Accelerated Fruit Libraries to Predict Storage Potential of ‘Hayward’ Kiwifruit Grower Lines

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Abstract

Reducing postharvest losses is a major challenge of the kiwifruit industry. Inherent variability between kiwifruit grower lines makes the prediction of postharvest storage quality a difficult task. This research aims to establish an Accelerated Fruit Library (AFL) rapid test methodology to collect data that would enable *a priori* segregation of 'Hayward' kiwifruit grower lines for storage potential. In the AFL, fruit losses were accelerated by storing at 20 °C and measured regularly at 3 day (d) intervals. The resulting pattern of losses in the AFL was assumed to reflect the losses in optimal storage (0 °C). Results from a preliminary study found that late harvested lines in the AFL displayed a more rapid decline in firmness than those harvested earlier, corresponding with the highest recorded ethylene contamination in the room. Therefore, later AFL attempts were refined by storing each grower line in a flow through system to maintain ethylene independence. The refined AFL methodology ensured expression of inherent loss patterns of each grower line. From the AFL data, parameters describing the distribution, variability and defect count were extracted. Number of fruit < 0.6 kg_f, 1st quartile, 3rd quartile firmness, mean and median firmness, SSC:firmness ratio and number of rots during AFL monitoring were slightly correlated ($r \geq |0.5|$) with fruit firmness at 126 d of optimal storage. None of the AFL parameters had consistent correlation ($r \geq |0.5|$ continuously at more than two measurement occasions) with storage firmness. Later, AFL softening curves were described with the Complementary Gompertz equation using the non-linear mixed effects procedure for fitting. Grower lines with higher fitted rate of firmness change parameter (κ) during AFL monitoring had a tendency to have low firmness at 100 and 126 d of optimal storage (r = -0.53 and -0.45 respectively). Using the fitted κ as a segregation guide, 60% of grower lines were successfully categorised into 1 of 3 storage potential categories (i.e. low, medium and high). Notably, κ successfully identified 90% of the low storage grower lines. Removing grower lines identified as low storing (65% of whole population) changed the proportion of observed low storing lines in the remaining population from 35% to 10%. However, in the next season where validation of the AFL methodology was conducted, using the fitted κ as a segregation tool resulted in only 53% of grower lines being correctly categorised. Meanwhile, 78% of grower lines with low storage potential were accurately predicted. However, removal of lines categorised as low storing (64.7%

of whole population) changed the proportion of observed low storing lines in the remaining population from 53% to 33.3%. Overall, the AFL methodology could have potential to segregate grower lines with different storage potentials but unfortunately higher proportion of low storing lines in the remaining population categorised as medium and high storage restrict its industrial application. Further development of the AFL methodology to predict storability of kiwifruit grower lines may be achieved with incorporation of pre-harvest information (change in fruit quality e.g. SSC and firmness on vine), compositional attributes (amount of minerals e.g. calcium), physiological indicators (e.g. respiration rate and ethylene production) and processes (e.g. cell wall changes and enzymatic activity) of fruit ripening during storage.

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List of Abbreviations

AFL	accelerated fruit library (s)
A_o	lower asymptote of softening curve
B	upper asymptote of softening curve
β	horizontal shift factor of softening curve
CG	complementary Gompertz
°C	degree Celsius
cm	centimetre (s)
CMM	complementary Michaelis–Menton
CO_2	carbon dioxide
d	day (s)
DM	dry matter (%)
EXP	exponential
FF	flesh firmness
g	gram (s)
GL	grower line (s)
h	hour (s)
IEP	inverse exponential polynomial
ISO	international organisation for standardisation
JMM	jointed Michaelis - Menten
κ	rate of softening
κ_α	threshold limit for higher κ value (s)
κ_β	threshold limit for lower κ value (s)
kg	kilogram (s)
kg_f	kilogram force
L	litre (s)
MAE	mean absolute error
MB	modular bulk (s)
Mg	magnesium
min	minute (s)
mm	millimetre (s)
µL	microlitre (s)

mL	millilitre (s)
n	number
N	newton
NZ	New Zealand
nL	nanolitre (s)
nlme	non-linear mixed effects
O	observed
P	predicted
ppb	part per billion (s)
ppm	part per million (s)
pptv	part per trillion volume (s)
%	percent
r	correlation coefficient
RH	relative humidity (%)
s	second (s)
SF	soft fractile
SSC	soluble solids content
t	time
τ	time shift

1 Introduction and Thesis Overview

1.1 INTRODUCTION

Kiwifruit (*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson cv. Hayward) is an important horticultural fruit crop for a number of countries. New Zealand is a leading kiwifruit exporter by selling more than 90% of production internationally (Burdon and Lallu, 2011). New Zealand kiwifruit earned almost NZ $1.1 billion from export in 2012/13 (Anon, 2013c).

Reducing postharvest losses of kiwifruit is a major challenge for the industry. One of the factors that contribute to these losses is inherent variability between batches, which is expressed as variable storage quality and causes storage life prediction to be a difficult task (Tijskens et al., 2003). While being a challenge, this variability provides an opportunity in fresh produce inventory management. The ability to categorise poor and good storing lines around the time of harvest could significantly contribute to inventory management. Which batches are sold earlier or later may have a significant impact on total losses across the stock (East, 2011). This may be particularly so for kiwifruit where over-soft fruit produce large amounts of ethylene that may accelerate softening of sound kiwifruit in the storage environment.

Kiwifruit firmness is an important quality determinant (Jackson and Harker, 1997). At-harvest kiwifruit are hard (6 - 10 kg_f), enabling robustness during picking and initial handling, and the fruit subsequently soften postharvest to eating soft stage (approximately 0.5 - 1.5 kg_f). Substantial variation in firmness at-harvest and during postharvest softening is known to exist between seasons (Woodward, 2006), districts (Kim, 1999) and within (Feng et al., 2003a) and between batches or orchards (MacRae et al., 1990b; Benge et al., 2000). Variation in at-harvest firmness has been associated with orchard management (Tombesi et al., 1993; Antognozzi et al., 1995; Pyke et al., 1996; Snelgar et al., 1998; Boyd and Barnett, 2011) and harvest timing (Mitchell et al., 1992; Costa et al., 1997; Boyd and Barnett, 2007).

Kiwifruit softening is a well documented process (MacRae et al., 1989b; MacRae et al., 1990a; Benge et al., 2000). Studies of kiwifruit firmness change during storage mainly follow different treatment (pre and postharvest) effects (Lallu et al., 1989; MacRae et al., 1989b; MacRae et al., 1990b; Davie, 1997; Hertog et al., 2004b) or compare different firmness measurement methods (Jackson and Harker, 1997; McGlone and Kawano, 1998; Schotsmans et al., 2008). Both Benge (1999) and White et al. (2005) suggested that initial firmness is an important factor affecting kiwifruit postharvest softening. In contrast, Harker et al. (1997) reported a poor relationship of at-harvest firmness with time to soften or rate of softening. Understanding firmness variability remains a challenge in the kiwifruit industry, particularly for making inventory decisions (Adams et al., 2010). Each year substantial costs (approximately $120 million) are incurred in removing soft fruit and re-packing the remaining fruit to meet export standards (Tanner et al., 2012). Understanding the variation between fruit populations is required for industry to be able to make more informed inventory decisions and consequently reduce product losses caused by over softening.

This research aims to develop an accelerated shelf life testing technique for the kiwifruit industry as a rapid test methodology to aid identification of ‘Hayward’ grower lines (GLs) for storage potential. Accelerated shelf life testing is a method to evaluate the remaining shelf life of a product by correlating the storage losses in an abusive environment with those observed in optimal storage conditions (Sewald and Devries, 2003). Therefore, evaluating the keeping quality of kiwifruit by using a fruit library maintained at abusive conditions can be referred to as ‘accelerated fruit library monitoring’. AFLs differ from conventional fruit libraries in that the conditions of the fruit library are altered deliberately to advance fruit losses in order to give predictive information about fruit storability under optimal storage conditions. In applying this approach, it is assumed that: 1) the variability between fruit batches that is routinely observed is captured in the fruit sampled for the AFL and 2) the losses observed in the accelerated conditions have a relationship to losses in optimal storage conditions.

In the case of kiwifruit, application of AFL is a new ideology to predict the storage potential of fruit batches and hence assist in making decisions in inventory management. Previously, changes in apple fruit quality at accelerated conditions (storage at 20 °C) have been used to determine fruit quality in optimal storage at 0 -

0.5 °C (Ingle and Morris, 1989; Iwanami et al., 2008). AFL of kiwifruit batches can be established by exposing fruit samples to high temperature or by application of ethylene (Antunes and Sfakiotakis, 2000; Antunes, 2007). Application of AFL to industry is only possible once protocols for accelerated storage and loss monitoring and their relationships to the performance of optimally stored produce have been established. Another important factor in the application of AFLs is the ease of monitoring of the AFL. Ultimately, the effort required to collect data from the AFL must be outweighed by the value of the information provided. A successful AFL methodology would help to identify good and poor storing batches of kiwifruit, reducing fruit losses and associated repacking costs and increasing the profitability of the industry. Given the above brief understandings, the following research objectives were aimed to achieve.

- Develop an AFL methodology for ‘Hayward’ kiwifruit GLs to predict the storage potential.
- Minimise the effort and cost of AFL establishment and data collection.
- Understand the relationship of losses in AFL with optimal storage potential.
- Validate the use of an AFL methodology to predict the storage potential of kiwifruit GLs.

1.2 THESIS OVERVIEW

The research was conducted at Massey University, Palmerston North, New Zealand. AFL was applied and tested for storage category (poor and good) prediction on more than 125 ‘Hayward’ kiwifruit lines. Kiwifruit samples were obtained after commercial grading processes. To accelerate ripening and losses, fruit were stored at 20 °C and compared to storage performance at 0 °C.

The literature review (Chapter 2) provides current knowledge of kiwifruit industry and its supply chain. It also comprises detailed review about different pre and postharvest factors affecting kiwifruit softening and other losses. This chapter includes aspects of quality management including fruit softening and its mathematical modelling. This literature review also explains potential application of AFL and its philosophy with relevant examples in other fruit. Lastly, this chapter states acceptable quality limits for soft defects of kiwifruit with reference to sample size.

Chapter 3 provides the insights gained from a preliminary establishment of AFL methodology. Interpretation of fruit losses in AFL by using simple and easy to calculate parameters as well as sample size manipulation to minimise effort and cost of AFL application and interpretation are investigated in this chapter.

Chapter 4 details the application of a refined AFL methodology based on the understandings developed from the previous study. Sample size of each GL for AFL losses assessment and number of measurement occasions for optimal storage has been reduced. This chapter also explains the interpretation of AFL to categorise kiwifruit grower lines for storage potential by using simple and easy to calculate AFL parameters. Parameters of AFL losses do not show any association with storage quality of GLs.

The fifth chapter re-interprets the AFL data with a mathematical modelling approach. This chapter explains model calibration to fit AFL softening data and GL dependent model parameter extraction. In addition, this chapter investigates the relationship between GL dependent model parameters and storage potential. The rate of firmness change of GLs during AFL monitoring assists in characterisation of GLs into storage categories.

Furthermore, chapter 6 demonstrates the validation study of AFL establishment in another season. This study also validates the use of fitted GL dependent model parameter to predict storage categories of GLs and discuss the chances of false categorisation.

The last chapter (7) formulates final discussion and recommendations including summarised outcomes and limitations of the AFL establishment and its interpretation. Options to accelerate softening loss in AFL and use of other modelling techniques are also discussed. In addition, this chapter also discusses the future research opportunities to include pre-harvest quality change information, assessment of physiological and compositional data of fruit during AFL.

2 Literature Review

2.1 OVERVIEW OF KIWIFRUIT INDUSTRY IN NEW ZEALAND

Kiwifruit was first known as Chinese gooseberry, *Actinidia chinensis* Planch., and/or Yang Tao, because of its Chinese origin. In New Zealand, kiwifruit was introduced by Mr. Allison of Wanganui in 1906 (Schroeder and Fletcher, 1965). Fortunately, both male and female plants were available and farmers started to produce fruit soon after the introduction and distributed over the country (Ferguson, 2005). The varieties grown today are originated from selective breeding of the original imported seedlings (Schroeder and Fletcher, 1965). The cultivar ‘Hayward’ (*Actinidia deliciosa*) was a selection, made from seedlings by the nurseryman Hayward Wright in 1920 (Ferguson and Bollard, 1990).

Commercial orchards of ‘Hayward’ kiwifruit were first established in New Zealand around 1930. Initially cultivation of ‘Hayward’ kiwifruit was restricted to the main growing areas in Bay of Plenty, because of suitable climatic conditions (Schroeder and Fletcher, 1965). In the early years, kiwifruit gained appreciation domestically mainly because of ease in management and attractive appearance and flavour (Anker-Kofoed, 2008). The popularity of the fruit increased gradually until the early 1970s. By that time, export market response was also an important cause of the increase in kiwifruit plantings in New Zealand (Woodward, 2006). Ultimately, demand for farmland for other agricultural purposes increased by almost 54% during 1983 - 1986. Therefore, growers felt the need to locate other suitable areas of the country to plant kiwifruit (Anker-Kofoed, 2008). Eventually, quick spread and high demand for kiwifruit resulted in its cultivation in many parts of the country.

Kiwifruit is being traded internationally almost all over the world although the numbers of exporting countries are very few. Based on production quantities, China, Italy, New Zealand and Chile are the largest kiwifruit producers (Burdon and Lallu, 2011). In the start, export of kiwifruit from New Zealand was started largely as a means of selling fruit that were surplus to the requirements of local market (Ferguson, 2005). In 1970, only 60% of total production was exported, mainly because of extensive domestic consumption. In the time, efforts to approach global markets were in process. By 1978, kiwifruit exports increased to over 80 percent of the total production and continued to rise rapidly during later years (Anker-Kofoed, 2008). Until now, export of New Zealand kiwifruit has been increased tremendously.

New Zealand is one of the major kiwifruit exporting countries by selling more than 90% of its crop internationally (Burdon and Lallu, 2011). New Zealand exports kiwifruit to most of the world (except Australia) from a single desk with all the marketing operations looked after by Zespri International Ltd. Zespri is a purely grower owned organisation, which strategically exports all the premium quality kiwifruit around the world with the brand name of ZESPRI^{TM} (Woodward, 2006). As kiwifruit export increased, the industry realised the importance of postharvest performance in maintaining the fruit quality during supply chain.

'Hayward' or green kiwifruit became an important cultivar because of better flavour and sustainability in long term storage and transportation to distant countries via ship (Ferguson and Bollard, 1990; Ferguson, 2005; Burdon and Lallu, 2011). Therefore, cultivation of 'Hayward' has been extended remarkably and this variety comprises major proportion of New Zealand grown kiwifruit. Whilst 'Hayward' is the dominant cultivar and marketed with the brand name of Zespri Green, other brands like Zespri Gold (Hort16A) and Zespri Sungold are also sharing the economic contribution made by New Zealand kiwifruit industry. The range of commercial kiwifruit cultivars other than 'Hayward' may increase in future. The better postharvest performance of 'Hayward' kiwifruit makes it the most preferred cultivar to be grown and exported worldwide (Burdon and Lallu, 2011).

In the past, industry usually differentiated kiwifruit based on external factors of quality, like fruit shape and size and absence of blemish and physical damage. Later, with the advent of quality assessment techniques, evaluation of fruit internal quality (i.e. dry matter, colour and firmness) also gained importance, mainly with the focus to increase product consistency (Banks, 2003). The green kiwifruit industry has to supply superior tasting fruit, as consumer perception has strong relationship with fruit dry matter (DM) levels (Burdon et al., 2004). Thus the entire supply chain for green kiwifruit has been aligned to produce, segregate, market and deliver a good tasting product (Banks, 2003), particularly for the highest-priced market, like Japan.

With the passage of time New Zealand kiwifruit industry became more specialised from fruit production until its consumption. Previously, orchards were responsible for growing, harvesting and packing. Continuous increase in fruit production and export potential divided the supply chain into specialised components to be able to manage successfully large quantities. Production of premium quality fruit became the only objective of orchard management. While pack houses and cool stores have the sole responsibility to consistently conduct all the later steps and processes (i.e. postharvest treatment, packing, cooling etc.; Anker-Kofoed, 2008). In recent times, supply chain of kiwifruit has been revolutionised to ensure the continuous supply of premium quality fruit. For this purpose, kiwifruit storage and efficient supply chain management has fundamental importance in the industry.

2.2 KIWIFRUIT SUPPLY CHAIN

Supply chain is defined as a management of events/tasks, which initiates the process of source, production and product delivery to the market in order to satisfy the needs of a customer (Hewett, 2003). For New Zealand, successful management of a supply chain of fresh fruit exports is very important. Distance of New Zealand from main markets (e.g. Europe and Asia) requires supply chain of fruit to be efficient (Aitken et al., 2004). Success of a fruit supply chain relies on harmonising requirements of consumer in a timely and cost effective manner (Praat et al., 2003). This requires a strong coordination between different sectors for efficient communication and control to develop a highly effective supply chain for any fruit. In New Zealand horticultural industry, kiwifruit and pipfruit are examples of sophisticated and successful supply chain systems (Hewett, 2003). For kiwifruit, Zespri International Ltd. also created global supply chain and systems department to ensure successful management of different aspects of planning, quality assurance and logistics. This system ensures the supply of premium quality fruit to more than 60 countries in the world (Anon, 2013a).

New Zealand kiwifruit industry is a combination of many integrated sectors of supply chain. Right from fruit growth to harvesting, curing, packing, storage, quality checking, repacking, and loading and unloading from ship all sectors work as an integrated supply chain. All of these sectors ensure the effective communication and understanding between each other to carry out specified processes (Mills, 2004a; Mills, 2005).

However, exchange of information is most important for better planning and in time decision making (Mills, 2005).

Exporting kiwifruit from New Zealand requires advance planning due to distance from the market. For example to reach long distance markets in Europe consignments may take four to five weeks (Lenting, 1992; Hewett, 2003). In this scenario, inventory decisions are very important to ensure transport and sale of right fruit at right time (Mills, 2004c). Industry has to take well in advance decisions to assimilate variability in batches of kiwifruit while maintaining balance between value and cost (Mills, 2005). Currently industry is making inventory decisions for kiwifruit batches on the basis of different variable factors e.g. previous year storage performance, orchard and at-harvest information and during storage quality check (Adams et al., 2010; Tanner et al., 2012). Unfortunately, these decisions still involve the chances of fruit losses as well as loss of profitability because of the variability in kiwifruit batches for quality. The industry is lacking a decision support system that can aid inventory decisions to prevent extensive fruit losses and associated costs.

A generalised scheme of kiwifruit supply chain explains the integrated process of packaging, storage, order processing, and decision making of fruit from different growers (Figure 2.1). Pack houses perform stock rotations and during storage fruit quality monitoring. Once the order is received, process of decision making starts to fulfil customer requirements (i.e. pack type, fruit quality and market location). Many factors like orchard information, harvest time in a season and condition check data influences the process of decision making for selling or holding particular grower or batch of fruit. Following the inventory decision for selling a grower or batch, fruit order is processed and sent to the customer or market place. Overall, inventory decisions for selling a grower or batch has significant impact on performance of a supply chain.

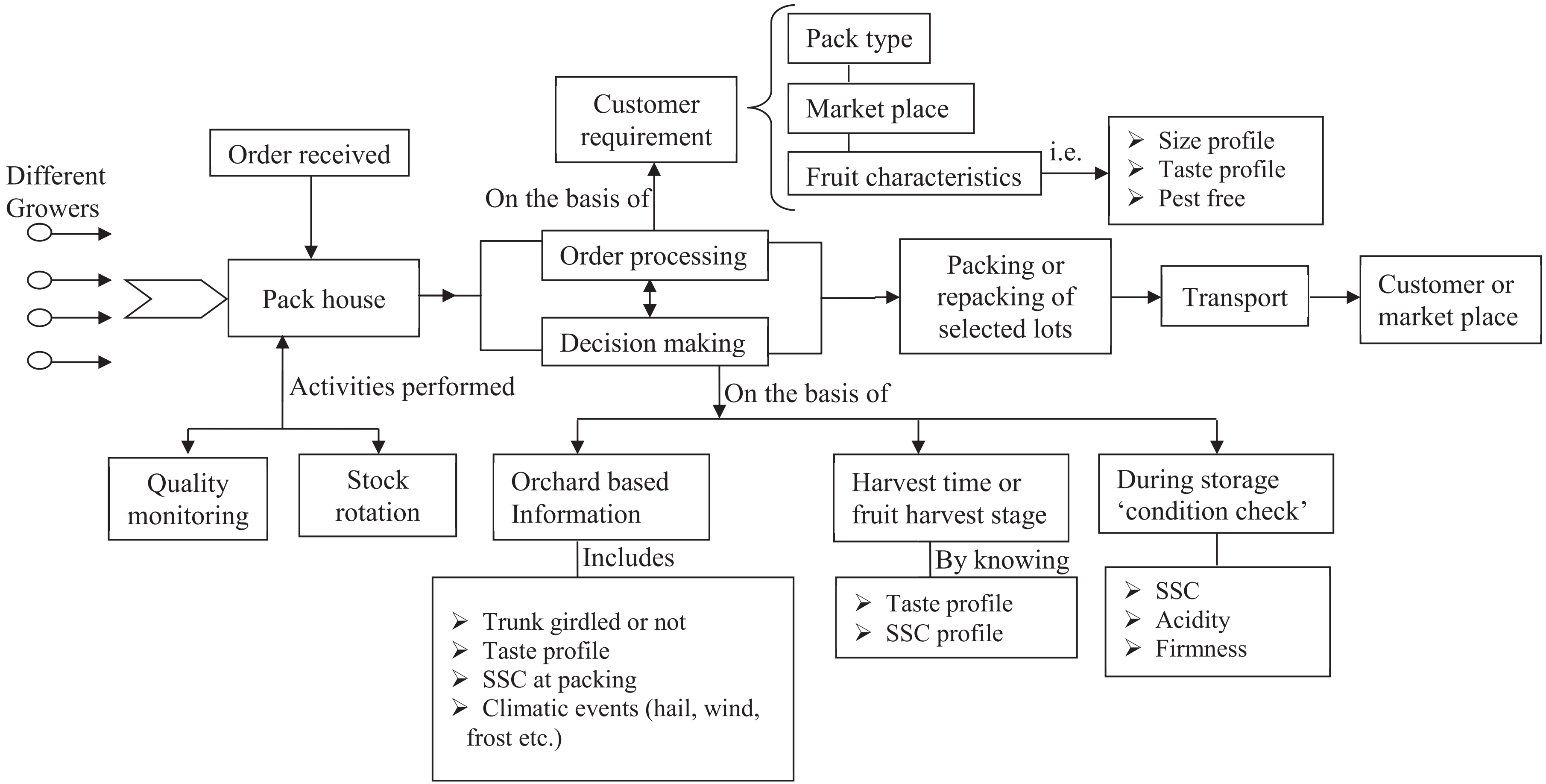

Figure 2.1: Generalised scheme of processes involved in kiwifruit supply chain.

2.2.1 Storage of kiwifruit

Fresh kiwifruit seldom are marketed immediately after harvest. Most of the fruit is usually warehoused for up to 4 - 6 months at 0 °C, with relative humidity 90 - 95% and no ethylene, during the storage (Harvey et al., 1983; McDonald, 1990; Ritenour et al., 1999; Burdon and Lallu, 2011). Kiwifruit slowly deteriorate over time even in cool store with softening being the major limiting factor of storage life (Feng et al., 2001). Immense losses occur if fruit soften prematurely in storage. Kiwifruit are very susceptible to small concentrations of ethylene (i.e. 0.01 - 0.03 ppm) during storage (Ben-Arie and Sonego, 1985; Mitchell, 1990; Hewett et al., 1999). So, one of the most important concerns for kiwifruit storage is management of ethylene in the atmosphere (Section 2.3.2.3). Factors such as frequency of opening the doors, location of storage facility, nearby roads and presence of decaying fruit in the vicinity of store rooms, all influences the concentration of ethylene in the atmosphere (Rushing, 2004). Rot incidence (Section 2.4.2) and low temperature breakdown (Section 2.3.2.2) are also important problems of kiwifruit and contribute to losses during storage (Hewett et al., 1999; Kim, 1999). Therefore, it is necessary to ensure correct temperatures with no accumulation of ethylene during storage (Mills, 2006; Burdon and Lallu, 2011). For successful management, application of strict regimes (temperature and humidity in an ethylene free environment) is needed to ensure fruit storage for long periods while maintaining good flavour and appearance. Effects of these factors may vary among different cultivars, orchards and seasons (Hopkirk and Clark, 1991; Benge, 1999).

The industry has focused on standardising its approaches to manage quality of fruit during storage. Fruit is routinely monitored in cool stores (known as condition checking) in order to track how each grower is storing and to effectively manage inventory (Mills, 2004b). To monitor quality, fruit samples from each grower are stored in the same standard conditions (e.g. temperature and humidity) and called operational fruit libraries. Industry usually assesses firmness and rots or disorders incidence in fruit libraries and attempt to define the storage quality of a batch or grower line (Benge and Kay, 2003). Incidence of soft and decaying fruit makes quality check a prerequisite. This can result in complete rejection or repacking of a batch prior to shipment if any of the quality parameters is below the threshold limits (Mills, 2004a). The results of losses and rejection are usually quantified and recorded according to criteria such as reason for rejection, growing district, fruit size, harvest date and length of storage (Hopkirk and Clark, 1991).

Efficient stock management requires that assessment of internal fruit quality should be done in advance of placing the batches in storage for long time. This would minimise subsequent costs of fruit monitoring and repacking (Mills, 2004b). High costs of fruit and repacking losses put pressure on the industry as well as postharvest researchers to develop a methodology whereby quality indicators can be used to identify batches having good or poor quality during storage (Hewett et al., 1999; Mills, 2004b). Many pre and postharvest factors influence fruit quality in storage. These factors contribute to variability in fruit quality between seasons and growers and make storage quality prediction a difficult task.

2.3 FACTORS INFLUENCING STORAGE QUALITY

There are many factors, which influence the storage quality and potential of fruit from different grower lines. These factors have prime importance to maintain supply of premium fruit at consumer end.

2.3.1 Pre and at-harvest factors

A number of pre-harvest factors affect fruit quality. Light is an important environmental factor that has the largest influence on quality of many tree crops, including apple, grape and kiwifruit. Intense canopy of kiwifruit vines can result in less light penetration to the bottom fruiting zones (Remorini et al., 2007). Position of fruit within canopy of the plant affects light exposure and thus can have marked influence on quality attributes (Cooper et al., 2007). For example, fruit exposed to high light intensities may have better quality (i.e. higher firmness) at-harvest and store for longer time with higher soluble solids content (SSC > 14%) compared to shaded fruit (Tombesi et al., 1993; Antognozzi et al., 1995; Tavarini et al., 2009). Canopy management (i.e. pruning practices) to increase light distribution is important to improve yield and fruit quality both at-harvest and postharvest (Gullo et al., 2013). Canopy management techniques to control crop load and trunk girdling to manipulate the vine carbon allocation (Boyd and Barnett, 2011) also have direct effects on fruit storage quality.

Water stress is another important factor that influences kiwifruit quality. In New Zealand, a kiwifruit vine may take water up to 150 L per day (Burdon and Lallu, 2011). Withholding irrigation during fruit development may reduce size (Reid et al., 1996), enhance Ca uptake (Davie, 1997) and increase SSC accumulation resulting in sweeter fruit in storage (Hewett et al., 1999). Reid et al. (1996) also reported that withholding irrigation until mid-January reduced the rate of firmness loss during storage and ultimately increased the time to reach 1 kg_f firmness by 30 d. Sustained periods of irrigation deficiency however should be avoided otherwise it may adversely affect fruit yield (Hewett et al., 1999).

Growth climate is a prime factor affecting crop yield and quality. Many researchers have reported the effect of temperature during growth period on kiwifruit quality (Hopkirk et al., 1989; Seager et al., 1996; Costa et al., 1997; Minchin et al., 2003; Richardson et al., 2004; Snelgar, 2004; Sfakiotakis et al., 2005; Snelgar et al., 2005; Hall et al., 2006; Burdon et al., 2007; Snelgar et al., 2007). Snelgar et al. (2005) reported maximum fruit growth at 17 °C. They also found that a rise in temperatures of 2 to 5 °C in the spring season may advance flowering date by 17 d and accelerate rate of shoot growth by 6 $mm.d^{-1}.°C^{-1}$ but fruit were less firm at-harvest. Snelgar et al. (2007) reported that average air temperature is a major climatic factor that affects kiwifruit dry matter (DM). Effect of temperature is variable during the growth season as Richardson et al. (2004) reported that rise in temperature (for 7 °C) in summer may increase vegetative growth, reduce fruit growth, reduce dry matter accumulation (e.g. from 22.6% to 14.2%) and reduce flesh firmness. In late autumn a rise in environment temperature can increase fruit growth and reduce SSC accumulation, ultimately delaying commercial maturity (Snelgar et al., 2005; Burdon and Lallu, 2011; Burdon et al., 2013). Thus, the temperature during growth and development influences fruit maturity. Low environmental temperatures in the week before harvest reduce fruit susceptibility to develop chilling injury in storage, possibly through an increased SSC (Francis et al., 2003; Sfakiotakis et al., 2005).

Plant nutrition is one of the most influencing factors for fruit quality. Higher concentrations of calcium (Ca) reduce the incidence of localised softening or soft patches, compared to the fruit with lower levels of Ca (Davie, 1997; Benge, 1999). Calcium nutrition has strong implications on storage behaviour of kiwifruit. Calcium concentration at-harvest has a negative correlation with rate of fruit softening during storage (Gerasopoulos et al., 1996). Calcium helps to maintain membrane integrity and cell wall structure. It inhibits activity of fruit softening enzymes like polygalacturonase and pectin methylesterease (Buxton, 2005; Boyd and Barnett, 2007). Applications of nitrogen (N) fertiliser significantly affect kiwifruit vine canopy and its vigour, size of fruit, DM (at-harvest) and storage potential. Higher inputs of nitrogen (100 kg N per hectare) may adversely affect fruit DM; which is particularly important because DM has direct link with consumer acceptability (Boyd et al., 2013). In general, many minerals (calcium, magnesium, potassium, and phosphorus) have relevance to physical, physiological and pathological storage disorders. Variation exists between orchards in New Zealand for availability of minerals to fruit vines, and that entirely depends on cultivation practices (Maguire and Mowat, 2003; Buxton, 2005). Some reports also suggested that organically grown kiwifruit remain firm longer with lower incidence of *Botrytis* compared to fruit from conventional production system, which usually have a tendency to soften rapidly and are more susceptible to develop soft patches (Benge, 1999; Hewett et al., 1999; D'Evoli et al., 2013).

Harvest date has a marked influence on fruit quality as well as storage performance. In New Zealand, kiwifruit picking of main crop starts in the beginning of May and continues until end of June or start of July (Sale, 1990). Fruit maturity at the time of harvest affects storage quality (Boyd and Barnett, 2007). A single maturity index cannot define physiological maturity. Instead, there are number of fruit development and ripening aspects, which collectively define fruit maturity (Burdon and Lallu, 2011). As fruit reach the mature stage, growth process slows down and then stops (in ‘Hayward’ approximately after 160 d of fruit set), although carbohydrate accumulation continuing. Breakdown of accumulated starch starts at 120 to 140 d after anthesis but timing can vary between orchards and seasons because of its dependence on cool nights (Burdon and Lallu, 2011). Maturity index of 6.2% SSC is used to determine the commercial harvest timing of kiwifruit (Hopkirk et al., 1986; Burdon and Lallu, 2011; Burdon et al., 2011; Burdon et al., 2013). Fruit harvested at SSC less than 6.2% may develop flesh breakdown (Crisosto and Crisosto, 2001). Fruit with higher SSC at the time of harvest can store well with satisfactory flavour and more sweetness at the ripe stage (Beever and Hopkirk, 1990; Hewett et al., 1999). Therefore, the SSC should be above 7 - 9% if the fruit are intended to be stored for longer period of time (Sale, 1990; Burdon et al., 2013). A delay in harvest can increase the risk of damage from frost or winter storms (Beever and Hopkirk, 1990).

Harvest time also effects fruit susceptibility to storage problems. Late harvested softer fruit are more prone to softening disorders than early harvested firmer fruit. On the other hand, firmness after storage can be greater if the fruit had high SSC at-harvest (Hewett et al., 1999). Early harvested firmer fruit tend to soften slightly faster than fruit harvested later in a season, which exhibit good storage potential (Crisosto et al., 1984; Abdala et al., 1996). Both very early and late harvested fruit become more susceptible to physiological disorders with shorter shelf life (Kader, 1999). The rate of softening also increases as fruit mature on vine. However, the firmness stage at which a change in softening rate occurs varies among orchards and seasons. There is no maturity index, which can accurately predict tendency of fruit to soften and develop storage disorders (Hewett et al., 1999; Burdon and Lallu, 2011).

Curing is an important at-harvest practice that influences kiwifruit quality in storage. After harvest, fruit are subjected to a curing treatment by keeping fruit bins at ambient temperature for 48 to 72 h in order to reduce incidence of rots (*Botrytis*) and to allow fruit to recover from harvesting injuries (Pennycook and Manning, 1992; Brigati et al., 2003). However, temperature and humidity levels during curing are difficult to control for different batches (Brigati et al., 2003). Duration of curing, temperature and humidity directly influence the batch quality (e.g. rots, weight loss) in storage (Bautista-Baños et al., 1997). Variation in curing temperature and duration at different pack houses can be a potential contributor in causing variability between batches for storage rots.

Overall, not one factor affects fruit quality but it is a conjunction of many growing conditions, orchard and vine management, and harvest maturity. All these factors contribute to variations in fruit quality at-harvest, during storage and transport.

2.3.2 Postharvest factors

2.3.2.1 Postharvest handling

Even though kiwifruit is hard at the time of harvest, it can be quite susceptible to postharvest handling problems. Any damage during handling can lead to premature softening due to ethylene production (Sale, 1990; Sale and Lyford, 1990). Physical or compression damage can occur because of fruit contact with each other or handling equipment. Soft fruit are more susceptible to compression damage and hard fruit can get impact damage resulting in symptoms of white lining in the outer pericarp, which later is evident as water soaking as softening progress (Burdon and Lallu, 2011; Ahmadi, 2012). During postharvest handling, speed of fruit movement on belts and drop heights (< 30 cm) are important aspects to avoid any mechanical damage (Budron and Lallu, 2011). Fruit susceptibility to physical damage and bruise injury can vary between different growers.

2.3.2.2 Temperature

After harvest, kiwifruit is packed and placed in a cool store at 0 °C for periods of 4 - 6 months (Sale, 1990; Cotter et al., 1991; Burdon and Lallu, 2011). During the storage period, temperature maintenance is of prime importance, as it has a direct effect on fruit quality, particularly flesh firmness. Fruit stored at 0 °C may develop low temperature breakdown or chilling injury, resulting in quality losses (Lallu, 1997; Sfakiotakis et al., 2005; Burdon et al., 2007; Lallu et al., 2011). Low temperature breakdown is usually characterised by the grainy appearance in outer pericarp of fruit as a result of deterioration of cellular membrane which ultimately leads to water soaking and then softening (Bauchot et al., 1999). Susceptibility to low temperature breakdown is related to exposure of kiwifruit to pre-harvest low temperature, maturity stage at harvest (Francis et al., 2003; Sfakiotakis et al., 2005; Burdon et al., 2007), cultural practices (i.e. summer pruning) and calcium contents (Gerasopoulos and Drogoudi, 2005). Ethylene production can start because of chilling injury during storage (Feng et al., 2003b) and can cause softening in adjacent healthy fruit.

Higher temperatures during storage advance the onset of autocatalytic ethylene production in kiwifruit and speed up the ripening process (Antunes and Sfakiotakis, 2000). Antunes and Sfakiotakis (2002a) stored kiwifruit at 0, 5 and 10 °C for 12 d and reported autocatalytic ethylene production upon removal to 20 °C. Changes in temperature affect the range of firmness levels. For example, Jeffery and Banks (1994) reported that kiwifruit with a firmness value of 20 N (~ 2 kg_f), sustained a loss of measured firmness of 35%, when fruit temperature was changed from 0 to 20 °C. Temperature can also influence expression of kiwifruit disorders during storage (Burdon and Lallu, 2011). Moreover, temperature variation during transit time to distant markets also leads to fruit quality deterioration. Maintenance of appropriate air temperature without fluctuation across the container can minimise the chance of fruit deterioration (Tanner and Amos, 2003).

2.3.2.3 Ethylene

Ethylene is an unsaturated hydrocarbon gaseous plant hormone and drives processes of ripening and senescence in climacteric fruits (Albert et al., 2013; Gapper et al., 2013). Kiwifruit has unique climacteric characteristics that at low temperature (i.e. 0 °C) healthy fruit produce ethylene very late during the ripening process. Meanwhile, at high temperature (i.e. 20 °C) kiwifruit exhibits a rapid rise in respiration rate and then in ethylene production to conclude climacteric ripening (Sfakiotakis et al., 1997; Antunes et al., 2000; Antunes, 2007). However, any damage or rot may trigger autocatalytic ethylene production in kiwifruit (Niklis et al., 1997; Sfakiotakis et al., 1997).

There are many studies which apply propylene to understand the autocatalysis activity and ripening response of kiwifruit. Application of propylene to kiwifruit when stored at 0, 10 and 20 °C revealed that fruit softening occurs at 0 and 10 °C but without significant rise in ethylene production. Meanwhile at 20 °C, ripening occurred with the increase in ethylene production (Sfakiotakis et al., 1989). Antunes et al. (2000) also tested the inhibition of autocatalytic ethylene production below temperatures of 11-14.8 °C (Stavroulakis and Sfakiotakis, 1993). Kiwifruit stored at 10 °C exhibited inhibition of ethylene production mainly because of low activity of both 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase as compared to fruit at 20 °C with propylene treatment (Antunes et al., 2000).

At the time of harvest, healthy kiwifruit produces negligible (< 0.01 $nL.kg^{-1}.h^{-1}$) ethylene (Jeffery and Banks, 1996; Park et al., 2006; Burdon and Lallu, 2011). During the early stages of storage (at 0 °C), ethylene production is very low (below 0.1 to 0.2 $\mu L.kg^{-1}.h^{-1}$). However, with passage of time, ethylene production accelerates as fruit firmness declines to less than 1 kg_f or 12 N and fruit reach the climacteric peak (Kim et al., 1999). The time to reach the climacteric peak typically ranges from 100 to 140 d of storage and can vary between harvest seasons and growers (Chiaramonti and Barboni, 2010; Atkinson et al., 2011).

Kiwifruit is very sensitive to exogenous ethylene exposure during storage (Antunes, 2007). Upon exposure, ethylene production increases along with respiration and firmness loss of kiwifruit (Park et al., 2006; Antunes, 2007). Green kiwifruit does not show any visible colour or external appearance change but ethylene addition causes an acceleration of fruit softening (Harman and McDonald, 1983). Even very low concentrations of ethylene (0.01 - 0.03 ppm) are sufficient to cause premature softening and disturb long term storage. Industry regularly monitors and maintains ethylene levels in cool rooms below the threshold of 0.03 ppm (Jeffery and Banks, 1996; Kim et al., 1999; Saltveit, 1999; Burdon and Lallu, 2011).

Fruit maturity stage and harvest date influences the time to onset of autocatalytic ethylene production. MacRae et al. (1989b) reported that kiwifruit harvested in late June and early July are advanced in their maturity and may prolong the time to produce internal ethylene enough to reach climacteric peak. Less mature kiwifruit (early harvested), held at 0 °C and then allowed to ripen at 20 °C, are more responsive to ethylene sources relative to very mature fruit (late harvested) stored at the similar conditions. This suggests that very mature kiwifruit produce sufficient ethylene via their own metabolism to saturate their potential responsiveness to exogenous ethylene. Thus late harvested fruit show very little response in terms of accelerated softening upon exogenous application of ethylene, once rapid softening has already been initiated by the endogenous ethylene as compared to early harvested (less mature) (Lallu et al., 1989).

Fruit growing location also influences ethylene production. Fruit from different growing locations or orchards produce varying concentrations of endogenous ethylene when stored in same conditions (Castillo et al., 1999). The endogenous ethylene concentration does not provide any correlation with quality change patterns of kiwifruit except for number of rots. Studies have reported poor correlation between firmness change in storage and endogenous ethylene production rates (Castillo et al., 1999; Feng et al., 2003b).

One of the most important factors that initiate the ethylene production is the incidence of postharvest diseases, especially *Botrytis cinerea*. Disease or rots can stimulate ethylene production in infected fruit and this may accelerate softening in neighbouring healthy fruit and lead to losses (Brook, 1991; Manning and Pak, 1993; Niklis et al., 1997; Feng et al., 2003b). Physical damage of kiwifruit can also enhance the production of endogenous ethylene (Sommer, 1989). Any damaged kiwifruit in a box can produce ethylene and cause premature softening (Jeffery et al., 1992).

Monitoring and techniques to reduce and eliminate ethylene production during storage are very important (Sfakiotakis et al., 2001; Atkinson et al., 2011). By controlling ethylene in the storage environment, degrading processes of quality, associated with ripening and senescence can be manipulated. Temperature control (storage at 0 °C) is one of the best options to minimise production and fruit sensitivity to ethylene in the storage environment (Kader, 2002). Highly sensitive ethylene detectors (e.g. ETD - 300) are available to monitor very minute concentrations (i.e. 300 part per trillion volume - pptv) produced by kiwifruit during storage (van den Dungen et al., 2011). Management personnel of storage facilities and pack houses should be aware of the possible sources of ethylene to minimise likelihood of contamination. An efficient ventilation system is very important to minimise the ethylene load produced in the store or pack house area (Banks, 1996).

