based on 3 different mechanisms; correlation to starch degradation, breakdown of cell wall structure, and development of chilling injury. The kinetic models were fitted to the experimental data demonstrated the potential to describe kiwifruit softening based on at-harvest attributes and time temperature information. Furthermore, estimation of the proportion of chilling injured fruit using an empirical approach based on time temperature information was established. The developed predictive model exhibits a reasonable fit on late maturity fruit firmness data but poorly predicted early and mid-maturity fruit, especially during the rapid softening phase. This suggests that fruit maturity has an important effect on the softening pattern. In conclusion, this thesis has evaluated the potential for using a mathematical approach to describe kiwifruit softening and predict fruit quality in subsequent storage. In order to improve the model prediction of fruit firmness, more studies are needed which include the influence of fruit maturity, ethylene production and concentration in the atmosphere, high temperature exposure, and pre-harvest treatment on kiwifruit softening.

9. References

Aitken, A. G. and Hewett, E. W. (2015). FreshFacts. The New Zealand Insitiute for Plant & Food Research, 2-11.

Albert, Z., Beh, M., Kuznyak, L. and Papp, I. (2013). Ripening of kiwifruits by ethylene treatment. *Acta Horticulturae*, 981, 699-703.

Alexander, L. and Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany*, 53(377), 2039-2055.

Antunes, M. D. C. (2007). The role of ethylene in kiwifruit ripening and senescence. *Stewart Postharvest Review*, 3(2), 9.

Antunes, M. D. C., Panagopoulos, T., Neves, N., Curado, F. and Rodrigues, S. (2005). The effect of pre- and postharvest calcium applications on 'Hayward' kiwifruit storage ability. *Acta Horticulturae*, 682, 909-916.

Antunes, M. D. C. and Sfakiotakis, E. M. (1997). Biochemical basis of thermoregulation of ethylene production and ripening of 'Hayward' kiwifruit. *Acta Horticulturae*, 444, 541-546.

Antunes, M. D. C. and Sfakiotakis, E. M. (2000). Effect of high temperature stress on ethylene biosynthesis, respiration and ripening of 'Hayward' kiwifruit. *Postharvest Biology and Technology*, 20(3), 251-259.

Antunes, M. D. C. and Sfakiotakis, E. M. (2002a). Chilling induced ethylene biosynthesis in 'Hayward' kiwifruit following storage. *Scientia Horticulturae*, 92(1), 29-39.

Antunes, M. D. C. and Sfakiotakis, E. M. (2002b). Ethylene biosynthesis and ripening behaviour of 'Hayward' kiwifruit subjected to some controlled atmospheres. *Postharvest Biology and Technology*, 26(2), 167-179.

Antunes, M. D. C. and Sfakiotakis, E. M. (2008). Changes in fatty acid composition and electrolyte leakage of 'Hayward' kiwifruit during storage at different temperatures. *Food Chemistry*, 110(4), 891-896.

Arpaia, M. L., Labavitch, J. M., Greve, C. and Kader, A. A. (1987). Changes in the cell wall components of kiwifruit during storage in air or controlled atmosphere. *Journal of the American Society for Horticultural Science,* 112(3), 474-481.

Arpaia, M. L., Mitchell, F. G. and Kader, A. A. (1994). Postharvest physiology and causes of deterioration. In: Hasey, J. K., Johnson, R. S., Grant, J. A. & Reil, W. O. (Eds.), Kiwifruit: growing and handling. University of California, California, 88-93.

Arpaia, M. L., Mitchell, F. G., Kader, A. A. and Mayer, G. (1985). Effects of 2 percent oxygen and carying concentrations of carbon dioxide with or without ethylene on the storage performance of kiwifruit *Actindia chinensis* cultivar Hayward. *Journal of the American Society for Horticultural Science*, 110(2), 200-203.

Arpaia, M. L., Mitchell, F. G., Kader, A. A. and Mayer, G. (1986). Ethylene and temperature effects on softening and white core inclusions of kiwifruit stored in air or controlled atmospheres. *Journal of the American Society for Horticultural Science*, 111(1), 149-153.

Bauchot, A. D., Hallett, I. C., Redgwell, R. J. and Lallu, N. (1999). Cell wall properties of kiwifruit affected by low temperature breakdown. *Postharvest Biology and Technology*, 16(3), 245-255.

Bautista-Banos, S., Long, P. G. and Ganesh, S. (2000). The role of relative humidity during early storage on Botrytis cinerea incidence of kiwifruit. *Revista Mexicana de Fitopatologia*, 18(2), 92-96.

Bautista-Baños, S., Long, P. G. and Ganesh, S. (1997). Curing of kiwifruit for control of postharvest infection by Botrytis cinerea. *Postharvest Biology and Technology*, 12(2), 137-145.

Bautista-Banos, S., Long, P. G. and Ganeshanandam, S. (1995). Physiological changes in kiwi fruit during a curing period and incidence of *B. cinerea* during storage. *Acta Horticulturae*, 398, 233-240.

Beever, D. J. (1992). Curing of kiwifruit after harvest. New Zealand Kiwifruit Feb, 11-12.

Beever, D. J. and Hopkirk, G. (1990). Fruit development and fruit physiology. In: Warrington, I. J. & Weston, G. C. (Eds.), Kiwifruit: Science and Management. Ray Richards Publisher, Auckland, 97 - 126.

Bellavi Jayashiva, S. P. (2012). Prediction of storage potential and firmness loss of 'Hayward' kiwifruit along the supply chains in India, Master of Food Technology, Massey University.

Ben-Yehoshua, S. (1987). Transpiration, water stress, and gas exchange. In: Weichmann, J. (Ed.), Postharvest Physiology of Vegetables. Marcel Dekker, New York, 113 - 170.

Benge, J. R., De Silva, H. N., Banks, N. H. and Jeffery, P. B. (2000). Empirical modelling of postharvest changes in the firmness of kiwifruit. *Postharvest Biology and Technology,* 19(3), 211-220.

Bhagyalakshmi, N., Prabha, T. N., Yashodha, H. M., Prasanna, V., Jagadeesh, B. K. and Tharanathan, R. N. (2002). Biochemical studies related to textural regulation during ripening of banana and mango fruit. *Acta Horticulturae*, 575, 717-724.

Biggs, M. S., Woodson, W. R. and Handa, A. K. (1988). Biochemical basis of high-temperature inhibition of ethylene biosynthesis in ripening tomato fruits. *Physiologia Plantarum*, 72(3), 572-578.

Biswas, P., East, A. R., Brecht, J. K., Hewett, E. W. and Heyes, J. A. (2012). Intermittent warming during low temperature storage reduces tomato chilling injury. *Postharvest Biology and Technology*, 74, 71-78.

Biswas, P., East, A. R., Hewett, E. W. and Heyes, J. A. (2016). Intermittent warming in alleviating chilling injury - a potential technique with commercial constraint. *Food and Bioprocess Technology*, 9(1), 1-15.

Bollen, A. F., Tanner, D. J., Soon, C. B., East, A. R., Dagar, A., Sharshevsky, H., . . . Pelech, Y. (2013). Wireless temperature monitoring system in a global kiwifruit supply chain. *Acta Horticulturae*, 1091, 205-212.

Bonghi, C., Pagni, S., Vidrih, R., Ramina, A. and Tonutti, P. (1996). Cell wall hydrolases and amylase in kiwifruit softening. *Postharvest Biology and Technology*, 9(1), 19-29.

Boquete, E. J., Trinchero, G. D., Fraschina, A. A., Vilella, F. and Sozzi, G. O. (2004). Ripening of 'Hayward' kiwifruit treated with 1-methylcyclopropene after cold storage. *Postharvest Biology and Technology*, 32(1), 57-65.

Bowen, J. H., Lowe, R. G. and Macrae, E. A. (1988). The effect of a pre-harvest treatment with Ethrel on the starch content of kiwifruit. *Scientia Horticulturae*, 35(3–4), 251-258.

Boyd, L. M. and Barnett, A. M. (2011). Manipulation of whole-vine carbon allocation using girdling, pruning, and fruit thinning affects fruit numbers and quality in kiwifruit. *Hortscience*, 46(4), 590-595.

Brook, P. J. (1990). *Botrytis cinerea* and fruit rot of kiwifruit, *Actinidia deliciosa*. Auckland, New Zealand: New Zealand Kiwifruit Marketing Board, Department of Scientific and Industrial Research, Plant Protection.

Brook, P. J. (1992). Botrytis stem-end rot and other storage diseases of kiwifruit. *Acta Horticulturae*, 2(297), 545-550.

Brosnan, T. and Sun, D.-W. (2001). Precooling techniques and applications for horticultural products — a review. *International Journal of Refrigeration*, 24(2), 154-170.

Brummell, D. A. (2006). Cell wall disassembly in ripening fruit. *Functional Plant Biology*, 33(2), 103-119.

Brummell, D. A., Dal Cin, V., Lurie, S., Crisosto, C. H. and Labavitch, J. M. (2004). Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. *Journal of Experimental Botany*, 55(405), 2041-2052.

Brummell, D. A., Harpster, M. H., Civello, P. M., Palys, J. M., Bennett, A. B. and Dunsmuir, P. (1999). Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *Plant Cell*, 11(11), 2203-2216.

Burdon, (2015). Soluble solids revisited: a maturity or harvest index for kiwifruit. *Acta Horticulturae*, 1096, 257-266.

Burdon, J. and Lallu, N. (2011). Kiwifruit (*Actinidia spp*.). In: Yahia, E.M. (Ed), Postharvest Biology and Technology of Tropical and Subtropical Fruits. Woodhead Publishing Limited, Cambridge, 326-360.

Burdon, J., Lallu, N., Francis, K. and Boldingh, H. (2007). The susceptibility of kiwifruit to low temperature breakdown is associated with pre-harvest temperatures and at-harvest soluble solids content. *Postharvest Biology and Technology*, 43(3), 283-290.

Burdon, J., Lallu, N., Pidakala, P. and Barnett, A. (2013). Soluble solids accumulation and postharvest performance of 'Hayward' kiwifruit. *Postharvest Biology and Technology*, 80, 1-8.

Burdon, J., McLeod, D., Lallu, N., Gamble, J., Petley, M. and Gunson, A. (2004). Consumer evaluation of 'Hayward' kiwifruit of different at-harvest dry matter contents. *Postharvest Biology and Technology*, 34(3), 245-255.

Burdon, J., Punter, M., Billing, D., Pidakala, P. and Kerr, K. (2014a). Shrivel development in kiwifruit. *Postharvest Biology and Technology*, 87, 1-5.

Burdon, J., Wohlers, M., Pidakala, P., Laurie, T., Punter, M. and Billing, D. (2014b). The potential for commonly measured at-harvest fruit characteristics to predict chilling susceptibility of 'Hort16A' kiwifruit. *Postharvest Biology and Technology*, 94, 41-48.

Burdon, J. N., Dori, S., Lomaniec, E., Marinansky, R. and Pesis, E. (1994). The post-harvest ripening of water stressed banana fruits. *Journal of Horticultural Science*, 69(5), 799-804.

Cantre, D., East, A., Verboven, P., Trejo Araya, X., Herremans, E., Nicolaï, B. M., . . . Heyes, J. (2014). Microstructural characterisation of commercial kiwifruit cultivars using X-ray micro computed tomography. *Postharvest Biology and Technology*, 92, 79-86.

Chen, C. R. and Ramaswamy, H. S. (2002). Color and texture change kinetics in ripening bananas. *Food Science and Technology*, 35(5), 415-419.

Chen, K., Li, F., Zhang, S. and Ross, G. S. (1999). Role of abscisic acid and indole-3-acetic acid in kiwifruit ripening. *Acta Horticulturae*, 26(2), 81-86.

Chen, K., Zhang, S., Lu, J. and Chen, Q. (1997). Variation of abscisic acid, indole-3-acetic and ethylene in kiwifruit during fruit ripening. *Scientia Agricultura*, 30(2), 54-57.

Chiaramonti, N. and Barboni, T. (2010). Relationship between the physicochemical parameters and the ethylene emission during cold storage of kiwifruits. *International Journal of Food Science & Technology*, 45(7), 1513-1516.

Civello, P. M., Powell, A. L. T., Sabehat, A. and Bennett, A. B. (1999). An expansin gene expressed in ripening strawberry fruit. *Plant Physiology*, 121(4), 1273-1279.

Clark, C. J., McGlone, V. A., De Silva, H. N., Manning, M. A., Burdon, J. and Mowat, A. D. (2004). Prediction of storage disorders of kiwifruit (*Actinidia chinensis*) based on visible-NIR spectral characteristics at harvest. *Postharvest Biology and Technology*, 32(2), 147-158.

Cosgrove, D. J. (2000). Loosening of plant cell walls by expansins. *Nature*, 407(6802), 321-326.

Costa, G., Noferini, M., Montefiori, M. and Brigati, S. (2003). Non-destructive assessment methods of kiwifruit quality. *Acta Horticulturae*, 610, 179-189.

Costa, G., Quadretti, R., Succi, A. and Morigi, M. (1997). Influence of harvest time and temperature on fruit quality and storage of kiwifruit (cv Hayward). *Acta Horticulturae*, 444, 517-522.

Crisosto, C. H. and Crisosto, G. M. (2001). Understanding consumer acceptance of early harvested 'Hayward' kiwifruit. *Postharvest Biology and Technology*, 22(3), 205-213.

Crisosto, C. H., Mitchell, F. G. and Ju, Z. G. (1999). Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. *Hortscience*, 34(6), 1116-1118.

Crisosto, C. H., Zegbe, J., Hasey, J. and Crisosto, G. M. (2011). Is dry matter a reliable quality index for 'Hayward' kiwifruit? *Acta Horticulturae*, 913, 531-534.

De Belie, N., Hallett, I. C., Harker, F. R. and De Baerdemaeker, J. (2000). Influence of ripening and turgor on the tensile properties of pears: A microscopic study of cellular and tissue changes. *Journal of the American Society for Horticultural Science*, 125(3), 350-356.

De Belie, N., Tu, K., Jancsók, P. and De Baerdemaeker, J. (1999). Preliminary study on the influence of turgor pressure on body reflectance of red laser light as a ripeness indicator for apples. *Postharvest Biology and Technology*, 16(3), 279-284.

De Ketelaere, B., Stulens, J., Lammertyn, J., Cuong, N. V. and De Baerdemaeker, J. (2006). A methodological approach for the identification and quantification of sources of biological variance in postharvest research. *Postharvest Biology and Technology*, 39(1), 1-9.

DeLong, J. M., Prange, R. K. and Harrison, P. A. (1999). Using the Streif Index as a final harvest window for controlled-atmosphere storage of apples. *Hortscience*, 34(7), 1251-1255.

Eaks, I. L. (1978). Ripening, respiration, and ethylene production of 'Hass' avocado fruits at 20 ° to 40 °C. *Journal of the American Society for Horticultural Science*, 103(5), 576-578.