2.4 MANAGEMENT OF KIWIFRUIT QUALITY

Management of kiwifruit quality at the orchard gate is difficult due to natural variability. Kiwifruit does not have any usual signals of homogeneity in quality unlike other many crops (e.g. apple) and hence when fruit are harvested in a single pick (Tanner et al., 2012) maturity is difficult to be discriminated. Currently industry is attempting to manage this variability through pre and at-harvest quality measures e.g. fruit weight, SSC, DM, firmness and other external characteristics (like cuts, bruises and rots etc.). Meanwhile in storage, variability in quality is managed by regularly monitoring change in firmness of a batch or grower line and incidence of rots and chilling injury development. Kiwifruit softening is an important factor linked to fruit storage performance and is also regarded as the primary indicator of eating ripeness and generally represents the main limiting factor for long term storage (Benge, 1999; Feng, 2003)

In industry, fruit rejection penalties put pressure on growers to manage cultural practices to yield a supply of uniform and premium fruit. Minimum average SSC (6.2%) and DM (14.5%) levels at-harvest are the threshold limits for New Zealand growers to ensure premium quality kiwifruit after storage (Burdon and Lallu, 2011; Burdon et al., 2011). To meet consumer acceptance standards for kiwifruit after 6 months storage, higher SSC levels (7 - 9%) and substantial amounts of DM at-harvest are preferred (Richardson et al., 1997; Jordan et al., 2000; Burdon et al., 2013). However, maturity criteria of minimum SSC levels have little relationship to fruit storage potential or firmness after storage (Mitchell et al., 1992; Burdon et al., 2011). This means that at the time of harvest, it is difficult to predict fruit firmness after storage. Variability in kiwifruit firmness remains a difficult task for the growers and industry to manage (Section 2.4.1). Likewise, rot or disease incidence also contributes to fruit losses (Section 2.4.2).

2.4.1 Firmness variability

Firmness is an important quality determinant of fruit (Jackson and Harker, 1997), and is also regarded as a primary indicator of eating ripeness and generally represents the main limiting factor for long term storage (Burdon and Lallu, 2011). At-harvest firmness of a mature but unripe kiwifruit may range from 6 to 11 kg_f. During the postharvest period, firmness decreases gradually as fruit reach the soft stage, suitable for eating (called eating soft stage at approximately ~ 0.5 - 1 kg_f) (MacRae et al., 1990a; Woodward, 2006). Firmness of kiwifruit varies substantially at-harvest and during postharvest softening between seasons, districts, within and between batches or growers (MacRae et al., 1990b; Kim, 1999; Benge et al., 2000; Feng et al., 2003a; Woodward, 2006). A number of orchard management and cultural practices are responsible for variation in firmness at-harvest that leads to variability in postharvest softening (Section 2.3.1). Kiwifruit storage potential is generally defined as the time after harvest required for the fruit to soften completely with appropriate flavour but without physiological disorders (Burdon and Lallu, 2011). For export, storage potential is the time to reach a firmness of less than 1 kg_f, as the minimum firmness threshold limit applied in industry.

The relationship of at-harvest firmness with postharvest softening is not clear. For example, Benge (1999) and White et al. (2005) stated that at-harvest firmness is a important factor affecting postharvest softening of kiwifruit. However, this does not mean that initial firmness has any relationship with time to soften or rate of softening (Harker et al., 1997). Thus, variability in the rate or extent of kiwifruit softening contributes to economic loss because of the inability to segregate poor storing batches. To reduce fruit loss and increase market returns, industry needs to understand softening variability between kiwifruit populations to make more informed inventory decisions (Adams et al., 2010).

2.4.1.1 Kiwifruit softening

Kiwifruit softening is a well studied process (MacRae et al., 1990a; Benge et al., 2000). Like melons and tomatoes, kiwifruit soften 75 - 100% during the ripening process to reach firmness levels of 0 - 1 kg_f (Johnston et al., 2002). Generally, kiwifruit softening during storage is considered as tri-phasic (Figure 2.2). First a slow phase of softening, followed by a second rapid phase and lastly a slow phase, which is accompanied by the start of internal ethylene production (Schröder and Atkinson, 2006; Atkinson et al., 2011; Burdon and Lallu, 2011). The first slow phase is also referred to as the initial lag period, in which very little change in firmness occurs. The second rapid phase represents the majority of change in fruit firmness usually until the fruit reach 2 kg_f. Following this, the third phase starts and shows decelerating softening until fruit becomes eating ripe (< 1 kg_f). After this, a slightly faster period of softening may start in the over-ripe stage; this has been termed the fourth phase of softening (Benge, 1999). For industry first, second and third phase of softening are most important to ensure the supply of acceptable quality fruit to the consumers. At fourth phase fruit is already very soft (firmness < 1 kg_f) and is considered as not exportable. Therefore, commercially, kiwifruit softening is usually taken as tri-phasic.

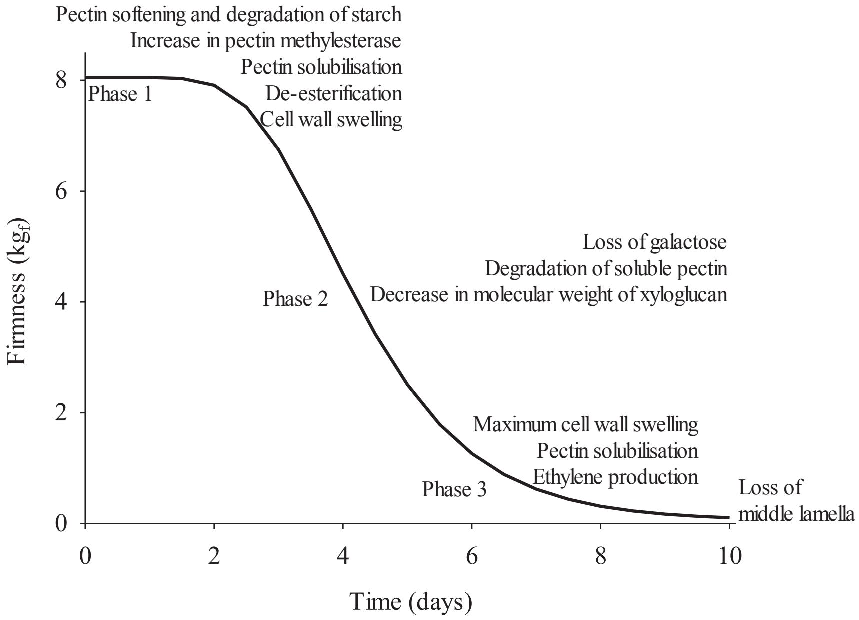

Figure 2.2: Kiwifruit softening phases in relation to different physiological processes (Schröder and Atkinson, 2006).

This tri-phasic softening pattern of kiwifruit is quite common with other fruit such as melons, tomatoes and apples (Johnston et al., 2002). On-vine softening follows the same pattern as it does off-vine. Softening process may exhibit swiftness on-vine around the time of fruit maturation (Burdon and Lallu, 2011). Thus, softening pattern in storage depends on firmness stage at the time of harvest. Firmness, at which the rapid softening (phase 2) occurs is not fixed and can vary for fruit within and between orchards and seasons (Burdon and Lallu, 2011). Rate of softening is an important factor, which determines the deterioration in fruit quality and ultimately influences the storage potential and losses (Brummell and Harpster, 2001).

The process of fruit softening involves coordinated changes of cell wall properties that make the structure weak (Brummell, 2006). Schröder and Atkinson (2006) extensively reviewed changes in cell wall structure during distinctive phases of kiwifruit softening (Figure 2.2). The process of softening starts (in the initial slow phase) with starch degradation, expression of cell wall swelling and increase in pectin methyl esterase activity, initiation of pectin solubilisation and de-esterification. Galactose loss, decrease in molecular weight of xyloglucan and depolymerisation of soluble pectin is believed to occur during rapid phase softening. In the third slow phase, along with internal ethylene production, cell wall swelling reaches its maximum (3 - 4 times increase from at-harvest) and pectin solubilisation continues. Finally, at over-soft or last stage of softening (fourth phase), middle lamella loss occurs with disintegration of cell walls and reduction of adhesion between cells (Schröder and Atkinson, 2006; Burdon and Lallu, 2011). Pectin solubilisation and depolymerisation occurs as a result of polygalacturonase (PG) activity, which requires de-methyl-esterification of pectin by the activity of pectin methylesterase (Brummell and Harpster, 2001). During the course of softening, the initial slow and secondary rapid phases (firmness change from ≈ 9 to 1 ~ 2 kg_f) occur with but low endogenous ethylene production (< 0.2 $\mu L.kg^{-1}.h^{-1}$). The third slow phase (firmness change from 1 ~ 2 to 0.6 ~ 0.8 kg_f) is always accompanied by the increase in autocatalytic ethylene production (Kim, 1999). During the third softening phase, kiwifruit also develops its aroma and respiratory peak (Atkinson et al., 2011).

2.4.1.2 Modelling kiwifruit softening

Describing and modelling variability in softening between batches of kiwifruit is rare. Two different modelling approaches i.e. mechanistic and empirical have potential to model kiwifruit softening. Mechanistic models are constructed as a description of the underlying phenomena, whose response (quality change) is being modelled. The approach of scientific reductionism (breaking a process or phenomenon into its constituents to understand and describe scientifically) is employed in mechanistic models (Thornley and France, 2007). Mechanistic models can simulate kinetics of physical and chemical changes occurring at the cellular level and interpret these as changes in product quality. Involvement of kinetic principles in the modelling procedure usually requires a solution with the application of (a series of) ordinary differential equations (ODEs). As a result, these models have an ability to incorporate the change of inputs (e.g. temperature and other atmospheric conditions). Mechanistic models are commonly used to describe the effects of different environmental conditions on produce quality.

Mechanistic models of quality change are most common in contemporary literature, e.g. for cucumber (Schouten et al., 2002b), apple (Tijskens et al., 1999; Hertog et al., 2001; Gwanpua et al., 2012), peaches (Tijskens et al., 1998) strawberry (Hertog et al., 1999; Schouten et al., 2002a), tomato (Schouten et al., 2004; Hertog et al., 2007; Van de Poel et al., 2012), avocado (Hertog et al., 2003) and including kiwifruit (Hertog et al., 2004b). In the example of mechanistic modelling of kiwifruit softening, Hertog et al. (2004b) characterised the quantitative relationship of gas exchange and fruit softening during modified atmosphere (MA) storage. The model combined an exponential decay (EXP) model of firmness with a Michaelis Menton model to account for the effects of atmosphere change, in addition to allowing all kinetics to be temperature dependent as a function of the Arrhenius equation. In another example, Schotsmans et al. (2008) also used a kinetics model along with EXP model to describe softening as a function of temperature, when firmness data of different measurement methods was compared. While in both examples (Hertog et al., 2004b; Schotsmans et al., 2008) a mechanistic modelling approach was applied, and an empirical equation (EXP) was used to describe kiwifruit softening. Later Feng (2010) proposed a kinetic model for kiwifruit softening based on underlying biochemical changes e.g. starch and cell wall degradation (Adams et al., 2010). Feng (2012) also modelled kiwifruit softening by estimating biological age of a batch from at-harvest firmness. This model followed the technique of modelling propagation of biological variation by the use of a logistic chain model based on biological age (Hertog et al., 2007).

Although mechanistic models may assist in developing understanding of kiwifruit softening, they require extensive understanding of fundamental factors affecting the response of interest to be truly mechanistic. A true mechanistic model based on evidence for the relationship between biochemical or physiological processes with modelled quality (softening) would take years to develop in the first instance. Even then, this may result in the requirement for many parameters each of which needs to be estimated from data to be a genuinely independent model input. For commercial application, it is important to ensure the value of results remains higher than effort and cost of data collection and losses. Any model used is required to be realistically applicable in industry.

Empirical modelling is an alternate approach to describe a response of a system by using mathematical equations without any mechanistic context. These models can characterise and compare biological systems accurately, depending on objectives (Thornley and France, 2007). Use of meaningless parameters in empirical models limits their applicability to the scenarios in which they were developed. A number of empirical models to describe kiwifruit softening have previously been used (Benge, 1999; Benge et al., 2000; White et al., 2005; Feng et al., 2006). In the most thorough work, Benge et al. (2000) compared the ability of five different models to fit mean firmness data of three harvests from one grower line during storage (at 0 °C). The authors found that Complementary Gompertz (CG), Jointed Michaelis-Menton (JMM) and Exponential Polynomial (IEP) models characterised softening accurately and had the potential to compare treatment effects in experimental studies. White et al. (2005) modelled softening profiles of different genotypes of kiwifruit according to Boltzmann function and/or simple EXP model. Later, Feng et al. (2006) also used EXP model to fit firmness data, to calculate storage life of batches at 0.5 °C. However, use of EXP model is not appropriate, as it is unable to describe initial lag (slow) phase when this occurs, during tri-phasic softening of kiwifruit. Johnston et al. (2006) also used an empirical sigmoid equation to fit apple softening in different controlled atmosphere conditions. Overall, the appropriate empirical models can characterise the tri-phasic softening of kiwifruit.

2.4.2 Postharvest diseases

In New Zealand, postharvest rots caused by *B. cinerea* contribute to fruit loss and reduced storage potential. Rots usually do not appear at-harvest but may eventually express symptoms during storage depending upon temperature (Burdon and Lallu, 2011). Storage rots are mainly the result of fruit infection in orchard, suggesting the prime importance of incidence control at orchard level. *B. cinerea* inoculum can be observed in wounded and dead leaves, flowers, and canes. A relationship exists between incidence of rot on fruit and *B. cinerea* presence on leaves in the orchard (Manning et al., 2010). Inoculum is prevalent in orchard but fruit carries only a latent infection because it has natural resistance until later in ripening (firmness of about 1 kg_f).

Storage rot caused by *B. cinerea* is also known as stem-end rot. Rot first appears at stem end and then progresses towards distal end. The rotten area of the fruit becomes water soaked and looks dark green compared to healthier parts. The spread of rot during storage occurs if fruit have disease and succumb to infection, allowing actively growing hyphae to invade neighbouring fruit (Brook, 1991; Michailides and Elmer, 2000).

Fungi from other species such as *Botryosphaeria, Diaporthe* and *Colletotrichum* etc. may not show symptoms during storage but upon removal to ambient conditions or when firmness reaches approximately 1 kg_f, they cause ripe rots (Brook, 1991; Michailides and Elmer, 2000). Any rotten fruit may start producing ethylene and can be the cause of premature softening of the whole tray (Brook, 1991; Niklis et al., 1997; Qadir et al., 1997). Substantially, storage rots contribute to fruit and economic losses incurred because of the requirement to remove rotten fruit and the subsequent repacking costs (Manning and Pak, 1993; Manning et al., 2010).

Incidence of *B. cinerea* can be minimised by effective pruning and canopy management of vines in orchard. Harvesting carefully to avoid any picking wound can also prevent rot incidence and spread during storage (Brook, 1991). Moreover, curing treatment (i.e. 48 hour at ambient temperature) after harvest can reduce incidence of rots in storage. Rot incidence also has relationship with fruit maturity. Mature fruit (at SSC > 7%) are also less susceptible to *B. cinerea* during storage (Michailides and Elmer, 2000; Manning et al., 2010).

2.5 ACCELERATED SHELF LIFE TESTING

The academic definition of shelf life is the amount of time a product can be stored without deterioration (Sewald and Devries, 2003). However, this concept creates a misconception for horticultural products, as quality deterioration starts immediately after harvest. However, for horticultural products, storage life is also defined as the time a product can be stored before reaching a quality standard, which is unacceptable, by the consumer. Accurate storage life prediction is desirable during the supply chain process to minimise the cost of product repacking. Usually storage life prediction tests are designed to validate the length of the time a product can remain as ‘acceptable’ (Kilcast and Subramaniam, 2000) and/or have no change in desired sensory characteristics over the remaining entire life of a product.

Shelf life testing under accelerated conditions is widely adopted by food producers (Sewald and Devries, 2003). This shelf life testing is known as ‘accelerated shelf life testing’. This method involves extrapolation of results from an accelerated environment to the expected results at optimal conditions. A few days or weeks at accelerated conditions required to reach a quality limit may correspond to months of shelf life at optimal conditions. For this form of testing, extreme environmental conditions (e.g. higher temperature and humidity) are used to accelerate the deterioration processes. Rise in storage temperature to create accelerated conditions is often assumed appropriate and interpreted by using Arrhenius model (Kilcast and Subramaniam, 2000). However, the pattern of deterioration (cause of product failure) of a product can differ between two storage conditions. A key assumption in accelerated shelf life testing is that pattern of deterioration in both conditions is correlated (Kilcast and Subramaniam, 2000). For each product, the correlation between the accelerated and optimal conditions will differ. Therefore, prior to industrial application, this approach requires establishment and validation of the relating correlation between storage and accelerated conditions (Kilcast and Subramaniam, 2000).

Accelerated shelf life testing of horticultural products differs from manufactured or processed food products (e.g. milk products) as many physiologically driven bio-chemical processes occur during storage. Another key feature is that one needs to perform this method for every batch of product. In the processed food industry, shelf life date is determined only once to print on the box. A carefully designed method of accelerated shelf life testing to predict the storage performance of fruit has potential to be adopted in the kiwifruit supply chain. However, application of this technique to fruit requires storage and monitoring of sub-samples of each batch of fruit in accelerated conditions. This approach may be referred to as accelerated fruit library monitoring.

2.5.1 Accelerated fruit library

An accelerated fruit library (AFL) is a technique to predict the storage life of fruit and assist in inventory management by identifying and enabling segregation of good or poor storing batches. AFL is different from conventional operational library monitoring, where fruit samples experience the same optimal storage conditions as the stored stock. The purpose of an operational library monitoring is to measure fruit loss at regular intervals to assess the amount and rate of loss in a whole lot (Benge and Kay, 2003). However, operational libraries do not predict the amount or rate of loss in advance. AFL may reflect loss patterns of a lot prior to the loss occurring during optimal storage, allowing mitigating actions to take place before the stock becomes unacceptable. By doing this AFL can facilitate in making efficient inventory decisions and enable reduction of total storage losses. The collection of losses information at accelerated conditions to correlate with normal storage environment may be possible, by doing careful observations of quality parameters that reflect the keeping quality of kiwifruit. For kiwifruit, flesh firmness is an important quality parameter along with number of rots (Section 2.4). In case of kiwifruit, application of ethylene and high temperature storage (or both) could be used to accelerate the softening losses.

Creation of an AFL requires loss assessment during a monitoring period to capture existing variability between fruit or batches. Data collected in AFL may combine the effect of many pre and at-harvest factors on storage potential. A robust relationship between AFL and storage data is required prior to industrial application (East, 2011). There are several examples of predicting storage potential or performance of fruit by following the pattern of losses at accelerated conditions. Ingle and Morris (1989) reported that softening rate in optimal storage conditions (0 °C) was correlated with softening at-harvest and at ambient conditions (20 °C). Later, after harvest flesh firmness change at 20 °C was used to indicate the storage potential of apple cultivars (Iwanami et al., 2004; Iwanami et al., 2008). East et al. (2008) proposed that rot incidence in mangoes stored at variable temperatures can be explained with Weibull distribution model. In addition, batch dependent model parameters at high temperature can indicate rot incidence in the same fruit at optimal (low temperature) conditions (East et al., 2008). East (2011) also predicted storage performance of strawberry batches as influenced by rot development from data collected in accelerated conditions (higher temperatures). In another example, at-harvest acceleration of degrading process by Mg^{2+} infiltration was used to predict the incidence of bitter pit in apple after storage (Burmeister and Dilley, 1993; Retamales et al., 2000; Sestari et al., 2009). These examples of AFL suggested that segregation of kiwifruit grower lines for good or poor storage potential may be achievable.

2.5.2 Options to accelerate kiwifruit softening

Both ethylene application and high temperature storage have the potential to accelerate kiwifruit softening and other losses (e.g. rots). Ethylene plays an important role in accelerating fruit ripening processes (Saltveit, 1999; Sfakiotakis et al., 2001; Section

2.3.2.3). Many researchers studied kiwifruit physiological and biochemical changes with application of ethylene at ambient conditions. Jeffery et al. (1992) stated that exposure of kiwifruit to 5 ppm ethylene at ambient conditions (20 °C) resulted in substantial reduction in firmness from 6.3 to 1.1 kg_f within 4 d of treatment. Wills et al. (2001) compared sensitivity of climacteric fruit to ethylene concentrations (i.e. < 0.005, 0.01, 0.1, 1 and 10 ppm). Banana and, kiwifruit were found highly sensitive to ethylene as compared to custard apple, mango, tomato, avocado and peach. Park et al. (2006) exposed kiwifruit to ethylene (100 ppm for 24 h) at 20 °C to study ethylene biosynthesis during ripening and observed a substantial decrease in flesh firmness at 2 d of treatment. Likewise, Korsak and Park (2010) also reported the same effect of ethylene exposure on kiwifruit to accelerate fruit softening. Therefore, ethylene can definitely accelerate the softening of kiwifruit in AFL. This high softening rate in accelerated environment could potentially be correlated with firmness loss in ethylene free low temperature storage. However, application of ethylene would not be as easy to apply in industry. Deliberate application of ethylene may entail a risk of contamination of the remainder of the fruit in the storage facility.

After packaging, kiwifruit are usually stored at low temperature (0 °C) for an extended period (Sale, 1990; Cotter et al., 1991; Burdon and Lallu, 2011) referred as optimal storage. During storage, temperature is of prime importance being a direct influencing factor on fruit quality, particularly loss of firmness (Ferguson and Stanley, 2003). Storage of kiwifruit at higher temperature (e.g. 20 °C) accelerates the ripening process because of the more rapid onset of autocatalytic ethylene production (Antunes and Sfakiotakis, 2000, 2002b). Given the examples of AFL application, use of high temperature could serve the purpose of accelerated fruit library establishment (Ingle and Morris, 1989; Iwanami et al., 2004; Iwanami et al., 2008; East, 2011). High temperature storage to accelerate fruit losses provides an opportunity for expressing uniform environmental conditions for all samples of many grower lines. Application of high temperature during AFL would be easy to adopt by industry with little additional cost or training.

2.6 QUALITY STANDARDS AND SAMPLING PLAN

The kiwifruit industry monitors quality by following cosmetic, shape, size, SSC accumulation, DM and time-variable defects (softening, rots and storage disorders) (Benge and Kay, 2003; Maguire and Mowat, 2003). The industry assesses fruit quality to meet in-market export and customer acceptable quality limits. For kiwifruit, the acceptable quality limits define the very small numbers of defects that consumers are willing to accept. These acceptable quality limits of defects ranges from 0 - 2% of the fruit lot but in some cases (e.g. packaging defects) it is up to 5% (Personal communication). For some quarantine related defects (diseases), there is a zero tolerance limit. However, an acceptable quality limit does not mean a desirable level. Consumers usually can tolerate a few more defects to a certain level higher than acceptable quality limit that is called threshold limit. However, the consumer is not willing to tolerate or accept the produce beyond the threshold limit (Reid and Sanders, 2007).

Product acceptability depends on quality and consumer’s acceptance limit of defects. Acceptance limit is influenced by economical and psycho-social limits of the consumer, while the product quality is defined by inherent properties (Tijskens and Polderdijk, 1996). For kiwifruit, acceptance limits for quality aspects are defined by the industry to take into account the consumer acceptance and fruit quality after harvest. Flesh firmness is the key determinant of kiwifruit quality for export and consumption. The firmness of ‘Hayward’ kiwifruit at harvest generally ranges from 6 to 11 kg_f, it considered to be eating soft at ~ 0.5 - 1.0 kg_f (Woodward, 2006). New Zealand kiwifruit industry standards are such that lines of fruit should not be exported if the mean firmness (measured by penetrometer) falls below the export threshold level of 11.8 N or 1.2 kg_f, and individual fruit firmness must be higher than 9.81 N or 1.0 kg_f (Jackson and Harker, 1997; Benge, 1999). However, with the recent understandings of variability within kiwifruit populations, use of average firmness to compare lines for storage potential is discouraged. It has been redefined that fruit with lowest firmness have importance to dictate the longevity or storability of a lot (Tanner et al., 2012).

The acceptable quality limit for the frequency of soft defects in lot of kiwifruit is standardised as 1.3% (as upper specification limit; personal communication). Upper limits are set to assure the quality of a lot is at a desired threshold level when product reaches the customer. Standards are designed to be easy to follow for making rapid decisions regarding rejection or acceptance of a fruit lot. Numerical (in proportion or percent) limits of defects to accept and reject depends entirely on the sample size as a function of population or lot. Sample size has huge importance for crops like kiwifruit that exhibit substantial variation for flesh firmness between fruit within a batch (Feng et al., 2003a). Acceptable quality limits for different defects also varies with sample size to accept or reject a lot. The discrimination power of a sampling size can be explained in a graphical form known as operative characteristics (OC) curve (Reid and Sanders, 2007). An OC curve displays the probability or chance (%) of accepting a good or bad (both) batch, given various proportions (%) of defects in the lot (Figure 2.3).

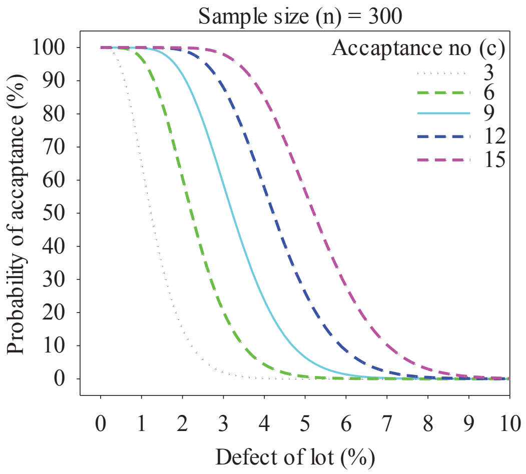

Figure 2.3: Operating characteristics curves for sample size of 300 with different acceptance numbers.

Hypothetical OC curves have defects of lots on x-axis and probability of acceptance on y-axis. Each curve represents probability of acceptance or rejection of a lot (sample size (n) = 300) at particular acceptance number (c) of defects (known as lot quality or proportion of defects). Proportion of defects represents the acceptable quality levels, which a consumer can accept in the whole lot of produce (Reid and Sanders, 2007). The steeper the slope of the OC curve, the more discrimination can be derived between ‘good’ and ‘bad’ by the chosen sample size. Probabilities of lot acceptance with given different levels of defects are obtained by using cumulative binomial distribution (Reid and Sanders, 2007).

For an acceptable limit of soft fruit (1.3%) in a lot, the OC curve can be interpreted for a sample size of 300, at acceptance number 9 (fruit with firmness less than 1 kg_f), there is 99% probability of rejecting a lot with more than 1.3% of soft fruit. For a different sample size, the acceptance number will be different to meet the standard of acceptable quality limit. Likewise, the acceptance number can vary for different kinds of defects. Increase in sample size would require higher acceptance number. However, a decrease in sample size would result in lower acceptance number for any defect and it can decrease the chance of accepting a lot with acceptable quality limits. An increase in the proportion of defects in any particular lot can decrease the chance of accepting it (Reid and Sanders, 2007). Different sample sizes can be tested for the potential to reject or accept a lot at any particular acceptable quality limit.

Kiwifruit industry follows ISO-2859 regulations of quality control to select sample size from fruit populations to apply standards of acceptable limits for different types of defects. Sample size of 300 is usually observed as per ISO standards to test a population or lot of fruit for export threshold limits (personal communication). For ease of application, acceptable quality limits for any defect of kiwifruit has been manipulated to decipher fractile of a sample. Fractile is an industrial approach of evaluating a fruit lot as per export standards and generally described as a defined fraction of population above or below a specific threshold limit (Jordan and Loeffen, 2013). Presence of soft defect or fruit has prime importance to accept or reject a population of kiwifruit for export. Fractile of soft defects in a population is called soft fractile (SF). SF shows the firmness value of that softest fruit beyond which the acceptable quality limit exceeds and lot can be rejected if SF is less than 1 kg_f. Acceptable quality limit of soft defect (1.3%) is assessed as SF, which becomes the acceptance number of 9th lowest firmness value of sample size of 300 fruit. This means for export threshold, any lot with 8 fruit having firmness less than 1 kg_f in a sample of 300, is considered acceptable while lots with 9 or more fruit with firmness less than 1 kg_f are rejected. SF represents the firmness of softest 3% fruit of a batch/line/lot/grower, which are the first to soften during storage. Industry usually monitors firmness losses during storage and uses SF information to compare different grower lines or batches for storage performance and to make inventory decisions (Adams et al., 2010).

2.7 SUMMARY

This chapter has provided an overview of kiwifruit industry in New Zealand. In addition, review of kiwifruit supply chain has highlighted the need by the industry for a robust system of storage quality prediction. Many pre and postharvest factors have been discussed which incorporates substantial variation in fruit quality during storage. Variability in the rate of firmness loss remains a challenge in the kiwifruit industry, particularly for making inventory decisions. AFL can be a potential approach to predict quality of fruit batches after storage. Nevertheless, development of an AFL to segregate kiwifruit grower lines for storage potential needs to be explored. This research attempts to test and validate the development of an AFL for kiwifruit grower lines to predict their storage potential. In addition, this study also focuses on minimising the cost and time of monitoring fruit quality in AFL and optimal storage. Lastly, this research also investigates the relationship between fruit losses in AFL and optimal storage to develop a predictive method of segregating GLs with good and poor storability.

3 Accelerated Fruit Library of Kiwifruit – A Preliminary Understanding

3.1 INTRODUCTION

One of the factors that contribute to postharvest losses is the inherent variability between grower lines (GLs) for storage potential or keeping quality of fruit. Many pre and postharvest factors are responsible for this variability between GLs. Previous season knowledge of fruit behaviour and at-harvest quality measures (i.e. SSC, DM and firmness) are not efficient to identify poor and good storing lines. Inventory decisions based on previous season and at-harvest information still involve the chances of fruit and profit losses.

Accelerated shelf life testing is a method to evaluate the keeping quality of a product by correlating losses in accelerated and optimal storage conditions (Sewald and Devries, 2003). Evaluating keeping quality of fruit using a fruit library kept at accelerated conditions is referred as 'Accelerated Fruit Library (AFL) monitoring'. Understanding of relationship between losses in AFL and optimal storage conditions is possible, through careful observations of parameters (e.g. firmness) that reflect the keeping quality of fruit. Two major assumptions are made when developing an AFL to segregate poor and good storing kiwifruit GLs. Firstly, the AFL sample is assumed to represent the variability observed in each GL. Secondly, losses observed in the AFL are assumed to have a relationship to losses in optimal storage conditions.

AFL monitoring is a technique to solve the problem of predicting shelf life of fruit batches and hence assist in making decisions in inventory management. A number of factors could be used to accelerate fruit losses during library monitoring. In the case of kiwifruit, application of ethylene and/or high temperature storage could accelerate the fruit ripening (Antunes and Sfakiotakis, 2000; Antunes, 2007). Kiwifruit are very sensitive to low concentrations (0.01 ppm) of ethylene in the storage environment (Ben-Arie and Sonego, 1985; Mitchell, 1990; Antunes, 2007). However, application of ethylene would not be as easy to apply in industry. Deliberately adding ethylene to the AFL represents a risk of contamination of the remainder of the fruit in the storage facility. At high temperature (i.e. 20 °C), kiwifruit behaves more like a climacteric fruit and start autocatalytic ethylene production, resulting in accelerated ripening (Antunes and Sfakiotakis, 2000; Antunes and Sfakiotakis, 2002a; Antunes, 2007). Use of high temperature to accelerate the ripening process of kiwifruit could potentially be effective in developing a correlation with shelf life at optimal storage conditions (i.e 0 °C). Application of high temperature during AFL would be easy to adopt by the industry with little additional cost or training. Therefore, for AFL of kiwifruit GLs, samples were stored at 20 °C.

Recently, Iwanami et al. (2008) used an accelerated shelf life test to define storage quality of different apple cultivars. Apple fruit of the same lot were stored in two batches at 20 °C and 0.5 °C. Fruit stored at 0.5 °C retained firmness and acidity for 8.9 and 3.7 times (respectively) longer than at 20 °C. Firmness and acidity at 0.5 °C were also predicted from the change in these parameters at 20 °C. Previously, Ingle and Morris (1989) also stored apple at two temperatures (0 °C and 20 °C), and proposed an equation to predict softening rate at 0 °C from 20 °C. However, this procedure was not useful for other apple varieties. These findings for apple suggested applicability of a similar AFL procedure for kiwifruit to distinguish GLs for different softening patterns.

Correlation of losses in AFL monitoring and optimal storage has potential to improve judgments about the storage potential of fruit. The method of assessment and manipulation of loss data may have a large impact on the success of AFL based predictions. One of the main objectives of AFL development for kiwifruit GLs is to adopt simple evaluation methods (i.e. firmness) that allow rapid assessment and interpretation of losses. These evaluation methods would help to make rapid and accurate predictions of storage potential and assist in inventory decisions. For loss assessment, change in fruit firmness is the most widely accepted criterion during storage along with rot incidence (Hewett et al., 1999; Ferguson and Stanley, 2003). To interpret loss data, there are several easy to calculate potential parameters of firmness distribution, average, variability and already established export thresholds, and defect counts.

This chapter describes the development of AFL method through evaluation of relationships between fruit losses observed during AFL and optimal storage. To generate the AFL data, 20 °C storage was used to accelerate fruit losses (i.e. firmness and rots). For optimal storage data, fruit was stored at 0 °C. The resulting data were analysed with the aim of differentiating GLs for storage potential whilst attempting to minimise AFL data collection effort. In a preliminary study, a large sample size of 300 fruit (approximately) per GL was used at each measurement occasion of AFL and optimal storage to capture representation of kiwifruit softening and other losses. Later sample size for AFL and number of measurement occasions for optimal storage were mathematically manipulated to reduce cost and effort of data collection.

3.1.1 Objectives

- Generation of a substantial data set of AFL and optimal storage data from different GLs.
- Development of an understanding about accelerated fruit losses (e.g. softening) during storage at 20 °C.
- Evaluate the relationship between losses of AFL and optimal storage, by using simple correlation of many easy to calculate AFL parameters.
- Mathematical manipulation of AFL sample size and assessment time in optimal storage to minimise the effort and cost of data collection.

3.2 MATERIALS AND METHODS

A preliminary AFL testing experiment was designed. To accelerate ripening of fruit a high temperature (20 °C) strategy was adopted, to be compared with fruit quality data recorded at optimal storage (0 °C). For each GL, 57 modular bulk (MB) packs (99 - 100 fruit/pack wrapped in polyliner) of count 36 ‘Hayward’ kiwifruit were sent in temperature control transport from a commercial packing facility in Bay of Plenty to the Postharvest Laboratory at Massey University, Palmerston North. In total 20 GLs were collected by receiving two lines per day from Tuesday to Friday over the harvest season of 16 d, started from May 5, 2010 (ISO day 125) to May 21, 2010 (ISO day 141). International Standard Organization (ISO) date/day is a system used by the industry for time keeping and to track fiscal years. In this study, ISO day was used to define day of each fiscal year.

Upon arrival, MB packs of each GL were randomly divided into three (3) groups of fruit measurement; at-harvest, optimal storage and AFL (Figure 3.1). MB packs of each GL were randomly allocated a pre-printed label that dictated the storage condition and time (day) of measurement.

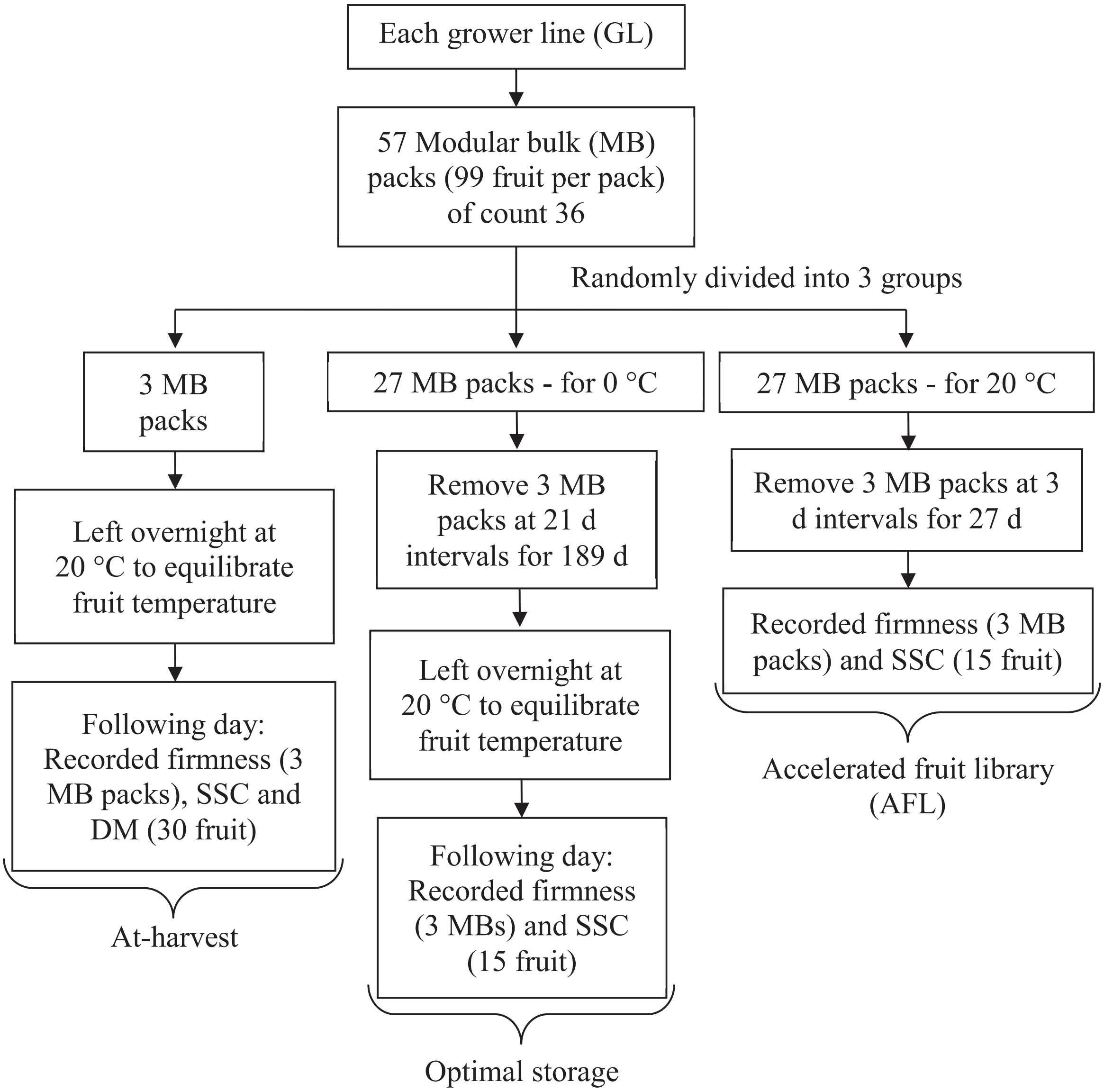

Figure 3.1: Fruit distribution and scheme of data collection for at-harvest, optimal storage and accelerated fruit library for each GL in 2010.

For at-harvest measurements, 3 MB packs per GL were left overnight at room temperature (20 °C) to equilibrate fruit temperature. Next day, firmness of all fruit in 3 MB packs, and soluble solids content (SSC) and dry matter (DM) of 30 fruit per GL were measured. For AFL, 27 MB packs per GL were stored at 20 °C for 27 d and fruit quality was monitored at 3 d interval. At each AFL measurement occasion, firmness of 3 MB packs and SSC of 15 fruit per GL were measured. For optimal storage, 27 MB packs per GL were stored at 0 °C for 189 d and fruit quality was assessed at 21 d interval. At each occasion, 3 MB packs per GL were removed from cool store and left overnight at room temperature (20 °C) for fruit temperature equilibration. Next day, firmness of all fruit in 3 MB packs and SSC of 15 fruit per GL were measured. For all measurement groups and occasions, incidence of rot, soft patches, bruise and any damage were also recorded.

Fruit temperature equilibration was performed for at-harvest and optimal storage data, to avoid errors in firmness measurement due to difference in temperature (Jeffery and Banks, 1994). During AFL and optimal storage monitoring, RH of >90% was maintained. Ethylene concentration in rooms was monitored intermittently during storage period. Room temperature was measured by thermocouples and was recorded in data loggers (Grant - 1200 Squirrel Digital Meter/Logger, Grant Instruments Ltd. Barrington, Cambridge, England). Large ethylene scrubbing sachets of Purafil® (Potassium permanganate - $KMnO_4$) were also placed in rooms in order to minimise ethylene accumulation during storage.

3.2.1 Firmness

Firmness of kiwifruit was assessed by QALink Penetrometer (Willowbank Electronics Ltd., Napier, New Zealand), attached to a computer. A standard 7.9 mm Effegi probe was fitted with penetrometer. Fruit skin of 1 - 2 mm (approximately) thickness was removed from two locations, at 90° angle to each other, around the equator. Fruit area without skin was then subjected to puncture at 8 mm depth with the speed of 20 $mm.s^{-1}$. Minimum measurable firmness was set at 0.1 kg_f. The average peak force of two measurements per fruit was recorded.

3.2.2 Soluble solids content (SSC)

SSC of fruit was measured by a digital pocket refractometer (PAL-1, Atago, Japan), after calibration to 0% by using distilled water. Fruit juice was extracted from the blossom and stem ends after a 15 mm cut at each end. Approximately equal amounts of fruit juice from both tissues were mixed on refractometer prism. Data were recorded as percentage soluble solids.

3.2.3 Dry matter (DM)

Fruit DM was determined by careful removal of water through drying. First marked empty petri dishes or weighing boats were weighed by using a balance (Mettler PG-503S, Toledo, Switzerland) with 0.001 g accuracy. A 2 - 3 mm equatorial slice was obtained from fruit. After placing fruit slice on the petri dish, it was re-weighed to record the fresh weight. Petri dishes were then placed in drying oven at 60 - 65 °C for 24 h. When weight of the dried sample was stable, each petri dish was weighed to record dry weight. DM was then calculated as percentage of fruit (Eq. 3.1).

$$\text{DM} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100 \qquad \text{Eq. 3.1}$$

3.2.4 Ethylene detection

For ethylene measurement ETD-300 (Sensor Sense, Nijmegen, Netherlands) was used, which is able to detect ethylene at very low concentrations (0.3 ppb). A sample pump (NMP 05B, KNF Neuberger GmbH, Germany) was used to suck air from different cool room locations and supply to the sensor at 5 L.h^{-1} using a continuous flow method. Sample air first passed through Drierite (self-indicating 8 mesh 2 mm granules, Acros Organics, Thermo Fisher Scientific, New Zealand) and soda lime to scrub moisture and CO_2, respectively. For ethylene detection from each location, continuous flow of sample was maintained until a consistent concentration of ethylene was achieved. Between each location or sample, ethylene free air was used to standardise the sensor.

3.3 DATA MANIPULATION AND ANALYSIS

3.3.1 At-harvest

To explore change in fruit quality characteristics as influenced by progression through the season, SSC, DM and firmness were assessed by the day of arrival (ISO d). Fruit for each GL was received within two days of harvest and we had no control over fruit storage between harvest and day of arrival.

3.3.2 Optimal storage

Firmness data from optimal storage were used to define the soft fractile (SF, 9th lowest firmness value of approximately 300 fruit, Table 3.1). SF is developed from the threshold limit of 1.3% failure, which means if 3% of population of 300 fruit have firmness value less than 1 kg_f, so it can be tolerated (Section 2.6). SF is a common form of expressing firmness data. SF are also used to understand and define the firmness trend and variability of GLs in industry (Adams et al., 2010). Time to reach SF of 1 kg_f is a common method to compare GLs for storage performance (Burdon et al., 2013). So, SF of GLs was defined over the storage period to estimate storage life. Storage life of a GL was the time (days) to reach a SF less than 1 kg_f. As firmness data were recorded at 21 d intervals, storage life was estimated by using linear interpolation between two data points (former and the first value when SF < 1 kg_f), to approximate a day when SF was less than 1 kg_f. From storage life, seasonal life of each GL was also calculated in ISO days. Seasonal life was defined as day of year (in ISO days), when each GL reached SF less than 1 kg_f. Calculating seasonal life of each GL rescaled storage life to the expected failure time during the year. Seasonal life may assign the same ISO day of year to GLs with different harvest times and storage life but it enables prioritisation of distribution decisions on a real time scale. Therefore, seasonal life of GLs was used for further data processing.

3.3.3 Accelerated fruit library (AFL)

Initial manipulation was performed to extract summary statistics and other parameters i.e. export threshold, distribution descriptors, average, variability and defect count; of AFL data sets (Figure 3.2, Table 3.1). Export threshold comprises a widely used parameter of SF, which is 9th softest fruit in a population of 300 fruit. Industry also uses distribution descriptors to assess and compare different batches quality during storage. Therefore, distribution descriptors like count of fruit with firmness less than 1, 2 or 3 kg_f, fruit at eating soft (~ 0.6 - 1 kg_f) and below eating soft (~ <0.6 kg_f) stage, and firmness value at 1st and 3rd quartile were also calculated. Distribution patterns of firmness values of a sample population were also assessed by skewness and kurtosis. Parameters related to averages comprise mean and median firmness while variability in observed firmness was evaluated by highest, lowest firmness, range (highest minus lowest), and standard deviation. Counts of fruit with rots, soft patches, bruise and defects were also recorded to evaluate losses in fruit quality other than softening during AFL.

Table 3.1: Parameters derived from AFL data at each measurement occasion. Each population was obtained from 3 MB packs of count 36 'Hayward' kiwifruit resulting in approximately 300 individuals.

Category	Parameters	Description
Export threshold	Soft fractile (SF, kg_f)	9th lowest value of firmness from 300 fruit
Distribution descriptors	Number of fruit <1 kg_f	
	Number of fruit <2 kg_f	
	Number of fruit <3 kg_f	
	Number of fruit within eating window (0.6 - 1 kg_f)	
	Number of fruit below eating window (< 0.6 kg_f)	
	1st quartile (kg_f)	Firmness value below which 25% of the firmness values are lying
	3rd quartile (kg_f)	Firmness value below which 75% of the firmness values are lying
	Skewness	Measure of symmetry
	Kurtosis	Measure of peakedness
Average	Mean (kg_f)	Average firmness
	Median (kg_f)	Middle firmness value
Variability	Minimum value (kg_f)	Minimum firmness value
	Maximum value (kg_f)	Maximum firmness value
	Range (kg_f)	Difference between highest and lowest fruit firmness
	Standard deviation (kg_f)	Measure of firmness variability
Defect count	Number of fruit with rot, soft patch, bruise and defect	

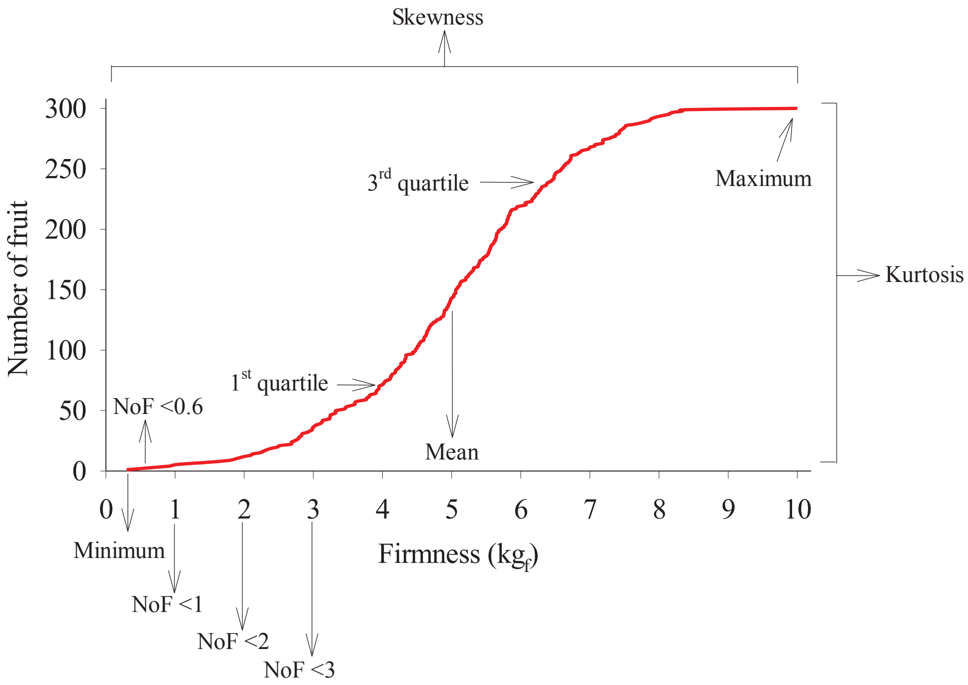

Figure 3.2: An hypothetical cumulative frequency graph to show different parameters of AFL firmness data. NoF: number of fruit.

Simplicity and ease of measurement was the main purpose to evaluate these AFL parameters. This was to make interpretation of results easy to follow at industrial scale with minimum calculation effort. AFL parameters were extracted for all measurement times separately with the propose to demonstrate storage potential of different GLs. Particular attention was given to parameters that had good prediction power at several adjacent measurement times (termed consistent parameters), because this should increase the likelihood of finding the same predictions in any data set. SSC:firmness ratio was also calculated from mean SSC and firmness values at each measurement occasion to follow the comparative change in both quality attributes during AFL.

3.3.4 Relating at-harvest and AFL to optimal storage

To investigate the usefulness of at-harvest and AFL monitoring data for generating information about storage potential of GLs in a season, calculated parameters were correlated with seasonal life (Figure 3.3). For this purpose, three different linear correlation techniques were followed. Firstly, AFL parameters were correlated to the observed seasonal life (Pearson correlation). Secondly, both AFL parameters and seasonal life were converted from the continuous ranges to ranked data sets ranging from 1 - 20, with one being the smallest value and 20 being the highest value recorded.

Ranked parameters were then correlated with ranked seasonal life (Spearman correlation). Thirdly, continuously variable AFL parameters were correlated with ranked seasonal life. Linear correlation gave an advantage that value or rank of GL in AFL monitoring could reflect value or rank of GL in optimal storage. This technique would be quick and easy to perform in an industrial environment. Obtaining a correlation from AFL parameters to ranked seasonal life of GL was advantageous because a simple continuous range of AFL parameters could reflect the rank of GLs throughout a season.

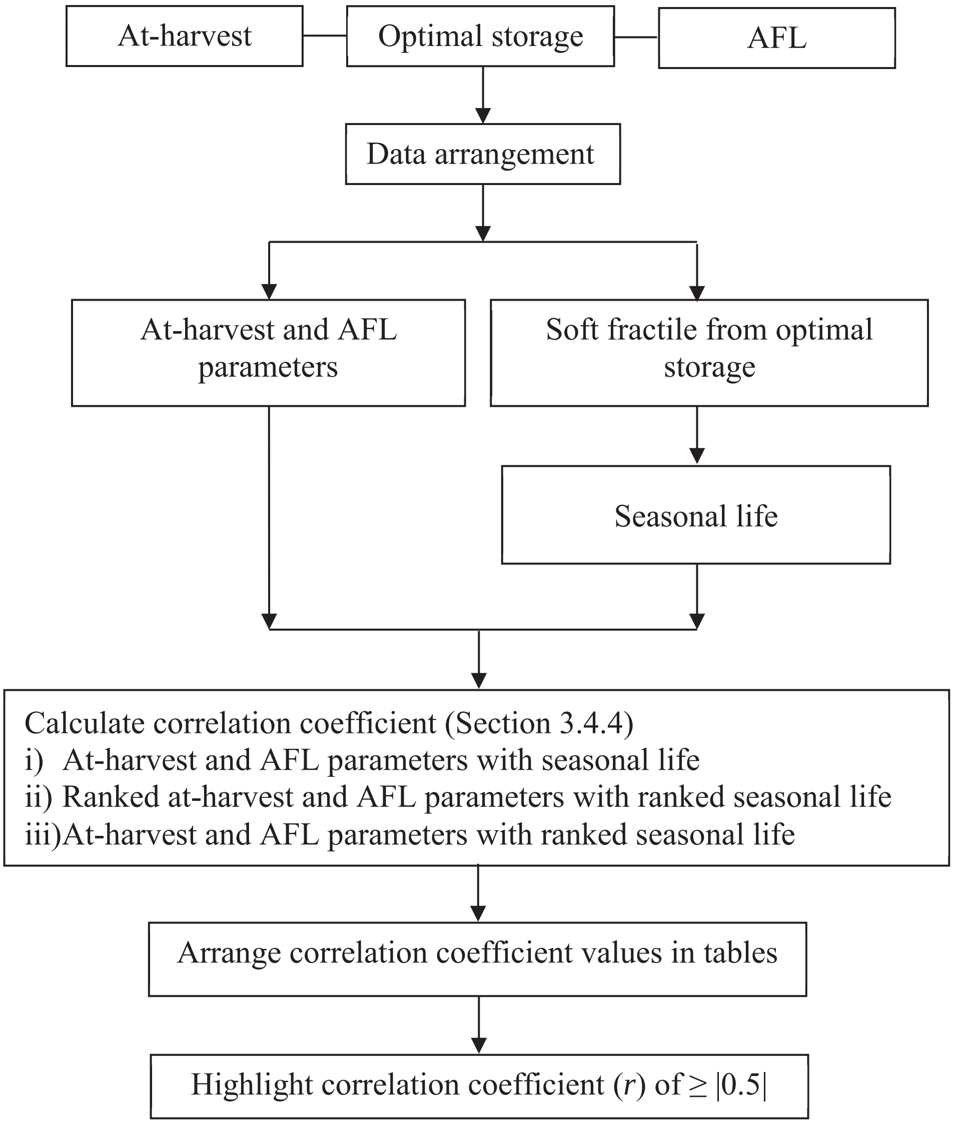

Figure 3.3: Scheme of data manipulation and calculation of correlation coefficients (*r*) for at-harvest and AFL parameters with storage performance of kiwifruit GLs.

3.4 RESULTS AND DISCUSSION

3.4.1 At-harvest

Fruit quality was expected to change over a harvest season. SSC increased as the season progressed ranging from 6.8 to 11.6% (Figure 3.4A). Increase in SSC over the harvest season was a result of starch conversion to sugars (Beever and Hopkirk, 1990; Burdon et al., 2011; Burdon et al., 2013). In New Zealand, SSC of 6.2 % is a minimum harvest index for export, however this is not optimum value and therefore, fruit are usually harvested at higher SSC of 7 - 9% (Richardson et al., 1997; Burdon et al., 2013). Results also confirmed that SSC of different GLs may vary even when harvested at the same time (Feng, 2003).

At-harvest DM decreased with the harvest season except one early harvested GL (with DM of 16.5%) and varied from 16 to 19.6% (Figure 3.4B). Observed DM values were in the expected range (12 - 20%) for kiwifruit. In New Zealand, the at-harvest minimum DM threshold is 14.5%. DM of kiwifruit is an important at-harvest quality criteria because higher DM assures better quality (high SSC) at eating ripe stage and therefore it has direct relation with consumer preference (Burdon and Lallu, 2011). More consumers like fruit harvested with DM equal or higher than 15.1% (Crisosto et al., 2012). In New Zealand, growers receive a premium for higher DM fruit and therefore commercially harvested kiwifruit usually have a DM of 14 - 17%. The DM range may vary depending upon season, orchard and time of harvest (Jordan et al., 2000; Burdon et al., 2004; Burdon and Lallu, 2011). Overall, DM of all GLs was in the expected range of commercial acceptability.

At-harvest mean firmness varied from 6.5 to 8.29 kg_f (Figure 3.4C). One GL had lower at-harvest mean firmness of 5.83 kg_f. Firmness of harvested kiwifruit was typical of commercial crop (MacRae et al., 1990a; Feng et al., 2003a; Feng, 2012; Burdon et al., 2013). However, soft fractile (SF, firmness of 9th softest fruit in 300) could also represent the variation in at-harvest firmness. SF varied from 4.6 to 6.3 kg_f (difference of 1.75 kg_f). Same GL with lower mean firmness (5.83 $kg_{f.}$) had lowest SF of 3 kg_f (Figure 3.4C). Overall firmness of different GLs harvested at different times was expected to decrease over the season as fruit ripe (MacRae et al., 1989a; MacRae et al., 1989b; Beever and Hopkirk, 1990). However, over the 16 d of harvest season, there was no substantial decrease in firmness.

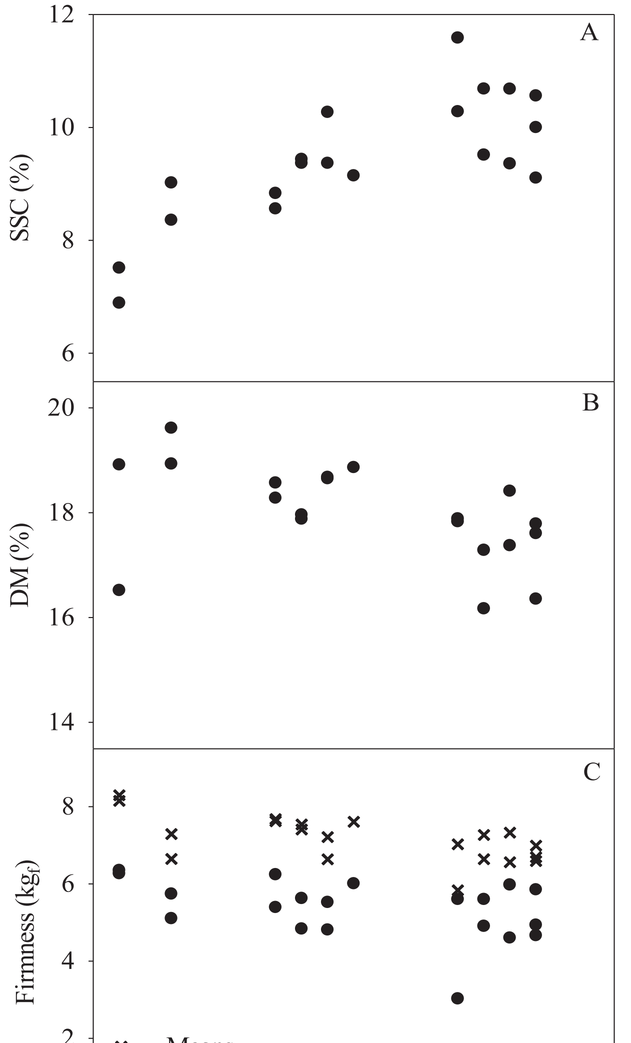

Figure 3.4: SSC (A), DM (B) and firmness (C) of GLs at harvest day in 2010. Each data point represents a GL. For SSC and DM each data point is an average of 30 fruit (10 fruit per MB pack), and for firmness an average of 297 fruit. Soft fractile represents firmness value of 9th softest fruit in population of 300 fruit for each GL.

Overall, at-harvest fruit quality (SSC, DM and firmness) of all GLs was in a commercially acceptable range. SSC of GLs increased with the progression of harvest season. However, DM and mean firmness did not show any trend over the short harvest season. Variation in fruit quality from different GLs obviously would result in variable storage quality. Changes occurring in fruit quality at the time of harvest affect the softening potential during storage, for example fruit having rapid accumulation of DM at-harvest can be softer after storage compared to fruit with slower rate of DM accumulation. Likewise, SSC accumulation rate at the time of harvest also contributes to substantial variation between orchards, for fruit firmness in storage (Adams et al., 2010).

3.4.2 Optimal storage

Softening curves of GLs during optimal storage exhibited a range (from 195 to 307 ISO d) of variability in time to reach 1 kg_f of SF (Figure 3.5). Variability in softening patterns of GLs was large that of 20 lines harvested within 16 d, some reached export threshold of 1 kg_f at 57 d of storage and for some it took approximately 180 d. This led to variation in seasonal life of GLs when rescaled on ISO day of year. The expression of storage potential in seasonal life will characterise GLs for their time to reach export threshold (SF < 1 kg_f) on a time scale of use to the industry and would give a comparison between GLs. Variation in softening curves demonstrated the behaviour of fruit from different growing locations and orchards with varying management and handling practices (Mitchell et al., 1992; Arpaia et al., 1994; Benge, 1999; Adams et al., 2010). Variation in softening patterns can also be affected by different at-harvest firmness (Crisosto et al., 1984; Benge et al., 2000; Ghasemnezhad et al., 2013). The observed variation in softening, demonstrates the magnitude of the challenge in making accurate predictive decisions about storage performance of different GLs. Ethylene concentration of the cool room remained below 0.03 ppm (industry tolerance limit) during whole storage period.

During optimal storage, SSC increased for all GLs over the first 42 - 63 d and then remained relatively stable in the range of 12 - 16% (Figure 3.6). SSC of fruit usually increases in optimal storage as process of ripening occurs (Kempler et al., 1992; Burdon et al., 2013). Overall, there was not much difference observed between GLs for patterns of SSC rise during storage and the increase in SSC did not reveal season timing effect. However, pattern of SSC accumulation may be similar between GLs but the timing of the rapid rise in SSC with ripening varies between GLs and season (Adams et al., 2010; Burdon et al., 2013).

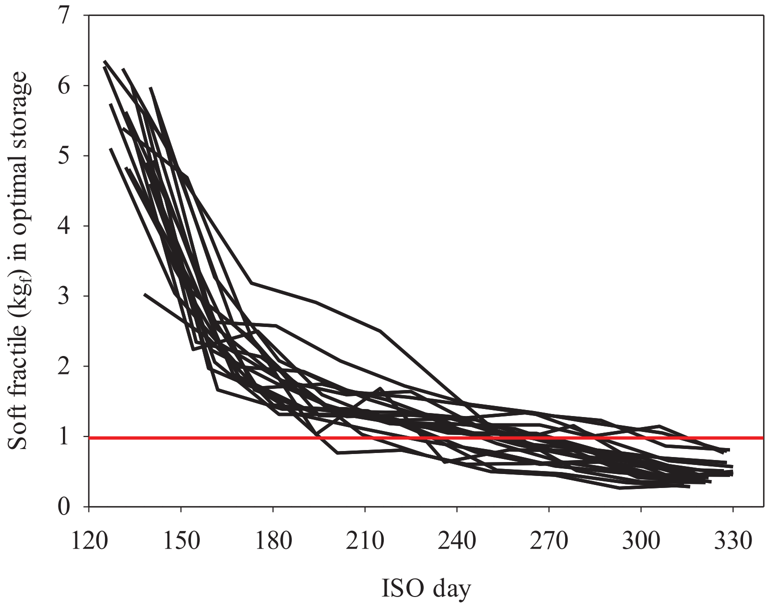

Figure 3.5: Change in soft fractile values of 20 GLs during optimal storage in 2010. Each firmness line represents a single GL and comprises data of 10 measurement occasions at 21 day intervals. Soft fractile is firmness of 9th softest fruit in a population of 300. Red line represents export threshold limit of 1 kg$_f$.

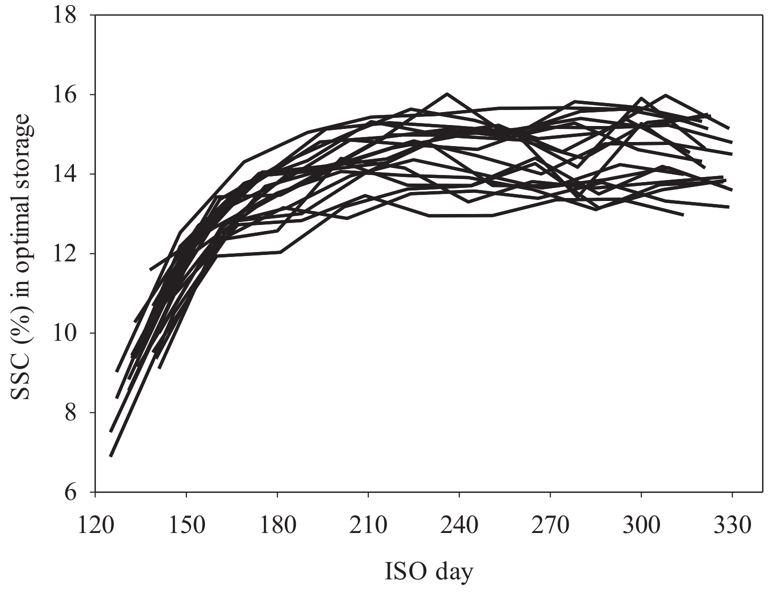

Figure 3.6: Change in SSC development during optimal storage for 20 GLs in 2010. Each line represents a single GL and comprises data of 10 measurement occasions at 21 day intervals. SSC at each measurement occasion comprise an average of 15 fruit.

From storage life (days to reach SF less than 1 kg$_f$), seasonal life of each GL was derived. Seasonal life (ISO d on which a GL reaches SF < 1 kg$_f$) is a rescaling of storage life on ISO days and helps to categorise GLs in a real time scale for time to fail. Seasonal life was found positively associated (*r* = 0.53) with ISO day of arrival (Figure 3.7), meaning that early harvested GLs had lower seasonal life as compared to those harvested later in a season. This result agreed with industry observation of late harvested fruit usually displaying longer storage life (Mitchell et al., 1992; Costa et al., 1997; Feng et al., 2006). An outlier GL at 137 ISO d of arrival reached the export threshold (SF < 1 kg$_f$) at 195 ISO d. Hence, it was clear that AFL would need to give better predictions of storage or seasonal life than simply following day of arrival of a GL.

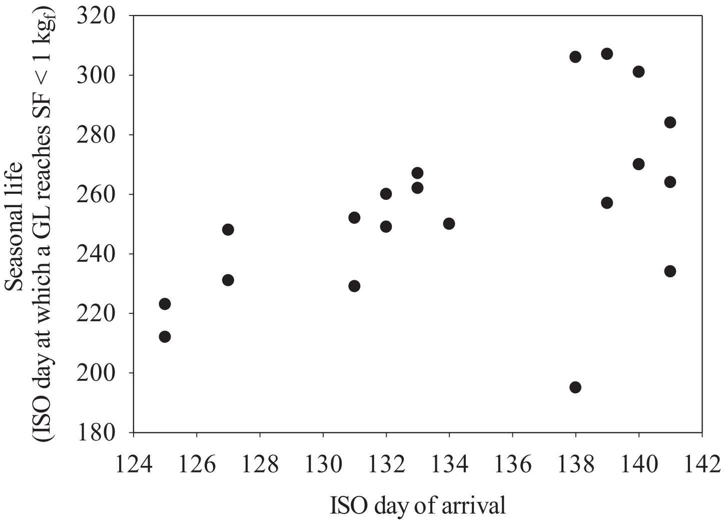

Figure 3.7: Pearson correlation of seasonal life of GLs with ISO day of arrival. Each data point represents a GL.

3.4.3 Accelerated fruit library (AFL)

Softening of GLs was rapid at 20 °C (Figure 3.8). Most notably early harvested GLs displayed longer storability before reaching a SF of 1 kg$_f$. These GLs generally displayed an appreciable lag phase of few days (6 - 9 d) before rapid softening. Contrastingly, later harvested GLs consistently displayed a rapid decline in firmness in the initial period of the AFL monitoring (the first 3 - 6 d). At the same time, ethylene within the AFL room was also monitored. Kiwifruit stored at 20 °C have substantial ethylene production as they soften (Antunes, 2007). In this work, the ethylene concentration was observed to build throughout the season in the AFL room, peaking at

0.8 ppm. Kiwifruit are known to be sensitive to low concentrations (i.e. 0.01 ppm) of ethylene even at 0 °C (Banks, 1996; Pranamornkith et al., 2012). The differences in softening patterns between earlier and late harvested GLs observed may be a manifestation of a seasonal effect. Alternatively, this rapid decline in firmness of later GLs may be a result of the ethylene concentration within the AFL room. Benge (1999) also observed biphasic rapid softening in fruit as consequence of ethylene contamination in a cool store and proposed that only in ethylene free conditions would fruit soften in their normal tri-phasic fashion.

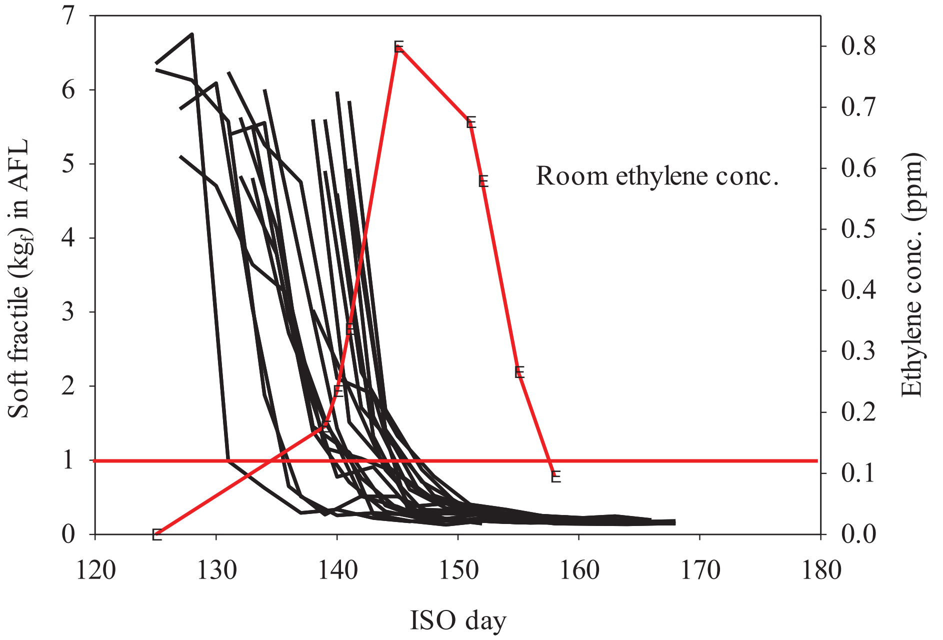

Figure 3.8: Change in soft fractile of 20 GLs during AFL monitoring in 2010. Each line represents a GL and comprises data of 10 measurement occasions at 3 day intervals. Soft fractile is firmness of 9th softest fruit in a population of 300. Red line represents export threshold limit of 1 kg_f.

The rise in SSC during AFL monitoring followed the at-harvest variation (ranging from 6.8 to 11.8) of 4.8% for all GLs. After a quick rise in SSC, the variation narrowed to 3.6% (ranges from 13.3 - 16.9, Figure 3.9). A few early harvested GLs displayed a relatively slow rise (in 6 - 9 d) in SSC compared to those harvested later (in 3 - 6 d) in the season. There are two possibilities for these differences. Firstly, late harvested fruit already had higher at-harvest SSC (10 - 11.8% in comparison to 6.8 - 8.8%). Hence, SSC was already increasing quickly when late harvested fruit were initially exposed to 20 °C. Alternatively, ethylene was already present in the room; produced by early harvested GLs may have also stimulated starch to sugar conversion in the late harvested fruit. The highest room ethylene concentration (0.8 ppm) coincided with the highest SSC peak in all GLs. A decline in SSC after the rapid rise was also evident. After complete degradation of all starch, fruit respiration reduces SSC (Smith and Clark, 1989; Burdon et al., 2013).

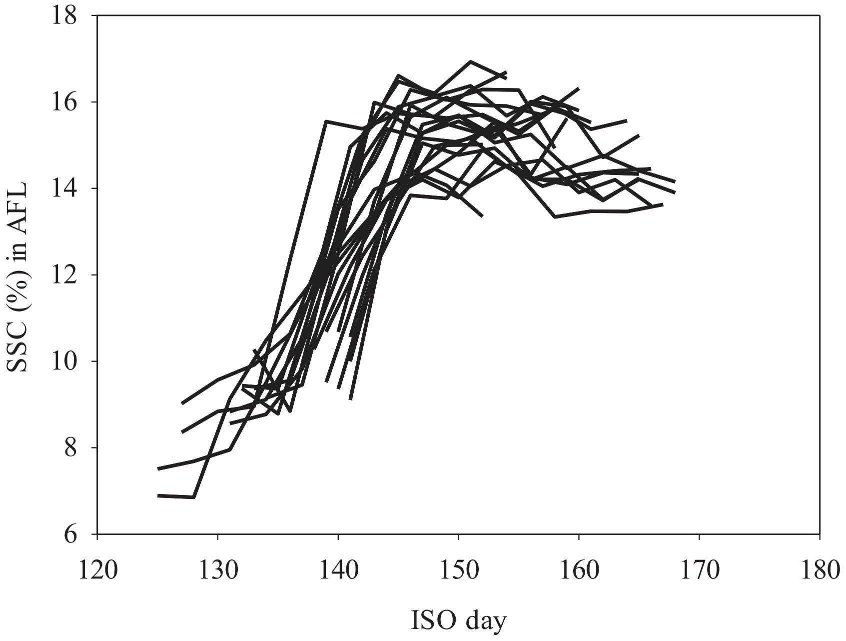

Figure 3.9: Change in SSC development during AFL monitoring for 20 GLs in 2010. Each line represents a single GL and comprises data of 10 measurement occasions at 3 day intervals. SSC at each measurement occasion comprises an average of 15 fruit.

3.4.3.1 Ethylene effect in AFL

Kiwifruit flesh firmness is best maintained by storage at 0 °C while maintaining very low levels of air ethylene (< 0.03 ppm) (McDonald, 1990; Cotter et al., 1991; Sfakiotakis et al., 2001). Kiwifruit do not produce much ethylene (< 0.01 $nL.kg^{-1}.h^{-1}$), particularly at the time of harvest (Park et al., 2006; Burdon and Lallu, 2011). However, at higher temperature (20 °C) kiwifruit behaves more like a typical climacteric fruit with autocatalytic ethylene production, leading to accelerated ripening and a decrease in firmness (Ming et al., 1992; Antunes and Sfakiotakis, 2002a; Chiaramonti and Barboni, 2010). Kiwifruit are sensitive to very low concentrations of ethylene (i.e. 0.01 ppm) during storage (Ben-Arie and Sonego, 1985; Mitchell, 1990). Therefore, ethylene produced by naturally ripening fruit may induce rapid ripening in adjacent unripe fruit

(Abeles et al., 1992). Quick decline in the firmness of later arrived GLs could be attributed to ethylene present in the AFL room (Figure 3.8).

To observe inherent softening behaviour, the AFL concept required the exposure of fruit to consistent abusive storage conditions. Inherent softening behaviour was essential in AFL monitoring to maintain relevance with optimal storage. Possibly the prevailing ethylene influence may have caused the quick decline in softening of late harvested GLs, reaching the threshold of 1 kg_f within 3 - 6 d of AFL monitoring. Early harvested fruit usually display an initial lag phase in softening after harvest (MacRae et al., 1990a) while for late harvested fruit the lag phase may disappear (MacRae et al., 1990a; Benge, 1999). The AFL softening data showed a similar pattern for early and late harvested GLs. However, on the other hand, the length of harvest season (16 d) used in this work was quite short, which suggest that the argument for ethylene causing the change in softening patterns was more likely the case. To overcome this uncertainty, future AFL monitoring efforts in this thesis were conducted to ensure an environment where ethylene produced by one GL cannot affect the softening pattern of other GLs. In achieving this condition, each GL could express its inherent softening trend and hence data collected from the AFL is more likely to be related with optimal storage.

3.4.4 Relationship of at-harvest and AFL parameters with seasonal life

Initially for parameter calculation, AFL firmness data were divided into two forms, with rots and without rots. “With rots” means, firmness of fruit having rot was included, whereas “without rot” means firmness of fruit with notable rot was excluded in parameter calculation. In industry during quality check, at certain threshold (e.g. proportion of rots in a selected sample from a population), repacking is performed to replace rotten or damaged fruit with healthy fruit. However, parameters from AFL firmness data (when calculated with or without rots) did not vary significantly in relation to seasonal life, suggesting that firmness values of rotten fruit (in AFL) may not affect prediction of storability of GLs (data not shown). Therefore, to ensure the AFL monitoring approach approximates industrial post-repack assessment, AFL firmness parameters without rots were calculated.

Correlation coefficient (*r*) values were calculated for seasonal life with parameters extracted from one at-harvest and nine AFL measurement occasions (Tables 3.2 - 3.4). Only correlation coefficients (*r*) of ≥ |0.5| were highlighted. This 0.5 threshold was adopted because seasonal life was previously correlated with ISO day of arrival (r = 0.53, Figure 3.7). Therefore, there would be no advantage if at-harvest or AFL parameters had a weaker association ($r \leq |0.5|$) with seasonal life. The resulting highlighted patches in table 3.2 - 3.4 indicate parameters of AFL that provided similar or better correlation with seasonal life. Continuously highlighted patches (with $r \geq |0.5|$) at two or more measurement occasions are more likely to be of value in different seasons. Conversely, highlighted patches that are isolated may appeared by chance and hence are unlikely to be consistent enough to be used as a storage life indicator.

Pearson correlation of at-harvest and AFL parameters (at 9 measurement occasions) with seasonal life revealed 27 combinations with $r \geq |0.5|$ (Table 3.2). The highest (*r*) value of -0.7 (with skewness) was found in only one instance (at 18 d of AFL monitoring). GLs with higher values for 3rd quartile firmness, maximum firmness value, firmness range and standard deviation at 9 - 15 d of AFL monitoring had tendency to have lower seasonal life ($r \geq -0.5$). GLs with higher number of rots at 24 d of AFL monitoring were more likely to have longer seasonal life ($r = 0.54$). For at-harvest quality indicators, GLs with higher SSC and SSC:firmness ratio had propensity to exhibit longer seasonal life (r = 0.6 and 0.5 respectively). DM at-harvest did not correlate with seasonal life.

Table 3.2: Pearson correlation coefficients (r) of at-harvest and AFL parameters with seasonal life (d) of 'Hayward' kiwifruit GLs. Yellow highlights represent negative correlation ($r \leq -0.5$) and green highlights show positive correlation ($r \geq 0.5$). Grey highlights represent unrounded coefficient values with $r \leq |0.5|$. NoF: number of fruit.

Parameters	At-harvest and AFL monitoring (day)									
	0	3	6	9	12	15	18	21	24	27
Soft fractile (SF)	-0.3	🟨 * -0.5	-0.3	-0.2	-0.1	-0.1	-0.2	-0.2	-0.1	-0.1
NoF < 1 kg$_f$	0.0	0.1	0.2	0.3	0.4	0.5	0.3	-0.2	-0.5	-0.2
NoF < 2 kg$_f$	0.3	0.1	0.2	0.4	0.4	0.5	0.1	-0.3	🟨 * -0.5	-0.2
NoF < 3 kg$_f$	0.3	0.2	0.4	* 0.5	0.4	🟩 * 0.5	-0.2	-0.3	🟨 * -0.5	-0.2
NoF at 0.6 - 1 kg$_f$	0.0	0.3	0.2	0.1	0.2	-0.1	-0.3	-0.3	-0.2	-0.2
NoF < 0.6 kg$_f$	0.0	-0.2	0.1	0.4	0.4	* 0.5	0.4	0.1	-0.3	-0.1
1st quartile	* -0.5	-0.4	-0.4	-0.4	-0.3	-0.4	-0.2	-0.2	-0.2	-0.3
3rd quartile	* -0.5	* -0.4	* -0.5	🟨 * -0.5	🟨 * -0.5	🟨 * -0.5	-0.4	-0.3	-0.4	-0.4
Skewness	0.1	0.3	0.4	0.2	-0.1	-0.3	🟨 *** -0.7	-0.3	-0.2	-0.1
Kurtosis	-0.2	-0.4	-0.4	0.1	0.2	0.1	🟨 * -0.5	-0.2	-0.1	-0.1
Mean	* -0.5	* -0.5	* -0.5	* -0.5	* -0.5	🟨 * -0.5	-0.4	-0.3	-0.3	-0.3
Median	* -0.5	* -0.4	* -0.5	* -0.5	* -0.4	🟨 * -0.5	-0.3	-0.2	-0.3	-0.4
Minimum value	-0.1	* -0.5	0.0	-0.2	0.0	-0.1	-0.1	0.1	0.0	0.1
Maximum value	🟨 * -0.5	-0.4	* -0.5	🟨 * -0.5	🟨 * -0.5	🟨 * -0.5	* -0.5	-0.4	* -0.5	-0.2
Range	-0.2	-0.2	🟨 * -0.5	🟨 * -0.5	🟨 * -0.5	🟨 * -0.5	* -0.5	* -0.5	🟨 * -0.5	-0.2
Standard deviation	0.0	-0.1	-0.4	🟨 * -0.5	🟨 * -0.6	🟨 * -0.5	* -0.5	* -0.5	🟨 * -0.5	-0.4
SSC:firmness ratio	🟩 * 0.5	0.4	0.3	0.4	* 0.5	0.4	0.4	0.4	0.3	0.4
Number of rots	0.4	0.0	0.2	0.3	0.0	0.1	0.2	0.3	🟩 * 0.5	0.3
SSC	🟩 * 0.6	* 0.5	* 0.5	* 0.5	0.3	0.1	-0.1	-0.2	-0.2	-0.1
DM	-0.1									

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; Values without * are non-significant ($P>0.05$)

Conversion of at-harvest and AFL parameter values at each measurement occasion, from continuous scale to their rank of 1 - 20 (1 being smallest and 20 as highest value in a population of 20 GLs) improved the correlation with the ranked seasonal life (1 being lowest and 20 as longest seasonal life in days). Application of Spearman correlation (ranked at-harvest and AFL parameters with ranked seasonal life) resulted in 67 combinations with $r \geq |0.5|$ (Table 3.3). GLs with higher rank for 3rd quartile firmness (from 3 - 15 d); mean and median (from 0 - 12 d); maximum firmness value (from 0 - 24 d); firmness range (from 6 - 24 d) and standard deviation (from 9 - 27 d) had tendency to have lower (rank for) seasonal life ($r \geq -0.5$). Meanwhile GLs having more number of fruit < 2 kg_f and more number of fruit < 3 kg_f (at 3 and 9 d); and more number of rots (at 21 - 24 d) during AFL monitoring had propensity to have longer seasonal life ($r \geq 0.5$). Furthermore, GLs with higher (rank) SSC and SSC:firmness ratio at-harvest and at 3 - 9 d of AFL monitoring tended to exhibit longer (higher rank) seasonal life ($r \geq 0.5$). Overall, 9 AFL parameters had consistent correlation ($r \geq |0.5|$ continuously at two or more measurement occasions) with ranked seasonal life of GLs. As in table 3.2 maximum firmness value, firmness range, standard deviation and 3rd quartile firmness showed extended periods of good correlation but now (in Spearman correlation) several other parameters also showed promise including mean, median, SSC and SSC:firmness ratio (Table 3.3).

Correlation of actual at-harvest and AFL parameters (continuous scale) with ranked seasonal life (1 being lowest and 20 as longest seasonal life in days) could make interpretation of AFL based information easy to define the rank of GL for storage potential. Correlation of at-harvest and AFL parameters with ranked seasonal life resulted in 63 combinations with $r \geq |0.5|$ (Table 3.4). GLs with higher values for 1st quartile firmness (from 0 - 3 d); 3rd quartile firmness, mean and median (from 0 - 15); maximum firmness value and firmness range (from 6 - 24 d) and standard deviation (from 9 - 24 d) tended to have a lower (rank of) seasonal life ($r \geq -0.5$). Meanwhile, GLs with more number of fruit < 2 kg_f and more number of fruit < 3 kg_f during AFL monitoring from 9 to 15 d had tendency to have longer (higher rank) seasonal life ($r \geq 0.5$). SSC at-harvest and during AFL monitoring also suggested that GLs with higher SSC (from 0 - 9 d) had tendency to have longer seasonal life ($r = 0.6$). Overall, 10 parameters were consistently associated ($r \geq |0.5|$ continuously at two or more measurement occasions) with seasonal life of GLs.

Table 3.3: Spearman correlation coefficients (*r*) of ranked at-harvest and AFL parameters with ranked seasonal life (d) of 'Hayward' kiwifruit GLs. Yellow highlights represent negative correlation ($r \leq -0.5$) and green highlights show positive correlation ($r \geq 0.5$). Grey highlights represent unrounded coefficient values with $r \leq |0.5|$. NoF: number of fruit.