East, A., Zhao, M., Jabbar, A., Samarakoon, H., Bollen, F., Adkins, M., . . . Heyes, J. (2016). Why is predicting kiwifruit quality in the cool chain so difficult? Paper presented at the 4th IIR Conference on Sustainability and the Cold Chain, Auckland, New Zealand.

East, A. R., Samarakoon, H. C., Pranamornkith, T. and Bronlund, J. E. (2015). A review of ethylene permeability of films. *Packaging Technology and Science*, 28(8), 732-740.

East, A. R., Tanner, D. J., Jobling, J. J., Maguire, K. M. and Mawson, A. J. (2008). The influence of breaks in storage temperature on 'Cripps Pink' apple physiology and quality. *Hortscience,* 43(3), 818-824.

Falk, S., Hertz, C. H. and Virgin, H. I. (1958). On the relation between turgor pressure and tissue rigidity. I. *Physiologia Plantarum*, 11(4), 802-817.

Famiani, F., Baldicchi, A., Farinelli, D., Cruz-Castillo, J. G., Marocchi, F., Mastroleo, M., . . . Battistelli, A. (2012). Yield affects qualitative kiwifruit characteristics and dry matter content may be an indicator of both quality and storability. *Scientia Horticulturae*, 146, 124-130.

Feng, J. (2003). Segregation of 'Hayward' kiwifruit for storage potential. Doctor of Philosophy, Massey University.

Feng, J., Mackay, B. R., Maguire, K. M., Tran, T., Bartle, M. and Wallace, G. (2010). Evaluation of differential energy X-ray analysis for non-destructive measurement of 'Hayward' kiwifruit (*Actinidia deliciosa*) attributes. *Acta Horticulturae*, 880, 291-295.

Feng, J. Q., MacKay, B. R. and Maguire, K. M. (2003a). Variation in firmness of packed Hayward kiwifruit. *Acta Horticulturae*, 610, 211-217.

Feng, J. Q., Maguire, K. M. and MacKay, B. R. (2003b). Factors affecting ethylene production of Hayward kiwifruit. *Acta Horticulturae*, 610, 203-209.

Findlay, J. S. and Combrink, J. C. (1996). Cold chain management. In: Combrink, J. C. (Ed.), Integrated Management of Post-Harvest Quality. INFRUITEC, Stellenbosch, 79-90.

Finney, E. E., Ben-Gera, I. and Massie, D. R. (1967). An objective evaluation of changes in firmness of ripening bananas using a sonic technique. *Journal of Food Science*, 32(6), 642-646.

Fonseca, S. C., Oliveira, F. A. R. and Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. *Journal of Food Engineering*, 52(2), 99-119.

Galvez, A. B., Garcia, M. V., Corrales, J. C., Lopez, A. C. and Valenzuela, J. A. L. (2010). Effect of gradual cooling storage on chilling injury and phenylalanine ammonia-lyase activity in tomato fruit. *Journal of Food Biochemistry*, 34(2), 295-307.

Gerasopoulos, D. and Drogoudi, P. D. (2005). Summer-pruning and preharvest calcium chloride sprays affect storability and low temperature breakdown incidence in kiwifruit. *Postharvest Biology and Technology*, 36(3), 303-308.

Goulao, L. F. and Oliveira, C. M. (2008). Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends in Food Science & Technology*, 19(1), 4-25.

Grierson, W. and Wardowski, W. F. (1978). Relative humidity effects on post-harvest life of fruit and vegetables. *Hortscience*, 13(5), 570-574.

Gwanpua, S. G., Verlinden, B. E., Hertog, M. L. A. T. M., Bulens, I., Van de Poel, B., Van Impe, J., . . . Geeraerd, A. H. (2012). Kinetic modeling of firmness breakdown in 'Braeburn' apples stored under different controlled atmosphere conditions. *Postharvest Biology and Technology*, 67, 68-74.

Hallett, I. C., Macrae, E. A. and Wegrzyn, T. F. (1992). Changes in kiwifruit cell wall ultrastructure and cell packing during postharvest ripening. *International Journal of Plant Sciences*, 153(1), 49-60.

Hallett, I. C., Wegrzyn, T. F. and MacRae, E. A. (1995). Starch degradation in kiwifruit: in vivo and in vitro ultrastructural studies. *International Journal of Plant Sciences*, 156(4), 471-480.

Harker, F. R., Carr, B. T., Lenjo, M., MacRae, E. A., Wismer, W. V., Marsh, K. B., . . . Pereira, R. B. (2009). Consumer liking for kiwifruit flavour: A meta-analysis of five studies on fruit quality. *Food Quality and Preference*, 20(1), 30-41.

Harker, F. R. and Hallett, I. C. (1992). Physicological-changes associated with development of mealiness of apple fruit during cool storage. *Hortscience*, 27(12), 1291-1294.

Harker, F. R. and Hallett, I. C. (1994). Physiological and mechanical properties of kiwifruit tissure associated with texture change during cool storage. *Journal of the American Society for Horticultural Science*, 119(5), 987-993.

Harman, J. E. (1981). Kiwifruit maturity. Orchardist of New Zealand, 54(4), 126-130.

Harman, J. E. and McDonald, B. (1989). Controlled atmosphere storage of kiwifruit. Effect on fruit quality and composition. *Scientia Horticulturae*, 37(4), 303-315.

Harpster, M., Brummell, D. and Dunsmuir, P. (2002). Suppression of a ripening-related endo-1,4-β-glucanase in transgenic pepper fruit does not prevent depolymerization of cell wall polysaccharides during ripening. *Plant Molecular Biology*, 50(3), 345-355.

Hashim, N., Bin Janius, R., Baranyai, L., Rahman, R. A., Osman, A. and Zude, M. (2012). Kinetic model for colour changes in bananas during the appearance of chilling injury symptoms. *Food and Bioprocess Technology*, 5(8), 2952-2963.

Hawkins, L. A. (1922). The effect of low-temperature storage and freezing on fruits and vegetables. *American Journal of Botany*, 9(10), 551 - 556.

Henriod, R. E., Gibberd, M. R. and Treeby, M. T. (2005). Storage temperature effects on moisture loss and the development of chilling injury in Lanes Late Navel orange. *Australian Journal of Experimental Agriculture*, 45(4), 453-458.

Hertog, M. L. A. T. M., Ben-Arie, R., Róth, E. and Nicolaï, B. M. (2004a). Humidity and temperature effects on invasive and non-invasive firmness measures. *Postharvest Biology and Technology*, 33(1), 79-91.

Hertog, M. L. A. T. M., Lammertyn, J., Desmet, M., Scheerlinck, N. and Nicolaï, B. M. (2004b). The impact of biological variation on postharvest behaviour of tomato fruit. *Postharvest Biology and Technology*, 34(3), 271-284.

Hertog, M. L. A. T. M., Lammertyn, J., Scheerlinck, N. and Nicolaï, B. M. (2007a). The impact of biological variation on postharvest behaviour: The case of dynamic temperature conditions. *Postharvest Biology and Technology*, 43(2), 183-192.

Hertog, M. L. A. T. M., Nicholson, S. E. and Jeffery, P. B. (2004c). The effect of modified atmospheres on the rate of firmness change of 'Hayward' kiwifruit. *Postharvest Biology and Technology*, 31(3), 251-261.

Hertog, M. L. A. T. M., Scheerlinck, N., Lammertyn, J. and Nicolaï, B. M. (2007b). The impact of biological variation on postharvest behaviour of Belgian endive: The case of multiple stochastic variables. *Postharvest Biology and Technology*, 43(1), 78-88.

Hertog, M. L. A. T. M., Verlinden, B. E., Lammertyn, J. and Nicolai, B. M. (2007c). OptiPa, an essential primer to develop models in the postharvest area. *Computers and Electronics in Agriculture*, 57(1), 99-106.

Hewett, E. W., Kim, H. O. and Lallu, N. (1999). Postharvest physiology of kiwifruit: The challenges ahead. *Acta Horticulturae*, 498, 203-216.

Heyes, J. A., Tanner, D. J. and East, A. R. (2010). Kiwifruit respiration rates, storage temperatures and harvest maturity. *Acta Horticulturae*, 880, 167-173.

Hodges, D. M., Lester, G. E., Munro, K. D. and Toivonen, P. M. A. (2004). Oxidative stress: Importance for postharvest quality. *Hortscience*, 39(5), 924-929.

Hopkirk, G., Harker, F. R. and Harman, J. E. (1990a). Calcium and the firmness of kiwifruit. *New Zealand Journal of Crop and Horticultural Science*, 18(4), 215-219.

Hopkirk, G., Lallu, N., Yearsley, C., Elgar, H., Cotter, J., Pennycook, S. and Manning, M. (1990b). Storage life and quality of kiwifruit held in bulk packaging. Report to the New Zealand Kiwifruit Marketing Board, 41.

Huber, D. J. (1992). The inactivation of pectin depolymerase associated with isolated tomato fruit cell-wall - Implications for the analysis of pectin solubility and molecular weight. *Physiologia Plantarum*, 86(1), 25-32.

Hyodo, H. and Fukasawa, R. (1985). Ethylene production in kiwifruit. *Journal of the Japanese Society for Horticultural Science*, 54(2), 209-215.

Jabbar, A. (2014). Accelerated fruit libraries to predict storage potential of 'Hayward' kiwifruit grower lines. Doctor of Philosophy, Massey University.

Jabbar, A. and East, A. R. (2016). Quantifying the ethylene induced softening and low temperature breakdown of "Hayward" kiwifruit in storage. *Postharvest Biology and Technology*, 113, 87-94.

Jabbar, A., East, A. R., Jones, G., Tanner, D. J. and Heyes, J. A. (2014). Modelling batch variability in softening of 'Hayward' kiwifruit from at-harvest maturity measures. *Postharvest Biology and Technology*, 90, 7-14.

Jackman, R. L., Gibson, H. J. and Stanley, D. W. (1992). Effects of chilling on tomato fruit texture. *Physiologia Plantarum*, 86(4), 600-608.

Jackman, R. L. and Stanley, D. W. (1992). Failure mechanisms of tomato pericarp tissue suggested by large and small deformation tests. *Journal of Texture Studies*, 23(4), 475-489.

Jaeger, S. R., Rossiter, K. L., Wismer, W. V. and Harker, F. R. (2003). Consumer-driven product development in the kiwifruit industry. *Food Quality and Preference*, 14(3), 187-198.

Jeffery, P. B. and Banks, N. H. (1996). Effects of ethylene on kiwifruit softening in coolstorage. *New Zealand Kiwifruit Journal*, 1(14), 9-10.

Johnston, J. W., Hewett, E. W., Hertog, M. L. A. T. M. and Harker, F. R. (2002). Temperature and ethylene affect induction of rapid softening in 'Granny Smith' and 'Pacific Rose™' apple cultivars. *Postharvest Biology and Technology*, 25(3), 257-264.

Johnston, J. W., Hewett, E. W., Hertog, M. L. A. T. M. and Roger Harker, F. (2001). Temperature induces differential softening responses in apple cultivars. *Postharvest Biology and Technology*, 23(3), 185-196.

Jordan, R. B., Walton, E. F., Klages, K. U. and Seelye, R. J. (2000). Postharvest fruit density as an indicator of dry matter and ripened soluble solids of kiwifruit. *Postharvest Biology and Technology*, 20(2), 163-173.

Kim, H. O., Hewett, E. W. and Lallu, N. (1999). The role of ethylene in kiwifruit softening. *Acta Horticulturae*, 498, 255-261

King, G. A., Henderson, K. G. and Lill, R. E. (1988). Shelf-life of stored asparagus is strongly related to post-harvest accumulated heat units. *Annals of Applied Biology*, 112(2), 329-335.

Klein, J. D. and Lurie, S. (1990). Prestorage heat treatment as a means of improving poststorage quality of apples. *Journal of the American Society for Horticultural Science*, 115(2), 265-269.

Klein, J. D., Lurie, S. and Benarie, R. (1990). Quality and cell-wall components of anna and granny smith apples treated with heat, calcium, and ethylene. *Journal of the American Society for Horticultural Science*, 115(6), 954-958.

Kojima, K., Sakurai, N. and Kuraishi, S. (1994). Fruit softening in banana: correlation among stress-relaxation parameters, cell wall components and starch during ripening. *Physiologia Plantarum*, 90(4), 772-778.

Koutsoflini, A., Gerasopoulos, D. and Vasilakakis, M. (2013). The effects of fruit maturation, delayed storage and ethylene treatment on the incidence of low-temperature

breakdown of 'Hayward' kiwifruit. *Journal of the Science of Food and Agriculture*, 93(2), 410-414.

Lafuente, M. T., Belver, A., Guye, M. G. and Saltveit, M. E. (1991). Effect of temperature conditioning on chilling injury of cucumber cotyledons - possible role of abscisic-acid and heat-shock proteins. *Plant Physiology*, 95(2), 443-449.

Lallu, N. (1997). Low temperature breakdown in kiwifruit. *Acta Horticulturae*, 2(444), 579-585.

Lallu, N., Manning, M. and Pak, H. (1997). Best curing practices for the orchard and packhouse. *New Zealand Kiwifruit Journal*, March/April, 35-36.

Lallu, N. and Webb, D. J. (1997). Physiological and economic analysis of precooling kiwifruit. *Acta Horticulturae*, 444, 691-697.

Lallu, N., Yearsley, C. W. and Elgar, H. J. (1992). Storage temperature affects the quality of kiwifruit. *Proceedings of the IV National Kiwifruit Conference*, 11-12.

Lallu, N., Yearsley, C. W. and Elgar, H. J. (2000). Effects of cooling treatments and physical damage on tip rot and postharvest quality of asparagus spears. *New Zealand Journal of Crop and Horticultural Science*, 28(1), 27-36.

Lammertyn, J., De Ketelaere, B., Marquenie, D., Molenberghs, G. and Nicolai, B. M. (2003). Mixed models for multicategorical repeated response: modelling the time effect of physical treatments on strawberry sepal quality. *Postharvest Biology and Technology*, 30(2), 195-207.

Lelievre, J. M., Latche, A., Jones, B., Bouzayen, M. and Pech, J. C. (1997). Ethylene and fruit ripening. *Physiologia Plantarum*, 101(4), 727-739.

Lewis, N. G. and Yamamoto, E. (1990). Ligin - occurrence, biogensis and biodegradation. *Annual Review of Plant Physiology and Plant Molecular Biology*, 41, 455-496.

Li, M., Verboven, P., Buchsbaum, A., Cantre, D., Nicolai, B., Heyes, J., . . . East, A. (2015). Characterising kiwifruit (*Actinidia sp.*) near skin cellular structures using optical coherence tomography. *Postharvest Biology and Technology*, 110, 247-256.

Lieberman, M. (1979). Biosynthesis and action of ethylene. *Annual Review of Plant Physiology and Plant Molecular Biology*, 30, 533-591.