Parameters	**At-harvest and AFL monitoring (day)**									
	0	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**
Soft fractile (SF)	-0.3	* -0.5	-0.4	-0.4	-0.2	-0.2	-0.3	-0.2	-0.2	-0.1
NoF < 1 kg_f	0.0	0.2	0.3	0.4	0.4	0.4	0.3	-0.3	** -0.6	-0.2
NoF < 2 kg_f	0.2	* 0.5	0.4	* 0.5	0.4	* 0.4	0.0	-0.4	** -0.6	-0.2
NoF < 3 kg_f	0.2	* 0.5	0.4	* 0.5	0.4	* 0.4	-0.2	-0.4	** -0.6	-0.2
NoF at 0.6 – 1 kg_f	0.0	0.3	0.3	0.2	0.0	-0.4	* -0.6	-0.4	-0.4	-0.2
NoF < 0.6 kg_f	0.0	-0.1	0.2	0.3	* 0.4	* 0.5	0.3	0.1	-0.3	-0.2
1st quartile	* -0.5	* -0.5	* -0.4	** -0.6	-0.4	-0.4	-0.4	* -0.5	-0.4	-0.3
3rd quartile	* -0.5	* -0.6	* -0.6	* -0.5	** -0.6	* -0.5	* -0.5	** -0.6	* -0.5	-0.4
Skewness	0.1	0.3	0.4	0.3	0.0	* -0.5	*** -0.8	0.0	-0.1	-0.1
Kurtosis	-0.2	* -0.4	-0.4	0.3	0.3	0.0	* -0.4	-0.3	0.3	-0.1
Mean	* -0.5	* -0.5	* -0.5	* -0.5	** -0.6	* -0.5	* -0.5	** -0.6	-0.4	-0.4
Median	* -0.5	* -0.5	* -0.5	** -0.6	* -0.5	* -0.5	-0.4	* -0.5	* -0.5	-0.4
Minimum value	-0.1	-0.4	0.0	-0.4	0.0	-0.1	-0.1	0.0	0.0	0.1
Maximum value	** -0.6	** -0.6	** -0.6	** -0.6	** -0.6	** -0.6	** -0.6	** -0.6	** -0.6	* -0.5
Range	-0.2	-0.2	** -0.6	** -0.6	** -0.6	*** -0.7	** -0.6	** -0.7	*** -0.7	* -0.5
Standard deviation	-0.1	-0.1	-0.4	** -0.6	** -0.6	*** -0.7	** -0.7	** -0.7	*** -0.7	* -0.5
SSC:firmness ratio	** 0.6	* 0.5	* 0.5	* 0.5	** 0.6	0.4	0.4	** 0.6	0.4	0.4
Number of rots	0.3	0.0	0.1	0.1	0.1	0.3	0.4	* 0.6	** 0.6	* 0.5
SSC	** 0.6	* 0.5	** 0.6	* 0.5	0.4	0.0	0.0	-0.1	-0.1	-0.1
DM	-0.3									

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; Values without * are non-significant ($P>0.05$)

Table 3.4: Correlation coefficients (*r*) of actual at-harvest and AFL parameters with ranked seasonal life (d) of 'Hayward' kiwifruit GLs. Yellow highlights represent negative correlation ($r \leq -0.5$) and green highlights show positive correlation ($r \geq 0.5$). Grey highlights represent unrounded coefficient values with $r \leq |0.5|$. NoF: number of fruit.

Parameters	At-harvest and AFL monitoring (day)									
	0	3	6	9	12	15	18	21	24	27
Soft fractile (SF)	-0.4	**-0.6	-0.4	-0.3	-0.2	-0.2	-0.2	-0.3	-0.2	-0.1
NoF < 1 kg_f	0.0	0.2	0.3	0.3	*0.5	**0.6	0.4	-0.2	*-0.5	-0.1
NoF < 2 kg_f	0.3	0.2	0.3	*0.5	*0.5	*0.6	0.1	-0.3	*-0.6	-0.1
NoF < 3 kg_f	0.3	0.3	*0.5	*0.6	*0.5	*0.6	-0.2	-0.3	*-0.6	-0.1
NoF at 0.6 - 1 kg_f	0.0	0.3	0.3	0.1	0.2	0.0	-0.3	-0.4	-0.3	-0.2
NoF < 0.6 kg_f	0.0	-0.1	0.3	*0.4	0.4	*0.5	*0.4	0.1	-0.3	-0.1
1st quartile	*-0.5	*-0.5	*-0.5	*-0.5	-0.3	*-0.4	-0.3	-0.3	-0.3	-0.3
3rd quartile	*-0.5	*-0.5	**-0.6	**-0.6	**-0.6	**-0.6	*-0.5	-0.4	*-0.5	-0.4
Skewness	0.2	0.3	*0.5	0.3	-0.1	-0.3	***-0.7	-0.3	-0.1	-0.1
Kurtosis	-0.2	-0.4	-0.4	0.2	0.2	0.1	*-0.5	-0.2	-0.1	-0.1
Mean	*-0.5	*-0.5	*-0.6	**-0.6	**-0.6	**-0.6	*-0.5	-0.4	*-0.4	-0.4
Median	*-0.5	*-0.5	**-0.6	**-0.6	*-0.5	**-0.6	-0.4	-0.4	-0.4	-0.4
Minimum value	-0.1	*-0.5	-0.1	-0.2	0.0	-0.2	-0.1	0.0	-0.1	0.1
Maximum value	**-0.6	*-0.5	**-0.6	**-0.6	**-0.6	**-0.6	**-0.6	*-0.5	*-0.5	-0.3
Range	-0.2	-0.2	**-0.6	**-0.6	**-0.6	**-0.6	**-0.6	*-0.6	**-0.6	-0.3
Standard deviation	0.0	-0.1	*-0.5	**-0.6	**-0.6	**-0.6	*-0.6	**-0.6	**-0.6	-0.4
SSC:firmness ratio	**0.6	0.4	0.4	*0.5	*0.6	*0.5	*0.5	*0.5	0.4	0.4
Number of rots	0.3	0.0	0.2	0.2	0.0	0.1	0.3	0.4	**0.6	*0.4
SSC	**0.6	*0.6	**0.6	*0.6	0.4	0.2	0.0	-0.2	-0.2	-0.1
DM	-0.1									

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; Values without * are non-significant ($P>0.05$)

Correlation of ranked parameters (at-harvest and AFL) with ranked seasonal life increased the number of combinations with $r \geq |0.5|$ (27 to 67) and resulted in more number (9) of consistent parameters ($r \geq |0.5|$ continuously at two or more measurement occasions (Table 3.2 and Table 3.3). Correlation of actual at-harvest and AFL parameters with ranked seasonal life slightly reduced the number of combinations (63) with $r \geq |0.5|$ (Table 3.3 and Table 3.4) but increased the number of consistent parameters (10). Interestingly, the period from 9 - 15 d in AFL monitoring (when fruit were rapidly softening) gave the best indications of seasonal life (ranked); but it is important to note that there were many quite good correlations ($r \geq |0.5|$) at-harvest as well (Table 3.4). Given that the kiwifruit softening curve is not linear; the relationship of AFL parameters to seasonal life was checked for linearity by drawing scatter plots. Overall in general, the Pearson correlations (Table 3.2), Spearman correlations (Table 3.3) and correlations of at-harvest and AFL parameters with ranked seasonal life (Table 3.4), exhibited a linear relationship between AFL parameters and seasonal life of GLs (e.g. Figure 3.10).

From all parameters maximum firmness value, firmness range, standard deviation, 3rd quartile, mean, median, SSC:firmness ratio and SSC showed promise as AFL parameters to indicate seasonal life of GLs. Even then, AFL data did not appear better than at-harvest correlations, which were measured without complexity of AFL. From all at-harvest promising parameters (in Table 3.2 - 3.4), SSC of GLs was most prominent parameter to indicate the storage potential (seasonal life). GLs with higher SSC at-harvest had longer seasonal life ($r = 0.63$, Table 3.2 - 3.4, Figure 3.10). These results agree with previous findings in which advanced maturity at-harvest as indicated by higher SSC (%) resulted in better storing fruit (Beever and Hopkirk, 1990; Feng et al., 2006). Hence, AFL technique should be better or be able to add more precision in predicting the storage potential of GLs in industry.

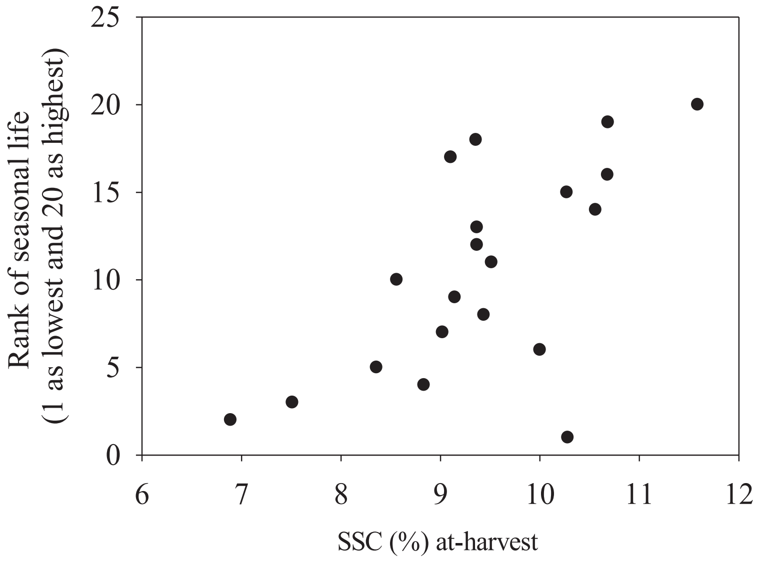

Figure 3.10: Correlation between at-harvest SSC with rank of GLs for seasonal life (d). SSC is mean of 30 fruit per GL. Highest rank means highest and lowest rank represents lower seasonal life of any GL. Each data point represents a GL.

3.5 IMPLICATIONS FOR FUTURE AFL EXPERIMENTS

3.5.1 Number of grower lines

The New Zealand kiwifruit industry has more than 2700 growers (Anon, 2013b). Despite the large amount of data collected in this first season, the use of only 20 GLs to develop AFL methodology for storage life prediction was limited. Therefore, in the next season more GLs with a wider variability in harvest timing (season length) were to be included in the study.

3.5.2 Softening potential evaluation

Amount of time required for quality assessment and availability of storage space were two major constraints of optimal storage of fruit. So to measure more GLs, solutions were required to remain within the working constraints of these two resources. One potential solution was to reduce the sampling frequency for fruit quality evaluation during optimal storage. In 2010, firmness of approximately 300 fruit (3 MB packs) was recorded at 21 d intervals on 9 measurement occasions during optimal storage. If number of measurements could be reduced, it would greatly facilitate examination of a large number of GLs. To achieve this seasonal life of GLs was correlated with SF values at each time of measurement (Table 3.5). Results showed that SF at 126 d of optimal storage was highly associated (r = 0.94) with seasonal life of GLs (Figure 3.11). This result suggested that evaluation of SF at 126 d of optimal storage could reflect the rank of GL for its softening potential. Therefore, for future study with the increase in number of GLs, sampling frequency for fruit quality evaluation could be reduced to just one or two assessments around 126 d of optimal storage.

Table 3.5: Correlation coefficients (*r*) of seasonal life with soft fractile of GLs at each measurement occasion during optimal storage.

Measurement occasion	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Day	0	21	42	63	84	105	126	147	168	189
Correlation coefficient (*r*)	-0.38	-0.30	-0.05	0.32	0.40	0.74	0.94	0.75	0.76	0.70

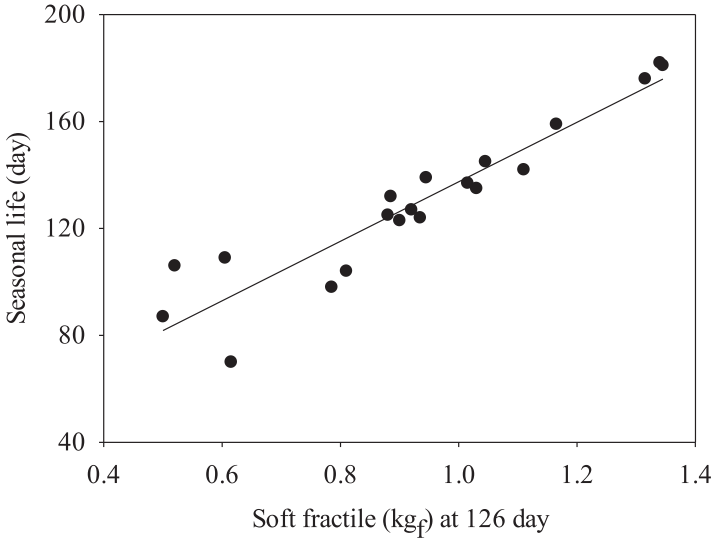

Figure 3.11: Correlation of seasonal life with soft fractile at 126 day of optimal storage. Each data point represents one GL.

3.5.3 AFL sample manipulation

During AFL monitoring, fruit quality of 300 fruit was assessed on each measurement occasion (at 3 d intervals for 27 d) for each GL (i.e. total 30,000 fruit per GL). This measurement protocol required substantial effort to collect the data during AFL monitoring period. In an industrial scenario, hundreds of GLs make their way into the supply chain every day during harvest season. Establishment of a similar AFL data collection approach would be difficult and might restrict the potential of this technique due to cost and time requirements. Therefore, AFL sample size manipulation was also performed mathematically to assess the effect of reduced sample size on resulting data sets. For this purpose, AFL firmness data recorded on 15 d of measurement for each GL was arbitrarily selected. Firmness values of 300 fruit for each GL were randomly divided into 10 sample groups of 30 values. Each sample group was further processed to calculate population description parameters. These population description parameters of AFL firmness (30 values) were then correlated with SF at 126 d of optimal storage.

For example, AFL average firmness calculated from a sample size of 30 was correlated with ranked SF (1 rank means lowest and 20 rank means highest value of SF) at 126 d of optimal storage (Figure 3.12). The same level of correlation (*r* = -0.55) was observed between AFL monitoring and seasonal life, as was observed when the parameter was extracted from 300 fruit. Hence, a 30 fruit sample at each measurement occasion during AFL monitoring could produce the same extent of correlation that was observed with 300 fruit. The extended horizontal bars represent the range of mean values from the 10 samples of 30 fruit. These bars also show the expected range of variability when mean firmness of 30 fruit instead of 300 was correlated with ranked SF at 126 d. This result suggested that sample size for each measurement occasion during AFL monitoring could be reduced to 30 fruit. Therefore, for future study with the increase in number of GLs, AFL sample size was reduced at each measurement occasion.

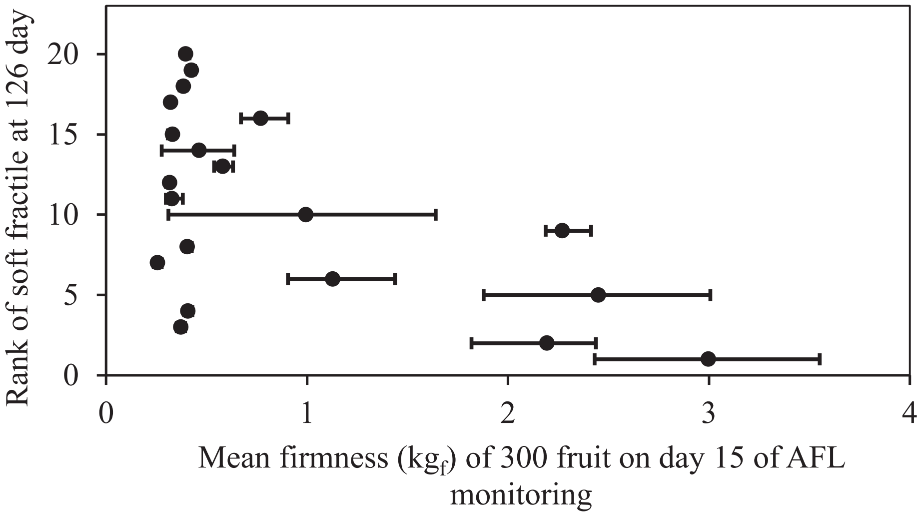

Figure 3.12: Mean firmness values at 15 day of AFL monitoring with rank of GL for soft fractile at 126 day of optimal storage. Each data point represents mean firmness of approximately 300 fruit for each GL. Extended horizontal bars represent the range of mean values for 10 samples of 30 fruit.

3.6 CONCLUSION

This chapter provides an introductory basis for AFL establishment and its capability to define the storage potential of ‘Hayward’ kiwifruit. Substantial data set for fruit quality during AFL and optimal storage monitoring was generated. Mathematical manipulation of selected sample size was also performed to minimise the effort and cost of AFL application. The following points were developed as conclusions of this preliminary study:

- Higher temperature (20 °C) accelerated kiwifruit softening and decay. However, effect of ethylene and harvest time in a season on softening of GLs during AFL monitoring requires further study, as late harvested GL showed faster decline in firmness, corresponding with the highest recorded ethylene contamination in the room.
- At-harvest SSC was associated with seasonal life of GLs in optimal storage.
- AFL firmness parameters of distribution (i.e. number of fruit < 2 and number of fruit < 3 kg$_f$); average (mean and median firmness); variability (maximum value, range and standard deviation); and fruit quality (SSC and SSC:firmness ratio) were associated with seasonal life of GLs.

- AFL monitoring should be conducted in an environment where ethylene produced by a GL should not affect the softening pattern of other GLs.
- During AFL data manipulation, removal of fruit “with rot” from parameter calculation did not improve the correlation of AFL parameters with seasonal life of GLs.
- Firmness assessment at 126 d of optimal storage could represent the rank of GL for softening potential.
- Reducing AFL sample size from 300 to 30 fruit did not affect the correlation of AFL parameters with storage potential of GLs.
- Including more GLs from later in the harvest season would better represent the industrial scenario.

4 Refined Accelerated Fruit Library

4.1 INTRODUCTION

The Accelerated Fruit Library (AFL) is a method being tested to segregate kiwifruit grower lines (GLs) for storage potential. One of the main assumptions of AFL method is that the occurrence of fruit losses at accelerated and optimal conditions is related. To achieve this expression of fruit losses should be inherent and independent of ethylene produced by other GL. In the first attempt of AFL development, acceleration of fruit losses later in the season may have occurred due to ethylene in the environment produced by other softer GLs (Figure 3.8). To eliminate this likelihood of ethylene contamination by softer GLs, refining of the AFL method was required. AFL establishment of each GL in a separate cool room has logistic and capital constraints which conflict with the method requirements of being robust and easy to apply industrially.

The scientific literature contains examples of independent storage conditions being established in the same cool room. For example, Antunes and Sfakiotakis (2000) studied the effect of ethylene on ripening by storing kiwifruit in 5 L jars, attached with a continuous air stream (100 $mL.min^{-1}$) with and without propylene. In another example, Pranamornkith et al. (2012) placed kiwifruit in 60 L sealed barrels with a flow through system used to expose fruit to four different ethylene concentrations during storage. A similar flow through system may serve the purpose of ripening each GL, independently of any external ethylene influence during AFL.

To minimise AFL establishment cost and to make data collection less labour intensive, sample size and number of measurement occasions needed to be reduced for AFL and optimal storage. In doing so, more GLs representing a longer harvest season could be studied for AFL based prediction of storage potential. For this purpose, sample size for AFL and optimal storage measurements were manipulated as concluded from the implications of AFL for future (Section 3.5). The sample size for AFL monitoring was reduced to 36 fruit which were evaluated at 3 d interval for 21 d. For optimal storage, fruit quality was evaluated on two occasions (100 and 126 d). More GLs were collected over an extended harvest period. Overall, this chapter describes the establishment of the refined AFL methodology to accelerate fruit losses and attempts to establish a relationship between AFL and storage potential of kiwifruit GLs.

4.1.1 Objectives

- Establish a flow through system for AFL monitoring to keep GLs independent of each other (ethylene).
- Establish a relationship of AFL losses with storage potential of GLs.

4.2 MATERIALS AND METHODS

Nine (9) modular bulk (MB) packs (99 - 100 fruit/pack wrapped in polyliner) per GL of count 36 ‘Hayward’ kiwifruit from 57 GLs were transported in a temperature controlled carriage from a commercial packing facility in Bay of Plenty to the Postharvest Laboratory at Massey University, Palmerston North. Three to four GLs were received per day from Tuesday to Friday over the harvest season of almost 5 weeks, starting from May 10, 2011 (ISO d 130) to June 14, 2011 (ISO d 165).

Upon arrival of each GL, 9 MB packs were randomly allocated a pre-printed label to divide them in two groups, 6 packs for optimal storage evaluation (3 at 100 d and 3 at 126 d) with the other 3 packs used for at-harvest and AFL measurements (Figure 4.1). For optimal storage, MB packs were immediately stored at 0 °C. From 3 MB packs (for at-harvest and AFL), eight polylined single layer trays of 36 fruit each were prepared. Out of the eight, one tray was assessed for at-harvest fruit quality. The remaining seven trays per GL were placed in a sealed 100 L plastic bag, and supplied with continuous air flow of 1.8 $L.min^{-1}$ delivered by air pumps (Bubbilo, DT800, Masterpet Corporation Ltd., Lower Hutt, New Zealand; Figure 4.2). Air was first passed through a jar filled with $KMnO_4$ ethylene scrubber (Purafil® Chemisorbant Media, Purafil, Inc., Georgia, USA) before delivering it to the bag, to ensure supply of ethylene free air. Bag outlet flow was delivered to the room ventilation, ensuring removal of any fruit generated ethylene from the room. Each bag required opening every 3 days to remove a tray for fruit quality assessment, potentially resulting in ethylene release into the cool room. To combat this likelihood further Purafil® was also placed within the room to reduce the risk of cross GL ethylene contamination.

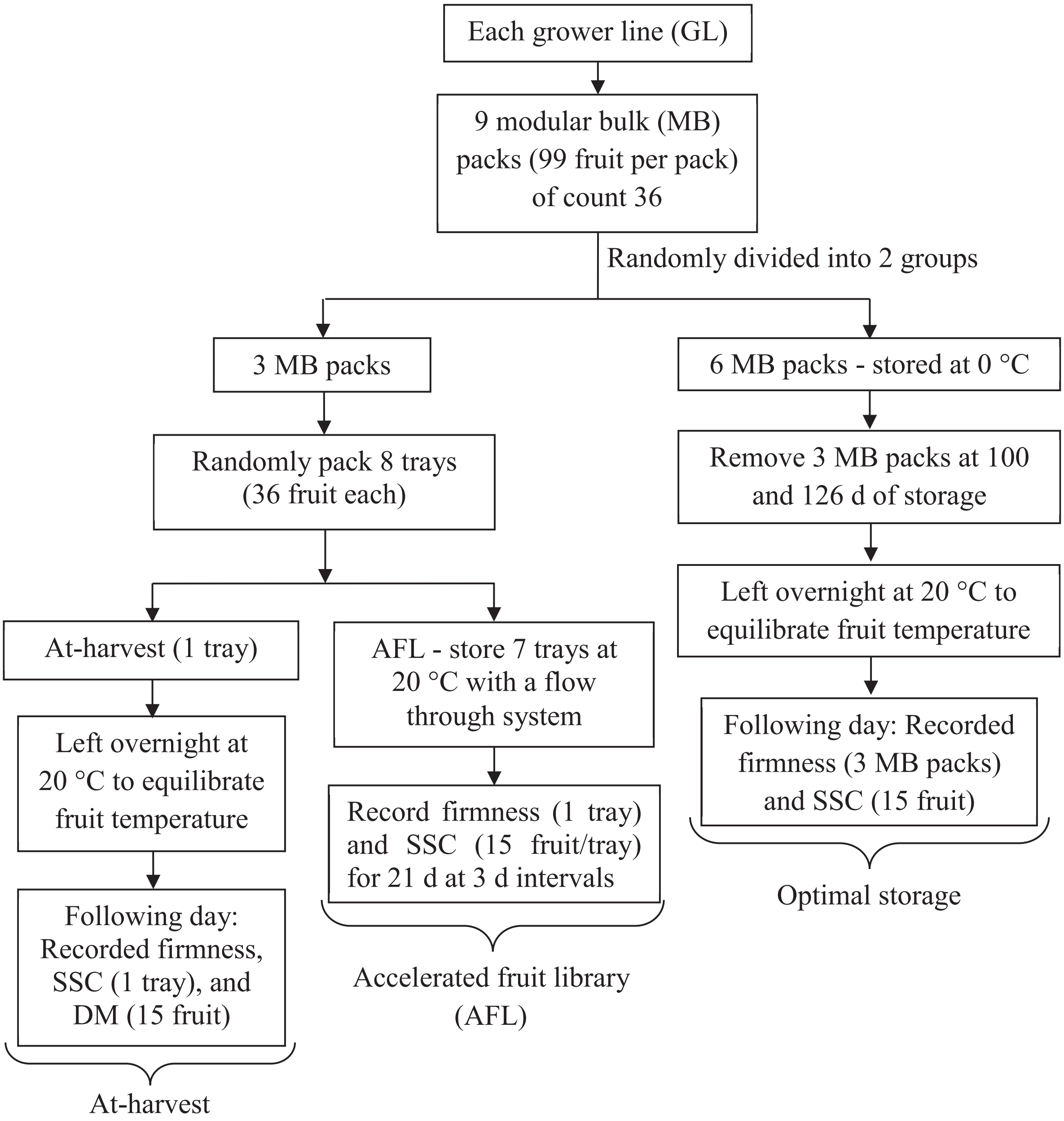

Figure 4.1: Fruit distribution and data collection for at-harvest, AFL and optimal storage measurements for each GL in 2011.

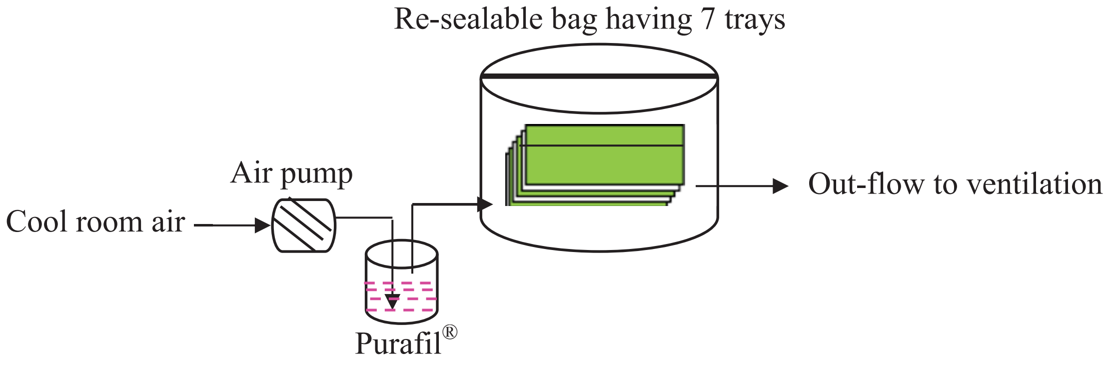

Figure 4.2: Diagram shows flow through system to keep each GL independent during AFL.

For at-harvest quality assessment, firmness and soluble solids content (SSC) of 36 fruit per GL (1 tray), and dry matter (DM) of 15 randomly selected fruit per GL were measured. For AFL measurements, firmness of 36 fruit (1 tray) and SSC of 15 randomly selected fruit per GL were recorded for 21 d with 3 d intervals. During optimal storage, firmness of 3 MB packs and SSC of 15 randomly selected fruit per GL were assessed at 100 and 126 d. Industry standard methods of firmness, SSC and DM evaluation were used as detailed in section 3.2.

Fruit temperature equilibration was performed for at-harvest and optimal storage measurements, to avoid errors in recorded firmness due to difference in fruit temperature (Jeffery and Banks, 1994). Room temperature was measured by thermocouples and recorded in data logger (Grant - 1200 Squirrel Digital Meter/Logger, Grant Instruments Ltd. Cambridge, England). For both AFL and optimal storage, RH of > 90% was maintained. Ethylene concentration in the rooms was monitored intermittently during the whole storage period and maintained below the industry threshold of 0.03 ppm (Banks, 1996; Kim, 1999).

4.2.1 Data manipulation

Manipulation of at-harvest and AFL data was performed to extract summary statistics and other parameters (i.e. descriptors of distribution, average, variability and defect count) as described in chapter 3 (Section 3.3, Table 3.1). From optimal storage firmness data, soft fractile (SF, 9th lowest firmness value of approximately 300 fruit) of each GL was defined at 100 and 126 d. SF represents a threshold limit of 1.3% failure for acceptable quality as per ISO-2859 regulations, which means if 3% of population of 300 fruit have firmness value less than 1 kg_f, which is acceptable (Section 2.6). SF is a common form of expressing firmness data, to understand and define the firmness trend and variability of GLs in the kiwifruit industry (Adams et al., 2010). In the first AFL attempt, no significant difference in estimating GL storage life was found between parameters calculated from populations with and without rots (Section 3.4.4). Therefore, in this season in order to further minimise data manipulation efforts, parameters were estimated from populations including fruit "with rots". In the first attempt, SF at 126 d was found to be representative of seasonal life of GLs (Section 3.5.2). Therefore, in this season work, SF at 126 d was used as the indicator of relative storage potential of GLs. Extracted parameters from at-harvest and AFL data were assessed for their correlation with SF of GLs after optimal storage. Calculated correlation coefficient (r) values were arranged in tables and only values $\geq |0.5|$ were highlighted (Section 3.4.4).

4.3 RESULTS AND DISCUSSION

4.3.1 Description of the data

4.3.1.1 At-harvest

At-harvest fruit quality of 57 GLs varied over the season. SSC increased from 6.5% to 14.5% over a harvest season of 5 weeks from 130 to 165 ISO days (Figure 4.3A). The rise in SSC of GLs during harvest season is a result of starch conversion to sugars with the progression of time (Beever and Hopkirk, 1990; Burdon et al., 2013; Burdon et al., 2014a). As per harvest maturity index of New Zealand kiwifruit industry, all GLs had SSC higher than 6.2% (Burdon et al., 2011). However, SSC may vary between GLs harvested at the same time of season (Feng, 2003).

Change in DM of GLs followed a decreasing trend (except one early harvested GL with DM of 15.5%) and overall varied from 14.5 to 17.5% over the harvest season (Figure 4.3B). In New Zealand, harvest DM usually ranges from 14 to 17%. Time of harvest in a season and orchard management practices both influence the amount of DM in kiwifruit (Burdon et al., 2004; Boyd and Barnett, 2011). At-harvest DM is usually taken as the indicator of the amount of carbohydrate (soluble and insoluble) in a fruit and has a direct relationship with the ripe fruit SSC and consumer acceptability (Crisosto et al., 2012).

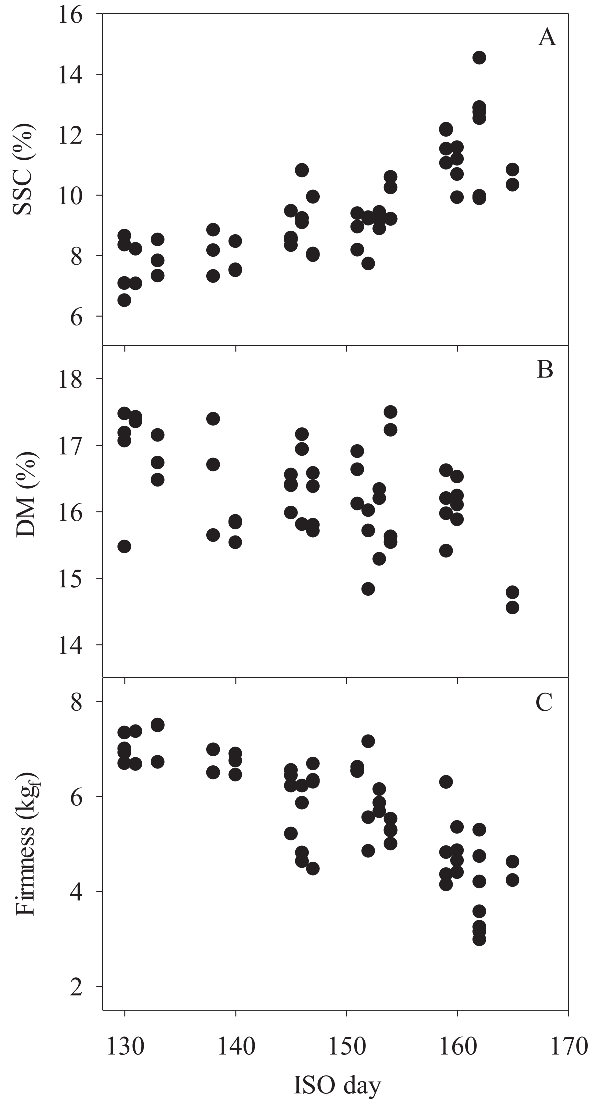

Figure 4.3: At-harvest SSC (A), DM (B) and firmness (C) of GLs in 2011. Each data point represents one GL. For SSC and firmness, each data point shows an average of 36 fruit and for DM average of 15 fruit.

At-harvest mean firmness decreased from 7.5 to 2.9 kg$_f$ with the progression in season (Figure 4.3C). In industry, at-harvest firmness is usually observed to decrease as the season progresses (MacRae et al., 1989a; MacRae et al., 1989b; Beever and Hopkirk, 1990; Feng et al., 2003a; Ghasemnezhad et al., 2013). Firmness of harvested fruit from different GLs was representative of commercial fruit (MacRae et al., 1990a; Feng et al., 2003a; Feng, 2012; Burdon et al., 2013).

Overall, as the harvest season progressed a clear trend of reduction in fruit firmness and increase in SSC was observed, while DM did not show any trend. The variety in the sampled GLs with different fruit quality at-harvest would allow observing and capturing the variability in postharvest softening and hence may provide robustness in AFL methodology development.

4.3.1.2 Optimal storage

Fruit quality (firmness and SSC) of 57 GLs was evaluated after 100 and 126 d at 0 °C. Soft fractile (SF) of GLs at 100 d varied from 0.69 to 1.57 kg_f. Out of 57 GLs, 20 had a SF below 1 kg_f and hence could be considered as un-exportable due to firmness of the lot (Figure 4.4A). These 20 un-exportable GLs were scattered over the harvest season suggesting no effect of harvest timing on GL storability. Later after 126 d of optimal storage, SF varied from 0.78 to 1.43 kg_f and 32 GLs had SF < 1 kg_f (Figure 4.4B). Overall, there was more tendency for early season fruit to fail (SF < 1 kg_f) given the 126 d data ($r = 0.26$).

SSC of GLs after 100 and 126 d of optimal storage ranged from approximately 12 to 15% (Figure 4.5A - B). Rise in SSC is typically observed during kiwifruit ripening in storage (Burdon and Lallu, 2011; Burdon et al., 2013). SSC of GLs after 100 and 126 d of optimal storage did not show any seasonality trend. Although at-harvest SSC increased during the harvest season, this effect was not evident in SSC after optimal storage.

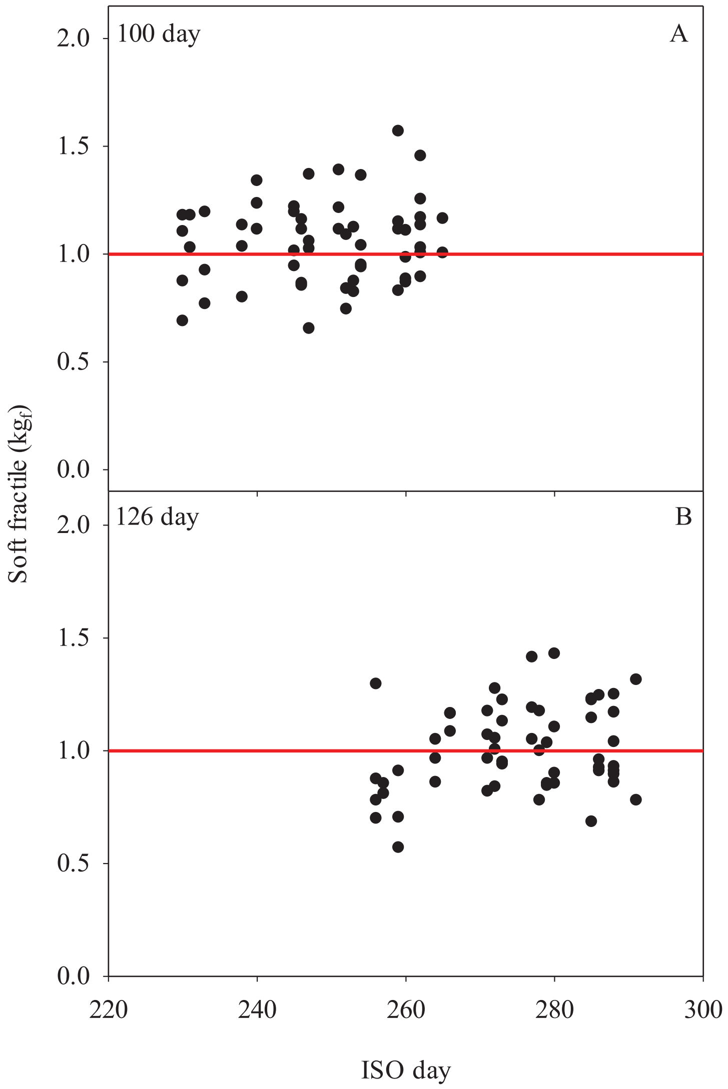

Figure 4.4: Soft fractile of 57 GLs after 100 (A) and 126 (B) day of optimal storage in 2011. Each data point is a GL. Red line represents an export threshold of firmness at 1 kg$_f$.

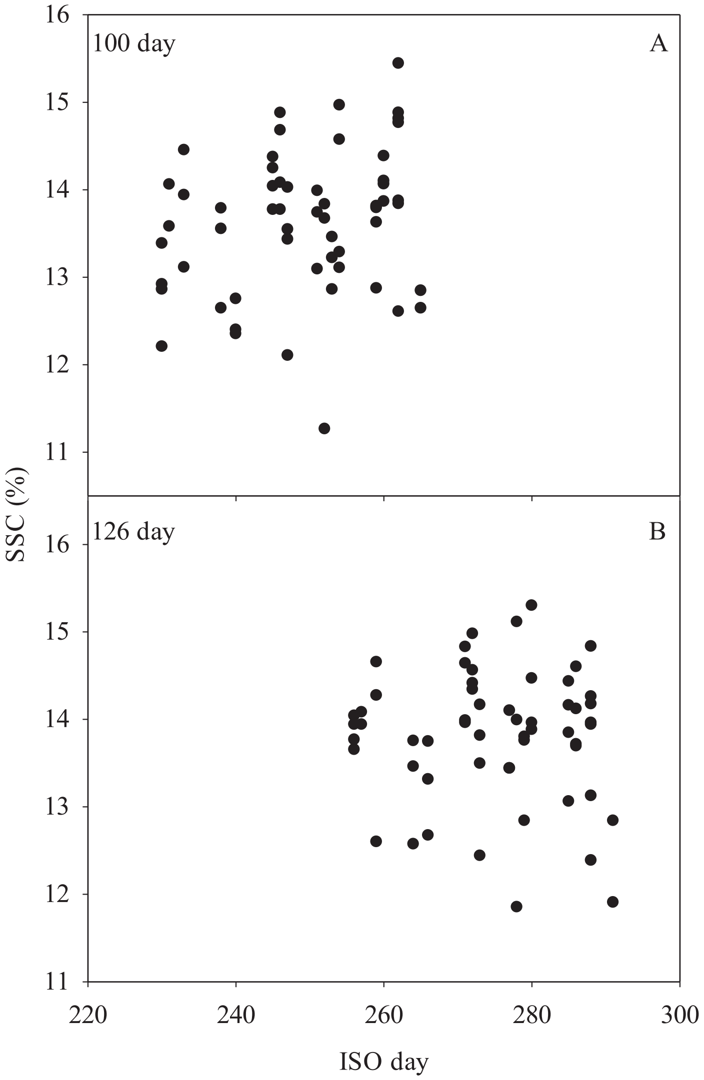

Figure 4.5: SSC of 57 GLs after 100 (A) and 126 (B) day of optimal storage in 2011. Each data point is an average of 15 fruit from a GL.

4.3.1.3 Accelerated fruit library (AFL)

Firmness change of GLs displayed a variety of curves during AFL monitoring, including those with and others without initial lag phases (Figure 4.6). The existence of an initial lag phase had no obvious link to harvest timing (ISO d) or initial (at-harvest) firmness. Some GLs at the start of the season, with an at-harvest firmness (> 6 kg_f) did not maintain a substantial initial lag phase, while other initially softer batches from the middle of the season seemed to have lag phases of 6 - 9 d at 20 °C prior to rapid softening. The longest lag phase observed in this data was 18 d.

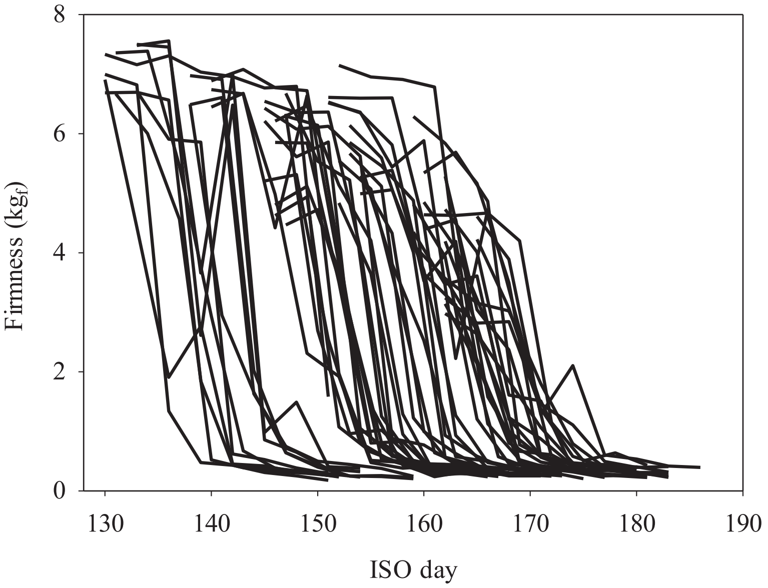

Figure 4.6: Firmness curves of 57 GLs during AFL monitoring in 2011. Each line represents a GL and comprises of 8 data points at 3 day intervals. Each data point is an average of 36 fruit.

The variability between different batches in softening behaviour under the same conditions is similar to previously reported effects of growing locations or orchards (Mitchell et al., 1992; Arpaia et al., 1994; Benge, 1999). The Observed variation in the softening trends among batches, demonstrates the industrial challenge of making inventory decisions based on the softening behaviour of kiwifruit.

In the previous AFL attempt, firmness of late harvested GLs decreased rapidly, possibly influenced by prevailing ethylene in the room produced by the early harvested GLs (Figure 3.8). In this attempt, GLs harvested late in the season showed initial lag phase patterns and did not express rapid decline in softening. GLs subsequently expressed their inherent and independent softening patterns during AFL monitoring. Therefore, use of the flow through system was seemingly appropriate for AFL loss data generation. Independent and inherent pattern of losses during AFL monitoring should further assure relevancy of assessing the relationship of AFL parameters with storage potential of GLs.

During AFL monitoring, SSC (%) of GLs accumulated from at-harvest range of 6.4 to 14.5% to ripe fruit SSC of 12 to 15.6% (Figure 4.7). SSC range of ripe fruit reduced and became consistent because of the conversion of all starch into soluble sugars (Jordan et al., 2000) during storage at high temperature. In some GLs, a slight decrease in SSC after initial rise was also observed, which could be because of complete starch depletion and fruit respiratory processes. This decrease in SSC after rising to a peak has been previously reported (Smith and Clark, 1989).