Lim, B.-S., Kim, J.-K., Gross, K. C., Hwang, Y.-S. and Kim, J.-H. (2005). Gradual postharvest cooling reduces blackening disorder in 'Niitaka' pear (*Pyrus pyrifolia*) fruits. *Journal of the Korean Society for Horticultural Science*, 46(5), 311-316.

Lin, T. T. and Pitt, R. E. (1986). Rheology of apple and potato tissue as affected by cell turgor pressure. *Journal of Texture Studies*, 17(3), 291-313.

Liu, H., Guo, W. and Yue, R. (2011). Non-destructive detection of kiwifruit firmness based on near-infrared diffused spectroscopy. *Chinese Society for Agricultural Machinery*, 42(3), 145-149.

Ma, Q. S., Suo, J. T., Huber, D. J., Dong, X. Q., Han, Y., Zhang, Z. K. and Rao, J. P. (2014). Effect of hot water treatments on chilling injury and expression of a new C-repeat binding factor (CBF) in 'Hongyang' kiwifruit during low temperature storage. *Postharvest Biology and Technology*, 97, 102-110.

Maclachlan, G. and Brady, C. (1994). Endo-1,4-[beta]-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. *Plant Physiology*, 105(3), 965-974.

Macrae, E., Quick, W. P., Benker, C. and Stitt, M. (1992). Carbohydrate-metabolism during postharvest ripening in kiwifruit. *Planta*, 188(3), 314-323.

Macrae, E. A. (1987). Development of chilling injury in New Zealand grown fuyu persimmon during storage. *New Zealand Journal of Experimental Agriculture*, 15(3), 333-344.

Macrae, E. A., Lallu, N., Searle, A. N. and Bowen, J. H. (1989). Changes in the softening and composition of kiwifruit (*Actindia-deliciosa*) affected by maturity at harvest and postharvest treatments. *Journal of the Science of Food and Agriculture*, 49(4), 413-430.

Manning, M. A., Meier, X. and Olsen, T. L. (2003). Fungi associated with fruit rots of *Actinidia chinensis* 'Hort16A' in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, 31(4), 315-324.

Manning, M. A., Pak, H. A. and Beresford, R. M. (2010). Non-fungicidal control of botrytis storage rot in New Zealand kiwifruit through pre- and postharvest crop management. In: Dov, P. & Gullino, M. L. (Ed), Postharvest Pathology. Springer, Netherlands, 183-196.

Manolopoulou, H. and Papadopoulou, P. (1998). A study of respiratory and physico-chemical changes of four kiwi fruit cultivars during cool-storage. *Food Chemistry*, 63(4), 529-534.

Marangoni, A. G., Palma, T. and Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biology and Technology*, 7(3), 193-217.

Matsui, T. and Kitagawa, H. (1990). Maturity evaluation of kiwifruit determined on the basis of starch and pectin contents and related enzyme activities. *Japanese Society for Food Science and Technology*, 37(3), 224-229.

Maxie, E. C., Mitchell, F. G., Sommer, N. F., Snyder, R. G. and Rae, H. L. (1974). Effects of elevated temperatures on ripening of 'Bartlett' pear. *Journal of the American Society for Horticultural Science*, 99(4), 344-349.

McAtee, P. A., Richardson, A. C., Nieuwenhuizen, N. J., Gunaseelan, K., Hoong, L., Chen, X. Y., . . . Schaffer, R. J. (2015). The hybrid non-ethylene and ethylene ripening response in kiwifruit (*Actinidia chinensis*) is associated with differential regulation of MADS-box transcription factors. *BMC Plant Biology*, 15, 16.

McDonald, B. (1990). Pre-cooling, storage, and transport of kiwifruit. In: Warrington, I. J. & Weston G. C. (Eds.), Kiwifruit: science and management. Ray Richards publisher, 429-459

McDonald, R. E., McCollum, T. G. and Nordby, H. E. (1993). Temperature conditioning and surface treatments of grapefruit affect expression of chilling injury and gas-diffusion. *Journal of the American Society for Horticultural Science*, 118(4), 490-496.

McGlone, V. A., Jordan, R. B., Seelye, R. and Martinsen, P. J. (2002). Comparing density and NIR methods for measurement of kiwifruit dry matter and soluble solids content. *Postharvest Biology and Technology*, 26(2), 191-198.

McGlone, V. A. and Kawano, S. (1998). Firmness, dry-matter and soluble-solids assessment of postharvest kiwifruit by NIR spectroscopy. *Postharvest Biology and Technology*, 13(2), 131-141.

McQueenmason, S. J. and Cosgrove, D. J. (1995). Expansin mode of action on cell-walls - analysis of wall hydrolysis, stress-relaxation, and binding. *Plant Physiology*, 107(1), 87-100.

Menesatti, P., Urbani, G. and Lanza, G. (2005). Spectral imaging VIS-NIR system to forecast the chilling injury onset on citrus fruits. *Acta Horticulturae*, 682, 1347-1354.

Meyer, B. S., Anderson, D. B. and Bohning, R. H. (1965). Introduction to plant physiology. Van Nostrand Company, New York.

Minas, I. S., Tanou, G., Karagiannis, E., Belghazi, M. and Molassiotis, A. (2016). Coupling of physiological and proteomic analysis to understand the ethylene- and chilling-induced kiwifruit ripening syndrome. *Frontiers in Plant Science*, 7, 19.

Mitchell, F. G. (1990). Postharvest physiology and technology of kiwifruit. *Acta Horticulturae*, 282, 291 - 307.

Mitchell, F. G., Mayer, G. and Biasi, W. (1992). Effect of harvest maturity on storage performance of 'Hayward' kiwifruit. *Acta Horticulturae*, 297, 617-625.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405-410.

Miyamoto, K., Mitani, Y., Soga, K., Ueda, J., Wakabayashi, K., Hoson, T., . . . Masuda, Y. (1997). Modification of chemical properties of cell wall polysaccharides in the inner tissues by white light in relation to the decrease in tissue tension in *Pisum sativum* epicotyls. *Physiologia Plantarum*, 101(1), 38-44.

Mohammadi, A., Rafiee, S., Emam-Djomeh, Z. and Keyhani, A. (2008). Kinetic models for colour changes in kiwifruit slices during hot air drying. *World Journal of Agricultural Sciences*, 4(3), 376-383.

Muskovics, G., Felföldi, J., Kovács, E., Perlaki, R. and Kállay, T. (2006). Changes in physical properties during fruit ripening of Hungarian sweet cherry (*Prunus avium L.*) cultivars. *Postharvest Biology and Technology*, 40(1), 56-63.

Nakamura, R., Inaba, A. and Ito, A. (1985). Effect of cultivating conditions and postharvest stepwise cooling on the chilling sensitivity of eggplant and cucumber fruits. *Scientific Reports of the Faculty of Agriculture, Okayama University*, 66, 19-29.

Newman, R. H. and Redgwell, R. J. (2002). Cell wall changes in ripening kiwifruit: 13C solid state NMR characterisation of relatively rigid cell wall polymers. *Carbohydrate Polymers*, 49(2), 121-129.

Nishitani, K. and Masuda, Y. (1981). Auxin-induced changes in the cell wall structure: Changes in the sugar compositions, intrinsic viscosity and molecular weight distributions of matrix polysaccharides of the epicotyl cell wall of *Vigna angularis*. *Physiologia Plantarum*, 52(4), 482-494.

Nourian, F., Ramaswamy, H. S. and Kushalappa, A. C. (2003). Kinetics of quality change associated with potatoes stored at different temperatures. *LWT - Food Science and Technology*, 36(1), 49-65.

Nunan, K. J., Sims, I. M., Bacic, A., Robinson, S. P. and Fincher, G. B. (1998). Changes in cell wall composition during ripening of grape berries. *Plant Physiology*, 118(3), 783-792.

Nunes, M. C. N., Emond, J. P. and Brecht, J. K. (2003). Quality of strawberries as affected by temperature abuse during ground, in-flight and retail handling operations. *Acta Horticulturae*, 604, 239-246.

Nunes, M. C. N., Morais, A., Brecht, J. K., Sargent, S. A. and Bartz, J. A. (2005). Prompt cooling reduces incidence and severity of decay caused by *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry. *Horttechnology*, 15(1), 153-156.

O'Sullivan, J., Ferrua, M. J., Love, R., Verboven, P., Nicolaï, B. and East, A. (2016). Modelling the forced-air cooling mechanisms and performance of polylined horticultural produce. *Postharvest Biology and Technology*, 120, 23-35.

Oz, A. T. and Eris, A. (2010). Influence of harvest time maturity and storage condition on internal ethylene production rate of 'Hayward' kiwifruits. *Journal of Food Agriculture & Environment*, 8(3-4), 185-187.

Pantastico, E. B., Grierson, W. and Soule, J. (1968). Chilling injury in tropical fruits. I. Bananas (*Musa paradisiaca var. sapientum* cv. Lacatan). *Proceedings American Society for Horticultural Science, Tropical Region*, 1967, 11, 82-91.

Patterson, K. J. and Currie, M. B. (2011). Optimising kiwifruit vine performance for high productivity and superior fruit taste. *Acta Horticulturae*, 913, 257-268

Paull, R. E. (1999). Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biology and Technology*, 15(3), 263-277.

Paull, R. E., Gross, K. and Qiu, Y. (1999). Changes in papaya cell walls during fruit ripening. *Postharvest Biology and Technology*, 16(1), 79-89.

Pelletier, W., Brecht, J. K., Nunes, M. C. D. and Emond, J. P. (2011). Quality of strawberries shipped by truck from California to Florida as influenced by postharvest temperature management practices. *Horttechnology*, 21(4), 482-493.

Pennycook, S. R. (1985). Fungal fruit rots of *Actinidia deliciosa* (kiwifruit). *New Zealand Journal of Experimental Agriculture*, 13(4), 289-299.

Pennycook, S. R. and Manning, M. A. (1992). Picking wound curing to reduce *botrytis* storage rot of kiwifruit. *New Zealand Journal of Crop and Horticultural Science*, 20(3), 357-360.

Percy, A. E., Melton, L. D. and Jameson, P. E. (1997). Xyloglucan and hemicelluloses in the cell wall during apple fruit development and ripening. *Plant Science*, 125(1), 31-39.

Percy, A. E., O'Brien, I. E. W., Jameson, P. E., Melton, L. D., MacRae, E. A. and Redgwell, R. J. (1996). Xyloglucan endotransglycosylase activity during fruit development and ripening of apple and kiwifruit. *Physiologia Plantarum*, 96(1), 43-50.

Perry, K. B. and Wehner, T. C. (1996). A heat unit accumulation method for predicting cucumber harvest date. *Horttechnology*, 6(1), 27-30.

Pinheiro, J., Alegria, C., Abreu, M., Gonçalves, E. M. and Silva, C. L. M. (2013). Kinetics of changes in the physical quality parameters of fresh tomato fruits (*Solanum lycopersicum, cv.* 'Zinac') during storage. *Journal of Food Engineering*, 114(3), 338-345.

Pinto, J. A. V., Schorr, M. R. W., Thewes, F. R., Ceconi, D. L., Both, V., Brackmann, A. and Fronza, D. (2015). Relative humidity during cold storage on postharvest quality of 'Niagara Rosada' table grapes. *Ciencia Rural*, 45(3), 386-391.

Plattaloia, K. A. and Thomson, W. W. (1987). Freeze-fracture evidence for lateral phase separations in the plasmalemma of chilling-injured avovado fruit. *Protoplasma*, 136(2-3), 71-80.

Prasad, M. and Spiers, T. M. (1992). The effect of nutrition on the storage quality of kiwifruit (a review). *Acta Horticulturae*, 297, 579-585.

Prasanna, V., Prabha, T. N. and Tharanathan, R. N. (2007). Fruit ripening phenomena - An overview. *Critical Reviews in Food Science and Nutrition*, 47(1), 1-19.

Purgatto, E., Lajolo, F. M., do Nascimento, J. R. O. and Cordenunsi, B. R. (2001). Inhibition of beta-amylase activity, starch degradation and sucrose formation by indole-3-acetic acid during banana ripening. *Planta*, 212(5-6), 823-828.

Pyke, N. B., Hopkirk, G., Alspach, P. A. and Cooper, K. M. (1996). Variation in harvest and storage quality of fruit from different positions on kiwifruit vines. *New Zealand Journal of Crop and Horticultural Science,* 24(1), 39-46.

Quevedo, R., Diaz, O., Ronceros, B., Pedreschi, F. and Aguilera, J. M. (2009). Description of the kinetic enzymatic browning in banana (*Musa cavendish*) slices using non-uniform color information from digital images. *Food Research International*, 42(9), 1309-1314.

Redgwell, R. J., MacRae, E., Hallett, I., Fischer, M., Perry, J. and Harker, R. (1997). In vivo and in vitro swelling of cell walls during fruit ripening. *Planta*, 203(2), 162-173.

Redgwell, R. J., Melton, L. D. and Brasch, D. J. (1990). Cell wall changes in kiwifruit following post harvest ethylene treatment. *Phytochemistry*, 29(2), 399-407.

Redgwell, R. J., Melton, L. D. and Brasch, D. J. (1991). Cell-wall polysaccharides of kiwifruit (*Actinidia deliciosa*): Effect of ripening on the structural features of cell-wall materials. *Carbohydrate Research*, 209, 191-202.

Redgwell, R. J., Melton, L. D. and Brasch, D. J. (1992). Cell wall-wall dissolution in ripening kiwifruit (*Actindia-deliciosa*) - solubilization of the pectic polyers. *Plant Physiology*, 98(1), 71-81.

Ricardo, V. and James, G. H. (2007). Reaction kinetics in food system. In: Dennis, R. H. & Daryl, B. L. (Eds.), Handbook of Food Engineering, Taylor & Francis Group, 125-286.

Ritenour, M. A., Crisosto, C. H., Garner, D. T., Cheng, G. W. and Zoffoli, J. P. (1999). Temperature, length of cold storage and maturity influence the ripening rate of ethylene-preconditioned kiwifruit. *Postharvest Biology and Technology*, 15(2), 107-115.

Rodeo, A. J. D. and Esguerra, E. B. (2013). Low temperature conditioning alleviates chilling injury in mango (*Mangifera indica L.* cv. Carabao) fruit. *Philippine Journal of Crop Science*, 38(1), 24-32.

Rose, J. K. C., Lee, H. H. and Bennett, A. B. (1997). Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proceedings of the National Academy of Sciences of the United States of America,* 94(11), 5955-5960.

Roth, E., Hertog, M. L. A. T. M., Kovacs, E. and Nicolai, B. (2008). Modelling the enzymatic softening of apples in relation to cultivar, growing system, picking date and season. *International Journal of Food Science and Technology*, 43(4), 620-628.

Rushing, J. W. and Huber, D. J. (1984). Invitro characterization of tomato fruit softening - the use of enzymically active cell-walls. *Plant Physiology*, 75(4), 891-894.

Rushing, J. W. and Huber, D. J. (1990). Mobiliy limitations of bound polygalacturonase in isolated cell-wall from tomato pericarp tissue. *Journal of the American Society for Horticultural Science*, 115(1), 97-101.

Ryall, A. L. and Lipton, W. J. (1979). Handling, transportation and storage of fruits and vegetables. AVI Publishing, Westport CT.

Samarakoon, H. (2013). Ethylene flux in postharvest kiwifruit. Master Degree, Massey University.

Schackel, K. A., Greve, C., Labavitch, J. M. and Ahmadi, H. (1991). Cell turgor changes associated with ripening in tomato pericarp tissue. *Plant Physiology*, 97(2), 814-816.

Scheerlinck, N., Peirs, A., Desmet, M., Schenk, A. and Nicolai, B. M. (2004). Modelling fruit characteristics during apple maturation: A stochastic approach. *Mathematical and Computer Modelling of Dynamical Systems*, 10(2), 149-168.

Schotsmans, W. C., Mackay, B. and Mawson, A. J. (2008). Temperature kinetics of texture changes in *Actinidia chinensis* 'Hort16A' during storage. *Journal of Horticultural Science & Biotechnology*, 83(6), 760-764.

Schotsmans, W. C., Nicholson, S., MacKay, B. and Mawson, A. J. (2005). Temperature dependent texture changes in storage of Zespri$^{(TM)}$ gold. *Acta Horticulturae*, 674, 599-603.

Schouten, R. E., Jongbloed, G., Tijskens, L. M. M. and van Kooten, O. (2004). Batch variability and cultivar keeping quality of cucumber. *Postharvest Biology and Technology*, 32(3), 299-310.

Schouten, R. E., Tijskens, L. M. M. and van Kooten, O. (2002). Predicting keeping quality of batches of cucumber fruit based on a physiological mechanism. *Postharvest Biology and Technology*, 26(2), 209-220.

Schroder, R. and Atkinson, R. G. (2006). Kiwifruit cell walls: Towards an understanding of softening? *New Zealand Journal of Forestry Science*, 36(1), 112-129.

Seager, N. G., Hewett, E. W. and Warrington, I. J. (1995). Manipulation of carbohydrate concentrations in kiwifruit. *New Zealand Journal of Crop and Horticultural Science*, 23(2), 213-218.

Sfakiotakis, E., Chlioumis, G. and Gerasopoulos, D. (2005). Preharvest chilling reduces low temperature breakdown incidence of kiwifruit. *Postharvest Biology and Technology*, 38(2), 169-174.

Shalom, N. B., Hanzon, J., Klein, J. D. and Lurie, S. (1993a). A postharvest heat treatment inhibits cell wall degradation in apples during storage. *Phytochemistry*, 34(4), 955-958.

Shalom, N. B., Hanzon, J., Pinto, R. and Lurie, S. (1996). Cell wall changes and partial prevention of fruit softening in prestorage heat treated 'Anna' apples. *Journal of the Science of Food and Agriculture*, 72(2), 231-234.

Shcherban, T. Y., Shi, J., Durachko, D. M., Guiltinan, M. J., McQueenmason, S. J., Shieh, M. and Cosgrove, D. J. (1995). Molecular-cloning and sequence-analysis of expansins - A highly conserved, multigene family of proteins that mediate cell-wall extension in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 92(20), 9245-9249.

Smith, G. S., Gravett, I. M., Edwards, C. M., Curtis, J. P. and Buwalda, J. G. (1994). Spatial analysis of the canopy of kiwifruit vines as it relates to the physical, chemical and postharvest attributes of the fruit. *Annals of Botany*, 73(1), 99-111.

Snelgar, W. P., Hall, A. J., Ferguson, A. R. and Blattmann, P. (2005). Temperature influences growth and maturation of fruit on 'Hayward' kiwifruit vines. Functional *Plant Biology*, 32(7), 631-642.

Snelgar, W. P., Hopkirk, G. and McPherson, H. G. (1993). Predicting harvest date for kiwifruit: Variation of soluble solids concentration with mean temperature. *New Zealand Journal of Crop and Horticultural Science*, 21(4), 317-324.

Soda, I., Hasegawa, T., Suzuki, T. and Ogura, N. (1987). Change of polyuronides in kiwifruit during ripening. *Agricultural and Biological Chemistry*, 51(2), 581-582.

Stavroulakis, G. and Sfakiotakis, E. (1993). Regulation by temperature of propylene induced ethylene biosynthesis and ripening in 'Hayward' kiwifruit. *Cellular and Molecular Aspects of the Plant Hormone Ethylene*, 16, 142-143.

Sugimoto, Y., Yamamoto, M., Abe, K. and Fuwa, H. (1988). Developmental changes in properties of kiwifruit (*Actinidia chinensis*) starches. *Journal of the Japanese Society of Starch Science*, 35(1), 1-10.

Taglienti, A., Massantini, R., Botondi, R., Mencarelli, F. and Valentini, M. (2009). Postharvest structural changes of Hayward kiwifruit by means of magnetic resonance imaging spectroscopy. *Food Chemistry*, 114(4), 1583-1589.

Tanner, D., Kay, C. and Mowat, A. (2012). Segregation technologies - Are they ready? *New Zealand Kiwifruit Journal*, July/August, 15-18.

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R. and Guidi, L. (2009). *Polygalacturonase* and *β-galactosidase* activities in Hayward kiwifruit as affected by light exposure, maturity stage and storage time. *Scientia Horticulturae*, 120(3), 342-347.

Terasaki, S., Sakurai, N., Kuroki, S., Yamamoto, R. and Nevins, D. J. (2013). A new descriptive method for fruit firmness changes with various softening patterns of kiwifruit. *Postharvest Biology and Technology*, 86, 85-90.

Thornley, J. H. M. and France, J. (2007). Role of mathematical models. In: Thornley. J. H. M. & France, J. (Eds.), Mathematical Models in Agriculture: Quantitative Methods for the Pant, Animal and Ecological Sciences. CABI, Oxfordshire, 1-18.

Tijskens, L. M. M. and Evelo, R. G. (1994). Modelling colour of tomatoes during postharvest storage. *Postharvest Biology and Technology*, 4(1-2), 85-98.

Tijskens, L. M. M., Heuvelink, E., Schouten, R. E., Lana, M. M. and van Kooten, O. (2005). The biological shift factor: Biological age as a tool for modelling in pre- and postharvest horticulture. *Acta Horticulturae*, 687, 39-46.

Tijskens, L. M. M., Konopacki, P. and Simcic, M. (2003). Biological variance, burden or benefit? *Postharvest Biology and Technology*, 27(1), 15-25.

Tijskens, L. M. M., Rodis, P. S., Hertog, M. L. A. T. M., Kalantzi, U. and van Dijk, C. (1998). Kinetics of polygalacturonase activity and firmness of peaches during storage. *Journal of Food Engineering*, 35(1), 111-126.

Tijskens, L. M. M., Unuk, T., Tojnko, S., Hribar, J. and Simčič, M. (2009). Biological variation in the colour development of Golden Delicious apples in the orchard. *Journal of the Science of Food and Agriculture*, 89(12), 2045-2051.

Tijskens, L. M. M., Vanoli, M., Dagar, A., Weksler, A., Lovati, F., Zerbini, P. E., . . . Lurie, S. (2012). Analysis and modelling of firmness and juiciness of peaches, subjected to chilling injury. *Acta Horticulturae*, 940, 439-445.

Tombesi, A., Antognozzi, E. and Palliotti, A. (1993). Influence of light exposure on characteristics and storage life of kiwifruit. *New Zealand Journal of Crop and Horticultural Science*, 21(1), 87-90.

Tomos, A. D., Leigh, R. A., Palta, J. A. and Willliams, J. H. H. (1992). Sucrose and cell water relations. In: Pollock, C. J., Farrar, J. F. & Gordon, A. J. (Eds), Carbon Partitioning Within and Between Organisms. BIOS Scientific Publishers, Oxford, 79-89.

Trejo Araya, X., Verboven, P., East, A. R., Cantre, D., Herremans, E., Nicolaï, B., . . . Heyes, J. (2013). Exploring kiwifruit surface characteristics using X-ray micro computed tomography. *Proceedings of the InsideFood Symposium*, Leuven, Belgium..

Tsuji, M., Harakawa, M. and Komiyama, Y. (1984). Changes in shelf life and quality of plum fruit during storage at high temperatures. *Journal of the Japanese Society for Horticultural Science*, 52(4), 469-475.

Van Buren, J. P. (1979). The chemistry of texture in fruit and vegetables. *Journal of Texture Studies*, 10(1), 1-23.

Van Den Dungen, R., Hekkert, S. T., Cristescu, S. M. and Harren, F. J. M. (2011). Highly sensitive ethylene detector for on-line measurements on kiwifruits. *Acta Horticulturae*, 913, 651-656.

Van Dijk, C., Boeriu, C., Peter, F., Stolle-Smits, T. and Tijskens, L. M. M. (2006a). The firmness of stored tomatoes (cv. Tradiro). 1. Kinetic and near infrared models to describe firmness and moisture loss. *Journal of Food Engineering*, 77(3), 575-584.

Van Dijk, C., Boeriu, C., Stolle-Smits, T. and Tijskens, L. M. M. (2006b). The firmness of stored tomatoes (cv. Tradiro). 2. Kinetic and Near Infrared models to describe pectin degrading enzymes and firmness loss. *Journal of Food Engineering*, 77(3), 585-593.

Van Pham, T., McConchie, R., Morris, S., Tanner, D. and Herbert, R. (2008). Prediction of firmness change in "Cripps Pink" apple during storage. *Acta Horticulturae*, 803, 319-325.

Von Lesecke, H. W. (1950). Bananas (2nd ed.). Wiley Interscience, New York.

Vonmollendorff, L. J., Jacobs, G. and Devilliers, O. T. (1992). Cold-storage influences internal characteristics of nectarines during ripening. *Hortscience*, 27(12), 1295-1297.

Wada, H., Shackel, K. and Matthews, M. (2008). Fruit ripening in *Vitis vinifera*: apoplastic solute accumulation accounts for pre-veraison turgor loss in berries. *Planta*, 227(6), 1351-1361.

Walton, E. F. and Dejong, T. M. (1990). Growth and compositional changes in kiwifruit berries from 3 californian locations. *Annals of Botany*, 66(3), 285-298.

Wang, C. Y. (1994). Chilling injury of tropical horticultural commodities. *Hortscience*, 29(9), 986-988.

Wang, C. Y. and Baker, J. E. (1979). Effects of 2 free-radical scavengers and intermittent warming on chilling and polar lipid-composition of cucumber and sweet pepper fruits. *Plant and Cell Physiology*, 20(1), 243-251.

Wegrzyn, T. F. and Macrae, E. A. (1992). Pectinesterase, polygalacturonase, and beta-galactosidase during softning of ethylene-treated kiwifruit. *Hortscience*, 27(8), 900-902.

Weibel, E. R. and Gomez, D. M. (1962). A principle for counting tissue structures on random sections. *Journal of Applied Physiology*, 17(2), 343-348.

White, A., de Silva, N., Requejo-Tapia, C. and Harker, F. R. (2005). Evaluation of softening characteristics of fruit from 14 species of *Actinidia. Postharvest Biology and Technology*, 35(2), 143-151.

Wills, R. B. H., Warton, M. A., Mussa, D. and Chew, L. P. (2001). Ripening of climacteric fruits initiated at low ethylene levels. *Australian Journal of Experimental Agriculture*, 41(1), 89-92.

Woodward, T. J. and Clearwater, M. J. (2011). Spatial variation in 'Hayward' kiwifruit dry matter content within a growing region across seasons. *New Zealand Journal of Crop and Horticultural Science*, 39(4), 241-249.

Woolf, A. B., Cox, K. A., White, A. and Ferguson, I. B. (2003). Low temperature conditioning treatments reduce external chilling injury of 'Hass' avocados. *Postharvest Biology and Technology*, 28(1), 113-122.

Wright, H. B. and Heatherbell, D. A. (1967). A study of respiratory trends and some associated physio-chemical changes of chinese gooseberry fruit *Actinidia chinensis* (Yang-tao) during the later stages of development. *New Zealand Journal of Agricultural Research*, 10(3-4), 405-414.

Wu, G. X., Zhou, H. W. and Wang, J. M. (1992). Biochemical mechanism and substances determination of enzymic browning of Yali pear (*Pyrus bretschneideri Rehd.*). *Acta Horticulturae Sinica*, 19(3), 198-202.

Xu, W. P., Wang, L., Yang, Q., Wei, Y. H., Zhang, C. X. and Wang, S. P. (2015). Effect of calcium and boron on the quality of kiwifruit. *Acta Horticulturae*, 1096, 317-320.

Yan, S., Li, L., He, L., Liang, L. and Li, X. (2013). Maturity and cooling rate affects browning, polyphenol oxidase activity and gene expression of 'Yali' pears during storage. *Postharvest Biology and Technology*, 85, 39-44.

Yang, H., Wu, F. and Cheng, J. (2011). Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. *Food Chemistry*, 127(3), 1237-1242.

Yang, Q., Rao, J., Yi, S., Meng, K., Wu, J. and Hou, Y. (2012). Antioxidant enzyme activity and chilling injury during low-temperature storage of kiwifruit cv. Hongyang exposed to gradual postharvest cooling. *Horticulture, Environment, and Biotechnology*, 53(6), 505-512.