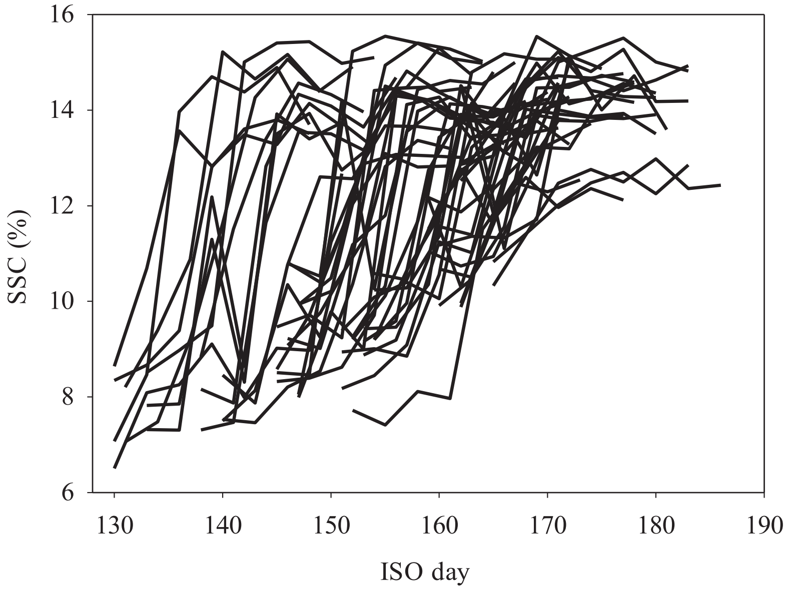

Figure 4.7: Change in SSC of 57 GLs during AFL monitoring in 2011. Each line represents a GL and comprises of 8 data points at 3 day intervals. Data point at-harvest represents average of 36 fruit. For AFL each data point is an average of 15 fruit.

4.3.2 Prediction of storage potential

4.3.2.1 Relationship of arrival day and storage potential

In this season data, harvest day did not show any trend with SF of GLs at 126 d (r = 0.26). However, in the previous season, seasonal life (ISO d on which a GL reaches SF < 1 kg_f) increased with day of arrival (r = 0.53, Figure 3.7), suggesting that early harvested GLs had less inherent storability. In the previous data, SF at 126 d was also correlated with day of arrival (r = 0.5) and suggested that early harvested GLs have a tendency to exhibit lower SF value after 126 d of optimal storage in comparison to late harvested GLs. Therefore, the two seasons of data are not in agreement, in terms of the relationship of season timing to fruit storability. Moreover, these results also conflict with previous understanding that late harvested fruit will have longer storage life than early harvested (Mitchell et al., 1992; Costa et al., 1997). Hence, it seems that day of arrival or harvest time does not always represent a trend of GL storability in a season.

4.3.2.2 Relationship of at-harvest and AFL parameters with storage potential

The relationship of seasonal life with SF at 126 d in the previous season data (Figure 3.11), guided the use of firmness at 126 d as representative of storability of a GL in this season. Assessment of fruit quality of 57 GLs at one time (126 d) during storage also assisted to overcome the constraints of time and labour, and storage space availability. SF at 126 d of the 20 GLs of the previous season ranged from 0.5 to 1.35 kg_f (Figure 3.11). This season SF of 57 GLs had a similar range of 0.57 to 1.43 kg_f at 126 d (Figure 4.4). This similarity in the range of SF at 126 d suggests that softening patterns during optimal storage were similar in both seasons.

SF at 126 d was used for further data analysis as an indicator of storage potential. Pearson correlation of at-harvest and AFL parameters with SF at 126 d resulted in only 7 combinations with $r \geq |0.5|$ (Table 4.1). GLs with high number of fruit < 1 kg_f and high number of fruit < 0.6 kg_f at 12 d of AFL monitoring had a tendency to have a low SF at 126 d (r = -0.5). Later in AFL monitoring at 21 d, GLs with high number of fruit < 2 and high number of fruit < 3 kg_f also tended to have high SF at 126 d (r = 0.52). Moreover, those GLs with higher values of SSC:firmness ratio at 12 and 18 d of AFL monitoring (r = 0.55 and 0.58 respectively) had higher SF value at 126 d. GLs with high number of rots at 21 d of AFL monitoring tended to have lower SF at 126 d of optimal storage (r = -0.58). At-harvest SSC and DM did not show any correlation with SF at 126 d. None of the at-harvest and AFL parameters had any consistent correlation (with $r \geq |0.5|$ continuously at two or more measurement occasions) with SF at 126 d.

Table 4.1: Pearson correlation coefficients (r) of at-harvest and AFL parameters with soft fractile at 126 day of optimal storage. Yellow highlights represent negative correlation ($r \leq -0.5$) and green highlights show positive correlation ($r \geq 0.5$). Grey highlights represent unrounded coefficient values with $r \leq |0.5|$. NoF: number of fruit

Parameters	At-harvest and AFL monitoring (day)							
	0	3	6	9	12	15	18	21
NoF < 1 kg_f	-0.1	-0.2	-0.4*	-0.5***	-0.5***	-0.2	0.0	0.5***
NoF < 2 kg_f	-0.2	-0.2	-0.3**	-0.4**	-0.4**	-0.3*	0.0	0.5***
NoF < 3 kg_f	-0.1	-0.1	-0.3**	-0.4**	-0.2*	-0.3*	0.1	0.5***
NoF at 0.6 - 1 kg_f	-0.1	-0.2	-0.4**	-0.2	0.1	0.0	0.0	0.3*
NoF < 0.6 kg_f	0.0	-0.1	-0.3*	-0.5***	-0.5***	-0.2	-0.1	0.4*
1st quartile	0.0	0.1	0.3*	0.4**	0.4**	0.4**	0.3*	0.4**
3rd quartile	-0.1	0.0	0.3*	0.4**	0.4**	0.4**	0.3*	0.4**
Skewness	0.0	0.0	-0.2	-0.3*	0.2	-0.1	0.0	0.0
Kurtosis	0.1	0.0	0.0	0.1	0.4**	0.1	0.3*	0.2
Mean	-0.1	0.0	0.3*	0.4**	0.4**	0.4**	0.3*	0.4**
Median	-0.1	0.0	0.3*	0.4**	0.4**	0.4**	0.3*	0.4**
Minimum value	0.0	0.1	0.3*	0.3*	0.4**	0.4**	0.4**	0.5**
Maximum value	-0.2	-0.1	0.3*	0.4**	0.5***	0.4**	0.4**	0.4*
Range	-0.2	-0.2	0.2	0.4**	0.4**	0.4**	0.4**	0.3*
SD	-0.2	-0.3*	0.2	0.4***	0.4**	0.4**	0.4**	0.3*
SSC:firmness ratio	0.1	0.0	-0.4**	-0.5***	-0.6***	-0.4**	-0.6***	-0.5**
Number of rots	-0.1	0.0	-0.2	-0.2	-0.2	-0.3*	-0.4***	-0.6***
SSC	0.1	0.2	-0.2	-0.2	-0.2	-0.2	-0.3	-0.1
DM	0.0							

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; Values without * are non-significant ($P>0.05$)

Conversion of continuous values of at-harvest and AFL parameters into rank of 1 - 57 (1 being lowest and 57 as highest value in a population) at each measurement occasion was conducted. Then ranked values of at-harvest and AFL parameters were tested for a correlation with the rank of GLs for SF value at 126 d (1 being lowest and 57 as highest SF value in kg_f). Application of Spearman correlation (rank of at-harvest and AFL parameters with ranked SF at 126 d) resulted in 24 combinations with $r \geq |0.5|$ (Table 4.2). GLs with higher mean, median, 1st and 3rd quartile firmness (at 9, 12 and 18 d); minimum and maximum firmness value (at 12 and 18 d); and number of fruit < 2 and number of fruit < 3 kg_f (at 21 d) during AFL monitoring tended to have higher SF value at 126 d ($r \geq 0.5$). GLs with higher SSC:firmness ratio (at 9, 12, and 18 d) and number of rots (at 18 and 21 d) in AFL monitoring tended to have lower SF value at 126 d of optimal storage ($r \geq 0.5$). At-harvest SSC and DM did not show any correlation with SF at 126 d. Overall, 6 AFL parameters (ranked values) had a consistent correlation ($r \geq |0.5|$ continuously at two or more measurement occasions) with rank of SF at 126 d of optimal storage.

Correlation of at-harvest and AFL parameters with the rank (1 - 57) of GLs for SF at 126 d (1 being lowest and 57 as highest SF value in kg_f) was assessed and resulted in 9 correlation combinations with $r \geq |0.5|$ (Table 4.3). GLs with higher number of fruit < 0.6 kg_f at 9 and 12 d of AFL had lower SF at 126 d (r = -0.52). GLs with higher SSC:firmness ratio (at 9, 12, 18 and 21 d) and number of rots (at 21 d) during AFL monitoring had a tendency to have lower SF at 126 d ($r \geq -0.5$). At-harvest SSC and DM had no correlation with SF at 126 d. Overall, only 2 parameters had consistent correlation ($r \geq |0.5|$ continuously at two or more measurement occasions) with SF at 126 d of optimal storage.

Table 4.2: Spearman correlation coefficients (*r*) of ranked at-harvest and AFL parameters with ranked soft fractile at 126 day of optimal storage. Yellow highlights represent negative correlation ($r \leq -0.5$) and green highlights show positive correlation ($r \geq 0.5$). Grey highlights represent unrounded coefficient values with $r \leq |0.5|$. NoF: number of fruit

Parameters	At-harvest and AFL monitoring (day)							
	0	3	6	9	12	15	18	21
NoF < 1 kg_f	-0.1	-0.2	* -0.3	*** -0.5	*** -0.5	0.0	0.3	*** 0.5
NoF < 2 kg_f	-0.2	-0.2	* -0.3	** -0.4	** -0.4	0.1	0.3	*** 0.5
NoF < 3 kg_f	-0.1	-0.1	* -0.3	** -0.4	* -0.3	0.1	* 0.3	*** 0.5
NoF at 0.6 - 1 kg_f	-0.1	-0.2	* -0.3	* -0.3	0.1	* 0.3	* 0.3	0.2
NoF < 0.6 kg_f	0.0	-0.1	** -0.4	*** -0.5	*** -0.6	-0.2	0.1	*** 0.5
1st quartile	0.0	0.1	** 0.4	*** 0.5	*** 0.6	** 0.4	*** 0.6	*** 0.5
3rd quartile	-0.1	0.0	** 0.4	*** 0.5	*** 0.5	** 0.4	*** 0.6	0.0
Skewness	0.0	-0.1	* -0.3	* -0.3	0.2	-0.1	0.1	0.0
Kurtosis	0.1	0.1	0.0	0.0	** 0.4	0.2	* 0.3	0.1
Mean	-0.1	0.0	** 0.4	*** 0.5	*** 0.6	*** 0.4	*** 0.6	*** 0.5
Median	-0.1	0.0	** 0.4	*** 0.5	*** 0.6	*** 0.4	*** 0.6	*** 0.5
Minimum value	0.1	0.2	** 0.4	*** 0.5	*** 0.6	** 0.4	*** 0.6	*** 0.5
Maximum value	-0.1	0.0	* 0.3	*** 0.5	*** 0.5	** 0.4	*** 0.6	*** 0.5
Range	-0.2	-0.2	0.2	*** 0.5	*** 0.5	** 0.4	*** 0.5	*** 0.5
SD	-0.2	-0.3	0.2	*** 0.5	*** 0.5	* 0.3	** 0.4	** 0.4
SSC:firmness ratio	0.1	0.0	** -0.3	*** -0.5	*** -0.6	** -0.4	*** -0.6	* -0.4
Number of rots	0.0	0.0	-0.2	-0.1	* -0.3	** -0.4	*** -0.6	*** -0.6
SSC	0.2	0.1	-0.2	-0.2	-0.1	-0.1	-0.2	-0.2
DM	0.0							

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; Values without * are non-significant ($P>0.05$)

Table 4.3: Correlation coefficients (r) of at-harvest and AFL parameters with ranked soft fractile at 126 day of optimal storage. Yellow highlights represent negative correlation ($r \leq -0.5$) and green highlights show positive correlation ($r \geq 0.5$). Grey highlights represent unrounded coefficient values with $r \leq |0.5|$. NoF: number of fruit

Parameters	At-harvest and AFL monitoring (day)							
	0	3	6	9	12	15	18	21
NoF < 1 kg_f	-0.1	-0.2	** -0.4	*** -0.5	*** -0.5	-0.2	0.0	** 0.4
NoF < 2 kg_f	-0.2	-0.2	** -0.4	** -0.4	** -0.4	-0.2	0.0	*** 0.5
NoF < 3 kg_f	-0.1	-0.1	** -0.4	** -0.4	-0.2	-0.2	0.1	*** 0.5
NoF at 0.6 - 1 kg_f	-0.1	-0.2	** -0.4	-0.2	0.1	0.1	0.1	0.2
NoF < 0.6 kg_f	0.0	-0.1	-0.4	*** -0.5	*** -0.5	-0.2	-0.1	* 0.4
1st quartile	0.0	0.1	* 0.3	** 0.4	** 0.4	* 0.3	* 0.3	** 0.4
3rd quartile	-0.1	0.0	* 0.3	** 0.4	** 0.4	* 0.3	* 0.3	** 0.4
Skewness	0.0	0.0	-0.2	* -0.3	0.2	-0.1	0.0	0.1
Kurtosis	0.1	0.1	0.0	0.1	** 0.4	0.1	* 0.3	0.2
Mean	-0.1	0.0	* 0.3	** 0.4	** 0.4	* 0.3	* 0.3	** 0.4
Median	-0.1	0.0	* 0.3	** 0.4	** 0.4	* 0.3	* 0.3	** 0.4
Minimum value	0.0	0.1	* 0.3	* 0.3	** 0.4	* 0.3	* 0.3	*** 0.5
Maximum value	-0.1	-0.1	* 0.3	** 0.4	** 0.4	* 0.3	** 0.3	** 0.4
Range	-0.2	-0.2	0.2	** 0.4	** 0.4	* 0.3	** 0.4	* 0.3
SD	-0.2	* -0.3	0.2	*** 0.4	** 0.4	* 0.3	** 0.4	* 0.3
SSC:firmness ratio	0.1	0.0	** -0.4	*** -0.5	*** -0.6	** -0.4	*** -0.6	** -0.5
Number of rots	0.0	0.0	-0.2	-0.2	-0.2	* -0.3	** -0.4	*** -0.6
SSC	0.1	0.1	-0.2	* -0.3	-0.2	-0.2	-0.3	-0.2
DM	0.0							

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; Values without * are non-significant ($P>0.05$)

Results from the three different correlation techniques (Table 4.1 - 4.3) demonstrated that AFL parameters representing population distributions (number of fruit < 2 kg_f, number of fruit < 3 kg_f, number of fruit < 0.6 kg_f, 1st and 3rd quartile firmness); average (mean and median firmness); variability (minimum and maximum firmness value); and fruit quality (SSC:firmness ratio and number of rots) at different measurement occasions were sporadically associated with SF at 126 d of optimal storage. Overall, number of fruit < 0.6 kg_f, 1st quartile, 3rd quartile, mean, median, SSC:firmness ratio and number of rots during AFL monitoring were somewhat consistently ($r \geq |0.5|$ continuously at two or more measurement occasions) associated with SF at 126 d. SSC at the time of harvest and during AFL did not show any correlation with storage firmness.

As a whole, less at-harvest and AFL parameters in this season data set were associated with storage potential of GLs as compared to the previous season. In previous season, maximum value, range, standard deviation, 3rd quartile, mean, median firmness and SSC:firmness ratio during AFL monitoring appeared as consistently associated with seasonal life (Section 3.4.4). All the consistent parameters had correlation coefficient value (r) higher than |0.5|, continuously at more than two measurement occasions. In this season, except 3rd quartile firmness, none of the other parameters of population distribution were found correlated for more than two measurement occasions with storage potential of GLs. Other somewhat promising parameters (like number of fruit < 0.6 kg_f, 1st quartile firmness, mean, median and SSC:firmness ratio) were correlated with SF at 126 d for two measurement occasions (only). SSC at-harvest was also associated ($r = 0.6$) with seasonal life in the previous season (Figure 3.10) but in this season at-harvest SSC did not show any correlation with SF at 126 d. Overall, in this season, at-harvest and AFL parameters did not show any improvement in association with storage potential of GLs.

At-harvest and AFL parameters did not show any continuous correlation for more than two measurement occasions. Correlation ($r \geq |0.5|$) of few AFL parameters at one or continuously at two measurement occasions with storage firmness could be a chance event and may not happen in different season. It would seem that manipulation of at-harvest and AFL data to extract quick and easy to calculate parameters in order to define storage potential of GLs is not a suitable approach.

An alternative approach of utilising the same set of AFL data would be to describe the complete softening curve behaviour of each GL during AFL monitoring. Softening curve fitting can be achieved by using a mathematical modelling technique (Benge, 1999; Benge et al., 2000; White et al., 2005). The subsequent model parameters such as rate of firmness change may also reflect softening pattern in optimal storage. Recently, rate of SSC change of kiwifruit before harvest was found to provide better information about fruit maturity in comparison to single data point of at-harvest SSC (Burdon et al., 2013). Hence, this approach has the advantage of using more data while attempting to predict GL storability, this curve fitting approach is ultimately more time consuming. However, use of complete softening curve information through modelling approach would require collection of enough data points in AFL monitoring. Softening curve description approach to predict storability of kiwifruit GLs is going to be attempted in chapter 5.

4.4 CONCLUSION

Grower lines were at commercially acceptable stage at the time of harvest. During AFL monitoring, each GL expressed an independent softening pattern. A wide range of softening patterns was observed across the 57 GLs during AFL monitoring. Few at-harvest and AFL parameters were correlated with SF at 126 d of optimal storage. To be using any of the at-harvest and AFL parameter for storage potential prediction, consistency in relationship of loss in AFL and optimal storage should be obligatory. Correlation of few parameters with storage firmness at one or two AFL measurement occasions may not appear in different season. An alternative method in which softening curves of GLs during AFL monitoring should be characterised by mathematical model may produce new parameters that relate to storage potential of GLs.

5 AFL Softening Curve Parameters to Predict Storage Potential (*)

5.1 INTRODUCTION

Kiwifruit firmness is a crucial quality criterion and determines commercial handling and trade threshold limits (Burdon and Lallu, 2011). Substantial variation exists between grower lines (GLs) for at-harvest firmness and postharvest softening patterns because of many pre and postharvest factors (Benge et al., 2000). Differences between GLs for softening patterns provide an opportunity to maintain fruit supply over an extended period. On the other hand, large variations in softening patterns make it difficult to predict the storage potential of GLs and contribute to a huge amount of fruit and profit losses every year because of over softening. To overcome this problem, the kiwifruit industry requires a robust approach to segregate GLs with low and high storage potential to make more informed inventory decisions.

This work describes the use of ‘Accelerated Fruit Library (AFL) method as a rapid test to categorise GLs for storage potential. AFL has potential to define the storage quality of fruit based on amount of losses in accelerated condition. Preliminary studies of AFL establishment showed that the expression of fruit losses needed to be independent of any external influence such as ethylene (Section 3.4.3.1). Later refined AFL methodology assured the expression of inherent loss pattern of kiwifruit GLs (Section 4.3.1.3). As ease of AFL establishment and robust interpretation has prime importance, several simple and easy to calculate AFL parameters were assessed for correlation with storage potential of GLs (Section 3.3.3 and Section 3.3.4). Parameters extracted from refined AFL methodology were not promising enough to express the relationship between AFL data and storage potential under optimal conditions (Section 4.3.2.2). An alternative approach is to follow the complete pattern of softening during AFL period to categorise GLs for storage potential.

Previously researchers have followed the complete patterns of quality change instead of using single data points to understand postharvest performance of fruit. Burdon et al. (2013) found rate of on-vine soluble solids content (SSC) accumulation more promising to define postharvest performance of kiwifruit rather a single SSC value at-harvest. For

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(*) This chapter includes material published in the paper:
Jabbar, A., East, A.R., Jones, G., Tanner, D.J., Heyes, J.A. (2014). Modelling batch variability in softening of ‘Hayward’ kiwifruit from at-harvest maturity measures. Postharvest Biology and Technology 90, 7-14.

apple, to understand tri-phasic nature of softening, Johnston (2001) followed the complete pattern of firmness loss. Kiwifruit softening is also a well studied process (MacRae et al., 1990a; Benge et al., 2000). Many studies followed complete softening of kiwifruit to study the effect of some pre- or postharvest treatments on fruit quality during storage (Benge et al., 2000; Feng, 2003; Hertog et al., 2004b; White et al., 2005; Feng et al., 2006). Therefore, rather than looking at a single data point, rate of firmness change of GLs during AFL monitoring may be a better indicator of storage potential of GLs. Obviously, this will add more data and confer greater value to the AFL based categorisation of GLs for storage potential but it will also increase the cost of establishment of proposed methodology.

Extraction of rate of firmness change for GLs requires curve fitting. Different mathematical methods have the potential to describe loss of kiwifruit firmness. The aim of study presented in this chapter was to model the AFL softening curves and determine if storability of GLs in optimal storage is predictable from the extracted model parameters.

5.2 MATERIALS AND METHODS

A summary diagram shows data collection and analysis processes used in this study (Figure 5.1). Data came from 57 kiwifruit GLs collected in 2011. During AFL monitoring, firmness data (at 3 d intervals for 21 d) were recorded from fruit stored at 20 °C in a flow through system (Section 4.2). In optimal storage, firmness data were recorded at 100 and 126 d (Section 4. 2). From firmness data of optimal storage, soft fractile (SF, 9th lowest firmness value of approximately 300 fruit) was defined for each GL (Section 4.2.1) and used as an indication of observed storage potential of GLs. AFL firmness data were used to predict the storage potential of GLs. For this purpose, AFL firmness data were mathematically modelled, to obtain softening parameters for each GL (Section 5.2.1) which were subsequently correlated with SF in optimal storage (Section 5.2.2). For best categorisation of GLs for storage potential, appropriate threshold values for AFL softening parameters of model were explored (Section 5.2.2.1). Categorisation results were presented in a contingency table (Section 5.3.3). The following sections provide the mathematical detail of each of the data processing steps that result in a prediction of GL storage potential.

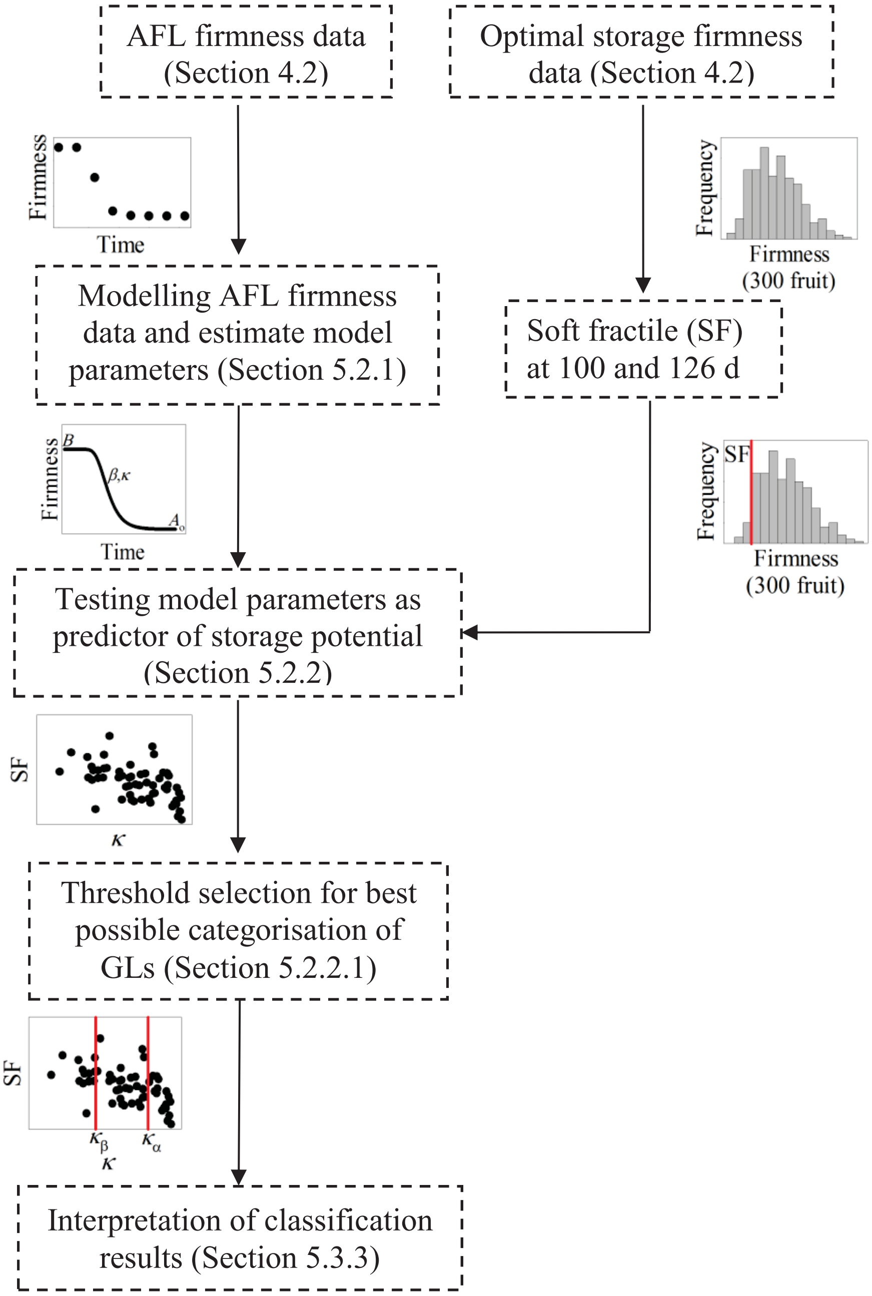

Figure 5.1: Scheme of methods performed to use AFL monitoring system to predict storage quality.

5.2.1 Modelling of AFL firmness data

Differences in firmness change of GLs can be described through a generic mathematical model that uses individualised parameters for each GL. A generalised kiwifruit softening pattern after harvest features an initial slow softening period (sometimes referred to as an initial lag phase), followed by an accelerated softening period and a later period of slow softening (Figure 4.6, Benge et al., 2000 and MacRae et al., 1990a).

The initial lag phase of fruit softening is not always observed (Figure 4.6, MacRae et al., 1990a). Two different modelling approaches (empirical and mechanistic) could be used to develop models of this trend. A number of existing models are also potential candidates to describe the softening curve of kiwifruit.

5.2.1.1 Choice of modelling approach

Softening of kiwifruit has been described by a number of empirical models (Benge et al., 2000; White et al., 2005; Feng et al., 2006; Schotsmans et al., 2008). Benge et al. (2000) applied different empirical models (Complementary Michaelis–Menton (CMM), Exponential Decay (EXP), Complementary Gompertz (CG), Jointed Michaelis–Menton (JMM) and Inverse Exponential Polynomial (IEP)) and compared their efficiency in describing softening of kiwifruit from three harvests of one grower line. These empirical models and their parameters provide little biological meaning and are constrained to applications with constant storage conditions, which is appropriate for this study, with the use of only constant 20 °C storage (in AFL). White et al. (2005) modelled softening profiles of different genotypes of kiwifruit with a Boltzmann function and simple exponential (EXP) model. The EXP model was also used by Feng et al. (2006) to calculate storage life of batches at 0.5 °C and Schotsmans et al. (2008) to describe temperature dependent softening of ‘Hort16A’ kiwifruit. However, kiwifruit softening follows a tri-phasic curve and the simplistic EXP model is inappropriate when there is a pronounced initial lag phase.

Limiting the model parameters required to describe the softening profile is advantageous, as each model parameter will require independent data to enable estimation. As a result, the number of model parameters required to be estimated has direct implications on the costs required to collect data and allow predictive use of a model. For a useful softening model, the value of results is required to remain higher than the effort of data collection and losses required to estimate model parameters. In an industrial scenario where many lines are required to be assessed, this puts pressure on minimising data collection effort. Among the different empirical models investigated by Benge et al. (2000) that describe the tri-phasic softening pattern, CG, JMM and IEP outperformed the other 2 models (CMM and EXP) assessed. However, the JMM is a 5 parameter model and the IEP model has 4 parameters, 3 of which seem to have no physical meaning. Alternatively the CG model has 4 parameters, one of which (A_o) defines the minimal firmness and hence can be assumed to be the global for all GLs. Given this, the CG model was chosen for its ability to express the three phases of kiwifruit softening, and with the knowledge that, at the most, only 3 parameters would be GL dependent.

Alternatives to using these empirical models are mechanistic models that may not only enable suitable prediction, but also further assist in developing the mechanistic understanding of a fruit quality change process (Section 2.4.1.2). Associating the modelled quality attribute (e.g. softening) with distinct physiological, biochemical and other processes or conditions is a more complex modelling process than the application of empirical models. Application of a mechanistic approach typically results in the requirement for multistage Ordinary Differential Equations (ODE) models that require many parameters. For example, in the recent tri-phasic apple softening model of Róth et al. (2008), 4 ODE and 1 algebraic equation were required, resulting in 5 batch-dependent parameters needing to be estimated. Therefore, while mechanistic models represent the contemporary field for mathematical models of fruit quality changes. This work used an empirical model due to the advantages of reducing the required data collection effort to find a solution that could be applied industrially.

5.2.1.2 Model application

CG has four fitted parameters that enable characterisation of kiwifruit softening (Eq. 5.1).

$$FF = A_o + B \left(1 - \frac{1}{\exp\left(\beta \exp\left(-\kappa\, t\right)\right)}\right) \qquad \text{Eq. 5.1}$$

FF is mean flesh firmness (kg_f) and A_o is a lower asymptotic value of mean firmness (kg_f). In applying the CG model to AFL firmness data A_o was set as a global parameter with a minimum value of 0.1 (kg_f) as this is the least detection limit of the penetrometer used to collect data. B is a scale parameter (kg_f) and κ (d^{-1}) represents rate of firmness decline with time (t, d). GLs have different at-harvest firmness that may affect rate of firmness change (κ). Therefore, B and κ needs to be GL dependent. β is a horizontal shift factor and assists in defining the lag phase of softening immediately after harvest.

5.2.1.3 Grower line dependent model parameter estimation

Each GL of kiwifruit has a different harvest maturity (i.e. firmness and SSC) and softens differently during AFL monitoring. Mixed effect models have been widely accepted and adopted in order to describe the variability of outcomes of seemingly similar units as a result of a number of factors (Lammertyn et al., 2003a; Lammertyn et al., 2003b; De Ketelaere et al., 2004; De Ketelaere et al., 2006; Aguirre et al., 2008; Tong et al., 2013). A mixed effects model is a statistical technique that simultaneously enables characterisation of main effects and the components causing variability (i.e. random effects) to the particular problem under observation (Pinheiro and Bates, 2000). Mixed effect models are appropriately applied to acknowledge the expected inherent variability present between batches of biological produce (De Ketelaere et al., 2003). Mixed effect models enable independent parameterisation for each GL within the framework of the same model, while still acknowledging that each individual GL is part of a larger population (i.e. the model describes generalised softening of 'Hayward' kiwifruit at 20 °C). The population of random effects parameters usually has a normal distribution, when considering the population as a set of independent batches. For the CG model, the main effects describe the average softening profile whereas the random parameters refer to GL dependent differences in AFL softening pattern.

Mixed effect models are either linear mixed effect (lme) or non-linear mixed effect (nlme) models. When derived empirically, nlme models use fewer parameters with physical interpretation than lme models (Pinheiro and Bates, 2000). Provided this nlme model can assure interpretation of GL dependent parameters of tri-phasic softening of kiwifruit. Therefore, the CG model (Eq. 5.1) was used to fit the firmness data, with non-linear mixed effects (nlme) by using the nlme procedure in R (version 2.15.1, cran.r-project.org; Pinheiro et al., 2013). CG model parameters were tested in a stepwise manner to assess if each parameter was best considered as GL dependent and globally fitted for all GLs. Model fitting was generated with maximum likelihood (Appendix 1) and an Akaike Information Criterion (AIC) was used to inform efficient model selection from different model formats. A smaller AIC number indicates a better overall model fit

(Pinheiro and Bates, 2000). Errors of fit between CG model and the data were assessed by calculating Mean Absolute Error (MAE, Eq. 5.2)

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}\left|O_i - P_i\right| \qquad \text{(Eq. 5.2)}$$

where n is the number of observations, i is the observation number, O is the observed value and P is the predicted value. Distribution of the resulting GL dependent parameter values of CG was assessed with an Anderson–Darling test in Minitab Version 16 (Minitab Inc., State College, PA, USA).

5.2.2 Testing CG model parameters as predictors of storage potential

GL dependent softening parameters were correlated with storage quality (firmness and rots) at 100 and 126 d. GL dependent softening parameters were further explored for best possible predictive categorisation of GLs for storage potential.

5.2.2.1 Threshold selection for best possible predictive categorisation

Categorisation of GLs based on AFL softening parameter (e.g. κ) was performed to segregate different lines for storage potential as determined from firmness (SF) in optimal storage. For this purpose, GLs were grouped into three categories for measured storage potential;

1) Low storage potential: GLs failed (SF < 1 $\mathrm{kg_f}$) at 100 d.
2) Medium storage potential: GLs acceptable at 100 d (SF > 1 $\mathrm{kg_f}$ at 100 d) and failed at 126 d (SF < 1 $\mathrm{kg_f}$ at 126 d).
3) High storage potential: GLs not failed at 126 d (SF > 1 $\mathrm{kg_f}$).

In order to predict storage behaviour κ was used to categorise GLs into three prediction groups. For this, two suitable threshold values were needed i.e. κ_α and κ_β (as upper and lower thresholds). Different combinations of κ_α and κ_β were tested from highest to lowest κ for three prediction categories (of GLs). Each combination started from high κ having a difference of 0.05 between κ_α and κ_β (e.g. $\kappa_\alpha = 0.93\ \mathrm{d}^{-1}$ and $\kappa_\beta = 0.88\ \mathrm{d}^{-1}$).

Clearly, the difference of 0.05 was arbitrary selection to create many combinations of κ_α and κ_β. While keeping the same κ_α, κ_β was changed to a low κ by decrements of 0.05 consecutively (e.g. κ_β = 0.83, 0.78 and so on). Each next combination started by reducing κ_α for value of 0.05 followed by κ_β selection (Appendix 2).

Prediction categories of GLs made by each combination of different κ_α and κ_β thresholds were compared in a contingency table, showing correct (for low, medium and high storage potential), missed (predicted as lower storage potential than measured) and false (predicted as higher storage potential than measured) categorisation (Table 5.1). Correct categorisation refers to number of GLs for 1 of 3 storage categories as measured (Table 5.1 green cells). Missed categorisation shows those lines which could have been stored safely for longer than predicted (Table 5.1 yellow cells). False categorisation refers to lines that were categorised as high storing but measured as low storing lines (Table 5.1 red cells).

Table 5.1: Example of contingency table to compare number of GLs measured and categorised by κ thresholds in three storage categories (low, medium and high). Green cells show correct categorisation. Yellow cells represent missed and red cells are for false categorisation. Row and column total shows number of GLs measured and categorised (respectively) in each storage category.

		Categorised by κ thresholds			Row total
		Low (κ_α)	Medium ($\kappa_\beta < \kappa < \kappa_\alpha$)	High (κ_β)	
Measured storage categories	Low (SF <1 kg_f at 100 d)	🟩	🟥	🟥	
	Medium (100 d) 1< SF <1 kg_f (126 d)	🟨	🟩	🟥	
	High (SF >1 kg_f at 126 d)	🟨	🟨	🟩	
Column total					

Ideally, number of GLs categorised by κ thresholds (column total) and measured (row total) in three storage categories should be same, if κ thresholds are best to categorise GLs for storage potential. Any line not correctly categorised, however could be in missed or false categorisations. Missed GLs represent the loss of an opportunity of keeping such lines for a bit longer. However, false categorisation creates a real risk of product losses, if they are stored for as long as the model predicts they can be stored.

From contingency table for tested combinations of κ_α and κ_β for 1 of 3 storage categories, the percentage of GLs in correct, missed and false categorisations was calculated. For each combination of κ thresholds, percentages of correct, missed and false categorisations were collected in separate tables (Appendix 2). From these percentage tables, best combination of thresholds (κ_α and κ_β) was selected that can result in minimum number of false categorisations to maximise benefits of AFL based prediction of storage potential.

5.3 RESULTS AND DISCUSSION

5.3.1 Modelling AFL firmness data

The CG model was used to define softening patterns of 57 GLs stored in AFL with non-linear mixed effects (nlme) model to estimate global and GL dependent parameters of CG. Each GL was considered as an independent experiment with at-harvest plus 7 measurement points over 21 d of AFL. First, CG was fitted to softening data, with A_o as a global parameter (due to constraint of lowest firmness measurement of 0.1 kg_{f},) while the other three parameters (B, β and κ) were GL dependent (CG_1) and output revealed an AIC of 1135 (Table 5.2).

Table 5.2: AIC of CG versions with different combinations of global and GL dependent parameters. Full details of outputs are provided in appendix 1.

Model version	Global parameters	GL dependent parameters	AIC no
CG_1	$A_o = 0.31$	$2.65 < B < 6.94$ $\beta = 72.87$ $0.23 < \kappa < 0.93$	1135
CG_2	$A_o = 0.31$, $\beta = 73.39$	$2.65 < B < 6.94$ $0.23 < \kappa < 0.93$	1129
CG_3	$A_o = 0.19$ $B = 5.74$ $\beta = 8.97$	$0.09 < \kappa < 0.74$	1319
CG_4	$A_o = -0.15$ $\beta = 4.61$ $\kappa = 0.23$	$3.05 < B < 9.35$	1466

Model parameters sensitivity analysis was assessed by changing the state of each parameter by 25% (Figure 5.2). For this purpose, parameter values were changed for an ordinary curve, whose firmness change with the rate (κ) of 0.8 d^{-1} and initial lag (β) of

80 from initial value (*B*) 8 kg_f to lowest (A_o) 0.1 kg_f over time (t) of 21 d. Sensitivity of one parameter was assessed at a time while keeping others same as for an ordinary curve. Model parameter B and κ accounted for different curve shapes as their values were raised and lowered (for 25%). Change in values of β and A_o did not affect the curve shape significantly. Hence, sensitivity analysis revealed that B and κ are GL dependent and can account for variability in different lines for initial firmness and rate of firmness change.

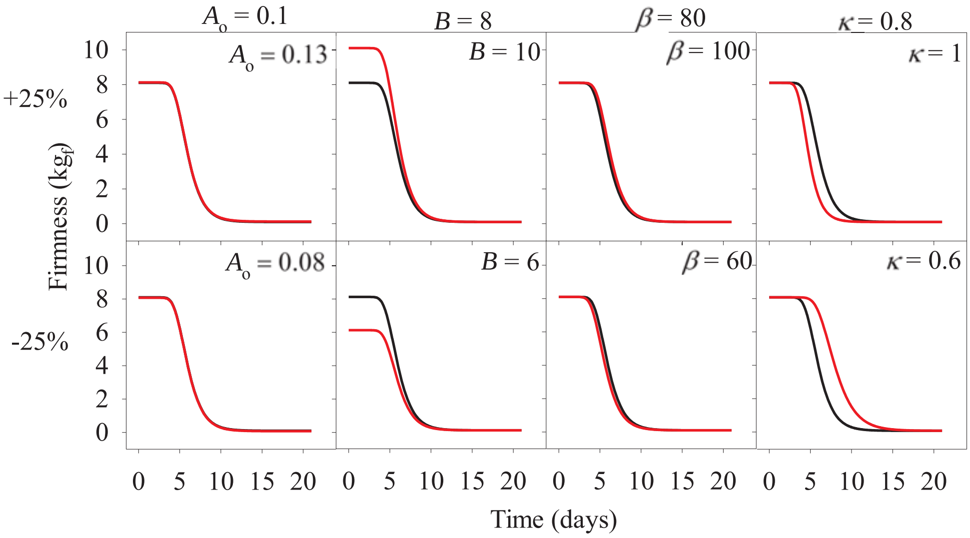

Figure 5.2: Sensitivity analysis of each CG parameter on the resulting model shape, when each parameter was changed by 25% (red) from values of an ordinary softening curve (black).

Given the sensitivity analysis, in addition to A_o, the horizontal shift factor (β) was also tested as global parameter for all GLs (CG_2, Table 5.2), given that this parameter had the least effect on model outcomes (Figure 5.2). CG_2 output revealed a reduced AIC of 1129 (meaning a better overall fit) as compared to CG_1. Further exploration of model options for lower AIC was also performed with only κ or B as the GL dependent parameters (CG_3 and CG_4 respectively). Both of these model options resulted in an increase in AIC, indicating poorer overall model fits (Table 5.2). Therefore, CG_2 was chosen as an appropriate model version for GL dependent model parameter extraction. Both the scale parameter (B) and rate of firmness change (κ) were important contributors to the description of GL dependent softening.

A second sensitivity analysis across the entire range of GL dependent parameters generated in CG_2 was conducted in order to observe the range of softening curves that were obtained (Figure 5.3). While keeping A_o (0.31 kg_f) and β (73) global, combinations of highest, lowest and average values of B and κ in the population of fitted softening curves, resulted in the nine curve shapes. These curves clearly show that CG_2 (when only B and κ were GL dependent) can describe a wide range of softening curves representative of the population of curves observed during data collection.

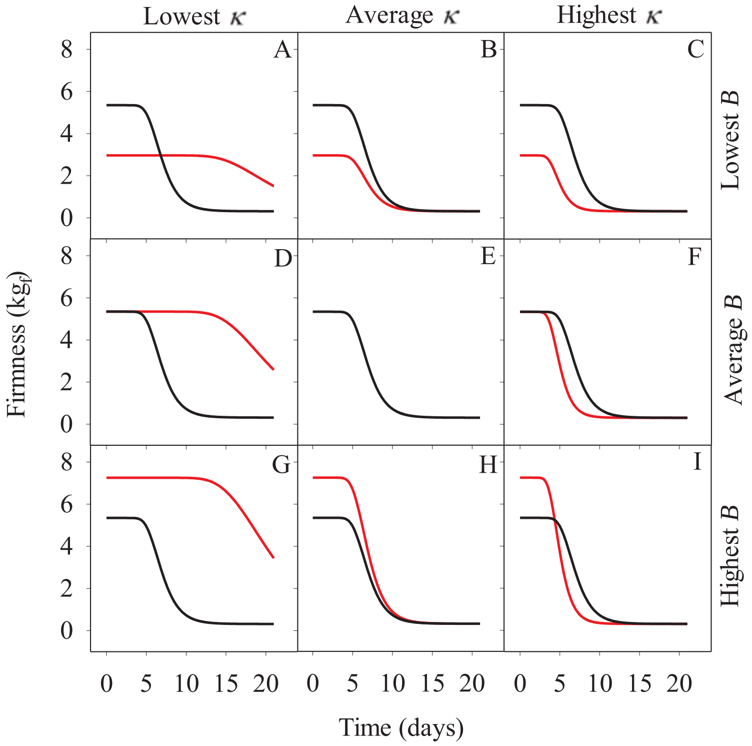

Figure 5.3: Sensitivity analysis for GL dependent CG parameters observed in population of GLs. The black line represents the model with average parameter values while red line shows model with different extremes of B and κ. For these curves, global parameters A_o of 0.31 kg_f and β of 73.39 were used.