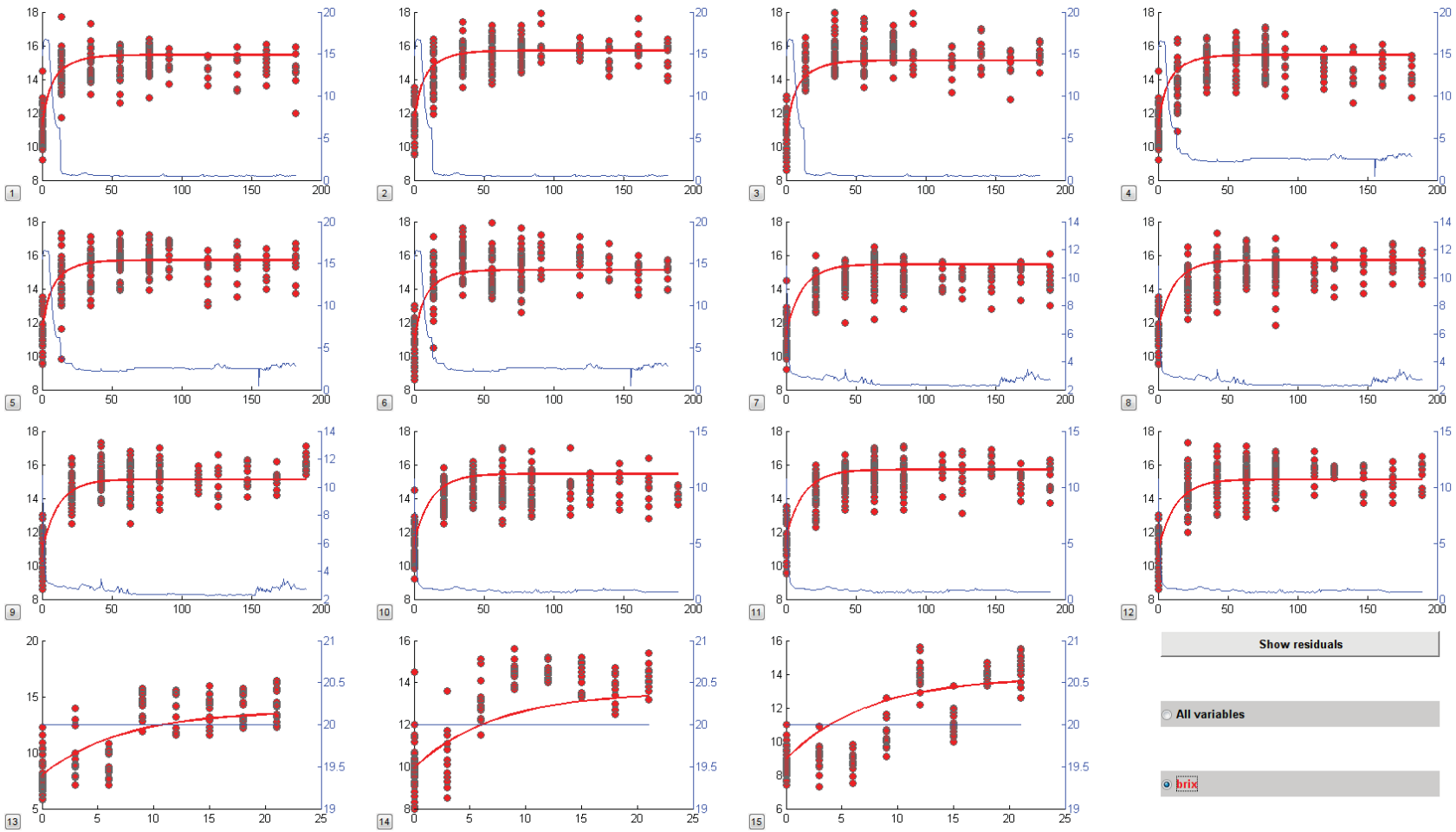
Yang, Q. Z., Zhang, Z. K., Rao, J. P., Wang, Y. P., Sun, Z. Y., Ma, Q. S. and Dong, X. Q. (2013). Low-temperature conditioning induces chilling tolerance in 'Hayward' kiwifruit by enhancing antioxidant enzyme activity and regulating en-dogenous hormones levels. *Journal of the Science of Food and Agriculture*, 93(15), 3691-3699.

Zhang, A. D., Hu, X., Kuang, S., Ge, H., Yin, X. R. and Chen, K. S. (2016). Isolation, classification and transcription profiles of the Ethylene Response Factors (ERFs) in ripening kiwifruit. *Scientia Horticulturae*, 199, 209-215.

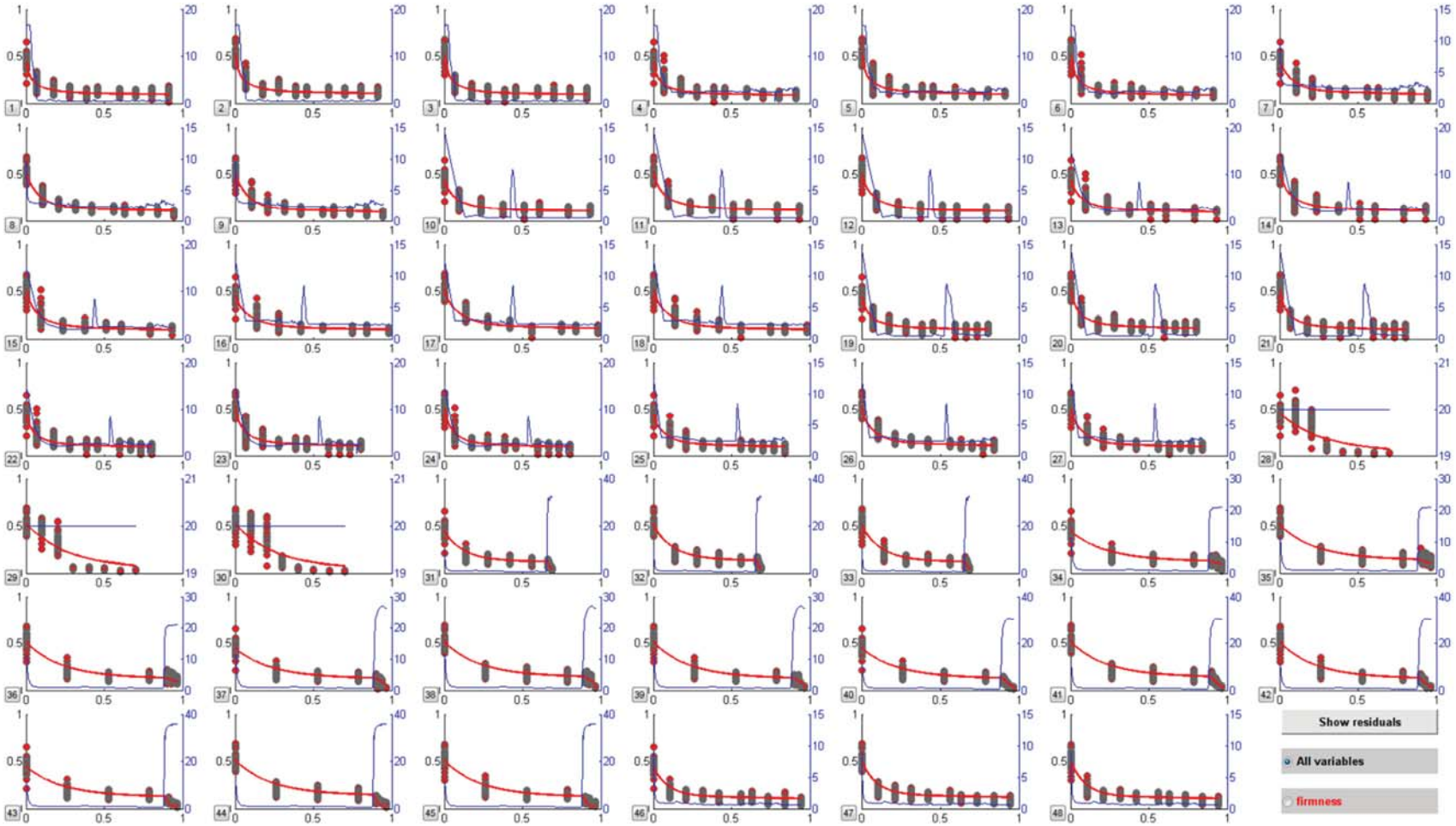
Appendices

At-harvest attributes	Factors	DF	Seq SS	Adj SS	Adj MS	F	P
Dry matter (D_{m0}), %	Fruit maturity	2	19.14	19.14	9.57	6.41	0.002
	Grower lines	2	116.81	124.00	62.00	41.54	0.000
	Fruit maturity x Grower lines	4	15.81	15.81	3.95	2.65	0.035
	Error	201	300.00	300.00	1.49		
	Total	209	451.76				
Soluble solids content (B_0), °Brix	Fruit maturity	2	1094.53	1094.53	547.26	378.86	0.000
	Grower lines	2	35.10	34.71	17.35	12.01	0.000
	Fruit maturity x Grower lines	4	8.85	8.85	2.21	1.53	0.194
	Error	201	290.34	290.34	1.44		
	Total	209	1428.82				
Fruit firmness (F_0), N	Fruit maturity	2	28250.90	28250.90	14125.50	219.84	0.000
	Grower lines	2	1716.00	1716.00	858.00	13.35	0.000
	Fruit maturity x Grower lines	4	1040.50	1040.50	260.10	4.05	0.003
	Error	201	16770.20	16770.20	64.30		
	Total	209	4777.60				

Appendix 1: ANOVA table of fruit maturity, grower lines and interaction effect on at-harvest attributes (dry matter content, soluble solids content and fruit firmness).



Appendix 2: Experimental data (red dots) and modelled (red solid line) soluble solids content of fruit (°Brix). Fruit were exposed to various temperature conditions. The blue solid lines represent the time temperature information of respective storage conditions.



Appendix 3: Experimental data (red dots) and modelled (red solid line) fruit firmness of kiwifruit (kgf). Fruit were exposed to various temperature conditions. The blue solid lines represent the time temperature information of respective storage conditions.

Appendix 4: Model formulation used to generate kiwifruit softening curves in Chapter 6. The codes were run using Matlab R2011b to simulate the softening curves based on the respective model inputs.

```
function odes=KiwiFun(t,D)

global kpo Ep kso Es Tref TempData
S=D(1);
bP=D(2);

i=find(TempData(:,1)>t,1);
T=TempData(i-1,2)+(TempData(i,2)-TempData(i-1,2))*(t-TempData(i-1,1))/(TempData(i,1)-
TempData(i-1,1));

ks=kso*exp(Es/8.314*(1/(Tref+273.15)-1/(T+273.15)));
kp=kpo*exp(Ep/8.314*(1/(Tref+273.15)-1/(T+273.15)));

odes=zeros(2,1);
odes(1)=-ks*S;
odes(2)=-kp*Fb;

global kpo Ep kso Es Tref TempData

kwo=0.07;
Ea,w=106850;
kso=0.14;
Ea,s=20105;
Tref=20;

TempData=xlsread('EgTempData0deg3d.xlsx','sheet1','A2:B1000');

a=0.76;
Ffix=0.1;

for n=1;
Fo=4.51;

Bo=11.3;

Dmo=18.2;

Be=-3.755+(1.057*Dmo);
So=(Be-Bo);

Fbo= Fi-a*So-c;

[t,D]=ode45('KiwiFun',[0:0.5:189],[So,Fbo]);
S(:,n)=D(:,1);
Fb(:,n)=D(:,2);
F=a*S(:,n)+Fb(:,n)+Ffix;
End
```

At-harvest attributes	Factors	DF	Seq SS	Adj SS	Adj MS	F	P
Dry matter (D_{m0}), %	Fruit maturity	2	19.14	19.14	9.57	6.41	0.002
	Grower lines	2	116.81	124.00	62.00	41.54	0.000
	Fruit maturity x Grower lines	4	15.81	15.81	3.95	2.65	0.035
	Error	201	300.00	300.00	1.49		
	Total	209	451.76				
Soluble solids content (B_0), °Brix	Fruit maturity	2	1094.53	1094.53	547.26	378.86	0.000
	Grower lines	2	35.10	34.71	17.35	12.01	0.000
	Fruit maturity x Grower lines	4	8.85	8.85	2.21	1.53	0.194
	Error	201	290.34	290.34	1.44		
	Total	209	1428.82				
Fruit firmness (F_0), N	Fruit maturity	2	28250.90	28250.90	14125.50	219.84	0.000
	Grower lines	2	1716.00	1716.00	858.00	13.35	0.000
	Fruit maturity x Grower lines	4	1040.50	1040.50	260.10	4.05	0.003
	Error	201	16770.20	16770.20	64.30		
	Total	209	4777.60				

Appendix 5: ANOVA table of fruit maturity, grower lines and interaction effect on at-harvest attributes (dry matter content, soluble solids content and fruit firmness).

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Zhao Junyu, Matthew

Name/Title of Principal Supervisor: Dr Andrew East

Name of Published Research Output and full reference:

1. Zhao, J. M., Bronlund, J. E. and East, A. R. (2015). Effect of Cooling Rate on Kiwifruit Firmness and Rot Incidence in Subsequent Storage. Acta Horticulturae, 1079, 313-318
2. East, A., Zhao, M., Jabbar, A., Samarakoon, H., Bollen, F., Adkins, M., Bronlund, J. Heyes, J. (2016). Why is predicting kiwifruit quality in the cool chain so difficult? Paper presented at the 4th IIR conference on sustainability and the cold chain, Auckland, New Zealand.

In which Chapter is the Published Work: Included in chapter 3 and 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  For first publication, the main author did experimental setup, data collection, analysis and writing the report. The co-authors edited the manuscripts and provided valuable suggestions while designing and conducting the work. The second publication, the candidate did experimental setup and data collection. The chief supervisor subsequently summarized this work and combined with other student and industry work to present an overview of his entire kiwifruit research programme.

Candidate's Signature — Date: 29/8/2016.

Principal Supervisor's signature — Date: 24/8/2016

Effect of Cooling Rate on Kiwifruit Firmness and Rot Incidence in Subsequent Storage

J.M. Zhao, J.E. Bronlund and A.R. East
Centre for Postharvest and Refrigeration Research
Massey University
Palmerston North
New Zealand

Keywords: *Actinidia deliciosa*, 'Hayward', direct cooling, gradual cooling, softening

Abstract

Temperature management is critical in maintaining quality and extending market life of 'Hayward' kiwifruit in the supply chain. In industry, harvested kiwifruit are pre-cooled using the forced draft cooling method to remove field heat. Removal of field heat reduces the rate of deterioration as fruit metabolism and growth of spoilage microorganisms are both lowered. Usually, rapid cooling is recommended to extend fruit storability and quality. This study investigates the change in kiwifruit firmness during storage as influenced by cooling profile to the storage temperature. Commercially produced 'Hayward' kiwifruit (*Actinidia deliciosa*) were either directly cooled (3 d) or gradually cooled (2 weeks) to storage temperature of 0°C and subsequently stored for up to 25 weeks. Fruit firmness and respiration rate measurements were conducted every 2 to 3 weeks across the storage period. Fruit that were cooled in 3 d had higher proportion of unmarketable fruit after 120 d of storage in comparison to fruit that were cooled in 2 weeks. After 40 d of storage, fruit cooled in 3 d displayed a more rapid average softening rate. An increase in respiration rate was observed in rapidly cooled fruit after 120 d, possibly due to the stress caused by chilling injury. Higher incidence of rotten fruit was found in fruit that were cooled in 2 weeks. This study suggests that gradual cooling allows kiwifruit to acclimatise to future low temperature storage conditions, implying that rapid cooling to storage temperature may not essentially improve the marketable life. On the other hand, gradual cooling increases the risk of rot development, possibly enabling the spoilage microorganism to advanced development initially. Controlled gradual cooling of fruit is impractical with the large harvest volumes of industry. Hence, there is a need to find an optimal cooling rate which removes field heat as applicable to industry.

INTRODUCTION

Kiwifruit (*Actinidia deliciosa* 'Hayward') are harvested at a minimum soluble solid content of 6.2% in New Zealand (Mitchell, 1990). The optimum storage conditions to store kiwifruit are 0°C and 95% relative humidity, resulting in a 4 to 6 months storage life (McDonald, 1990). Fruit firmness is used as a primary indication of kiwifruit quality. During ripening, firmness decreases until it reaches optimal eating range of 6 to 10 N and continues to reduce below the optimal eating range becoming too soft for consumption (McDonald, 1990). Kiwifruit is also susceptible to decay by *Botrytis cinerea*. Usually, *B. cinerea* attacks the stem end of unwounded fruit and slowly moves toward the distal end (Michailides and Elmer, 2000). Excessive softening and rotten fruit lead to fruit losses and additional labour cost for sorting and re-packing in industry.

Ripening and senescence can be retarded by removing the field heat of harvested produce and thus maintaining good keeping quality of fruits and vegetables. Cooling kiwifruit within 24 to 48 h after harvest is recommended to maintain optimum quality and storage life (McDonald, 1990). Precooling is a process applied to rapidly cool fruit before storage, shipping, or processing. Forced air cooling is an effective method in removing heat rapidly by passing cold air through packed and palletised fruit by establishing a pressure drop (Findlay and Combrink, 1996). This method is commonly used in industry

to cool fruit quickly when the storage rooms do not have sufficient cooling capacity and/or uniform cooling is restricted due to the type of packaging (Burdon and Lallu, 2011). Previously, kiwifruit precooled within 9 to 12 h to storage temperature were found to be firmer, but with higher incidence of rots and chilling injury than in passively cooled kiwifruit (Lallu and Webb, 1997).

Kiwifruit softening generally consists of three distinct softening phases. An initial lag phase is followed by a rapid decline and later gradual softening phase towards a lower asymptote (Jabbar et al., 2014). In addition, Benge et al. (1997) proposed that the rate of softening may increase again during the gradual softening phase. White et al. (2005) suggested that the initial lag phase is correlated to the time taken to become fully ripen, and thus affected by the fruit maturity.

The objective of this work was to evaluate the storability of kiwifruit as influenced by the rate of cooling to storage temperature. Understanding the role of the cooling profile on the subsequent storage performance will aid in the development of a predictive model for kiwifruit quality during storage given time-temperature information.

MATERIALS AND METHODS

Commercially produced 'Hayward' kiwifruit from the Bay of Plenty region were harvested in late May 2012 from 3 different growers. Fruit were commercially graded with count 36 size delivered to Massey University, Palmerston North in modular bulk boxes and subsequently randomly packed into single layer trays with polyliners. Care was taken to separate growers as each grower was used as a replicate. Fruit were cooled using two different cooling methods, direct or gradual cooling (Fig. 1). Direct cooling was achieved by placing the trays of fruit into a cold room set at 0°C and 95±5% RH. Gradual cooling was achieved by placing the trays of fruit into a cold room with decreasing set point temperature from 16 to 0°C over a period of 2 weeks. The fruit were subsequently stored in a cold room at 0°C and 95±5% RH for 25 weeks. Firmness and respiration rate measurements were conducted at intervals of 2 to 3 weeks across the storage period.

Fruit Firmness Measurement

Kiwifruit were equilibrated to 20°C before measurements. A QALink penetrometer (Willowbank Electronics Ltd., Napier, New Zealand), fitted with a standard 7.9 mm round Effegi probe and integrated to a computer was used. A 2-mm slice of skin was removed from an equatorial region before measurement. The probe was set to penetrate the flesh to a depth of 8 mm at 20 mms^{-1} with the minimum measurement being 1 N. Two measurement locations perpendicular to each other were used for each fruit. Firmness readings were calculated based on an average of 36 fruit.

Respiration Measurement

Individual kiwifruit were placed in a 500-ml sealed glass jar. Carbon dioxide concentrations were measured upon sealing and after 3 h at 0°C. Headspace gas was sampled using a 1-ml syringe and injected to a carbon dioxide transducer (Analytical Development Company, Hoddesdon, UK) which was interfaced to an integrator (HP3396A, Hewlett Packard, USA). The respiration rate was calculated based on the accumulation of carbon dioxide concentration over time considering the fruit weight and remaining free volume of the jar. The respiration rate determined was an average of 10 individual fruit.

Decay Incidence

Decay incidence was assessed visually by inspecting for symptoms of rots which developed on the side or stem end. The incidence of decay was calculated as a percentage of the total fruit population (36 fruit per grower).

Statistical Analysis

The experiments were conducted using a complete random design, with each

grower line representing a replicate. All statistical analysis was performed using Minitab (v15, Minitab Inc, USA). Data were subjected to a General Linear Model, with cooling method, grower and storage time as fixed factors. Comparison of means was undertaken using Tukey's test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Kiwifruit ripening consists of phases of softening, an initial lag phase, a secondary rapid phase and a long gradual final phase (White et al., 2005). The rate of cooling of kiwifruit to storage temperature had a significant effect on the subsequent fruit firmness in storage ($p<0.05$). Direct cooling of kiwifruit to storage temperature resulted in a more rapid decrease in fruit firmness in the long gradual softening phase, after 40 d of storage (Fig. 2A). This more rapid softening was not observed in fruit that were gradual cooled, maintaining the firmness at about 10 N after 120 d of storage. Previously, precooling of kiwifruit using forced air cooling resulted in firmer fruit during the first 70 to 105 d of storage compared to passive cooling (Lallu and Webb, 1997). In this work, there was no distinct difference in firmness of fruit that were either directly or gradually cooled before 120 d of storage (Fig. 2A).

A possible reason for better storability in gradual cooled fruit is that slow cooling allows fruit to build resistance against cold temperature, similar to temperature conditioning. The more rapid softening in directly cooled fruit may be a result of the development of chilling injury. Previously, Lallu (1997) observed that shorter cooling time to storage temperature resulted in higher incidence of chilling injured fruit while Yang et al. (2013) found that kiwifruit kept at 12°C for 3 days before storage at 0°C was observed to result in firmer fruit from 80 d of storage in comparison to directly cooled fruit. Gradual cooling of kiwifruit to storage temperature may act as a period of acclimatisation, and thus enable fruit resistance to low temperature (Burdon and Lallu, 2011). Similarly, quality of 'Hass' avocado can be improved by applying temperature conditioning at 6 or 8°C for 3 to 5 days prior to storage (Woolf et al., 2003).

The New Zealand kiwifruit industry standard requires fruit to be not less than 10 N at time of export. Direct cooling resulted in a higher percentage of fruit with firmness less than 10 N after 120 d (Fig. 2B). Presentation of the firmness data in this format further suggests that direct cooling of kiwifruit to storage temperature does not improve the long term market life of kiwifruit.

Respiration rate can be used to indicate the fruit metabolic activity. Directly cooled fruit had a higher respiration rate compared to gradually cooled fruit during storage (Fig. 3A). There is an increase in respiration rate after 120 d of storage which corresponds to the time when directly cooled fruit become softer than gradually cooled fruit. It is unknown if the increase in respiration rate observed after 120 d is a cause or effect of the more rapid softening observed towards the end of storage for the directly cooled fruit. This result may suggest that the fruit were undergoing chilling stress as fruit respiratory response can be altered when under chilling stress. Previously Macrae (1987) found that the respiration rate of chilling injured was higher than non-chilling injured fruit.

Although directly cooled fruit results in more rapid softening in the slow softening phase, the incidence of decay was lower compared to gradually cooled fruit. Significant incidence of decay was only observed for directly cooled fruit at the end of storage (Fig. 3B). Similarly, the incidence of fruit decay in strawberry was found to be reduced with prompt cooling (Nunes et al., 2005). The growth of pathogens that cause rots on fresh produce is temperature dependent, and thus it is recommended to store fresh produces at low temperature to reduce development. It is possible that the higher incidence of rotten fruit in gradually cooled fruit is a result of the delay in cooling allowing the pathogen to advance in development during this time. However, the longer cooling period of gradually cooled fruit does not always lead to more rot development, and in fact may allow fruit to build resistance against decay. Pennycook and Manning (1992) showed that a delay in packing and cooling of kiwifruit for up to 7 d resulted in a lower incidence of subsequent rots.

While results for gradual cooling in this work suggest potential for long storage life of kiwifruit, application of the applied cooling profile to industry is challenging. The industry handles a large volume of fruit over a 2 month harvesting window, and thus a controlled gradual cooling is impractical. Subsequently more work is required to develop an industry relevant cooling method which enables firmer fruit after long storage periods whilst also achieving a low incidence of rotten fruit.

CONCLUSIONS

The time taken to cool kiwifruit to storage temperature influenced fruit firmness during the gradual softening phase and incidence of rotten fruit during storage. Gradual cooling of kiwifruit to storage temperature resulted in firmer fruit towards the end of storage with higher incidence of rotten fruit throughout storage. As controlled gradual cooling of fruit is impractical with the large harvest volumes of industry further work is required to explore the optimal cooling rate which removes field heat whilst resulting in firm kiwifruit with low rot incidence.

ACKNOWLEDGEMENTS

This work is an output of the Food Structure Design Theme of Fonterra/Zespri™ Primary Growth Partnership research project funded by the Ministry of Primary Industries, New Zealand.

Literature Cited

Benge, J.R., De Silva, H.N., Banks, N.H. and Jeffery, P.B. 2000. Empirical modelling of postharvest changes in the firmness of kiwifruit. Postharvest Biol. Technol. 19:211-220.

Burdon, J. and Lallu, N. 2011. Kiwifruit (*Actinidia* spp.). p.326-360. In: E.M. Yahia (eds.), Postharvest Biology and Technology of Tropical and Subtropical Fruits, Vol. 3, Cocona to mango. Woodhead, Cambridge.

Findlay, J.S. and Combrink, J.C. 1996. Cold chain management. p.79-90. In: J.C. Combrink (eds.), Integrated Management of Post-Harvest Quality, Paarl Printing, Paarl.

Jabbar, A., East, A., Jones, G., Tanner, D. and Heyes, J. 2014. Modelling batch variability in softening of 'Hayward' kiwifruit from at-harvest maturity measures. Postharvest Biol. Technol. 90:7-14.

Lallu, N. and Webb, D.J. 1997. Physiological and economic analysis of precooling kiwifruit. Acta Hort. 444:691-698.

Macrae, E.A. 1987. Development of chilling injury in New Zealand grown 'Fuyu' persimmon during storage. New Zeal. J. Exp. Agri. 15:333-344.

McDonald, B. 1990. Pre-cooling, storage, and transport of kiwifruit. p.429-459. In: I.J. Warrington and G.C. Weston (eds.), Kiwifruit: Science and Management, Ray Richard, New Zealand.

Michailides, T.J. and Elmer, P.A.G. 2000. Botrytis gray mold of kiwifruit caused by *Botrytis cinerea* in the United States and New Zealand. Plant Dis. 84:208-223.

Mitchell, F.G. 1990. Postharvest physiology and technology of kiwifruit. Acta Hort. 282:291-307.

Nunes, M.C.N., Morais, A., Brecht, J.K., Sargent, S.A. and Bartz, J.A. 2005. Prompt cooling reduces incidence and severity of decay caused by *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry. HortTechnology 15:153-156.

Pennycook, S.R. and Manning, M.A. 1992. Picking wound curing to reduce Botrytis storage rot of kiwifruit. New Zeal. J. Crop Hort. 20:357-360.

White, A., De Silva, N., Requejo-Tapia, C. and Harker, F.R. 2005. Evaluation of softening characteristics of fruit from 14 species of *Actinidia*. Postharvest Biol. Technol. 35:143-151.

Woolf, A.B., Cox, K.A., White, A. and Ferguson, I.B. 2003. Low temperature conditioning treatments reduce external chilling injury of 'Hass' avocados.

Postharvest Biol. Technol. 28:113-122.
Yang, Q.Z., Zhang, Z.K., Rao, J.P., Wang, Y.P., Sun, Z.Y. and Ma, Q.S. 2013. Low-temperature conditioning induces chilling tolerance in 'Hayward' kiwifruit by enhancing antioxidant enzyme activity and regulating endogenous hormones levels. J. Sci. Food Agric. 93:3691-3699.

Figures

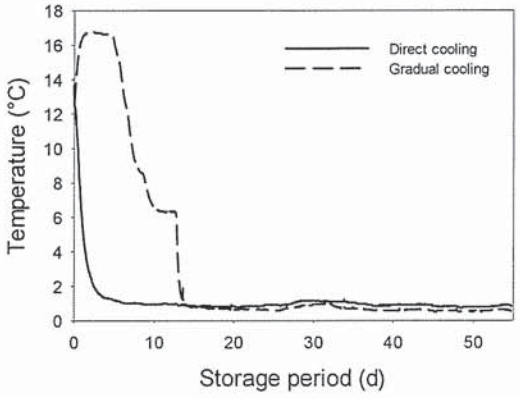


Fig. 1. Time temperature profile of direct and gradual cooling treatments respectively. The temperature data represents the temperature of the air within a single tray.

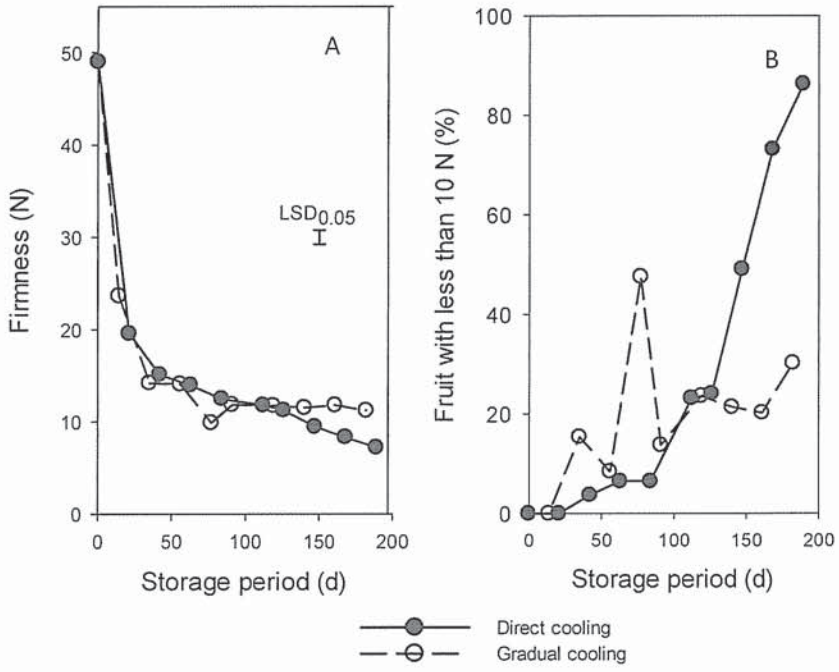


Fig. 2. Effect of cooling profile on softening (A) and percentage of fruit with firmness of less than 10 N (B) of 'Hayward' kiwifruit during storage. The data set points represent 3 replicate growers of 36 fruit and hence for (A) n=108 and (B) n=3.