Both parameters (B and κ) combine to create different softening shapes and are explicitly capable of accounting for the variation in initial firmness value and rate of firmness change over time for a range of curves. Lowest κ resulted in extended lag phase regardless of variation in B, suggesting that slow rate of firmness change results in expression of initial lag for longer time (Figure 5.3A, D and G). This suggests that κ is not only defining the rate of firmness change but it also influences the initial lag phase. Likewise different upper asymptotes in combination with fastest κ still expressed an initial lag phase (Figure 5.3C, F and I). Expression of initial lag phase even with highest κ and lowest *B* (Figure 5.3I) is because of β influence on κ in defining shape of the curve.

The CG_2 (A_o = 0.31 kg_f, β = 73.39 as global; *B* and κ as GL dependent) appropriately characterised the softening of 57 GLs of kiwifruit (Figure 5.4B). The CG_2 fitted well while expressing all the softening stages to look alike the data (Figure 5.4A). Change in initial firmness over harvest season (ISO d) was also evidential in fitted curves. The fitted curves also confirmed that expression of initial lag phase was GLs inherent characteristic (MacRae et al., 1990a). However, few (14%) fitted curves showed an initial lag phase in softening, even it was not present in raw curves. Mean absolute error (MAE) of fitted curves varied from 0.07 to 1.20 kg_f, with an average of 0.31 kg_f (Figure 5.5). Approximately, 84% (48) of GLs had MAE less than 0.5 kg_f. MAE of 7 GLs further ranged from 0.53 to 0.91 kg_f. Only 2 GLs had MAE higher than 1 kg_f and ranged from 1.09 to 1.20 kg_f.

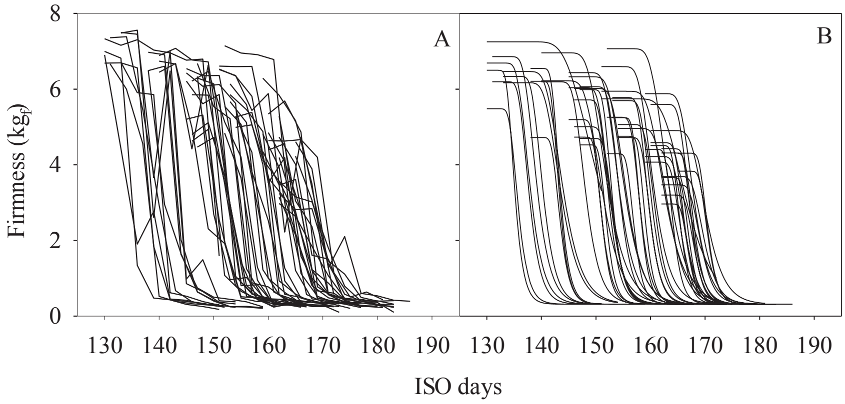

Figure 5.4: Raw (A) and fitted (B) softening curves of 57 GLs during AFL monitoring in 2011. CG_2 with global A_o (0.31 kg_f) and β (73.39), and GL dependent *B* (range of 2.65 to 6.94 kg_f) and κ (range of 0.23 to 0.93 d^{-1}) parameters was used to fit the firmness data.

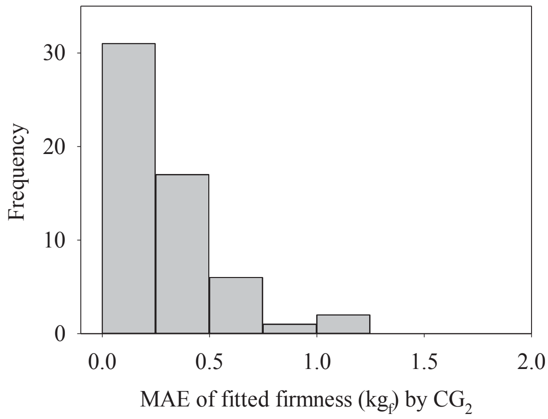

Figure 5.5: Histogram of mean absolute error (MAE) of fits by CG$_2$ for the firmness date of 57 GLs during AFL monitoring in 2011.

Nine curves that represent the range of softening patterns including the GLs with minimum and maximum MAE of CG$_2$ fits are shown in figure 5.6. The CG$_2$ described most stages of softening in the example curves except on occasions in which a less dominant lag phase was present in the data (Figure 5.6B and I). This result is partly due to the use of a global β in curve fitting process. Sensitivity analysis of GL dependent parameters (B and κ) showed that shape and rate of firmness change of the curves are not only described by κ but also affected by β (Figure 5.3). This effect of the horizontal shift parameter (β) resulting in too much initial lag phase on some occasions was also observed by Benge et al. (2000). The highest MAE of 1.20 kg$_f$ was observed in GL7 with B of 5.88 kg$_f$ and κ of 0.44 d^{-1} (Figure 5.5C). Alternatively, GL40 with B of 4.75 kg$_f$ and κ of 0.7 d^{-1} had lowest MAE of 0.07 kg$_f$ (Figure 5.6G). The higher MAE was found for GLs that demonstrated an inconsistent decline in firmness over the storage time (i.e. an observed increase in average firmness between two measurement points), a result that can occur on occasions when destructive methods of firmness measurement are used. Longer initial lag phase was observed in GL2 than any other GL (Figure 5.6A). Presence of such types of softening with extended initial lag phase has been related to fruit maturity, as immature kiwifruit from three genotypes showed delay in onset of softening during storage at 20 °C (White et al., 2005).

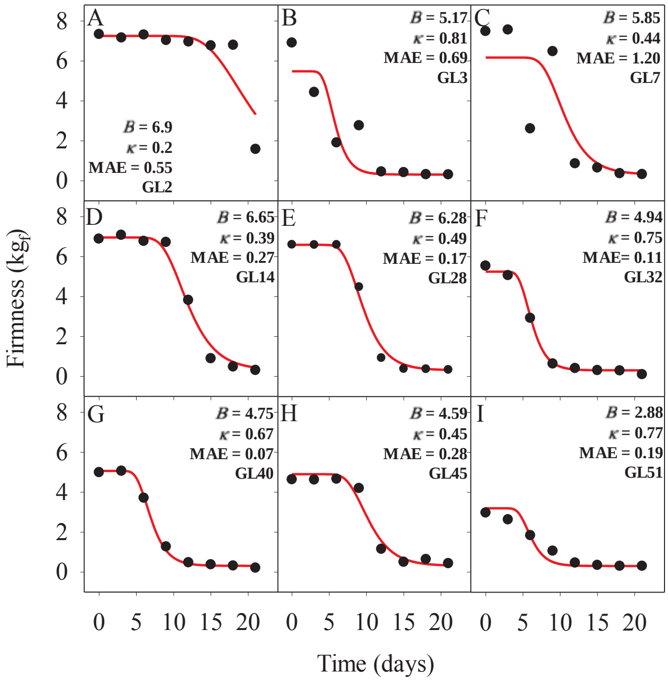

Figure 5.6: Comparison of raw (dots) and fits (red) of firmness data by CG_2 during AFL monitoring in 2011. GLs were selected to demonstrate the range of softening patterns including lines with minimum and maximum mean absolute error (MAE) of fits. Each dot point represents an average of 36 fruit. B represents upper asymptote (kg_f) and κ is rate of firmness change (d^{-1}). GL number shows the ascending order in which lines were collected over a harvest period of 5 weeks.

The model parameter values from application of CG_2 resulted in B ranging from 2.65 to 6.93 kg_f with a mean of 5.03 kg_f (Figure 5.7A). Meanwhile κ varied from 0.23 to 0.93 d^{-1} with a mean of 0.68 d^{-1} (Figure 5.7B). The nlme procedure attempts to estimate GL dependent parameters with a normal distribution. Both B and κ populations were not significantly different from a normal distribution ($P > 0.05$). GL dependent parameters B and κ were independent of each other and found not to be strongly associated (r= -0.50) with each other (Figure 5.7C). However, GLs with low initial firmness had a tendency to be coupled with a high rate of firmness decline. This indicates that lines with low initial firmness (usually the ones harvested late in a season) soften faster during storage at 20 °C. However, GLs with a high initial firmness ($B > 4.5$ kg_f) showed a wide range of rates of firmness decline ($0.23 < \kappa < 0.93$ d^{-1}). This relationship indicates that lines harvested at firm stage can express a range of softening patterns during storage (at 20 °C).

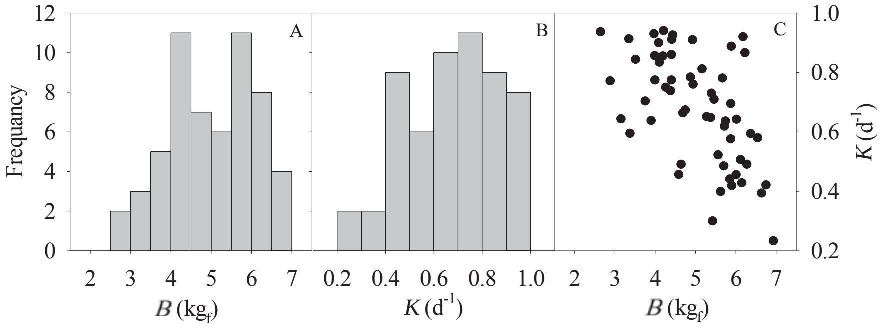

Figure 5.7: Histogram of GL dependent CG$_2$ parameters, B (A) and κ (B), estimated from 2011 AFL softening data and correlation between them (C).

5.3.2 Testing CG model parameters as predictor of storage potential

Correlation coefficient values show the extent of association between extracted parameter values (B and κ) from AFL softening curves with storage quality (firmness and rots) measured at 100 and 126 d of optimal storage (Table 5.3). With the exception of number of rots at 126 d in all correlation combinations, κ was found more associated with storage quality than B. This result is not surprising given that B is an upper asymptote and represents the initial firmness. At-harvest firmness was found not to be a good predictor of storage quality of GLs (Table 4.1 - 4.3). GLs with a high value for B and low rate of firmness change (κ) during AFL tended (r = -0.47 and 0.41 respectively) to have a low number of rots at 126 d of optimal storage. Rate of firmness change (κ) during AFL monitoring was negatively associated with both mean firmness and SF during optimal storage. This result indicated that GLs with higher rate of firmness change (κ) during AFL monitoring had tendency to have lower SF at 100 (Figure 5.8) and 126 d of optimal storage (r = -0.53 and -0.45 respectively). SF was used to express storage potential of a GL because in industry it is the preferred and extensively followed measurement for storage quality evaluation. Rate of firmness change (κ) during AFL monitoring was further characterised to categorise GLs for storage potential.

Table 5.3: Correlation coefficients (*r*) of fruit quality (firmness and rots) in optimal storage with AFL softening model parameters *B* (kg$_f$) and κ (d^{-1}) in 2011.

Optimal storage		Correlation coefficient (*r*) with AFL parameters	
		B (Upper asymptote)	κ (Rate of firmness change)
At 100 d	SF	0.12	-0.53
	Mean firmness	0.41	-0.55
	Number of rots	-0.37	0.47
At 126 d	SF	0.03	-0.45
	Mean firmness	0.23	-0.38
	Number of rots	-0.47	0.41

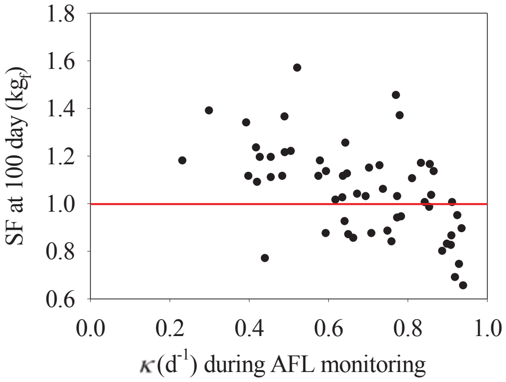

Figure 5.8: Correlation of rate of firmness change parameter (κ) during AFL monitoring with soft fractile at 100 day of optimal storage in 2011. Each data point is a GL. Red line represents an export threshold of 1 kg$_f$.

5.3.3 Threshold selection for best possible categorisation

Through investigating different combinations of κ_α and κ_β, it was found that 0.63 and 0.53 were the best threshold values respectively (Appendix 2). This threshold combination resulted in the lowest (5.3%) number of GLs in false categorisation, predicted as having a higher storage potential than measured (Table 5.4). Meanwhile 59.6% of GLs were correctly categorised into their respective storage categories (i.e. low, medium and high), leaving 35.1% of lines as missed categorisation (having lower predicted storage potential than observed).

Thirty seven (37) GLs (64.9%) were categorised as having low storage potential while only 18 of these were measured as low, 8 were found to be medium and a further 11 had high storage potential. Only 5 (8.7%) GLs were categorised as medium storage (0.53< κ <0.63), of which 3 were measured as medium and 1 was measured in each of the low and high storage potential categories. Fifteen (15) GLs (26.3%) were categorised as high storage potential with 13 of these actually measured as high and 1 was measured in each of low and medium storage potential categories. Overall, κ successfully identified 90% of the low storage GLs. Removing GLs categorised as low storing (65% of whole population) changed the proportion of observed low storing lines in the remaining population from 35% to 10%.

Table 5.4: Contingency table compares the number of GLs measured and categorised by κ thresholds (κ_α = 0.63 and κ_β = 0.53) in three storage categories (low, medium and high) in 2011. Green cells show correct categorisation. Yellow cells represent missed and red cells are for false categorisation. Row and column total shows number of GLs measured and categorised respectively in storage categories.

		Categorised by κ thresholds			Row total
		Low ($\kappa_\alpha \geq 0.63$)	Medium ($0.53< \kappa<0.63$)	High ($\kappa_\beta \leq 0.53$)	
Measured storage categories	Low (SF <1 kg$_f$ at 100 d)	18	1	1	20
	Medium (100 d) 1< SF <1 kg$_f$ (126 d)	8	3	1	12
	High (SF >1 kg$_f$ at 126 d)	11	1	13	25
Column total		37	5	15	57

In worst case, AFL may categorise more than 65% of the population as low storage and may not identify substantial proportion as high storage. Having said that, it may well be that many lines need to be sold early in the season anyway to meet commercial demand, so correctly identifying a small number of lines with longer storage potential may be the most important practical outcome. Careful comparison of industrial sale patterns with AFL based categorisation would help to assess the benefit of AFL methodology to minimise fruit loss.

5.3.4 Application of κ thresholds in future

Selection of κ thresholds (κ_α and κ_β) for best categorisation of GLs to each storage category was possible because of the availability of storage information. However, application of this threshold in an industrial scenario without prior storage information has the potential to be influenced by seasonal differences. Huge variability exists between seasons for kiwifruit maturity and postharvest softening patterns, and makes storage life prediction a difficult task (Mitchell et al., 1992; Benge, 1999; Snelgar et al., 2007; Adams et al., 2010; Burdon and Lallu, 2011). Therefore, category thresholds may need to be adapted for each season. Two potential options may be used to adapt κ thresholds to season differences, including:

1. Use the same κ thresholds in every year. This can be advantageous by employing a consistent approach of AFL based categorisation of GLs every year and would provide an opportunity of easy interpretation. On the other hand, application of same κ thresholds every year may not be good and may result in increased losses.
2. Take the 60% of GLs in a whole population with highest κ values in AFL monitoring and sell them before 100 d in optimal storage. This option involve an assumption that 60% values of the highest κ in AFL monitoring could define the low and medium storage GLs (SF < 1 kg_f before or at 126 d). The remaining proportion of lines can be considered as high storage potential and assigned to be sold after 126 d of storage. However, there will be chances of false categorisation of GLs and may result in fruit loss.

Each of these options should be assessed for benefit and amount of losses before industrial application. Therefore, these options will be evaluated in the validation study of AFL based categorisation of kiwifruit GLs for storage potential (Chapter 6).

5.4 CONCLUSION

This chapter has demonstrated curve fitting of AFL firmness data by CG to enable description of GL dependent softening for the model parameter extraction. GLs with high value of κ during AFL monitoring had tendency to have low SF after 100 and 126 d in optimal storage. For best possible categorisation of GLs for 1 of 3 storage categories, thresholds of κ were required. Through investigating different combinations of κ_α and κ_β, 0.63 and 0.53, respectively, were the best threshold values. With this threshold combination, minimum numbers of GLs were categorised as false, i.e. predicted as having a higher storage potential than observed. Removal of GL categorised by AFL κ thresholds as low storage potential decreased the proportion of low storing lines in the remaining population. Overall, AFL method was successful in predicting 90% of the lines with low storage potential. Comparison of AFL based categorisation with industrial sale patterns was recommended to evaluate the benefit of AFL methodology in minimising fruit loss. Application of the same κ thresholds in industrial scenario without prior storage information may be influenced by seasonal change; therefore, annual assignment of thresholds needs to be robust. To ensure the robustness of AFL based categorisation of kiwifruit GLs for storage potential, it should be re-assessed in a different season before industrial application. Therefore, validation study of the developed approach in new season of 2012 was performed (Chapter 6).

6 Validation of Accelerated Fruit Library (*)

6.1 INTRODUCTION

Existence of inherent variability for softening patterns between kiwifruit grower lines (GLs, Figure 3.5 and Figure 4.6) leads to variation in storage potential (time to reach soft fractile (SF) of < 1 kg_f). This variability contributes to fruit losses because of the inability to differentiate between poor or good storing lines. There are many pre and postharvest factors, which contribute to softening variability during optimal storage (Section 2.3). An 'Accelerated Fruit Library (AFL) can be a potential methodology to segregate GLs for storage potential, based on the rate of softening losses in abusive conditions (Section 2.5.1 and Section 5.3.3). For successful development of AFL, one of the main assumptions is that softening losses in accelerated conditions are predictive of losses under optimal storage.

Storage of kiwifruit at higher temperature (20 °C) accelerates fruit softening (Antunes and Sfakiotakis, 2000, 2002a and Antunes, 2007; Figure 3.8 and Figure 4.6). Previous findings show that fruit loss parameters at any individual time point during AFL monitoring were not correlated with firmness loss in optimal storage (Chapter 4, Section 4.3.2.2). Evaluation of a complete softening pattern during AFL monitoring may yield an improved relationship with firmness loss in optimal storage. In contemporary literature, many examples were found to describe kiwifruit softening curves (Benge et al., 2000; Feng, 2003; Hertog et al., 2004b; White et al., 2005; Feng et al., 2006). The Complementary Gompertz (CG_2) model has potential to efficiently fit AFL firmness data (Benge et al., 2001, Figure 5.4B and Figure 5.5) with two global (A_o and β) and two GL dependent (B and κ) parameters (Figure 5.2, Section 5.3.1). Correlation of firmness change parameter (κ) with SF in optimal storage (at 100 d) showed that GLs with faster decrease in firmness during AFL monitoring (high κ) had a tendency to have lower SF in optimal storage (r = -0.53, Table 5.3). Further characterisation of κ showed that two thresholds of κ ($\kappa_\alpha = 0.63$ and $\kappa_\beta = 0.53$, Section 5.3.3) can appropriately categorise approximately 60% of GLs into 1 of 3 storage potential categories (i.e. low, medium and high).

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

Validation of the calibrated AFL methodology is essential before attempted application in industry to segregate GLs for storage potential. Demonstration of acceptable predictive accuracy of a model or a method with the specified domain is called validation (Thornley and France, 2007). Softening of kiwifruit from the same orchard can vary between different seasons because of many environmental and orchard management practices (Benge, 1999). Prior to industrial application, a validation study is required for a different season for the AFL based categorisation of storage potential. It is important to consider that different seasons may require different thresholds (κ_α and κ_β) to segregate GLs for storage potential categories. Therefore, the study presented in this chapter was conducted with an objective to validate the developed AFL methodology and explore the use of κ thresholds to segregate GLs of a different season for storage potential categories.

6.2 MATERIALS AND METHODS

Nine modular bulk (MB) packs (99 to 100 fruit per pack wrapped in a polyliner) per GL of count 36 ‘Hayward’ kiwifruit from 51 GLs were sent in temperature controlled transport from a commercial packing facility (in Bay of Plenty) to Massey University, Palmerston North, New Zealand. GLs were received over a harvest season of almost 5 weeks, from May 10 (ISO day 131) to June 14, 2012 (ISO day 166). Upon arrival of each GL, MB packs were randomly divided into two groups for fruit measurement; 6 for optimal storage and 3 for at-harvest and AFL (Figure 6.1).

Six MB packs for optimal storage were immediately stored at 0 °C. Eight polylined single layer trays of 36 fruit each were prepared from the remaining 3 MB packs for at-harvest and AFL measurements. Out of eight, one tray was assessed for at-harvest fruit quality. The remaining seven trays were stored in a flow through system at 20 °C for AFL measurements (Figure 4.2). To ensure acceleration of fruit losses without influence of any external ethylene, the same method and control measures were followed as explained in chapter 4 (Section 4.2).

At-harvest, firmness and soluble solids content (SSC) of 36 (1 tray), and dry matter (DM) of 15, randomly selected fruit per GL were measured. For AFL measurements, firmness of 36 fruit per GL was recorded over 21 d at 3 d intervals. During optimal storage, firmness of 3 MB packs per GL was assessed at 100 and 126 d. Standard methods for assessment of firmness, SSC and DM were used as detailed in chapter 3 (Section 3.2).

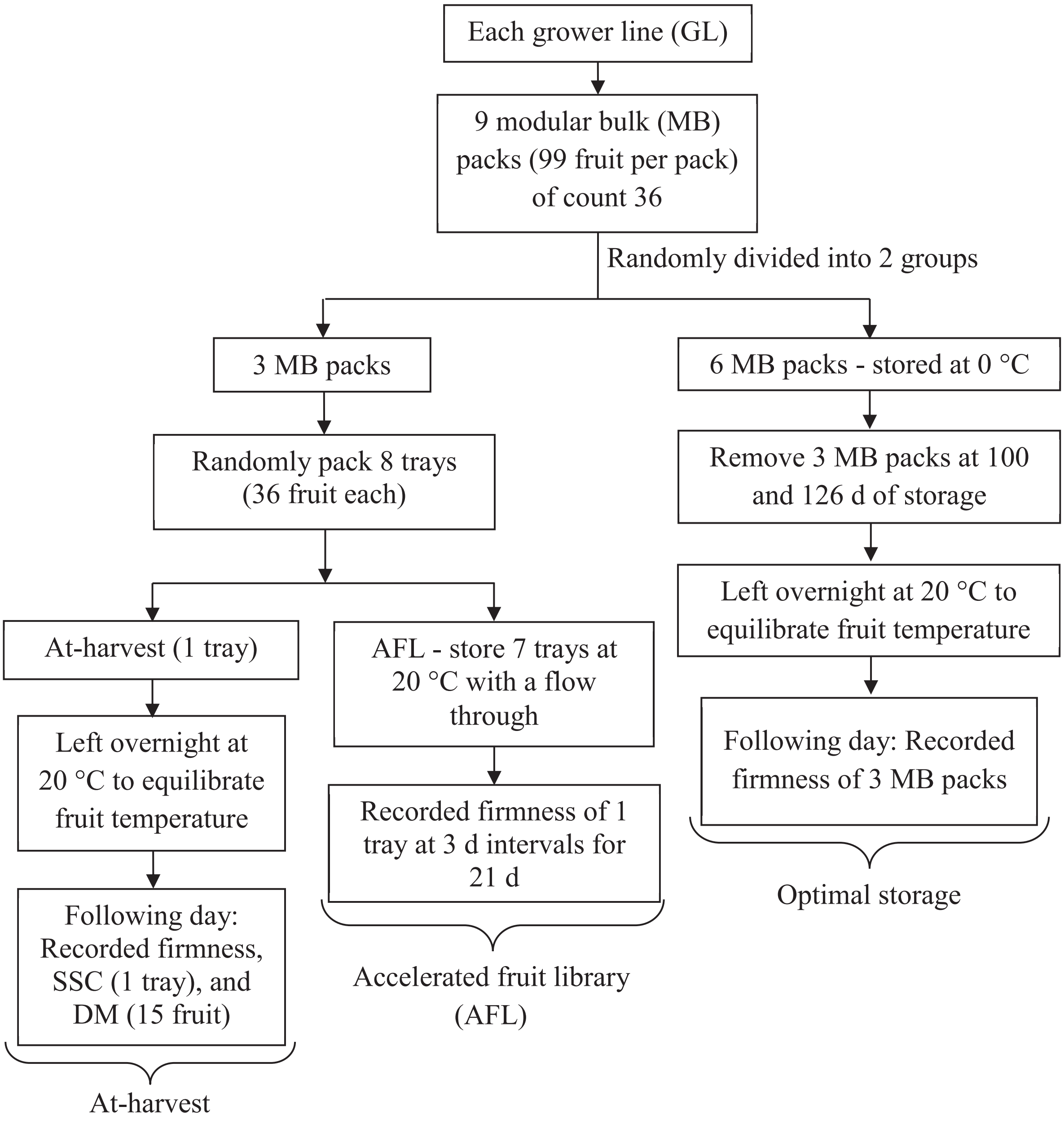

Figure 6.1: Fruit distribution and data collection for at-harvest, AFL and optimal storage measurements for each GL in 2012.

Fruit temperature equilibration was performed for at-harvest and optimal storage measurements, to avoid errors in firmness data due to difference in fruit temperature (Jeffery and Banks, 1994). Thermocouples were used to measure temperature and data were recorded in data loggers (Grant - 1200 Squirrel Digital Meter/Logger, Grant Instruments Ltd. Barrington, Cambridge, England). During both optimal storage and AFL monitoring, RH of > 90% was maintained in cool rooms. Ethylene concentration in the cool room was monitored intermittently during the whole storage period and maintained (by placing Purafil® ($KMnO_4$) sachets) below the industry standard 0.03 ppm. The same procedure of ethylene concentration evaluation was adopted as explained in chapter 3 (Section 3.2.4).

6.3 DATA ANALYSIS

From the firmness data in optimal storage, the SF value (kg_f, 9th lowest firmness value of approximately 300 fruit) for each GL at 100 and 126 d was defined. From at-harvest data, means of SSC, DM and firmness were calculated for each GL. From AFL data of each GL means of firmness were calculated at each measurement occasion. AFL firmness data were fitted with the Complementary Gompertz equation (CG_2) with non-linear mixed effects by using nlme procedure in R (version 2.15.1, cran.r-project.org, Pinheiro et al., 2013, Eq. 5.1, Section 5.2.1.2 and Section 5.2.1.3). Fitted GL dependent parameters (B and κ) were estimated for each GL as explained in chapter 5 (Section 5.3.1). Errors of fits by CG_2 to AFL firmness data were assessed as Mean Absolute Error (MAE, Eq. 5.2)

Estimated values of firmness change parameter (κ) of CG_2 model were used to predict the storage potential category (low, medium and high) by adopting the previously defined thresholds for κ_α and κ_β (Chapter 5, Section 5.3.3). While considering that seasonal differences may occur, thresholds of κ (κ_α and κ_β) were also re-defined for the validation data set to predict storage potential. For validation purpose, SF values at 100 and 126 d were used to define observed storage potential categories of GLs (Section 5.2.1.2). A contingency table was used to compare observed and predicted GLs for storage potential categories (Table 5.1).

6.4 RESULTS AND DISCUSSION

6.4.1 Description of the data

6.4.1.1 At-harvest

At-harvest fruit quality of 51 GLs varied over the 5 week harvest season (131 to 166 ISO d). SSC of GLs gradually increased from 6.9 to 13.9%. Only one GL, harvested late in the season (158 ISO d) had higher at-harvest SSC of 15.8% (Figure 6.2A). Increase in SSC during harvest season was observed because of starch conversion to sugars with the passage of time (Beever and Hopkirk, 1990; Burdon et al., 2013). All GLs had SSC higher than of 6.2%, a maturity index for New Zealand growers to harvest kiwifruit for export (Burdon et al., 2011).

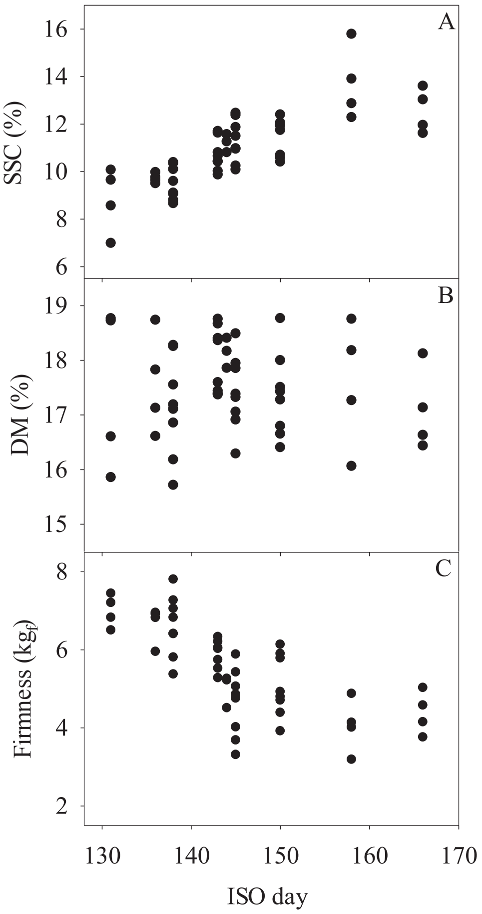

Figure 6.2: At-harvest SSC (A), DM (B) and firmness (C) of 51 GLs in 2012. Each data point represents a GL. For SSC and firmness, each data point shows an average of 36 fruit and for DM average of 15 fruit per GL.

At-harvest DM of 51 GLs varied from 15.7 to 18.8% and did not followed any clear trend over the harvest season of 5 weeks (Figure 6.2B). At-harvest DM of kiwifruit varies from 12 to 20%. DM is an important determinant of fruit quality when fruit ripen. Consumer preferences for kiwifruit have a relationship with at-harvest DM. Higher DM at-harvest results in better fruit quality with high SSC when ripe (Burdon et al., 2004; Burdon and Lallu, 2011).

At-harvest mean firmness followed a decreasing trend as the season progressed. Firmness gradually decreased from 7.8 to 3.2 kg_f (Figure 6.2C). During fruit development, kiwifruit are very hard and firmness starts decreasing while it is on vine. In industry, at-harvest fruit firmness usually decreases as the season progresses (MacRae et al., 1989a; MacRae et al., 1989b; Beever and Hopkirk, 1990; Burdon et al., 2014a). At-harvest kiwifruit firmness of all GLs was representative of commercial maturity. When harvested at same time, GLs vary in initial firmness, which represents the variability in fruit maturity (Benge, 1999).

Overall, a clear trend was observed for the decrease in firmness and increase in SSC at the time of harvest, as the season progressed. At-harvest DM did not show any clear trend over the harvest season. Variation in at-harvest quality between GLs suggests the potential for variability in postharvest softening patterns to be observed. Thus, this variability between GLs provides an opportunity of having robustness in the validation study of AFL based storage potential predictions.

6.4.1.2 AFL softening

Softening of 51 GLs during AFL monitoring displayed a variety of patterns (Figure 6.3A). GLs exhibited softening curves with and without initial lag phase. The differences in the initial lag phase between batches were independent of harvest timing (ISO d) or initial firmness. Some lines at the start of season, with an at-harvest firmness > 6 kg_f did not maintain a substantial initial lag phase. Meanwhile many GLs displayed an initial lag phase (3 - 9 d) even when the at-harvest firmness was low (< 4 kg_f). Overall, five GLs displayed extended initial lag phase of 18 to 21 d. The longest lag phase (21 d) observed in this data was for a GL with at-harvest firmness of 4.8 kg_f. The softening variability between different lines when stored in the same conditions represent the effects of growing locations or orchards (Mitchell et al., 1992; Arpaia et al., 1994; Benge, 1999). At-harvest firmness seemed to have no effect on fruit softening patterns (Ghasemnezhad et al., 2013).

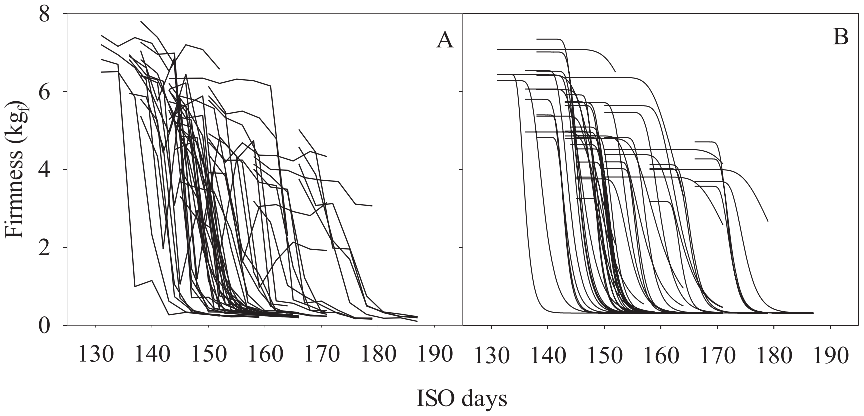

Figure 6.3: Raw (A) and fitted (B) softening curves of 51 GLs during AFL monitoring in 2012. CG$_2$ with global A_o (0.31 kg$_f$) and β (125), and GL dependent B (range of 2.86 to 7.03 kg$_f$) and κ (range of 0.19 to 1.07 d^{-1}) parameters was used to fit the firmness data.

CG$_2$ was fitted to firmness data of 51 GLs in AFL monitoring with nlme procedure, while having two global (A_o and β) and two GL dependent (B and κ) model parameters. Softening curve of each GL comprised 8 measurement points (1 at-harvest and 7 during AFL monitoring at 21 d). Model output resulted with an AIC value of 1118. Fitted curves with time (ISO d) suggested that CG$_2$ characterised all softening phases of GLs (Figure 6.3B) and exhibited similar patterns to those observed in raw data (Figure 6.3A).

Mean absolute error (MAE) of fitted curves ranged from 0.06 to 1.61 kg$_f$ with an average of 0.34 kg$_f$ for all GLs (Figure 6.4). In total, 82% (42) of GLs had MAE less than 0.5 kg$_f$. MAE of 6 GLs ranged from 0.54 to 0.86 kg$_f$ while 2 GLs further had MAE higher than 1 kg$_f$ and ranged from 1.10 to 1.20 kg$_f$. One GL with inconsistent softening pattern during AFL monitoring had highest MAE of 1.61 kg$_f$. Overall, CG$_2$ fitted the AFL softening of GLs aptly.

CG$_2$ was fitted to softening curves with global parameter value of 0.31 kg$_f$ for A_o and 125 for β (Appendix 3). Estimated GL dependent model parameter values of B varied from 2.86 to 7.03 kg$_f$ with mean of 4.79 kg$_f$ (Figure 6.5A). Values of κ varied from 0.19 to 1.07 d^{-1} with mean of 0.67 d^{-1} (Figure 6.5B). Minimum κ values (0.19 - 0.25 d^{-1})

were observed in GLs that did not completely soften and maintained a long lag phase in the AFL monitoring.

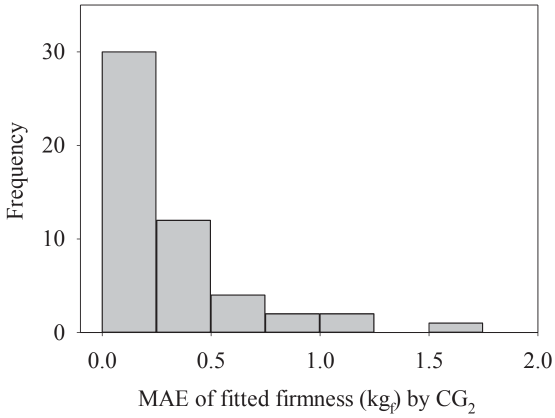

Figure 6.4: Histogram of mean absolute error (MAE) of fits by CG$_2$ for the firmness data of 51 GLs during AFL monitoring in 2012.

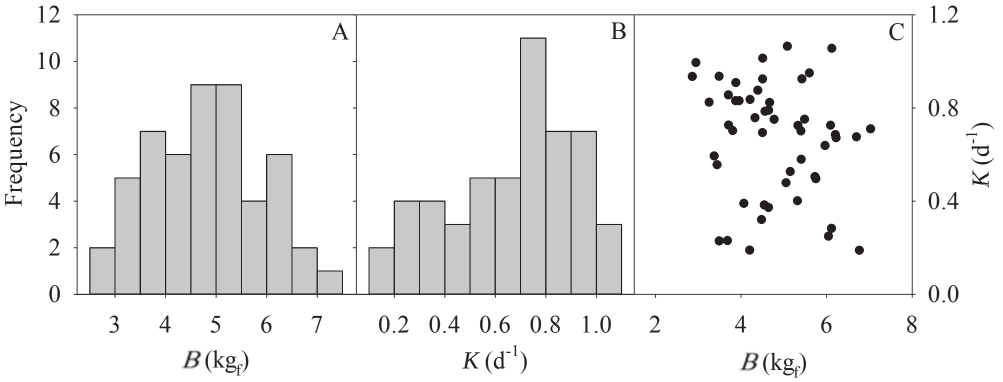

Figure 6.5: Histogram of GL dependent CG$_2$ parameters, B (A) and κ (B), estimated from 2012 AFL softening data and correlation between them (C).

Normality tests revealed that B values were not significantly different from a normal distribution ($P > 0.05$), meanwhile, κ values were significantly different from a normal distribution ($P < 0.05$). B and κ were largely independent ($r = -0.18$) of each other (Figure 6.5C). Results showed that upper asymptote (B) did not influence the rate of firmness change (κ) during AFL monitoring. GLs (with initial firmness $3 < B < 7$ kg$_f$) can exhibit a wide range for rate of firmness change ($0.19 < \kappa < 1.06$ d^{-1}) during storage at 20 °C.

In 2011 AFL firmness data were fitted by global parameter of $A_o = 0.31$ kg$_f$ and $\beta = 73$ (Section 5.3.1). In 2012, fitted value of A_o was same (at 0.31 kg$_f$) but global value for β increased to 125. Range of fitted values for GL dependent parameter of upper asymptote (B) did not differ substantially between two seasons, while varying from 2.65 to 6.93 kg$_f$ for 2011 (Figure 5.7A) and 2.86 to 7.03 kg$_f$ for 2012 (Figure 6.5A). However, range of fitted values for rate of firmness change (κ) varied for both seasons, while ranging from 0.23 to 0.93 d^{-1} in 2011 (Figure 5.7B) and 0.19 to 1.07 d^{-1} in 2012 (Figure 6.5B). Most importantly the range for fastest (with difference of 0.14 d^{-1} in higher κ) softening GLs varied more in two seasons than slow (with difference of 0.04 d^{-1} in lower κ) softening lines. Different range of κ values in validation season may possibly require new thresholds for best categorisation of GLs for storage potential.

6.4.2 AFL based prediction of storage potential category

6.4.2.1 Use of same κ thresholds

In the calibration study (in 2011), thresholds of $\kappa_\alpha = 0.63$ d^{-1} and $\kappa_\beta = 0.53$ d^{-1} were used to categorise GLs to 1 of 3 storage potential categories (Section 5.3.3). Firstly, for validation, the same κ_α and κ_β thresholds of AFL softening were used to predict storage potential (in 2012). SF values at 100 and 126 d were used to compare the predicted results for 3 storage potential categories. Validation results showed that κ thresholds ($\kappa_\alpha = 0.63$ d^{-1} and $\kappa_\beta = 0.53$ d^{-1}) appropriately categorised 53% of GLs storage potential (Table 6.1). Meanwhile 25.5% of lines were predicted to have a lower storage potential than observed (missed categorisation). Overall, 21.5% of GLs were predicted as higher storage potential than observed (false categorisation).

Thirty-three (33) GLs (64.7%) were predicted as low storage while only 21 of these were observed as low and 6 were found to be medium and a further 6 had high storage potential. Only 3 lines (5.9%) were predicted as having medium storage potential while 2 of these were observed as low and 1 as high storage. Fifteen (15) GLs (29.4%) were predicted as high storing, while only 6 of these were actually observed as high and 4 were found to be low and 5 had medium storage potential. Overall, κ thresholds ($\kappa_\alpha =$ 0.63 d^{-1} and $\kappa_\beta = 0.53$ d^{-1}) successfully predicted only 77.8% (21) of observed low storage GLs (27). Removal of GLs (33) predicted as low storing (64.7% of whole population) changed the proportion of observed low storing lines in the remaining population from 52.9% to 33.3%.

Table 6.1: Contingency table compares the number of GLs measured and categorised by κ thresholds ($\kappa_\alpha = 0.63$ and $\kappa_\beta = 0.53$) in three storage categories (low, medium and high) in 2012. Green cells show correct categorisation. Yellow cell represents missed and red cells are for false categorisation. Row and column total shows number of GLs measured and categorised (respectively) in each storage category

		Categorised by κ thresholds			Row total
		Low ($\kappa_\alpha \geq 0.63$)	Medium ($0.53< \kappa <0.63$)	High ($\kappa_\beta \leq 0.53$)	
Measured storage categories	Low (SF <1 kg_f at 100 d)	🟩 21	🟥 2	🟥 4	27
	Medium (100 d) 1< SF <1 kg_f (126 d)	🟨 6	🟩 0	🟥 5	11
	High (SF >1 kg_f at 126 d)	🟨 6	🟨 1	🟩 6	13
Column total		33	3	15	51

Previously in 2011, the same κ thresholds categorised only 5.2% of lines in false categorisation while in validation study (in 2012) 21.5% of lines were false categorised. In 2011, removal of predicted low storing lines reduced the proportion of observed low storing lines from 35% to 10% in the remaining population (Table 5.4). In the validation study, there were higher proportions (33.3%) of low storing lines remaining in the population of medium and high storage potential categories, which may contribute to fruit losses in the supply chain. This can be because of seasonal difference in range of κ values. Use of same κ thresholds every year may not be appropriate to predict GLs for storage potential categories whilst minimising the number of lines in false categorisation. Moreover, seasonal differences in optimal storage softening may also contribute to the difficulty in applying same κ thresholds to categorise GLs for storage potential. Seasonal difference in optimal storage softening was evident, as there were only 20 (35%) out of 57 GLs failing at 100 d (SF < 1 kg_f) in 2011 (Table 5.4) while in 2012, 27 (53%) out of 51 GLs had low storage potential (Table 6.1). Perhaps, each season may require new κ thresholds for best categorisation of GLs for storage potential.