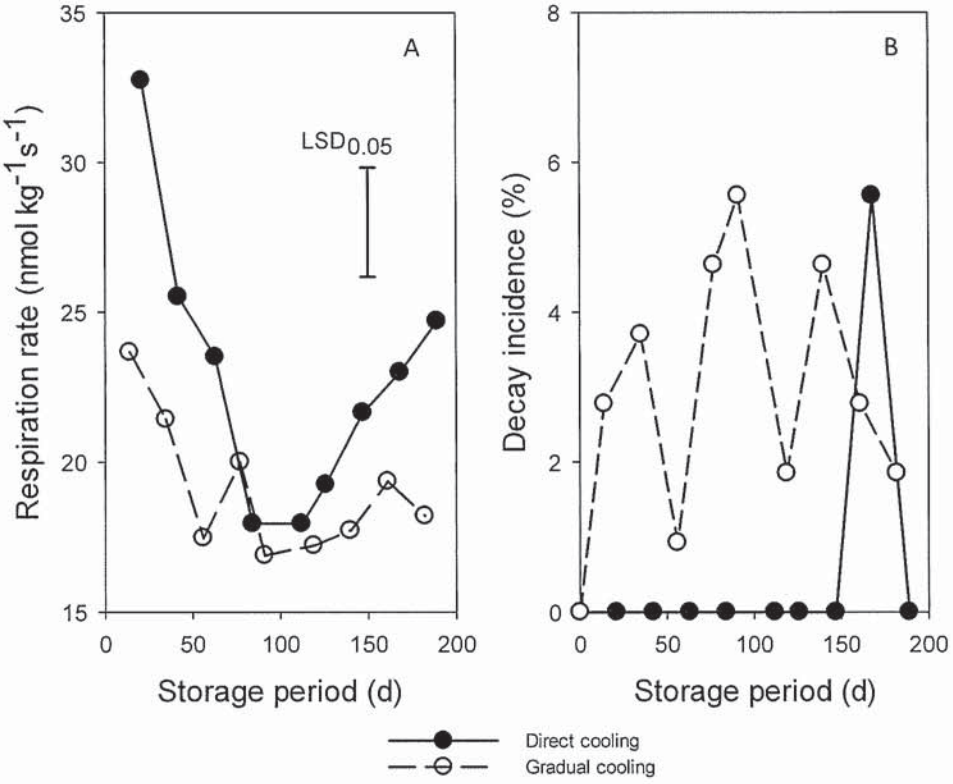


Fig. 3. Effect of cooling profile on fruit respiration rate (A) and decay incidence (B) during storage. Respiration rate was measured at storage temperature (0°C) and each data point represents the mean of 3 grower replicates of 10 fruit per grower (n=30). Decay incidence data represent the percentage of rotten fruit found across 3 replicates of 36 fruit per grower line (n=3).

WHY IS PREDICTING KIWIFRUIT QUALITY IN THE COOL CHAIN SO DIFFICULT?

Andrew East[(a)], Matthew Zhao[(a)], Abdul Jabbar[(a)], Himani Samarakoon[(a)], Frank Bollen[(b)], Matt Adkins[(b)], John Bronlund[(a)], Julian Heyes[(a)].

[(a)]Centre for Postharvest and Refrigeration Research, Massey University, Palmerston North, New Zealand. a.r.east@massey.ac.nz
[(b)]Zespri International, Mt Maunganui, New Zealand. matt.adkins@zespri.com

ABSTRACT

For seasonal produce such as kiwifruit, to enable all year supply a considerable portion of the stock is stored. For kiwifruit, despite best efforts a proportion of this stock's quality diminishes to a point of being unsalable resulting in fruit losses. In theory these quality changes in the cool chain should be predictable given the knowledge of the at-harvest condition and time-temperature history of the stock, and applying the time-temperature-tolerance hypothesis. Unfortunately this has not proven to be the case with a number of factors contributing to proliferation of variability in the cool chain. This paper discusses evidential data of the additional prediction complications introduced by grower line differences, cooling rate, storage temperature and ethylene. Only when each of these effects can be qualitatively measured and described will Hayward kiwifruit quality be able to be adequately predicted and hence industry storage losses reduced.

Keywords: *Actinidia deliciosa*, storability, fruit losses, ethylene.

1. INTRODUCTION

The New Zealand kiwifruit industry exports approximately $1B worth of stock annually represented as approximately 100 million trays of kiwifruit. The vast majority of this industry remains the "traditional" brown furry skinned, green fleshed, 'Hayward' (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson) cultivar. The harvest season for this cultivar extends from mid April to mid June, with the vast majority of the fruit harvested in the month of May. Fruit are harvested at a mature (> 6.5 °brix) yet inedible (> 40 N) firmness, which enables robustness during subsequent handling and packaging procedures.

During the harvest season the majority of fruit are graded and packed into corrugated fibreboard packaging with internal polyethylene polyliners, while a portion of fruit are stored in harvest bins (either wooden or plastic) either in air or controlled atmosphere (2% O_2, 2% CO_2) conditions. Stocks of fruit remain instore in New Zealand prior to shipping to global markets over the subsequent 6 month period or are supplied to the local market (Australia and New Zealand) over an even longer period of time. During this storage phase of the supply chain, a proportion of fruit which was graded as first class at the time of harvest inevitably reduces quality to a point where it becomes unacceptable for marketing due to any of decay, shrivel or chilling injury development or in the case of access to export markets, excessive softening (< 10 N). These losses tend to be minor during the first 3 months of storage but can rapidly accelerate beyond 4 months when approximately 30% of the stock remains in storage.

These losses represent a significant financial risk to kiwifruit postharvest operations and growers as clearly fruit that falls below quality standards becomes fruit that is not sold. Additional costs are incurred in managing these losses through resorting for recovery of acceptable product and repacking operations. Clearly as losses accelerate when 70% of the stock has already been sold, one approach is to predictively identify poor storing stock and prioritise dispatch of this stock before its "use-by" date. In order to achieve this, a method to predict quality later in the storage period at the time of harvest is

required. Over the past 5 years Massey University as supported by Zespri International has conducted a series of research projects with the overarching aim of being able to predict storability of 'Hayward' kiwifruit. Unfortunately, no "magic bullet" processes have been found, but in the process of this work, we have elucidated why defining storage life of Hayward kiwifruit, especially with respects to softening and chilling injury development is such a difficult task. Grower line differences, cooling profiles, temperature control in storage and ethylene in the environment all play a crucial role in exacerbating the variability within the system. The influences of each of these factors are discussed in detail in the remainder of this paper.

2. GROWER LINE DIFFERENCES

It is well established that conditions the plant is exposed to during fruit development not only influence fruit maturity development and quality at harvest, but also subsequent storage performance (e.g. Zaffoli et al., 2015). Weather conditions during development and the harvest season alone are the major contributor to seasonal differences in postharvest storage. Beyond these orchard location factors, such as latitude, light exposure and soil nutrients are likely to influence storage (Woodward, 2007). Similarly, orchard management practices that vary across the industry including fruit crop load (fruit to leaf ratio), canopy management, girdling, light manipulation (e.g. reflective mulches) and more recently moisture manipulation (e.g. adaptation of covered cropping systems) all have the potential to influence softening or chilling injury development in subsequent storage. The result is a vast inherent variability among grower lines within industry.

A random sample of 20 grower lines from a Te Puke packhouse and stored them in the same small coolroom, the variability in the time for 3% of the fruit to pass below the export threshold of 10 N (≈ 1 kg_f) firmness is wide (Figure 1; Jabbar, 2014). The poorest storing grower line takes just 52 days for 3% of its population to be < 1 kg_f while the best was took 168 days. Hence, the variability entering into the postharvest phase represents a potential shelf life of 110 ± 50 days. This figure alone demonstrates the challenge of predicting performance of individual grower lines.

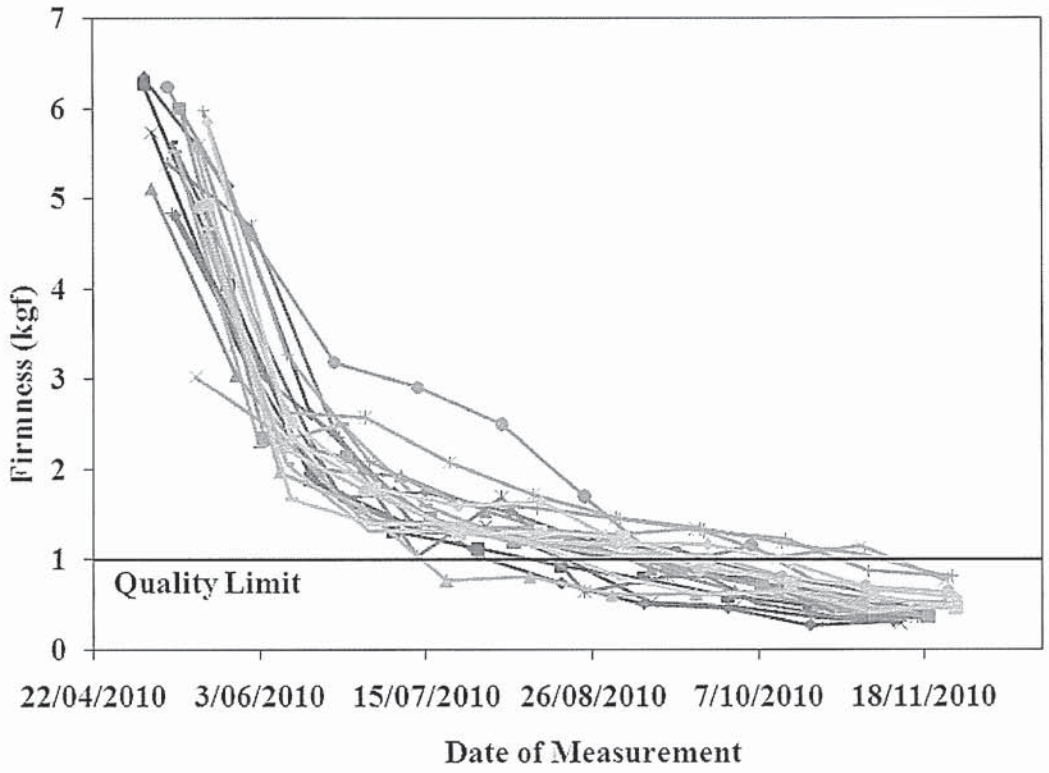


Figure 1. 3rd percentile firmness of 20 grower lines of Hayward kiwifruit stored at 0°C in air. Data presented represents the 9th softest fruit of a 300 fruit measurement sample measured at 21 day intervals for each grower line. The horizontal line at 1 kg_f represents the firmness quality limit for export (Jabbar, 2014).

These same variabilities in growing location and management practice result in the difference in maturity development and fruit quality (DM, brix, firmness) at harvest. Consequently there are many attempts at using these data to provide indications of useful storage life predictions. Other work, including our own (Jabbar, 2014) tried to add to the available data, through developing an accelerated fruit library technique, and describing these differences in softening profiles at 20 °C as a source of information (Jabbar et al., 2014). Alternatively non-destructive measurement technologies such as NIR (Li et al., 2015) or fringe projection (East et al., 2014) provide another potential source of information that may describes these grower line difference and assist as data input into individualised grower line prediction models.

3. COOLING PROFILE IMPLICATIONS

Temperature control is important in maintaining the quality of all food products and the first part of this process is reducing the fruit temperature from harvest condition in a process referred to as cooling. Cooling is an energy intensive process, commonly achieved in industry by either forced draft cooling, room cooling or a combination of both while the product is in a palletised state. Achieving optimal storage temperatures rapidly has advantages in that the product physiological rate is reduced and hence "reserves" available for maintenance of product quality remain for storage. However, rapid and subsequently prolonged changes in temperature to those not experience by the product during development can result in quality reducing disorders referred to as chilling injuries. For kiwifruit one of the forms of chilling injury is referred to as Storage Breakdown Disorder (SBD) which is characterised by an initially grainy outer pericarp appearance (in a pre-failure state), which develops into dark translucent and watery pericarp in severe cases (Fig. 2). This same disorder is often referred to as Low Temperature Breakdown (LTB) in scientific forums. First described by Lallu and Webb (1997), more rapid cooling rates have been found to consistently influence subsequent development of SBD after long periods of storage (Zhao et al., 2015; Fig. 3b).

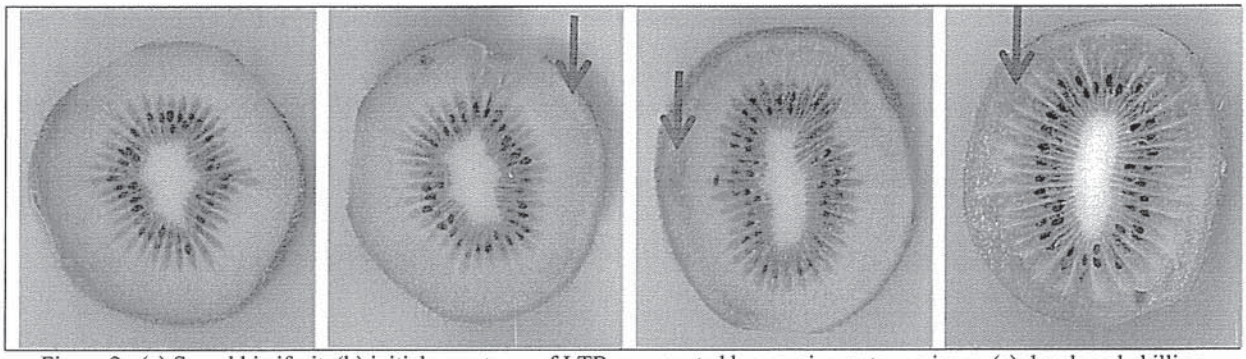


Figure 2. (a) Sound kiwifruit, (b) initial symptoms of LTB represented by a grainy outer pericarp, (c) developed chilling injury represented by darkened outer pericarp ring (d) severe chilling injury represented by dark translucent pericarp.

In the authors opinion it is still unclear whether LTB is an independently developed injury or an injury which accompanies advanced ripening. What LTB does do is differentiate soft fruit from soft and inedible fruit (LTB). However it is also true that LTB does not occur when fruit remain above eating firmness (< 10 N).

In addition to LTB development, more recently however cooling rate has also been associated with rate of softening during advanced storage (Zhao et al., 2015). Fruit cooled (within 3 days of harvest) to 0 °C has consistently been observed to soften more rapidly during later storage (100-200 days) than those which take 1-2 weeks to cool to the storage temperature (Fig. 3a). The response of fruit to soften more rapidly when cooled rapidly is counter intuitive, and prohibitive to successful long term storage. As a result, the procedures to transfer fruit temperature from at-harvest temperature to optimal storage temperatures create vast variability of subsequent softening rates within the industry and consequently challenges for prediction of quality change.

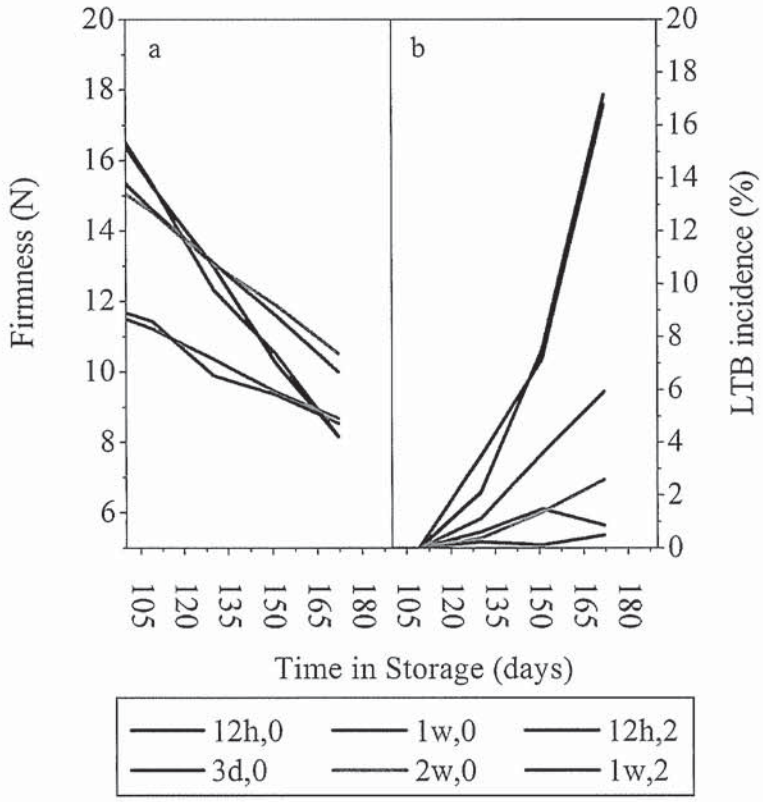


Figure 3. (a) Average firmness change and (b) LTB incidence of Hayward kiwifruit during long term (>100 d) cool storage after being cooling to the storage temperature at different rates. Data represents measures of 90 fruit each of 3 grower lines at 21 day intervals. Treatment labels are descriptions of time to reach storage temperature and the subsequent storage temperature, being (12h,0) 12 hours to cool to 0 °C, storage at 0 °C; (3d,0) 12 days to cool to 0 °C, storage at 0 °C; (1w,0) 1 week to cool to 0 °C, storage at 0 °C; (2w,0) 2 weeks to cool to 0 °C, storage at 0 °C; (12h,2) 12 hours to cool to 0 °C, storage at 2 °C; (1w,2) 1 week to cool to 2 °C, storage at 2 °C.

4. STORAGE TEMPERATURE IMPLICATIONS

Industrial refrigerated coolrooms are large spaces in which evaporator equipment located somewhere within that room cools the air and subsequently maintains product temperature. The room temperature is controlled to a set point which will be defined by temperature measurement at 1 or more locations within the room. However, laws of physics and a desire to control operational cost of refrigeration equipment results in constant temperatures not usually being maintained. It is not unusual for the application of a "deadband" of 1 to 2 °C temperature differential to control room temperature. For example if a set-point of 1 °C is desired, the refrigeration system may turn on at 2 °C and off at 0 °C, causing the room to oscillate between these control points with time.

Beyond the temporal oscillation described above, positional variation within industrial coolrooms is an unavoidable reality. Relatively warm air (2 °C) is cooled (to 0 °C) by the evaporator located at one location in the room and thrown to the far side of the facility. As the air finds its way back to the evaporator it collects heat from the fruit, roof, walls and floors, heating to 2 °C (for example) before returning to the evaporator. Where the fruit is located within this system, with respects to the air as is flows around the room and heats up, will dictate small positional differences in the storage temperature. The fruit located close to the evaporator air flow will be cooler than those located at other locations.

Beyond both positional and temporal temperature variation, a range of set-point temperatures are used in industry as a method to account for room differences or as a management strategy to attempt to

extend storage life or alternatively avoid LTB development described in the previous section. The combined result of this set-point, temporal and positional variability is the reality that within industry the bulk of the fruit after cooling will be stored somewhere in the range of 0 to 2 °C, with this scale of variability being not necessarily unusual even within a single room.

So given that we accept that a 0 – 2 °C storage temperature difference is normal, unavoidable and potentially even best practice, what impact does this temperature variability have on storage performance of kiwifruit over long storage periods? Over the past few seasons we have conducted trials with deliberate storage differences of 2 °C. The 2 °C condition does result in more rapid softening in the initial storage period (first 100 days, Fig. 3a), but as storage extends, 2 °C becomes beneficial as LTB is substantially lower and softening rate is slower (Fig. 3b). However in storage decay (*Botrytis cinerea*) incidence can be higher at 2 °C (data not shown). Given that there are initial firmness and decay benefits of storing at 0 °C and LTB and later firmness benefits of storing at 2 °C, it is understandable that set point changes during the storage season have been considered by some postharvest operators as a solution to getting the best of both worlds. In a study in which kiwifruit were shifted from 0 °C to 2 °C or vice versa during the season, we did not find any consistent results (data not shown). What is clear is that subtle temperature differences (in the 0-2 °C range) during storage, over long time frames (i.e. > 100 days) can have substantial influence on the quality outcomes of kiwifruit.

5. STORAGE ROOM ETHYLENE IMPLICATIONS

The plant hormone ethylene is associated with ripening and deterioration in many fruit. However, it is well known that kiwifruit are especially sensitive to ethylene. Kiwifruit are a climacteric fruit, meaning that as part of its natural ripening process, fruit produce ethylene themselves at dramatically increased rates. For most fruit this occurs at the onset of ripening (i.e. as they begin to soften or change colour) but for kiwifruit this pronounced production of ethylene only occurs once fruit soften to approximately eating firmness (Fig 4; Samarakoon, 2012). What is even more unusual is the magnitude of the ethylene production increase when it occurs, being a 100,000 times increase. Putting this in perspective, this dramatic increase means that a single very soft kiwifruit potentially produces the same amount of ethylene as 10 pallets of hard kiwifruit.

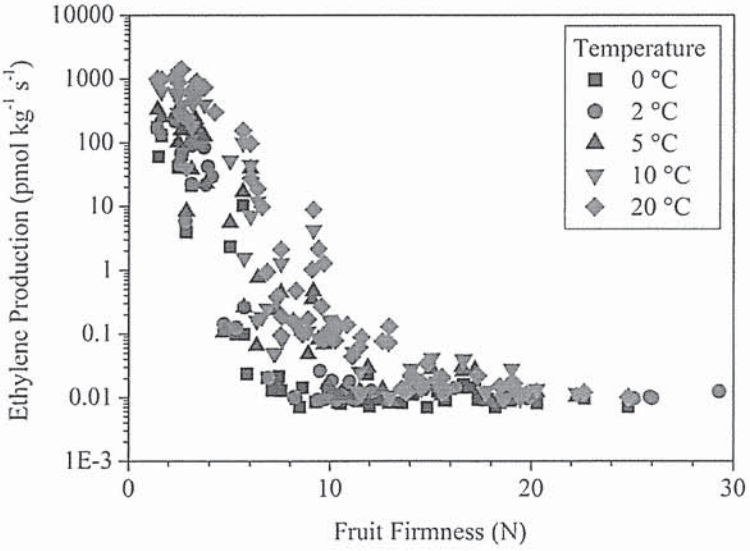


Figure 4. Ethylene production of 'Hayward' kiwifruit as a function of firmness (N) and temperature (°C) for the firmness range from 30 to 1.5 N. Each data point represents ethylene production obtained from a single experimental jar with either seven, three or one fruit depending on the rate of ethylene production of kiwifruit. (Samarakoon, 2013)

Ethylene can be sourced not only from the fruit but from other points of contamination including combustion gases, smoke and decaying material. However in the modern supply chains external sources of contamination can and are controlled through the use of scrubbing systems. But given the ability of soft fruit to produce ethylene then it only takes a small number to produce a significant amount.

So just how much ethylene is in the environment at storage temperatures is important? The industry standard of 30 parts per billion (ppb or nL L^{-1}) is widely used as a gauge for when issues are occurring. However recent development in ethylene sensing technology has allowed reinvestigate of this threshold with an ability to measure ethylene at ppb levels accurately. Fig 5 summarises the subsequent results of controlling ethylene concentrations in the environment at 1, 10, 100 and 1000 ppb (Jabbar and East, 2016). Immediately after harvest exposure to as little as 10 ppb can reduce storage life by approx. 4 weeks. Later in storage, 10 ppb is little different to 1 ppb, but 100 ppb does result in both substantial softening and LTB development. The big unknown in the industrial system is just how much ethylene are the fruit actually exposed to while sitting in their polylined boxes. This is difficult to estimate due to the uncertainties of the folded polyliner system used in industry and difficult to measure on mass, which is often complicated by the rapid absorption of ethylene by other fruit in the box. What we do know is that as one fruit gets soft, it can produce substantial amounts of ethylene and the remainder of the fruit within that micro environment of the box are likely to be sensitive to it. It's our opinion that this combination of factors leads to the vast box to box variability observed in industry where 1 box of very soft fruit can be located next to another of perfectly saleable kiwifruit on the same pallet.

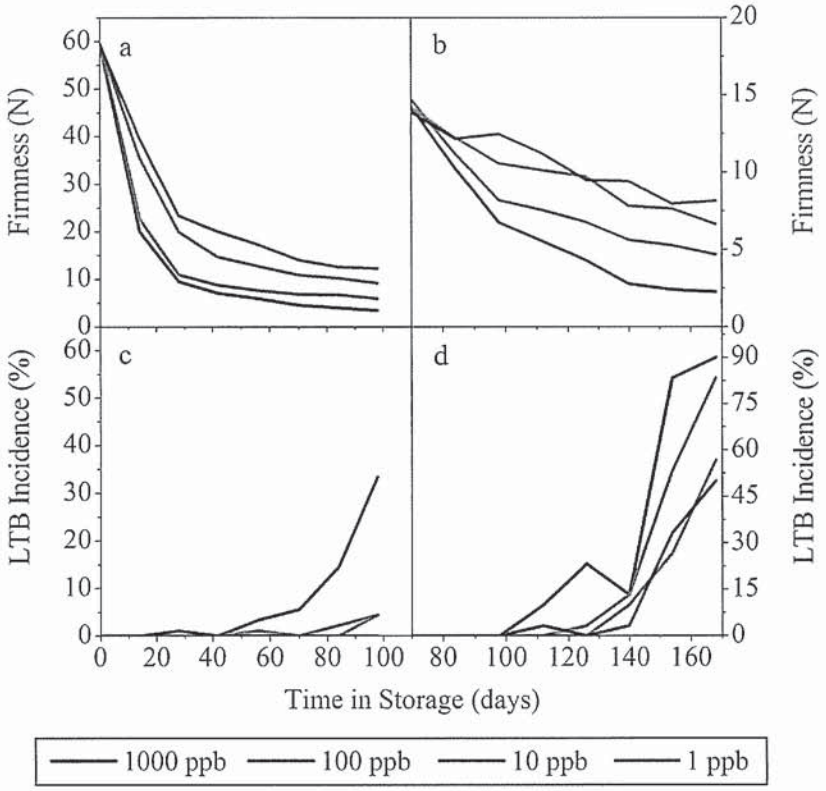


Figure 5. (a-b) Average firmness and (c-d) incidence of LTB development of 'Hayward' kiwifruit in response to exposure to exogenous ethylene during cool storage at 0 °C in air. For (a) and (c) exogenous ethylene was applied at initiation of storage, whereas for (b) and (d) exogenous ethylene was applied after 70 days storage in 1 ppb in air at 0 °C. Data represents measures of 30 fruit each of 3 grower lines at 14 day intervals.

CONCLUSIONS

Product storability of individual kiwifruit grower lines is currently very challenging as the quality changes during storage are influenced by pre-harvest factors, initial cooling rate, and supply chain temperature and ethylene conditions. Consequently in order to predict kiwifruit quality, information of all of these factors will be required as inputs for modelling. Time-temperature logs from harvest within the supply chain (as detailed previously by Bollen et al., 2014) provide information to interpret cooling and storage condition differences. However information that usefully describes grower line differences and the ethylene environment will also be required for successful quality prediction. Subsequently, our current research programs at Massey University focus on non-destructive data collection methods and assessing ethylene effects.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contribution of Dr David Tanner and Alistair Mowat who support and guided much of the Massey University research over the past 5 years which has resulted in much of the evidence used to and referred to in this paper. Research that contributed to this paper has been funded by Zespri International Ltd and the Ministry of Primary Industries through and Primary Growth Partnership research project.

REFERENCES

Bollen, A.F., Tanner, D.J., Soon, S.B., East, A.R., Dagar, A., Sharshevsky, H., Mowat, A., Heyes, J.A., and Pelech, Y. Wireless temperature monitoring system in a global kiwifruit supply chain. Acta. Hort. 1091, 205-212.

East, A., Bloomfield, C., Trejo Araya, X., and Heyes, J. 2014. Evaluation of fringe projection as a method to provide information about horticultural product surfaces. Proceedings of the 19th International Horticultural Congress, Brisbane, Australia, August 2014.

Jabbar A. 2014. Accelerated fruit libraries to predict storage potential of 'Hayward' kiwifruit grower lines. PhD thesis, Massey University, New Zealand.

Jabbar, A., East, A.R. 2016. Quantifying the ethylene induced softening and low temperature breakdown of 'Hayward' kiwifruit in storage. Postharvest Biology and Technology, 113, 87-94.

Jabbar, A., East, A., Jones, G., Tanner, D., Heyes, J. 2014. Modelling batch variability in softening of 'Hayward' kiwifruit from at-harvest maturity measures. Postharvest Biology and Technology, 90, 7-14.

Lallu, N., Webb, D.J. 1997. Physiological and economic analysis of precooling kiwifruit. Acta Hort. 444. 691-698.

Li, M., Pullanagari R.R., Pranamornkith, T., Yule, I.J., East A.R. 2015. Applying visible-near infrared (Vis-NIR) spectroscopy to classify cv. 'Hayward' kiwifruit firmness after storage. Proceedings of the 5th Model-It Conference, Wageningen, Netherlands, October 2015.

Samarakoon, H. 2013. Ethylene flux in postharvest kiwifruit systems. MPhil thesis. Massey University, New Zealand.

Woodward T. 2007. Variation in 'Hayward' kiwifruit quality characteristics. MPhil thesis. University of Waikato, New Zealand.

Zhao, M., Bronlund, J.E., East, A.R. 2015. Effect of cooling rate on kiwifruit firmness and rot incidence in subsequent storage. Acta. Hort. 1079. 313-318.

Zoffoli, J.P., Naranjo, P. and Cuevas, A. 2015. Understanding postharvest quality of kiwifruit in relation to shoot and cane vigor. Acta Hort. 1096. 333-340