6.4.2.2 Re-defined thresholds of κ for validation data set

To re-define κ thresholds, different combinations of κ_α and κ_β were tested from highest to lowest κ for three prediction categories of GLs. Each combination started from high κ having a difference of 0.04 between κ_α and κ_β (e.g. $\kappa_\alpha = 1.07$ d^{-1} and $\kappa_\beta = 1.03$ d^{-1}). The difference of 0.04 was an arbitrary selection to create many combinations of κ_α and κ_β. While keeping the same κ_α, κ_β was changed to a low κ by a decrement of 0.04 consecutively (e.g. κ_β = 0.99, 0.95 and so on). Each next combination started by reducing κ_α by 0.04 d^{-1} followed by κ_β selection. Prediction categories of GLs made by each combination of κ thresholds were compared in a contingency table (Section 5.2.2.1, Table 5.1). Percentages of GLs in correct, missed and false categorisation were collected in accordance to different combinations of κ_α and κ_β (Appendix 4).

The resulting combination of re-defined κ thresholds ($\kappa_\alpha = 0.67$ d^{-1} and $\kappa_\beta = 0.59$ d^{-1}) for validation data set appropriately categorised 56.8% GLs into 1 of 3 storage potential categories (Table 6.2). This threshold combination of κ categorised 21.6% of GLs in each of missed (predicted low storage potential than observed) and false categorisation (predicted higher storage potential than observed).

Table 6.2: Contingency table compares the number of GLs measured and categorised by κ thresholds ($\kappa_\alpha = 0.67$ and $\kappa_\beta = 0.59$) in three storage categories (low, medium and high) in 2012. Green cells show correct categorisation. Yellow cells represent missed and red cells are for false categorisation. Row and column total shows number of GLs measured and categorised (respectively) in each storage category.

		Categorised by κ thresholds			Row total
		Low ($\kappa_\alpha \geq 0.67$)	Medium ($0.59< \kappa <0.67$)	High ($\kappa_\beta \leq 0.59$)	
Measured storage categories	Low (SF <1 kg$_f$ at 100 d)	21	1	5	27
	Medium (100 d) 1< SF <1 kg$_f$ (126 d)	5	1	5	11
	High (SF >1 kg$_f$ at 126 d)	6	0	7	13
Column total		32	2	17	51

Overall, the new thresholds ($\kappa_\alpha = 0.67$ d^{-1} and $\kappa_\beta = 0.59$ d^{-1}) of validation AFL softening data increased correct categorisation for only 2 GLs as compared to using previous season thresholds ($\kappa_\alpha = 0.63$ d^{-1} and $\kappa_\beta = 0.53$ d^{-1}). However, new κ thresholds did not reduce the number of GLs in false categorisation. Given these results, application of the same κ thresholds every season can be suggested but chances of false categorisation may alter significantly between seasons. Moreover, in industry calculation of κ thresholds every season is impossible as storage data is required to enable selection of κ thresholds.

6.4.2.3 60% of GLs with highest κ

Instead of using κ threshold every season to categorise GLs for storage potential, an alternative option was proposed (Section 5.3.4) to take 60% of GLs with higher κ values and consider these as low storing. This option involved an assumption that 60% values of the highest κ in AFL could define the low storage GLs (SF < 1 kg$_f$ at 100 d). Based on κ values, GLs were categorised in two categories, i.e. 60% with highest κ and remaining 40% of lines. To compare with observed storage potential, SF values at 100 d were used (Table 6.3), because using κ thresholds predicted around 60% of population as failed (SF < 1 kg$_f$) at 100 d in both seasons.

Table 6.3: Contingency table compares the number of GLs measured and categorised by higher 60% of κ in two storage categories (low and high) in 2012. Green cells show correct categorisation. Yellow cells represent missed and red cells are for false categorisation. Row and column total shows number of GLs measured and categorised (respectively) in each storage category.

		Categorised by κ		
		60% GLs with highest κ	40% GLs	Row total
Measured storage potential	Low (SF <1 kg$_f$ at 100 d)	20	7	27
	High (SF >1 kg$_f$ at 100 d)	10	14	24
	Column total	30	21	51

Results showed that 34 (66%) GLs were accurately categorised for low and high storage potential. Removal of 60% of low storing lines with highest κ values in AFL, changed the proportion of low storing lines in the remaining population from 52.9% to 33.3%.

The proportion of low storing lines in the remaining lot is the same as when different thresholds of κ were used to categorise for storage potential. Given these results, 60% of lines with higher κ can be considered as low storing every season instead of using different thresholds, but chances of low storing lines in the remaining population can be high.

6.5 CONCLUSION

The objective of the study presented in this chapter was to validate the AFL methodology and use of κ thresholds to predict GLs of different season for storage potential. Establishment of AFL by using flow through system to keep each GL independent for ethylene effect was validated successfully. GLs displayed a variety (with and without initial lag phase) of softening patterns in AFL monitoring (Figure 6.3A). The CG_2 model (A_o and β as global, and B and κ as GL-dependent) appropriately characterised the softening of GLs. Using previous season AFL κ thresholds ($\kappa_\alpha = 0.63$ and $\kappa_\beta = 0.53$) resulted in 21.5% false categorisation, meanwhile approximately half (52.9%) of GLs were appropriately categorised. While considering different season effect on softening patterns of GLs during AFL (different κ) and optimal storage monitoring, κ thresholds were re-defined for validation data set. New κ thresholds ($\kappa_\alpha =$ 0.67 and $\kappa_\beta = 0.59$) were unsuccessful in reducing the proportion of GLs in false categorisation (21.6%) with a marginal increase in accurate predictions (56.9%). Both new and previous season κ thresholds resulted in the same proportion of low storing lines (33.3%) in the remaining population after removing the GLs predicted as having low storage potential. Even categorisation of 60% of GLs with highest κ as low storing (failed at 100 d) also resulted in 33.3 % of low storing lines in the remaining population. Hence, it would seem that similar κ thresholds can be used every season to segregate lines for storage potential but chances of false categorisation may vary due to seasonal variation for softening patterns. Overall, the validation study in different season concluded that AFL monitoring system could be used to predict the storage potential of GLs. However, chances of predicting lines as higher storage than observed can be high and may contribute to fruit and profit loss in the supply chain, and thus restrict its industrial application.

7 Discussion and Recommendations (*)

7.1 INTRODUCTION

Postharvest fruit losses are one of the biggest concerns of the kiwifruit industry. Inherent variability exists between grower lines (GLs) for storage potential (time to reach firmness threshold of 1 kg_f, Figure 3.5). This variability results in difficulty to categorise different GLs for storage potential. Currently industry relies on previous season information and at-harvest fruit quality measures to differentiate good or poor storing GLs. Fruit loss costs approximately $ 120 million per year to the industry which includes monitoring and re-packing cost to eliminate the over soft and rotten fruit to meet export standards (Tanner et al., 2012). This research attempted to develop a rapid testing methodology of accelerated fruit libraries (AFLs) to predict the storage potential of 'Hayward' kiwifruit GLs. AFL predicts the storage potential of GLs based on the amount or rate of losses in accelerated conditions, while assuming that the pattern of losses is similar to optimal storage.

In this work, establishment of AFL methodology was first understood by conducting a preliminary study (Chapter 3). For AFL monitoring, fruit were stored at 20 °C. From learning of the initial trial, AFL methodology was refined before applying in a new season (Chapter 4). Correlations of at-harvest and AFL parameters of losses with storage potential of GLs were found to not be prospective indicators of storage potential (Section 4.3.2.2). Later, softening curves to describe AFL behaviour were fitted by using the Complementary Gompertz (CG) equation and GL dependent model parameters were used to segregate GLs for storage potential categories (Chapter 5). A validation study of the CG modelling approach was performed in an additional new harvest season (Chapter 6). This chapter summarises the outcomes and learning for AFL establishment and resulting data interpretation to assist in segregation of kiwifruit GLs for storage potential while considering limitations of the study, industrial implications and further research opportunities.

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

7.2 ESTABLISHMENT OF AFL

Results from preliminary study (first season) revealed that storage at 20 °C (for AFL monitoring) accelerated the softening process and other losses (e.g. rots) of kiwifruit GLs (Figure 3.8 and Figure 4.6). Storage of kiwifruit at 20 °C also had a consequence of endogenous ethylene production potentially leading to further fruit softening (Section 7.2.1). Expression and number of rotten fruit influenced the amount and value of softening data (Section 7.2.2). Type of fruit packaging (i.e. modular bulk (MB) pack) was considered to have a significant role in expression and spread of rots during storage at 20 °C (Section 7.2.3). The cost and labour required to conduct AFL study for each GL can be reduced through limiting the storage duration and number of measurement occasions used to collect softening data at 20 °C (Section 7.2.4). The following sections discuss the challenges of addressing these observations.

7.2.1 Ethylene effect

At the time of commercial harvest, kiwifruit produce negligible ethylene (< 0.01 $nL.kg^{-1}.h^{-1}$, Burdon and Lallu, 2011). At high temperature (20 °C) kiwifruit behave as a climacteric fruit, resulting in autocatalytic ethylene production, leading to accelerated ripening and decrease in firmness (Ming et al., 1992; Antunes and Sfakiotakis, 2002a; Chiaramonti and Barboni, 2010). Ethylene accumulation was observed in the AFL room during the first season, when all samples were placed collectively in the same room (Figure 3.8). Kiwifruit are sensitive to very low concentrations of ethylene (i.e. 0.01 ppm) during storage (Ben-Arie and Sonego, 1985; Mitchell, 1990). Higher ethylene concentrations (0.8 ppm) were measured in the room and were coupled with rapid decline in firmness of GLs arriving late in the season. Ethylene produced by ripening early harvested GLs potentially contaminated and caused more rapid softening of those arriving later in the season. To overcome this ethylene contamination issue, each GL was stored in a flow through system during future AFL attempts (Figure 4.2). GLs demonstrated a variety of softening patterns when stored without ethylene cross-contamination (Figure 4.6 and Figure 6.3A). Hence, to express the inherent pattern of losses, storage of AFL samples should be in an environment where GLs cannot be influenced by ethylene produced by any other GL.

7.2.2 Rotten fruit effect

Keeping kiwifruit at higher temperature enhances expression of rots (McDonald, 1990). During AFL monitoring, firmness of fruit having rot was also measured by avoiding the rot area. However, this procedure could not assure that the firmness measurement still does not carry a rot effect. Inclusion of rotten fruit firmness in data affects the distribution of fruit population at any measurement occasion. Therefore, data manipulation was also conducted when firmness of fruit "with rot" was excluded. However, AFL parameters did not vary significantly in relation to predicting the storage potential when rotten fruit data were omitted, suggesting that firmness values of rotten fruit may not affect prediction of storage potential of GLs (Section 3.4.4). Removal of fruit firmness data "with rot" does not eliminate the potential rot effect on healthy fruit. Once kiwifruit develop a rot, they become soft and start producing ethylene that may also accelerate the ripening of other healthy fruit within the box (Pennycook, 1985). Additionally, any damage caused during handling may result in premature softening by the action of ethylene accumulation within the box or tray (Davie, 1997).

Incidence of rot is unpredictable and highly variable not only from year to year and from orchard to orchard but even between fruit picked from the same vines on different days (Pennycook, 1985). However, GLs with higher number of rots at 18 and 21 d of AFL had a tendency to have lower SF at 126 d of optimal storage (r = -0.6, Table 4.2). Perhaps, number of fruit that develop rot can be important to define storability of GLs and hence cannot be ignored. Current system (i.e CG based modelling) ignores rot specific information and just assumes that this is captured indirectly as a soft fruit. Alternatively for AFL firmness data set, upon detection of any rotten fruit, exclusion of the whole tray as a data can be an option to eliminate the effect of rots. For instance, for AFL monitoring, extra trays could be stored than what is required to be measured and could be utilised as replacement trays if a tray is lost due to rot development.

7.2.3 Fruit packaging

In the first season, fruit were stored in modular bulk (MB) packs with three MBs (99-100 fruit per pack) assessed per measurement occasion during AFL monitoring. Later, sample size was reduced to 30 fruit to reduce the substantial effort required to collect the data (Section 3.5.3; Figure 3.12). Reduced sample size suited packing the fruit in a single layer tray for each measurement occasion in later attempts of AFL monitoring.

When a fruit gets a rot or bruise injury it produces more ethylene (Pennycook, 1985). As rotten fruit has the potential to affect the inherent ripening pattern of the remaining healthy fruit in a box. Prevalence of rotten fruit in a MB pack can affect almost all 99 - 100 fruit. For example, on one occasion, 3 MB packs of a GL varied ($P < 0.05$) in mean firmness, corresponding to the recorded rot incidence in each pack (i.e. Pack 1, 6.49 kg_f with 0 rots; Pack 2, 3.39 kg_f with 1 rotten fruit; and Pack 3, 1.99 kg_f with 4 rotten fruit; Figure 7.1). Variation in packs (of same GL at one occasion) for mean firmness was generally observed in relation to rot incidence. Hence, variability caused by rot incidence in packs for firmness can result in false representation of a GL mean firmness at any measurement occasion.

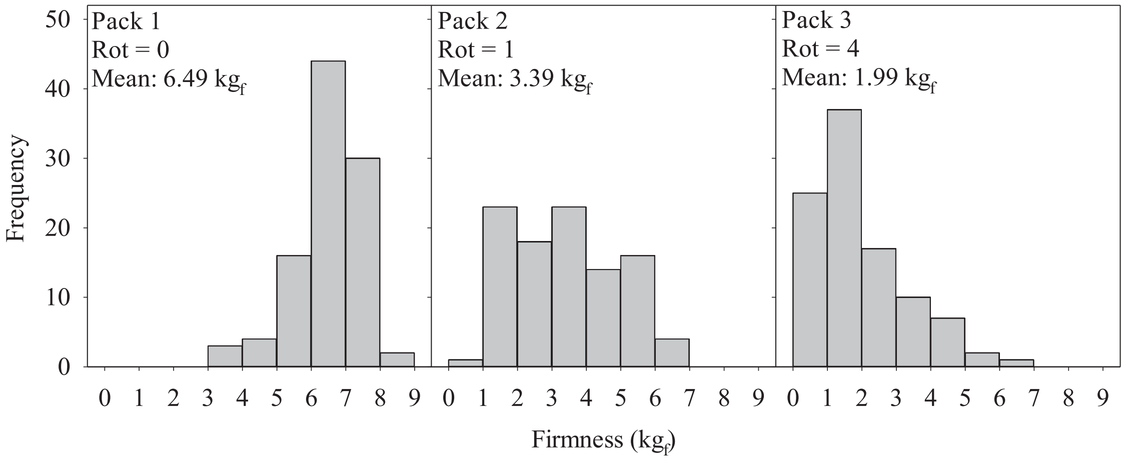

Figure 7.1: Histogram of fruit firmness in 3 modular bulk (MB) packs. Packs represent same GL at one measurement occasion during AFL monitoring in 2010. Each pack contains 99 fruit.

Alternatively, in single layer tray ethylene from rotten or over ripe fruit may only influence a reduced number (e.g. 30) of fruit in comparison to MB packed fruit. In MB pack, any rotten fruit could easily spread decay among other fruit in the same box during storage (Sommer et al., 1983; Figure 7.2A) and could result in more rotten fruit per pack. More rotten fruit in a pack can result in increase in ethylene production that would not only affect healthy fruit inside the pack, but are likely to influence the inherent ripening of fruit in the neighbouring packs. However, in a single layer tray each fruit has its own location with less contact with other fruit (Figure 7.2B) which leads to less or no spread of decay within tray and can result in less influence on healthy fruit. Therefore, for future study involving high temperature storage, use of single layer tray of fruit can minimise rot affect and its spread on data.

A: Spread of rot in a MB of kiwifruit B: Kiwifruit in a tray

Figure 7.2: Kiwifruit in a modular bulk (MB) pack (A) demonstrating the spread of rot in comparison with fruit in single layer tray (B).

7.2.4 Reduction in AFL data collection

In the first season, during AFL monitoring fruit losses assessments were made on 9 measurement occasions (at 3 d intervals over 27 d). A substantial number of rotten fruit were recorded after 21 d and firmness loss was difficult to assess. Therefore, in later seasons the number of measurement occasions were reduced to 7 (at 3 d intervals over 21 d) to minimise the fruit waste at later stages of AFL monitoring. A usual kiwifruit softening pattern expresses three phases, an initial slow phase, a secondary rapid phase and final slow phase (Benge et al., 2000). Enough data points are required to be collected to exhibit all phases of softening to enable later CG model fitting. Firmness data in later seasons showed that 8 data points (from 0 - 21 d at 3 d intervals) were enough to express the three softening phases with the exception of 6 GLs (in both seasons) which had an extended initial lag phase (18 - 21 d, Figure 5.4 and Figure 6.3).

A reduction in the number of data points required to describe that complete softening curve can be advantageous to minimise the effort and cost required for AFL application. The initial slow and secondary rapid phases differentiate GL softening patterns.

However, differences between GLs decrease, as fruit become very soft (below 1 kg_f, Figure 5.4 and Figure 6.3). Hence, to characterise the softening change at 20 °C, storage duration could perhaps be reduced to 18 or 15 d instead of 21 d. Alternatively, the time interval between measurement occasions could be extended (e.g. to 6 d instead of 3 d) to reduce the number of data points describing the GL softening patterns. Fortunately, we are able to process existing data sets to determine the impact of these potential modifications.

AFL softening data of 57 GLs collected in 2011 (Section 4.2) was used to compare different data collection patterns *post-hoc* (after the fact). From the data set collected over 21 d at 3 d intervals, data points at 21 and 18 d were obscured to follow the influence of using AFL storage durations of 18 and 15 d respectively. Alternatively, to analyse the effect of collecting data at extended intervals, data points collected at 6 d intervals were used (i.e. 0, 6, 12 and 18 d). The CG equation was again fitted with non-linear mixed effects to the modified AFL softening data (Section 5.2.1.2 and Section 5.2.1.3). Global (A_o and β) and GL dependent (B and κ) parameters were extracted for each modified data collection pattern and compared. Rate of firmness change (κ) of curves for different data collection patterns were also assessed for correlation with SF at 100 and 126 d of optimal storage.

Fitted values of B for softening curves in all four data collection patterns (21, 18, 15 d with 3 d interval and 18 d with 6 d interval) did not substantially vary ($P > 0.05$, Table 7.1 Appendix 5). Rate of firmness change (κ) of curves representing data for 18 and 15 d were statistically different from 21 d ($P < 0.05$, Table 7.1). Both average and range of κ decreased as the storage duration was reduced. The highest κ value decreased more than the lowest value with the reduction in storage duration, meaning that for most rapidly softening GLs, the fitted κ was most influenced. Overall, GL dependent parameters (B and κ) for curves representing duration of 18 and 15 d were highly correlated ($r = 0.99$) with the fitted values for softening data collected in 21 d with 3 d interval (Appendix 5).

Table 7.1: Comparison of GL dependent parameters of CG fitted to softening data representing different data collection patterns.

Data collection pattern (day)	GL dependent			
	B		κ	
	Means	Range	Means	Range
21	5.03A	2.65 to 6.93	0.67A	0.23 to 0.94
18	5.23A	2.93 to 6.94	0.49C	0.12 to 0.74
15	5.40A	3.20 to 7.16	0.43D	0.12 to 0.61
18 (with 6 day intervals)	5.32A	2.90 to 6.92	0.62B	0.15 to 0.90
$LSD_{0.05}$	0.38		0.056	

Different letters in columns represents significant differences between population of parameters.

Average κ for softening curves representing data for 18 d collected at 6 d intervals were slightly different ($P < 0.05$) from κ values representing duration of 21 d at 3 d intervals (Table 7.1). GL dependent parameters (B and κ) for data collected over 18 d (at 6 d intervals) remained strongly correlated (for B, $r = 0.94$; for κ, $r = 0.93$) with the fitted values for 21 d data collected at 3 d intervals (Appendix 5). Overall, comparison of the mean curves show a similar shape for 21 and 18 d collected at 3 d intervals and 18 d with 6 d intervals (Figure 7.3A, B and D). Reduction in storage duration to 15 d with 3 d intervals slightly reduced the expression of lag phase of softening curve (Figure 7.3C).

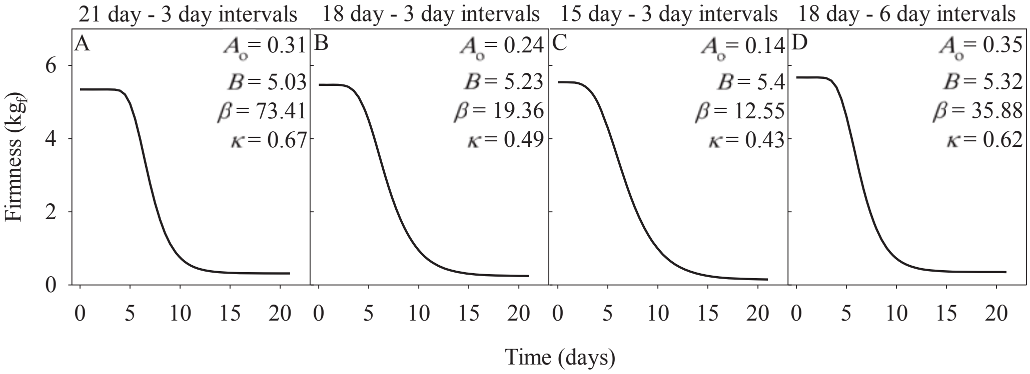

Figure 7.3: CG fitted mean curves of AFL softening data collected in 21 (A), 18 (B), 15 (C) day with 3 day intervals and 18 day with 6 day intervals (D).

Different data collection patterns did not substantially change the correlation of firmness change parameter (κ) in AFL with firmness (SF value) after 100 and 126 d of optimal storage (Table 7.2). These results suggested that AFL monitoring duration could be reduced without affecting the relationship of firmness change parameter with firmness loss in optimal storage. Reducing storage duration can affect the GL dependent parameter values and their ranges for fitted softening curves. The different ranges of κ for data collected in reduced AFL monitoring durations indicated that new thresholds (κ_α and κ_β) to categorise GLs for 1 of 3 storage potential categories will be required.

Table 7.2: Correlation coefficients (r) of firmness change parameter (κ) of curves representing different data collection patterns in AFL monitoring with firmness in optimal storage.

κ of curves representing different data collection patterns (day)	Correlation coefficient (r) with SF values in optimal storage	
	100 day	126 day
21	-0.53	-0.45
18	-0.54	-0.46
15	-0.51	-0.45
18 (with 6 d intervals)	-0.51	-0.47

7.3 AFL SOFTENING LOSSES

Storage of kiwifruit lines at 20 °C substantially accelerated the softening losses during 21 d (Section 4.3.1.3 and Section 6.4.1.2). GLs demonstrated variability in their softening patterns. In two seasons, some GLs maintained an extended initial lag phase (18 - 21 d) while others did not (Figure 5.4 and Figure 6.3). The initial lag phase of kiwifruit softening has usually been assumed to be influenced by fruit maturity at-harvest (White et al., 2005). For example very immature fruit (very early harvest) may not soften completely to eating firmness of 0.6 - 0.8 kg_f until treated with ethylene (Burdon and Lallu, 2011). Such lines raised a question whether the arbitrary chosen 20 °C was the optimum way to accelerate softening losses in AFL (Section 7.3.1). Application of ethylene concentration could be a potential alternative to accelerate the softening of GLs (Section 7.3.2). To represent AFL data, calculation of mean firmness could be avoided by using population distribution descriptors (Section 7.3.3). This section further discusses the options to generate and analyse losses information.

7.3.1 High temperature

Kiwifruit maturity stage and storage temperature influences the softening rate (Ritenour et al., 1999). Use of 20 °C was chosen in this study due to the ease of application in comparison to other options like gas manipulation. It is possible that both rapid (Figure 5.6B) and very slow declines (Figure 5.6A and Figure 6.3) in firmness observed at 20 °C in some GLs may not reflect softening behaviour in optimal storage. Higher temperature (> 20 °C) can reduce the time differences between prematurely soft and healthy fruit to reach the same final firmness (Davie, 1997). A lower AFL temperature (e.g. 15 °C) would comparatively slow down the softening process (Schotsmans et al., 2008). Alternatively, GLs with extended initial lag phase may require longer time or temperature higher than 20 °C to completely soften. Early harvested fruit may exhibit significant difference in softening patterns when stored at different temperatures, while late harvested fruit may not exhibit substantial differences in softening patterns (Schotsmans et al., 2008). Expression of all softening phases depends upon capacity of fruit to soften in relation to storage conditions (e.g. temperature) and time. Use of high temperature (> 20 °C) could accelerate the softening loss of early to late harvested GLs, including those which do not complete softening curve during AFL monitoring. Meanwhile it may reduce the differences between GLs for slow and rapid softening. Therefore, amount and rate of losses for early to late harvested kiwifruit can be studied at different temperatures to define optimum temperature for AFL establishment.

7.3.2 Ethylene application

Storage of kiwifruit at higher temperature (20 °C) results in initiation of autocatalytic ethylene production and accelerated ripening (Antunes and Sfakiotakis, 2000; Antunes, 2007). The magnitude of ethylene production also depends on the number of very soft, damaged or rotten fruit in a box (Section 7.2.3). Perhaps, rapidly softening GLs may have produced more ethylene than others.

Applied ethylene could potentially accelerate fruit softening during AFL monitoring. The magnitude of acceleration will be a function of concentration, duration of application and temperature. Correct concentration of ethylene perhaps accelerates the softening process (from 2 - 3 weeks to 6 - 7 d) and would provide an opportunity to reduce the AFL monitoring duration. Previously, application of 0.5 ppm ethylene reduced the time to reach firmness of 2.6 kg_f by 75% at 10 °C (Arpaia et al., 1986). Application of 10 ppm ethylene for 24 h at 20 °C resulted decrease in firmness to 1.36 kg_f within 4 - 5 d (Crisosto et al., 1997). At higher temperatures (e.g. > 15 °C), ethylene application can cause kiwifruit to reach their climacteric peak more rapidly (Antunes, 2007) and can result in accelerated softening. However, it seems highly likely that ethylene application would over-ride inherent differences between GLs in softening rate, such as we saw in first attempt of AFL monitoring in 2010 (Section 3.4.3.1). Ethylene application protocol (concentration and duration) in AFL would have to be tested to see if it could accelerate softening losses of kiwifruit while retaining the differences between GLs.

7.3.3 Data manipulation

In this study, mean firmness of 36 fruit in the AFL data was used to follow softening patterns of GLs. However, means of population do not express the within batch fruit variability for firmness. Feng et al. (2003a) reported that substantial variation exists within batches for fruit firmness. Variability between fruit of any batch or population can be because of differences in growth conditions, mineral concentrations, flowering time and physiological maturity (Feng et al., 2003a; Jordan and Loeffen, 2013). While use of mean is effective for locating a population on a particular axis of quality, unfortunately, averages are of limited use in characterising acceptability of an entire population. Depending upon variability among members of a population, it is quite possible that a significant proportion of a population's individuals are unacceptable even if the average seems quite acceptable (Banks, 2003). Two populations with identical average firmness just above the soft threshold of 1 kg_f may have different numbers of soft fruit (Adams et al., 2010). Use of averages to compare biological populations is also criticised by De Ketelaere et al. (2003). Moreover, in an industrial scenario where during fruit quality checking, rotten fruit are usually removed from the population, interpretation of batch quality based on mean value would be biased because of variable population sizes at different measurement occasions.

In the first season, along with average firmness, other parameters like maximum firmness value, firmness range, standard deviation (representing variability) and 3rd quartile (representing distribution) also showed potential to indicate seasonal life of GLs (Table 3.2 - 3.4). Such parameters are more representative of extremes (e.g. maximum firmness value and range of firmness) in a population. In industry the number of fruit with low firmness (< 1 kg_f) in a population directly influences fruit loss leading to higher re-packing cost while achieving export standards (SF > 1 kg_f). Perhaps a change to parameters which describe the wider firmness distribution and variability during softening could provide a better insight about the fruit population behaviour and hence a better prediction of GL storability. However, means are the most stable parameter, whereas extremes become less stable to estimate. In addition, population size is also important to estimate variability parameters. Smaller sample size (36 fruit at each measurement) used in later attempts (in 2011 and 2012) of AFL methodology may not truly represent existing extremes and could restrict the potential of using variability parameters to describe population behaviour or pattern in storage. The larger sample size (300 fruit for each measurement occasion) used by industry, as per ISO-2859 regulations, provide an opportunity of using population extremes (e.g. SF) to differentiate batches. However, use of a small sample size could be appropriate for observing average firmness change of kiwifruit GLs during AFL monitoring.

7.4 MODELLING OF SOFTENING

Kiwifruit softening in AFL was described using an empirical model having GL dependent parameters assisting interpretation of the differences between GLs softening patterns (Section 7.4.1). Alternatively, in contemporary research, biological age is used to explain differences in fruit quality traits (e.g. firmness) between batches or individuals (Section 7.4.2). This section discusses the use of empirical models and biological age to describe softening of kiwifruit GLs.

7.4.1 Empirical modelling

The CG model appropriately characterised kiwifruit softening (Figures 5.4 - 5.5 and 6.3 - 6.4). Use of a model of tri-phasic nature was required. The CG model expressed all softening stages. Example CG fits for nine batches demonstrate how the GL dependent parameters B and κ can accurately characterise the range of softening patterns observed in the data (Figure 5.6). CG described most stages of softening except on occasions in which a less dominant lag phase was present in the data (Figure 5.6B, I). This result is partly due to the use of a global β in curve fitting process. The effect of the horizontal shift parameter (β) resulting in too much initial lag phase on some occasions was also observed by Benge et al. (2000).

Others have used the exponential decay (EXP) model to describe kiwifruit softening curves (Benge et al., 2000; White et al., 2005; Feng et al., 2006; Schotsmans et al., 2008). However, in all of these studies, the expression of an initial lag phase was not evident in the softening data. Use of EXP model to fit AFL softening could be inappropriate, as it is unable to describe the initial lag (slow) phase when this occurs during tri-phasic softening of kiwifruit. Along with CG, JMM and IEP were also investigated by Benge et al., (2000) to describe softening of kiwifruit (Section 2.4.1.2). The JMM model has 5 parameters and IEP is a 4 parameters model. Parameter sensitivity analysis of JMM and IEP can be proposed to define global and GL dependent parameters. Providing the minimum number of GL dependent parameters of JMM and IEP, such models may help to describe the softening patterns of GLs without excessive initial lag phase where it does not occur in data.

7.4.2 Modelling biological age of kiwifruit

Assuming all kiwifruit go through similar development, from fruit set to ripening and senescence, the softening behaviour should be similar for each fruit. However, at any point in time, substantial differences in firmness either within or between populations of fruit are observed. These differences could be attributed to fruit differing in developmental stage or otherwise referred to as biological age (Tijskens et al., 2005). This concept of biological age has already been applied to describe the variation in within batch postharvest firmness of apple (Shmulevich et al., 2003) and colour of tomato (Tijskens and Evelo, 1994), cucumber (Schouten et al., 1997) and avocado (Hertog, 2002). These examples demonstrate that differences between individual fruit at any time point can be described by a standard maturation curve, in which each individual is located at a different point on the curve as a result of differences in timing of maturity.

Applying biological age to the CG equation required the assumption that the 4 CG parameters remain constant for all batches with the differences explained by a time shift parameter (τ, d). Mathematically, addition of τ to the CG equation (Eq. 5.1) results in Equation 7.1.

$$FF = \mathrm{A_o} + \mathrm{B}\left(1 - \frac{1}{\exp\,(\exp\,(-\kappa\,(\mathrm{t}-\tau)))}\right) \qquad \text{(Eq. 7.1)}$$

Where *FF* is flesh firmness ($\mathrm{kg_f}$), $\mathrm{A_o}$ is lower and B upper asymptote ($\mathrm{kg_f}$), κ represents rate of firmness change ($\mathrm{d^{-1}}$) in time (t, d) and τ is the shift in time (d). The equation assumes that all fruit start from the same firmness (B) although some softening may have occurred prior to harvest. Given that B defines the upper asymptote for firmness, the highest initial firmness measured across the set of kiwifruit batches can be taken as constant value (i.e. $\mathrm{A_o}$ + B). This approach assumes that providing the same environmental conditions after harvest, all fruit have the same rate of firmness change (κ). Therefore, applying equation 7.1 to describe batch dependent softening, $\mathrm{A_o}$ and B remained as set constants; κ was fitted as a global parameter while τ was fitted as a batch dependent parameter.

The time shift CG model was tested to fit firmness data of GLs collected in two seasons (Figure 7.4C). The time shift CG model resulted in a single shape model being applied to all GLs with variability in softening trends being described by the shift in time. The resulting model was able to describe the difference in timing of rapid softening of each GL. However, the time shift CG model has clear deficiencies in the ability to describe the observed lag phase in softening immediately after harvest, especially in the later season fruit (>150 ISO d) which were less firm at harvest (Figure 7.4A).

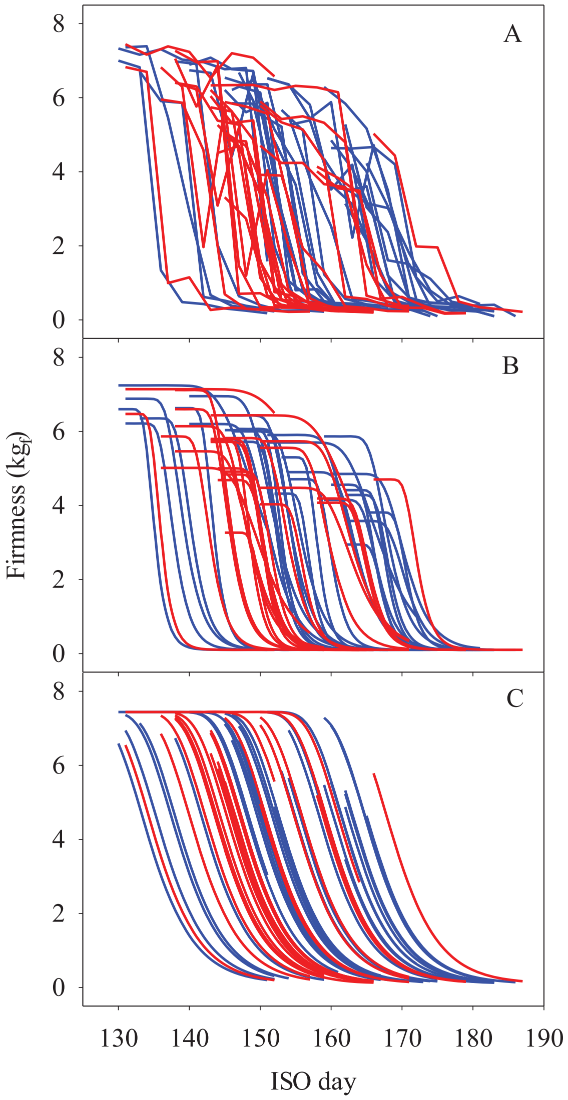

Figure 7.4: Raw data (A), CG fitted (B) and time shift CG fitted (C) softening curves of 54 kiwifruit GLs randomly selected from harvest season of 2011 (blue) and 2012 (red). Raw data comprises of 8 data points (mean of 36 fruits) at 3 day interval.

Fits by time shift model demonstrated a complete lack of description of a lag phase in those GLs in which the initial firmness < 7 kg$_f$. In addition, slow speed of transition between the upper and lower asymptotes (κ) results in a consistent under prediction of firmness initially and over prediction of firmness at the later period of softening. The nature of the time shift CG model results in the GLs with the initially highest firmness taking the longest time to soften to the lower asymptote and hence makes the assumption that initial firmness and time to soften (to a desired point) are correlated, which was not the case. Overall, time shift CG did not characterise well the softening phases of kiwifruit GLs. Hence, time shift or biological age based modelling of kiwifruit softening to study variability between GLs may not be an appropriate approach to use.

7.5 INTERPRETATION OF AFL TO PREDICT STORAGE POTENTIAL

Complementary Gompertz described the AFL firmness data and GL dependent parameters were extracted. Rate of firmness change (κ) categorised GLs for 1 of 3 storage potential categories by applying two thresholds as κ_α and κ_β (Section 7.5.1). Softening trend of GLs during AFL monitoring influences the storability prediction by κ thresholds (Section 7.5.2). Seasonal differences between GLs for fruit quality at-harvest and during storage can also influence loss in AFL and optimal storage (Section 7.5.3). Comparison of AFL based prediction for storage potential with industrial sale pattern would help to assess the benefit of AFL methodology (Section 7.5.4). Following sections discusses these aspects of AFL interpretation to predict storage potential.

7.5.1 Storage potential prediction

In calibration season, GLs with higher rate of firmness change (κ) during AFL monitoring had a tendency to have low firmness at 100 and 126 d of optimal storage (r = -0.53 and -0.45 respectively, Section 5.3.2). Thresholds $\kappa_\alpha = 0.63$ and $\kappa_\beta = 0.53$, categorised 60% of GLs accurately for low (SF <1 kg_f at 100 d), medium (at 100 d 1< SF <1 kg_f at 126 d) and high (SF >1 kg_f at 126 d) storage potential. Importantly 90% of the low storage GLs were predicted successfully (Section 5.3.3). Only 5.2% of GLs were predicted as having a better storability than observed. In the validation season, the same thresholds $\kappa_\alpha = 0.63$ and $\kappa_\beta = 0.53$ segregated 53% of GLs correctly (Section 6.4.2.1), while successful prediction of low storage GLs reduced to 78%. Meanwhile, proportion of GLs predicted as having a better storability than observed increased to 21.5%. New thresholds ($\kappa_\alpha = 0.67$ and $\kappa_\beta = 0.59$) for the validation data set were sought but had little effect on GL segregation. Overall, AFL methodology can segregate GLs of low storage potential but number of GLs predicted as having better storability than observed may lead to fruit and profit loss.

7.5.2 Grower lines predicted as longer storage than observed

Rate of firmness change (κ) thresholds (κ_{α} = 0.63 and κ_{β} = 0.53) during AFL monitoring predicted 5 and 21% of GLs to have longer storability than observed in both seasons (Section 5.3.3 and Section 6.4.2.1 respectively). Visually observing data of these GLs during AFL monitoring reveals that 7 (2 in calibration and 5 in validation season) of these GLs had inconsistent softening pattern (i.e. an observed increase in average firmness between two measurement points, Figure 7.5). Another (3) GLs also showed extended initial lag phases (18 - 21 d) and did not complete softening during 21 d of AFL monitoring.

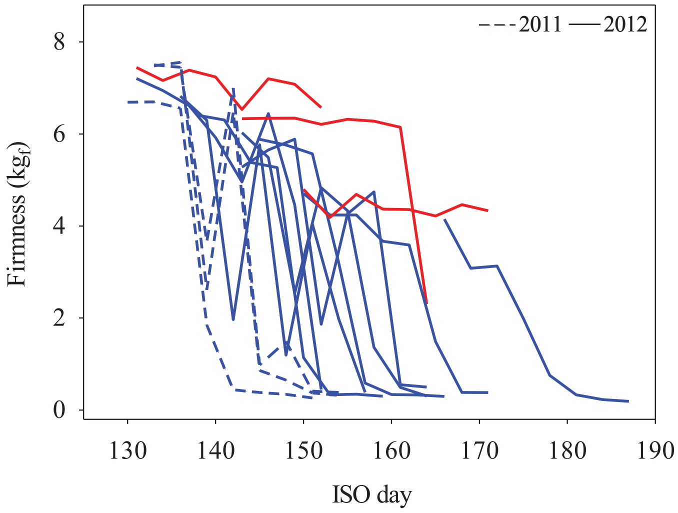

Figure 7.5: Inconsistent (blue) and incomplete (red) softening of GLs during AFL monitoring predicted by rate of firmness change (κ) thresholds (κ_{α} = 0.63 and κ_{β} = 0.53) to have longer storage potential than observed in 2011 and 2012.

Removal of data showing an increase in mean firmness between two measurement points of AFL monitoring would change the fitted rate of firmness change (κ). The resulting new fitted values of κ may improve the prediction of storability. To test this hypothesis *post-hoc* (after the fact), firmness data of GLs predicted as having longer storage potential than observed in both seasons was cleansed of unusual data. This *post-hoc* correction of softening curves was performed by visually identifying a substantial rise in firmness value between two measurements and eliminating the data showing firmness rise. The new fitted rate of firmness change parameter (κ) reduced the number of GLs categorised as having a higher storage than observed from 3 to 1 in calibration and 11 to 9 in the validation season. Inconsistent softening patterns of such GLs during

AFL monitoring may be the cause of false categorisation for storage potential. Inconsistency in softening curve can also occur due to the presence of any rotten or very soft fruit in a box producing ethylene and stimulating healthy fruit to soften (Section 7.2.2). Given the fact that inconsistency in softening pattern cannot be avoided in data collection, perhaps such GLs can be considered as likely to be wrongly predicted by AFL κ thresholds. Therefore, considering such lines (with inconsistent softening during AFL monitoring) as low storing could be recommended.

7.5.3 Seasonal differences between GLs

In the first season, 20 different GLs were selected over a 2 week harvest period while in later seasons 57 and 51 GLs were studied over a harvest period of 5 weeks. Differences in fruit maturity with harvest time are expected between seasons. At-harvest SSC rise with the progression of harvest time was more rapid in 2010 and 2012 in comparison to 2011 (Figure 7.6A). Increase in SSC occurs because of starch conversion to sugars with rate of starch conversion being dependent on the environmental temperature. Converted sugars are also then utilised in respiration (Beever and Hopkirk, 1990). A rise in temperature during late autumn (mid April to late May) slows down SSC accumulation and delays commercial harvest maturity (Benge, 1999; Snelgar et al., 2005). Cool nights (low temperature) are usually required for soluble solids development (Burdon et al., 2007; Burdon et al., 2013). Daily temperature records of Te Puke region by the National Institute of Water and Atmosphere (NIWA), New Zealand reveals that there was higher minimum temperature for longer time in late autumn during 2011 in comparison to 2010 and 2012 (Figure 7.7), explaining the slower accumulation of SSC in 2011 (Figure 7.6A).

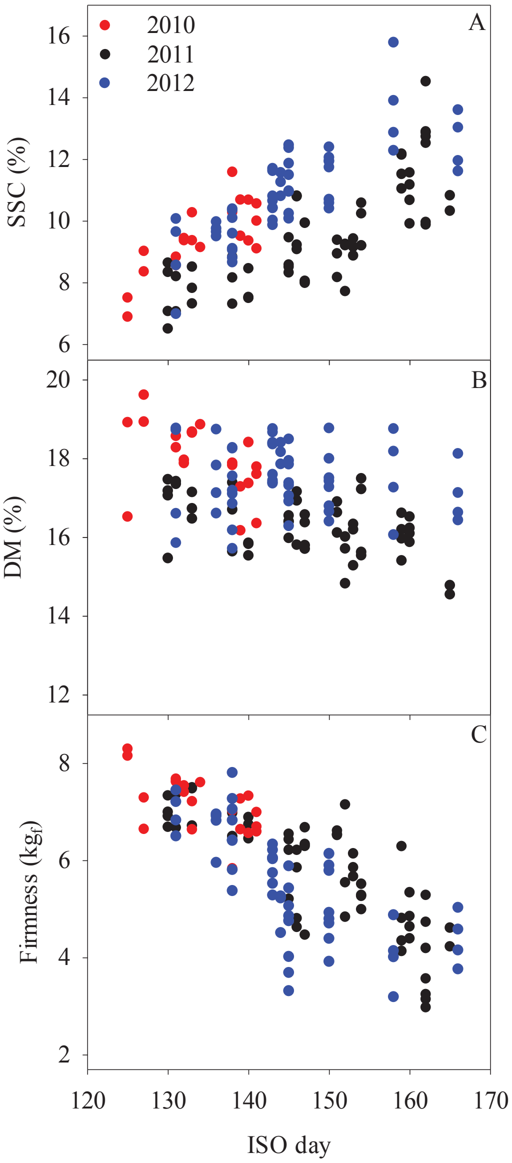

Figure 7.6: At-harvest SSC (A), DM (B) and firmness (C) of three seasons. Each data point represents an average for a GL. SSC shows an average of 36 fruit in 2010 and 30 fruit per GL in each of 2011 and 2012. Firmness represents an average of 297 fruit in 2010 and 36 fruit per GL in each of 2011 and 2012. DM shows an average of 30 in 2010 and 15 fruit per GL in each of 2011 and 2012.

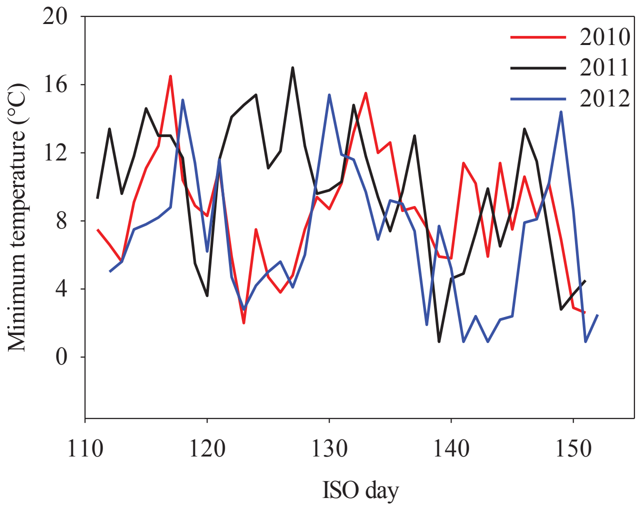

Figure 7.7: Minimum temperature of Te Puke region in 2010 (red), 2011 (black) and 2012 (blue) during late autumn (21st April to 31st May) starting from ISO day of 111 to 152. Data were collected by National Institute of Water and Atmosphere, New Zealand.

At-harvest DM was relatively lower in 2011 as compared to 2010 and 2012 (Figure 7.6B). At-harvest DM largely represents the total reserved carbohydrates including starch (40 - 70%) plus soluble sugars in the fruit (Burdon et al., 2004; Crisosto et al., 2012). Growth temperature also has direct influence on kiwifruit DM at-harvest. High average temperature during summer and autumn can reduce the DM content and delay starch degradation (Richardson et al., 2004). Lower DM levels in 2011 can be the result of relatively higher autumn temperature. DM at-harvest has direct relation with soluble sugars once the fruit are ripe (Jordan et al., 2000) and thus defines the consumer liking for the fruit after storage. Hence, variation in DM at-harvest leads to variability in eating quality of the fruit after storage. At-harvest DM (%) for 2010 and 2011 expressed a decreasing trend over a harvest period.

Firmness followed a similar decreasing trend in all three years (Figure 7.6C). At the stage of final maturation of fruit (around 160 d after fruit set or from mid April), decrease in flesh firmness usually occurs on the vine. However, at-harvest firmness of three seasons did not express the seasonal differences between GLs.

Overall, variability in fruit quality existed for at-harvest SSC and DM between three seasons. At-harvest variability in fruit quality between seasons demonstrates the potential for variation in softening pattern in AFL and storage potential. There was 1 GL in 2011 and 5 GLs in 2012, that did not soften (maintained a lag phase of 18 - 21 d) during the AFL monitoring. Seasonal differences were also evident in firmness change during storage. It was found that 12 of 20 (60%), 32 of 57 (56%) and 38 of 51 (74%) GLs had a SF < 1 kg_f after 126 d of optimal storage in each of the experimental years. This type of seasonal variation may influence predictions made by κ thresholds during AFL monitoring.

7.5.4 Comparison of required sale with industry

To apply the developed AFL technique is constrained by the rate in which kiwifruit are dispatched to market. For the AFL technique to be adoptable, the amount of fruit identified as required to be sold, must be less than that required by the market. Cumulative sale (%) rate required by the identification of failed GLs, in the AFL was compared with the industrial sale rate of 'Hayward' main crop fruit (Figure 7.8). Industrial sale patterns are usually consistent in different years. AFL based predicted sale pattern represents cumulative failure of GLs until 126 d of optimal storage.

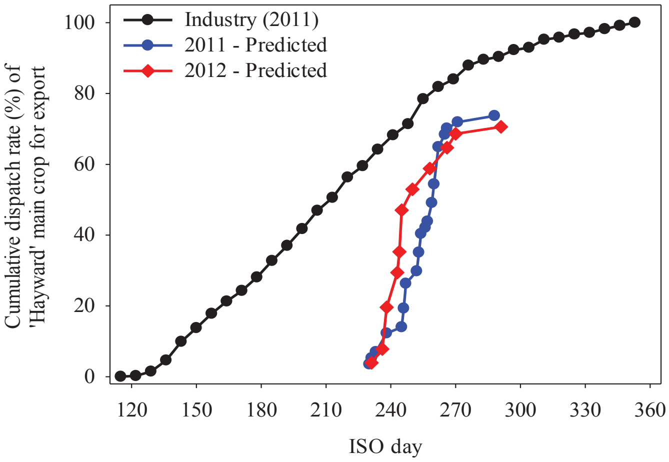

Figure 7.8: Comparison of cumulative dispatch rate (%) of 'Hayward' main crop by industry and predicted by AFL method in 2011 and 2012. Sale pattern was predicted until 126 day of optimal storage for both seasons.

For both AFL trail years a total of 73 and 70% of GLs were predicted to be required to sell by 290 ISO d. Industrially the sale pattern indicates that by 290 ISO d approximately 90% of the main crop is sold. This comparison demonstrates that the predicted rate of sale of GLs dictated by the AFL method is less than the current industrial rate and hence the method is not constrained by the requirement to sell fruit at a rate that is higher than current.

The fruit harvest season ranges from approximately 120 to 180 ISO d. The length of the harvest season may vary between seasons depending upon environmental factors (e.g. temperature) influencing fruit maturity ($SSC > 6.2$ %). AFL method are not needed for very early harvested GLs (before 150 ISO d) as this fruit is targeted for immediate release to market. Fruit harvested in mid to late harvest season (after 150 - 180 ISO d) largely goes to storage prior to market. AFL methodology could be more useful for mid to late harvest season crop to segregate GLs for their softening pattern to devise sale plan by selling the low storage lines early and keeping the longer storage ones for later supply.

7.6 RESEARCH OPPORTUNITIES

7.6.1 Collection of pre-harvest data

At-harvest fruit quality was found to have a weak relationship ($r = 0.2$) with fruit firmness during storage (Table 4.1 - 4.3). Pre-harvest or on vine changes in quality have direct impact on postharvest behaviour of fruit during storage. For example, fruit having relatively rapid accumulation of DM at-harvest will be more soft. Likewise, rate of SSC accumulation at-harvest contributes to substantial variation between orchards (Adams et al., 2010). Kiwifruit completes three phases of softening on vine and after harvest until fully ripe (0.6 - 0.8 kg_f). The rapid decline stage can occur on vine and the relativity of this stage to harvest time is very important to understand postharvest softening pattern of particular GL (Adams et al., 2010; Burdon and Lallu, 2011). Moreover, Burdon et al. (2013) reported that rate or pattern of SSC accumulation is potentially a robust indicator of fruit quality after storage, instead using a single at-harvest value of SSC. Hence, combining rate of change in fruit quality on-vine (firmness, SSC and DM) with softening pattern in AFL may result in more accurate characterisation of GLs for storage potential. The kiwifruit industry has the usual practice of collecting fruit quality data (SSC, firmness and DM) at regular intervals prior to commercial harvest. Pre-harvest fruit quality data of the two seasons (2011 and 2012) can be accessed in future to characterise GLs for storage potential. Given the use of pre-harvest data may improve predictability of GLs by AFL method, combination of on-vine fruit quality data with AFL monitoring can be suggested in future.

7.6.2 Use of at-harvest data to predict model parameters

In contemporary postharvest research, models of quality combine two approaches, deterministic and stochastic modelling (Tijskens et al., 2003). The deterministic models use interpretable model parameters for underlying processes (Schouten et al., 1997; Hertog et al., 1999), while stochastic models estimate parameters through fitting a model on data (Hertog et al., 2004a; Schouten et al., 2004). Data is required to infer deterministic and stochastic effects, resulting in a *post-hoc* (after the fact) modelling procedure. However, for successful adoption of a model that considers batch specific differences as a tool in industry, there is a need to determine batch specific model parameters *a priori* (before the fact). The most likely source of information that can be used to determine these model parameters is the at-harvest attributes of each batch. For example, Johnston, (2001) modelled variability in softening of apple caused by different orchard sources with an empirical sigmoid model, where the model parameter of firmness change (κ) was predicted from at-harvest firmness, skin greenness and starch concentration. Van de Poel et al., (2012) used fruit mass and colour to determine the biological age of tomatoes and subsequently perform model based classification.

In this study, CG with two GL dependent parameters (B and κ) was used to characterise the softening curves of GLs. Fitting the CG model to characterise different softening patterns required extensive firmness data collection. Being able to define GL dependent model parameters from at-harvest fruit quality (e.g. SSC, firmness, DM and ISO day of arrival), before softening occurs would be advantageous. Data collected in the calibration season (2011) was used, to test the idea of *a priori* prediction of GL dependent model parameters from at-harvest fruit quality. GL dependent model parameters (B and κ) were correlated to at-harvest fruit quality data (Table 7.3).

Table 7.3: Correlation coefficients (*r*) of at-harvest fruit quality with GL dependent parameters of CG fitted for AFL data in 2011.

GL dependent parameters	At-harvest fruit quality			
	SSC (%)	Mean firmness (kg_f)	DM (%)	ISO day of arrival
B (kg_f)	-0.80	0.92	0.24	-0.75
κ (d^{-1})	0.32	-0.45	-0.19	0.26

GLs with higher B values tended to be harvested early in a season (early ISO days) and had lower SSC at the time of harvest (*r* = -0.80 & -0.75 respectively, Table 7.3). GLs with higher B values also had higher at-harvest mean firmness (*r* = 0.92). As B represents the scale parameter of the CG curve, the correlation with at-harvest firmness is not surprising. Rate of firmness change (κ) did not show good correlation with at-harvest fruit quality. However, rate of firmness change (κ) in AFL monitoring has the potential to predict storage potential of GLs in optimal storage. The scale parameter (B) does not help to predict storage potential (Table 5.3). Hence, use of at-harvest quality to make *a priori* prediction of model parameter (κ), required to segregate GLs for storage potential cannot be recommended.

7.6.3 Assessment of fruit quality

7.6.3.1 Physiological status of fruit

Flesh firmness is an important indicator of quality and widely used in industry to define storage performance of kiwifruit. Therefore, in this study, during both AFL and optimal storage monitoring, to evaluate storage performance of GLs fruit firmness was used. However, change in fruit physiological parameters like rate of respiration and ethylene production during ripening could be an indicator of storage performance. Different GLs may show different patterns of respiration rate and ethylene production when stored in the same conditions. Although kiwifruit produce negligible ethylene (< 0.01 $nL.kg^{-1}.h^{-1}$) with low respiration at the time of harvest but during ripening substantial amounts of ethylene with an increased rate of respiration occur (Antunes and Sfakiotakis, 2000). Kiwifruit stored at 20 °C produce ethylene coinciding with the rise in respiration rate (Antunes et al., 2000). However, kiwifruit of the same quality characteristics may vary in subsequent ripening because of the mechanisms regulating ethylene production (Davie, 1997). Assessment of change in respiration and ethylene production during AFL monitoring representing differences in GLs to reach climacteric peak may indicate the pattern of fruit deterioration and may improve the ability of the currently studied quality indicators to predict fruit quality during optimal storage.

7.6.3.2 Compositional attributes

Along with fruit quality indicators, assessment of fruit mineral composition (e.g. calcium and nitrogen) may also assist in categorisation of GLs for storage performance. Availability of sufficient calcium and nitrogen is essential for better fruit quality at-harvest and during storage (Davie, 1997; Benge, 1999). Kiwifruit lines with low calcium and high nitrogen store less long than fruit with high calcium and low nitrogen (Davie, 1997; Benge, 1999; Feng, 2003; Maguire and Mowat, 2003; Feng et al., 2006). Calcium may not influence at-harvest firmness but it has strong effect on later stages of fruit softening because of its role in maintaining cell wall strength (Davie, 1997). Assessment of calcium and nitrogen of kiwifruit at-harvest along with fruit quality changes (i.e. firmness) in AFL may result in better segregation of GLs for storage performance. Assessment of minerals of many GLs coming into a supply chain may have a practical constraint of time and cost. Assessment of calcium or nitrogen from a commercial facility would cost approximately NZ $40 per sample with a turn-around time of approximately 2 - 3 weeks. Given the opportunity to overcome time and financial constraints, mineral analysis could be conducted to add to the AFL data set to develop better prediction models of storage potential.

7.6.4 Physiological process of kiwifruit softening

Fruit ripening process accompanies a change in texture that happens largely because of disassembly of the cell wall (Brummell, 2006). Kiwifruit softening occurs in three temporal phases of slow, fast and slow marked with internal ethylene production (Schröder and Atkinson, 2006). Softening in three distinct phases may provide an opportunity to study the cell wall changes during the whole softening process. Mostly outer pericarp of fruit has been studied to understand cell wall changes during kiwifruit softening. However, complete disintegration of cell wall during softening occurs because of number of events such as pectin solubilisation and degradation, cell wall swelling, and reduction in molecular weight of xyloglucan and dissolution of middle lamella (Harker and Hallett, 1994; Redgwell and Harker, 1995; Redgwell, 1996; Redgwell et al., 1997; Newman and Redgwell, 2002; Brummell, 2006; Schröder and Atkinson, 2006). Occurrence of these events in relation to kiwifruit softening phases has already been described in detail (Schröder and Atkinson, 2006; Burdon and Lallu, 2011). However, most of the studies on cell wall disintegration during softening use ethylene treated fruit (Burdon and Lallu, 2011). Timing of these cell wall changes during normal or accelerated softening without ethylene application may be different. Following differences between kiwifruit GLs for timing of occurrence of such events during accelerated ripening could provide better insights of softening changes during optimal storage.

Cell wall modification during softening occurs by the action of several enzymes with specific roles, for example polygalacturonase, β-galactosidase, pectin methyl esterase, and xyloglucan endotransglucosylase or hydrolase and mannan transglycosylase (Redgwell et al., 1992; Ross et al., 1993; Bonghi et al., 1996; Schröder et al., 1998; Brummell and Harpster, 2001; Ciardiello et al., 2004; Schröder and Atkinson, 2006; Atkinson et al., 2011). Although, activity of these enzymes at different softening phases of kiwifruit is well explained, what controls the timing of ripening is still unclear (Burdon and Lallu, 2011). During accelerated softening, activity of these enzymes may express differently for each GL. Differences in enzyme activity between GLs during AFL monitoring may differentiate them for softening patterns during storage.

Application of knowledge on cell wall disintegration events and activity of different enzymes relevant to softening processes would be constrained by additional costs and time required. In accelerated softening, occurrence of any event of cell wall disintegration and expression of any enzyme activity may be quick. However, amount of laboratory time required to collect data of any cell wall disintegration process and enzyme activity could be a constraint to accommodate substantial number of GLs for AFL study.

7.6.5 Use of novel non-destructive techniques

In this work, standard industry quality indices were used to observe GL dependent softening during AFL and optimal storage monitoring. Techniques beyond the standard measures of pre and postharvest fruit assessment such as NIR (McGlone and Kawano, 1998; McGlone et al., 2002; Costa et al., 2003; McGlone et al., 2007; Costa et al., 2011; Lee et al., 2012; Blanke, 2013), X-ray (Mondragon et al., 2011; Trejo-Araya et al., 2013; Cantre et al., 2014), MRI (Taglienti et al., 2009; Alfatni et al., 2013; Burdon et al., 2014b) and often non-destructive measurements (Feng et al., 2013) could be applied to gather additional data for better characterisation of GLs for softening differences. Subsequent involvement of such techniques with AFL methodology may better reflect the internal changes occurring during ripening of different GLs. Detailed information provided by these techniques may aid accuracy in AFL based segregation of GLs for storage potential.

7.7 RECOMMENDATIONS

Given the discussion about potential aspects of AFL establishment, assessment and modelling of fruit softening losses during AFL monitoring, interpretation of AFL based predictions and suggested future research opportunities, the following are the recommendations given this research.

- High temperature storage (at 20 °C) of 'Hayward' kiwifruit batches harvested at different time in a season should be in an environment where inherent ripening of batches cannot be influenced by ethylene produced by any other batch.
- Following change in average firmness at 20 °C requires exclusion of whole tray upon detection of any rot, to avoid the influence rot may have caused on healthy fruit by triggering premature softening. Storage of extra trays is recommended to be used as replacement if any tray is lost due to rots. For study involving high temperature storage (at 20 °C) of kiwifruit, instead modular bulk packs use of single layer tray of fruit is recommended to minimise rot affect and its spread on data.
- For AFL based predictions of storage potential for kiwifruit GLs, monitoring duration at 20 °C can be reduced to 18 d for minimising cost and effort involved in AFL establishment.
- To study the average firmness change during storage at 20 °C, sample size of 30-36 fruit can be appropriate to use. However, in industrial scenario population distribution parameters (e.g. SF) have significant information for differentiating

poor and good storing GLs. Hence use of a large sample size (i.e. 300 fruit) is recommended if following change in population distribution parameters for firmness of kiwifruit.

- The possibility of incorrectly predicting GL storability from AFL monitoring system can be reduced by identifying GLs with inconsistent softening behaviour during AFL monitoring (e.g. an observed increase in average firmness with storage time). Considering GLs with inconsistent softening during AFL monitoring as low storing by default is recommended.
- Seasonal variation for softening patterns could influence predictions made by rate of firmness change (κ) observed during AFL monitoring. To develop a robust AFL based prediction system, it is recommended to consider the seasonal differences.
- AFL methodology can be recommended to use for mid to late harvest season crop to predict storability of kiwifruit GLs. Comparison of industrial and predicted sale patterns suggests that AFL based predictions are not required to be recommended for early harvested crop.
- Collection and inclusion of on-vine fruit quality data (pre-harvest) with AFL data is an area that remains unexplored but may further improve predictions of storability for kiwifruit GLs.
- At-harvest quality data provides no indication of the model parameter of firmness change (κ).
- During AFL monitoring, following change in fruit physiology (respiration and ethylene production) may further allow differentiation of GL fruit ripening behaviour and deterioration patterns. Therefore, for future study, assessment of respiration and ethylene changes during AFL monitoring is recommended to evaluate the potential of these two physiological indicators for predicting storability of GLs.

7.8 CONCLUSION

Reduction in supply chain losses of kiwifruit is one of the biggest tasks for New Zealand industry. This research attempted to use an AFL methodology to segregate kiwifruit lines for storage potential based on amount and rate of losses in abusive conditions. It was found that storage of fruit samples at 20 °C could accelerate the softening losses. Fitting the firmness data with Complementary Gompertz (with non-linear mixed effects) has potential to interpret differences in softening patterns between kiwifruit GLs. GL dependent parameter of firmness change (κ) from AFL monitoring has potential to successfully segregate 53 - 60% of GLs into three storage categories. AFL based categorisations for low storage lines reduced the proportion of such lines in the remaining population, ranging from 10 to 33.3%. In conclusion, this thesis has shown the potential for AFL methodology to predict GLs with different storage potentials. The continued presence of low storing GLs in the remaining population may be a limitation for AFL methodology application in industry. Application of AFL based prediction of storage potential may require further information such as pre-harvest fruit quality, compositional or physiological indicators of fruit ripening. However, this may introduce additional cost that conflicts with the benefits of successful segregation methodology. The understandings generated in this thesis may allow AFL method to become a component of a successful system for segregating kiwifruit by their storage potential.

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Appendix

Appendix 1

Complementary Gompertz input and generated output files for use in nlme procedure in R (version 2.15.1, cran.r-project.org; Pinheiro et al., 2013). Blue script indicates model formulation in R (input), required for successful operation of model. All other script represents generated output of model. All scripts this section represents model out of calibration data set in 2011.

A: A_0 as global while β, B and κ as GL dependent (CG$_1$)

```
> AFL.nlmeG1<-nlme(Y ~ A0 + B*(1-exp(-b*exp(-k*X))),fixed=A0+B+b+k~1,
random= B+b+k~1,start=c(A0=0.1,B=5,b=100,k=0.7),data=AFL.grp)
> summary(AFL.nlmeG1)
```

```
Nonlinear mixed-effects model fit by maximum likelihood
  Model: Y ~ A0 + B * (1 - exp(-b * exp(-k * X)))
 Data: AFL.grp
        AIC          BIC          logLik
        1135.209     1180.557     -556.6046
Random effects:
 Formula: list(B ~ 1, b ~ 1, k ~ 1)
 Level: GNo
 Structure: General positive-definite, Log-Cholesky parametrization
        StdDev                Corr
B       1.110920e+00          B             b
b       4.757192e-05          -0.326
k       1.911034e-01          -0.492        0.662
Residual 6.048093e-01
Fixed effects: A0 + B + b + k ~ 1
        Value        Std.Error    DF     t-value       p-value
A0      0.31204      0.043936     396    7.102110      0e+00
B       5.03630      0.164397     396    30.635036     0e+00
b       72.70377     19.518938    396    3.724781      2e-04
k       0.67415      0.045661     396    14.764265     0e+00
 Correlation:
        A0           B            b
B       -0.280
b       0.319        -0.173
k       0.325        -0.385       0.806
Standardised Within-Group Residuals:
Min          Q1           Med          Q3           Max
-5.840       -0.256       -0.003       0.225        3.633
Number of Observations: 456
Number of Groups: 57
```

B: A_0 and β as global while B and κ as GL dependent (CG$_2$)

```
> AFL.nlmeG2<-update(AFL.nlmeG1,random=B+k~1)
summary(AFL.nlmeG2)
```

```
Nonlinear mixed-effects model fit by maximum likelihood
  Model: Y ~ A0 + B * (1 - exp(-b * exp(-k * X)))
```

```
 Data: AFL.grp
            AIC             BIC             logLik
            1129.132        1162.111        -556.5658
Random effects:
 Formula: list(B ~ 1, k ~ 1)
 Level: GNo
 Structure: General positive-definite, Log-Cholesky parametrization
                    StdDev          Corr
B                   1.1108189       B
k                   0.1914069       -0.492
Residual            0.6046775
Fixed effects: A0 + B + b + k ~ 1
            Value           Std.Error       DF      t-value         p-value
A0          0.31245         0.043907        396     7.116017        0e+00
B           5.03533         0.164358        396     30.636371       0e+00
b           73.41876        19.760825       396     3.715369        2e-04
k           0.67550         0.045762        396     14.761075       0e+00
 Correlation:
            A0              B               b
B           -0.279
b           0.319            -0.173
k           0.325           -0.384          0.806

Standardised Within-Group Residuals:
Min                 Q1              Med             Q3              Max
5.841               0.257           -0.004          0.225           3.636
Number of Observations: 456
Number of Groups: 57
```

C: A_o, B and β as global while κ as random (CG_3)

```
> AFL.nlmeG3<-nlme(Y ~ A0 + B*(1-exp(-b*exp(-k*X))),fixed=A0+B+b+k~1,
+ random=k~1,start=c(A0=0.1,B=5,b=75,k=0.7),data=AFL.grp)
> summary(AFL.nlmeG3)
```

```
Nonlinear mixed-effects model fit by maximum likelihood
  Model: Y ~ A0 + B * (1 - exp(-b * exp(-k * X)))
 Data: AFL.grp
            AIC             BIC             logLik
            1319.621        1344.356 -      653.8105
Random effects:
 Formula: k ~ 1 | GNo
                k           Residual
StdDev: 0.1566241           0.8742985
Fixed effects: A0 + B + b + k ~ 1
            Value           Std.Error       DF      t-value         p-value
A0          0.192821        0.0713610       396     2.70206         0.0072
B           5.746305        0.1293244       396     44.43327        0.0000
b           8.974205        1.4582541       396     6.15407         0.0000
k           0.421169        0.0333482       396     12.62942        0.0000
Correlation:
```

```
        A0          B           b
B       -0.611
b       0.336       -0.600
k       0.389       -0.417      0.714
Standardised Within-Group Residuals:
Min         Q1          Med         Q3          Max
-3.383      -0.342      0.061       0.307       3.481
Number of Observations: 456
Number of Groups: 57
```

D: A_0, β and κ as global while B as random (CG_4)

```
> AFL.nlmeG4<-nlme(Y ~ A0 + B*(1-exp(-b*exp(-k*X))),fixed=A0+B+b+k~1,
+ random=B~1,start=c(A0=0.1,B=5,b=75,k=0.7),data=AFL.grp)
> summary(AFL.nlmeG4)
```

```
Nonlinear mixed-effects model fit by maximum likelihood
  Model: Y ~ A0 + B * (1 - exp(-b * exp(-k * X)))
 Data: AFL.grp
        AIC         BIC         logLik
        1466.674    1491.409    -727.3369
Random effects:
 Formula: B ~ 1 | GNo
          B         Residual
StdDev: 1.528778    1.063458
Fixed effects: A0 + B + b + k ~ 1
        Value       Std.Error   DF    t-value     p-value
A0      -0.154684   0.1555570   396   -0.994386   0.3206
B       5.711825    0.3340124   396   17.100636   0.0000
b       4.611645    0.8500591   396   5.425088    0.0000
k       0.230550    0.0223751   396   10.303869   0.0000
 Correlation:
        A0          B           b
B       -0.638
b       0.578       -0.656
k       0.783       -0.658      0.917
Standardised Within-Group Residuals:
Min         Q1          Med         Q3          Max
-2.360      -0.446      0.016       0.259       5.922
Number of Observations: 456
Number of Groups: 57
```

Appendix 2: Tables showing percentages of correct, missed and false categorisations at different tested combinations of κ_α and κ_β thresholds in 2011.

A: Table for percentage of correct categorisation with different κ_α and κ_β combinations.

κ_β	Percentage of correct categorisation													
0.88	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	49.1
0.83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	57.9	47.4
0.78	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	56.1	57.9	47.4
0.73	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	57.9	57.9	59.6	49.1
0.68	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	57.9	56.1	56.1	57.9	47.4
0.63	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	52.6	52.6	50.9	50.9	52.6	42.1
0.58	0.0	0.0	0.0	0.0	0.0	0.0	0.0	59.6	56.1	56.1	54.4	54.4	56.1	45.6
0.53	0.0	0.0	0.0	0.0	0.0	0.0	57.9	59.6	56.1	56.1	54.4	54.4	56.1	45.6
0.48	0.0	0.0	0.0	0.0	0.0	47.4	49.1	50.9	47.4	47.4	45.6	45.6	47.4	36.8
0.43	0.0	0.0	0.0	0.0	43.9	43.9	45.6	47.4	43.9	43.9	42.1	42.1	43.9	33.3
0.38	0.0	0.0	0.0	40.4	38.6	38.6	40.4	42.1	38.6	38.6	36.8	36.8	38.6	28.1
0.33	0.0	0.0	38.6	40.4	38.6	38.6	40.4	42.1	38.6	38.6	36.8	36.8	38.6	28.1
0.28	0.0	36.8	36.8	38.6	36.8	36.8	38.6	40.4	36.8	36.8	35.1	35.1	36.8	26.3
0.23	35.1	35.1	35.1	36.8	35.1	35.1	36.8	38.6	35.1	35.1	33.3	33.3	35.1	24.6
κ_α	0.28	0.33	0.38	0.43	0.48	0.53	0.58	0.63	0.68	0.73	0.78	0.83	0.88	0.93

B: Table for percentage of missed categorisation with different κ_α and κ_β combinations.

κ_β	Percentage of missed categorisation													
0.88	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	5.3
0.78	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.3	8.8	7.0
0.73	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8	14.0	10.5	8.8
0.68	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.8	19.3	17.5	14.0	12.3
0.63	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.6	29.8	26.3	24.6	21.1	19.3
0.58	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	31.6	29.8	26.3	24.6	21.1	19.3
0.53	0.0	0.0	0.0	0.0	0.0	0.0	38.6	35.1	33.3	31.6	28.1	26.3	22.8	21.1
0.48	0.0	0.0	0.0	0.0	0.0	49.1	47.4	43.9	42.1	40.4	36.8	35.1	31.6	29.8
0.43	0.0	0.0	0.0	0.0	52.6	52.6	50.9	47.4	45.6	43.9	40.4	38.6	35.1	33.3
0.38	0.0	0.0	0.0	59.6	59.6	59.6	57.9	54.4	52.6	50.9	47.4	45.6	42.1	40.4
0.33	0.0	0.0	61.4	59.6	59.6	59.6	57.9	54.4	52.6	50.9	47.4	45.6	42.1	40.4
0.28	0.0	63.2	63.2	61.4	61.4	61.4	59.6	56.1	54.4	52.6	49.1	47.4	43.9	42.1
0.23	64.9	64.9	64.9	63.2	63.2	63.2	61.4	57.9	56.1	54.4	50.9	49.1	45.6	43.9
κ_α	0.28	0.33	0.38	0.43	0.48	0.53	0.58	0.63	0.68	0.73	0.78	0.83	0.88	0.93

C: Table for percentage of false categorisation with different κ_α and κ_β combinations.

κ_β	Percentage of false categorisation													
0.88	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.9
0.83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	35.1	47.4
0.78	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.6	33.3	45.6
0.73	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.3	28.1	29.8	42.1
0.68	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.3	24.6	26.3	28.1	40.4
0.63	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8	17.5	22.8	24.6	26.3	38.6
0.58	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	12.3	14.0	19.3	21.1	22.8	35.1
0.53	0.0	0.0	0.0	0.0	0.0	0.0	3.5	5.3	10.5	12.3	17.5	19.3	21.1	33.3
0.48	0.0	0.0	0.0	0.0	0.0	3.5	3.5	5.3	10.5	12.3	17.5	19.3	21.1	33.3
0.43	0.0	0.0	0.0	0.0	3.5	3.5	3.5	5.3	10.5	12.3	17.5	19.3	21.1	33.3
0.38	0.0	0.0	0.0	0.0	1.8	1.8	1.8	3.5	8.8	10.5	15.8	17.5	19.3	31.6
0.33	0.0	0.0	0.0	0.0	1.8	1.8	1.8	3.5	8.8	10.5	15.8	17.5	19.3	31.6
0.28	0.0	0.0	0.0	0.0	1.8	1.8	1.8	3.5	8.8	10.5	15.8	17.5	19.3	31.6
0.23	0.0	0.0	0.0	0.0	1.8	1.8	1.8	3.5	8.8	10.5	15.8	17.5	19.3	31.6
κ_α	0.28	0.33	0.38	0.43	0.48	0.53	0.58	0.63	0.68	0.73	0.78	0.83	0.88	0.93

Appendix 3: CG_2 output with global (A_0 and β) and random (B and κ) for validation data set in 2012.

Complementary Gompertz input and generated output files for use in nlme procedure in R (version 2.15.1, cran.r-project.org; Pinheiro et al., 2013). Blue script indicates model formulation in R (input), required for successful operation of model. All other script represents generated output of model.

A_0 and β as global while B and κ as GL dependent (CG_2)

```
> AFL.nlmeG<-nlme(Y ~ A0 + B*(1-exp(-b*exp(-k*X))),fixed=A0+B+b+k~1,
+ random=B+k~1,start=c(A0=0.1,B=6,b=50,k=0.3),data=AFL.grp)
> summary(AFL.nlmeG)
Nonlinear mixed-effects model fit by maximum likelihood
  Model: Y ~ A0 + B * (1 - exp(-b * exp(-k * X)))
 Data: AFL.grp
          AIC           BIC                 logLik
           1118.914     1151.004            -551.4568
Random effects:
 Formula: list(B ~ 1, k ~ 1)
 Level: GNo
 Structure: General positive-definite, Log-Cholesky parametrization
         StdDev         Corr
B        1.1129189      B
k        0.2495674      -0.134
Residual 0.6636544
Fixed effects: A0 + B + b + k ~ 1
          Value         Std.Error     DF      t-value        p-value
A0         0.31408      0.05390       354     5.826621       0.0000
B         4.79808       0.17668       354     27.156204      0.0000
```

```
b        125.62757      45.39463       354    2.767455      0.0059
k        0.66681        0.05838        354    11.421048     0.0000
 Correlation:
         A0             B              b
  B      -0.312
  b      0.308          -0.170
  k      0.297          -0.203         0.780
Standardised Within-Group Residuals:
Min             Q1             Med            Q3            Max
-4.51           -0.212         -0.044         0.228         3.607
Number of Observations: 408
Number of Groups: 51
```

Appendix 4: Tables showing percentages of correct, missed and false categorisation at different tested combinations of κ_α and κ_β thresholds in 2012.

A: Table for percentage of correct categorisation with different κ_α and κ_β combinations.

κ_β	Percentage of correct categorisation																					
1.03																						25.5
0.99																					29.4	25.5
0.95																				33.3	29.4	25.5
0.91																			35.3	35.3	31.4	27.5
0.87																		37.3	31.4	31.4	27.5	23.5
0.83																	37.3	37.3	31.4	31.4	27.5	23.5
0.79																43.1	39.2	39.2	33.3	33.3	29.4	25.5
0.75															49.0	45.1	41.2	41.2	35.3	35.3	31.4	27.5
0.71														52.9	49.0	45.1	41.2	41.2	35.3	35.3	31.4	27.5
0.67													51.0	49.0	45.1	41.2	37.3	37.3	31.4	31.4	27.5	23.5
0.63												56.9	52.9	51.0	47.1	43.1	39.2	39.2	33.3	33.3	29.4	25.5
0.59											54.9	56.9	52.9	51.0	47.1	43.1	39.2	39.2	33.3	33.3	29.4	25.5
0.55										54.9	52.9	54.9	51.0	49.0	45.1	41.2	37.3	37.3	31.4	31.4	27.5	23.5
0.51									54.9	52.9	51.0	52.9	49.0	47.1	43.1	39.2	35.3	35.3	29.4	29.4	25.5	21.6
0.47								51.0	51.0	49.0	47.1	49.0	45.1	43.1	39.2	35.3	31.4	31.4	25.5	25.5	21.6	17.6
0.43							52.9	51.0	51.0	49.0	47.1	49.0	45.1	43.1	39.2	35.3	31.4	31.4	25.5	25.5	21.6	17.6
0.39						51.0	51.0	49.0	49.0	47.1	45.1	47.1	43.1	41.2	37.3	33.3	29.4	29.4	23.5	23.5	19.6	15.7
0.35					52.9	52.9	52.9	51.0	51.0	49.0	47.1	49.0	45.1	43.1	39.2	35.3	31.4	31.4	25.5	25.5	21.6	17.6
0.31				54.9	52.9	52.9	52.9	51.0	51.0	49.0	47.1	49.0	45.1	43.1	39.2	35.3	31.4	31.4	25.5	25.5	21.6	17.6
0.27			58.8	56.9	54.9	54.9	54.9	52.9	52.9	51.0	49.0	51.0	47.1	45.1	41.2	37.3	33.3	33.3	27.5	27.5	23.5	19.6
0.23		58.8	60.8	58.8	56.9	56.9	56.9	54.9	54.9	52.9	51.0	52.9	49.0	47.1	43.1	39.2	35.3	35.3	29.4	29.4	25.5	21.6
0.19	52.9	54.9	56.9	54.9	52.9	52.9	52.9	51.0	51.0	49.0	47.1	49.0	45.1	43.1	39.2	35.3	31.4	31.4	25.5	25.5	21.6	17.6
κ_α	0.23	0.27	0.31	0.35	0.39	0.43	0.47	0.51	0.55	0.59	0.63	0.67	0.71	0.75	0.79	0.83	0.87	0.91	0.95	0.99	1.03	1.07

B: Table for percentage of missed categorisation with different κ_α and κ_β combinations.

κ_β	Percentage of missed categorisation																					
1.03																						0.0
0.99																					0.0	0.0
0.95																				0.0	0.0	0.0
0.91																			0.0	0.0	0.0	0.0
0.87																		5.9	3.9	3.9	3.9	3.9
0.83																	5.9	5.9	3.9	3.9	3.9	3.9
0.79																5.9	5.9	5.9	3.9	3.9	3.9	3.9
0.75															7.8	5.9	5.9	5.9	3.9	3.9	3.9	3.9
0.71														11.8	9.8	7.8	7.8	7.8	5.9	5.9	5.9	5.9
0.67													19.6	17.6	15.7	13.7	13.7	13.7	11.8	11.8	11.8	11.8
0.63												21.6	19.6	17.6	15.7	13.7	13.7	13.7	11.8	11.8	11.8	11.8
0.59											23.5	21.6	19.6	17.6	15.7	13.7	13.7	13.7	11.8	11.8	11.8	11.8
0.55										25.5	25.5	23.5	21.6	19.6	17.6	15.7	15.7	15.7	13.7	13.7	13.7	13.7
0.51									27.5	27.5	27.5	25.5	23.5	21.6	19.6	17.6	17.6	17.6	15.7	15.7	15.7	15.7
0.47								31.4	31.4	31.4	31.4	29.4	27.5	25.5	23.5	21.6	21.6	21.6	19.6	19.6	19.6	19.6
0.43							31.4	31.4	31.4	31.4	31.4	29.4	27.5	25.5	23.5	21.6	21.6	21.6	19.6	19.6	19.6	19.6
0.39						33.3	33.3	33.3	33.3	33.3	33.3	31.4	29.4	27.5	25.5	23.5	23.5	23.5	21.6	21.6	21.6	21.6
0.35					33.3	33.3	33.3	33.3	33.3	33.3	33.3	31.4	29.4	27.5	25.5	23.5	23.5	23.5	21.6	21.6	21.6	21.6
0.31				35.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	31.4	29.4	27.5	25.5	23.5	23.5	23.5	21.6	21.6	21.6	21.6
0.27			35.3	35.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	31.4	29.4	27.5	25.5	23.5	23.5	23.5	21.6	21.6	21.6	21.6
0.23		37.3	35.3	35.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	31.4	29.4	27.5	25.5	23.5	23.5	23.5	21.6	21.6	21.6	21.6
0.19	43.1	41.2	39.2	39.2	37.3	37.3	37.3	37.3	37.3	37.3	37.3	35.3	33.3	31.4	29.4	27.5	27.5	27.5	25.5	25.5	25.5	25.5
κ_α	0.23	0.27	0.31	0.35	0.39	0.43	0.47	0.51	0.55	0.59	0.63	0.67	0.71	0.75	0.79	0.83	0.87	0.91	0.95	0.99	1.03	1.07

C: Table for percentage of false categorisation with different κ_α and κ_β combinations.

κ_β	Percentage of false categorisation																					
1.03																						74.5
0.99																					70.6	74.5
0.95																				66.7	70.6	74.5
0.91																			64.7	64.7	68.6	72.5
0.87																		56.9	64.7	64.7	68.6	72.5
0.83																	56.9	56.9	64.7	64.7	68.6	72.5
0.79																51.0	54.9	54.9	62.7	62.7	66.7	70.6
0.75															43.1	49.0	52.9	52.9	60.8	60.8	64.7	68.6
0.71														35.3	41.2	47.1	51.0	51.0	58.8	58.8	62.7	66.7
0.67													29.4	33.3	39.2	45.1	49.0	49.0	56.9	56.9	60.8	64.7
0.63												21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.59											21.6	21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.55										19.6	21.6	21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.51									17.6	19.6	21.6	21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.47								17.6	17.6	19.6	21.6	21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.43							15.7	17.6	17.6	19.6	21.6	21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.39						15.7	15.7	17.6	17.6	19.6	21.6	21.6	27.5	31.4	37.3	43.1	47.1	47.1	54.9	54.9	58.8	62.7
0.35					13.7	13.7	13.7	15.7	15.7	17.6	19.6	19.6	25.5	29.4	35.3	41.2	45.1	45.1	52.9	52.9	56.9	60.8
0.31				9.8	13.7	13.7	13.7	15.7	15.7	17.6	19.6	19.6	25.5	29.4	35.3	41.2	45.1	45.1	52.9	52.9	56.9	60.8
0.27			5.9	7.8	11.8	11.8	11.8	13.7	13.7	15.7	17.6	17.6	23.5	27.5	33.3	39.2	43.1	43.1	51.0	51.0	54.9	58.8
0.23		3.9	3.9	5.9	9.8	9.8	9.8	11.8	11.8	13.7	15.7	15.7	21.6	25.5	31.4	37.3	41.2	41.2	49.0	49.0	52.9	56.9
0.19	3.9	3.9	3.9	5.9	9.8	9.8	9.8	11.8	11.8	13.7	15.7	15.7	21.6	25.5	31.4	37.3	41.2	41.2	49.0	49.0	52.9	56.9
κ_α	0.23	0.27	0.31	0.35	0.39	0.43	0.47	0.51	0.55	0.59	0.63	0.67	0.71	0.75	0.79	0.83	0.87	0.91	0.95	0.99	1.03	1.07

Appendix 5: Correlation of GL dependent parameters of CG fitted to softening data collected in different storage durations.