Characterising Texture and Cellular Level Responses of ‘Centurion’ Blueberries during Storage in Different Weight Loss Conditions

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Abstract

Postharvest blueberry softening hinders consumer acceptance and correlates with high moisture loss during storage. Such textural variations have been attributed to factors such as turgor, cell wall modifications and other microstructural changes in the outer cell layers of the fruit. This thesis investigated the impact of moisture loss on blueberry quality, as well as the structure/function relationships associated with fruit texture characteristics during postharvest using an integrated physical and microstructural approach.

Four different weight loss conditions (62%, 76%, 93% and 98% RH) were evaluated over a three week postharvest storage period to assess blueberry texture parameters using a texture analyser, where microstructural changes were assessed by light microscopy and optical coherence tomography (OCT).

Under high weight loss conditions there was an increase in berry softening and a decrease in texture characteristics whereas an increase in berry firmness, hardness and gumminess was observed during storage under low weight loss conditions.

Light microscopy clearly illustrated microstructural differences among ‘Centurion’ blueberries stored in different weight loss conditions, in retention of cell shape, degree of cell to cell wall contact, the amount of space between cells and cell wall integrity. When berries lost moisture during storage, epidermal and subepidermal cells retained their integrity, and parenchyma cells lost integrity leading to collapse which may contribute to overall fruit quality during postharvest.

3D OCT images showed no obvious differentiation between large cells at each weight loss treatment, however significant differences were observed in the microstructure between each storage period. In general the microstructure of medium to large cells in the parenchyma tissue showed an increase in average surface area and total surface area after each storage period.

In summary, low weight loss storage conditions help to preserve blueberry texture and quality, whilst maintaining cellular structure and integrity during postharvest storage. It is recommended blueberries are stored between 95 – 99% RH and at a low temperatures to prevent moisture loss during postharvest.

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List of Abbreviations

CO_2	Carbon Dioxide
E-OCT	Endoscopic Optical Coherence Tomography
FD-OCT	Fourier Domain Optical Coherence Tomography
FFT	Fast Fourier Transform
GLM	General Linear Matrix
H_2O	Water
HSD	Honest Significant Difference
HIS	Hyperspectral Imaging
MIFST	Massey Institute of Food Science and Technology
MRI	Magnetic Resonance Imaging
MSI	Multispectral Imaging
NIRS	Near Infra-Red Spectroscopy
O_2	Oxygen
OCT	Optical Coherence Tomography
PLS	Partial Least Squares
Rf	Radio Frequency
RH	Relative Humidity (%)
SEM	Scanning Electron Microscopy
SLD	Super Luminescent Diode
SNR	Signal to Noise Ratio
SS-OCT	Swept Source Optical Coherence Tomography
TD-OCT	Time Domain Optical Coherence Tomography
TEM	Transmission Electron Microscopy
TPA	Texture Profile Analysis
USDA	United States Department of Agriculture
VP	Vapour Pressure

Table of Contents

Appendices

List of Figures

List of Tables

Chapter 1.
Introduction

Blueberries originate from North America and belong to the genus *Vaccinium,* the family Ericaceae and the subfamily *Vacciniodiae* (Eck *et al.,* 1990). Species of major economic importance include the rabbiteye blueberry *V. ashei* Reade, the lowbush blueberry *V. angustifolium* Aiton, and the highbush blueberry *V. corymbosum* L. (Hancock *et al.,* 1996). Highbush blueberries can be separated into northern and southern highbush types. Northern highbush varieties ripen late in the season, whereas southern highbush cultivars produce early in the season (Lyrene, 1990). Rabbiteye blueberries have a pink spot at the calyx end, resembling the eye of a wild rabbit whilst ripening, and derived its name from wild *V. ashei* berries.

A large number of cultivars are available to blueberry growers, with a large variability in growing conditions and postharvest behaviour (Strik, 2007). Commercially grown highbush and rabbiteye blueberry cultivars are used for the fresh market, while lowbush blueberry cultivars are orientated towards food processing (Du *et al.,* 2011). Highbush cultivars have larger stem scars than rabbiteye cultivars and tend to soften faster during the postharvest chain. Rabbiteye cultivars have more seeds, a thicker epidermis and are generally smaller than highbush berries (Makus and Morris, 1993).

Increasing demand for blueberries worldwide resulting from high nutritional value and health benefits has been driven by extensive marketing campaigns, resulting in high stability of prices within the fresh and processed blueberry market. Increasing health and wellness trends are fuelling market growth, where the global growth rate in volume (metric tonnes) increased 139% from 2008 to 2016 (Brazelton and Young, 2017). According to Strik (2007) global blueberry plantations increased by 90% between 1995 – 2005 with approximately 43,200 hectares producing 195,000 tonnes. While North America produces more than 70% of the global production of blueberries, equal amounts of fresh and processed blueberries are produced (Brazelton and Young, 2017). Southern Hemisphere growers supply the Northern Hemisphere during the offseason (Strik, 2007). South America and the Asia and Pacific region account for 25% and 7.5% of global highbush blueberry production respectively (Brazelton and Young, 2017). New Zealand blueberry exports doubling since 2010 to $41 million in 2016, primarily spurred by research and consumer recognition of their health benefits (The New Zealand Institute for Plant & Food Research Ltd, 2017).

Blueberries have a high nutritional value and contain beneficial nutrients, phyto-nutrients, polyphenols, carotenoids, fibre, folate, Vitamin C and E as well as iron to name a few. They are one of the richest sources of antioxidant phyto-nutrients, with higher levels of anthocyanins and phenolics than many other fruits and vegetables (Prior *et al.,* 1998). Antioxidants neutralise free radicals which are unstable molecules that cause the development of a number of diseases such as cardiovascular diseases and cancer (Prior *et al.,* 1998). These naturally occurring compounds have been recognised as having the potential to reduce the risk of disease (Prakash *et al.,* 2010). These health benefits have been increasingly marketed driving the demand globally for blueberries with highbush blueberry growth predicted to be over 904,000 metric tonnes by 2021 (Brazelton and Young, 2007).

Fresh blueberries rapidly deteriorate as a result of decay, shrivelling and softening (Forney, 2009). Consequently, they have a limited shelf life between 7 – 14 days, therefore year round availability and extended storage life for export to distant markets is important to meet increasing consumer demands. By storing blueberry fruit at 90 – 95% relative humidity (RH) and 0 ºC acceptable berry quality can be maintained for up to 2 and 4 weeks in highbush and rabbiteye cultivars respectively increasing postharvest storability (Kader, 2001; Perkins-Veazie, 2004). Increasingly exports from the Southern Hemisphere are achieving an extension of postharvest shelf life by using controlled atmosphere (CA) (Ehlenfeldt and Martin, 2002). The temperature the berries are stored at during postharvest can lead to blueberry losses therefore it is recommended postharvest temperature throughout the supply chain remain between 0 – 5 ºC to maintain commercial quality (Kader, 2001; Mitcham *et al.,* 2002; Perkins-Veazie, 2004).

It is known that relative humidity (RH) effects the rate of moisture loss within blueberries (Paniagua *et al.,* 2013). The RH conditions during postharvest are extremely important in maintaining the moisture content of blueberries. Increased moisture loss measured as weight loss has correlated to a decrease in blueberry firmness which is strongly associated with texture (Paniagua *et al.,* 2013). A decrease in weight loss of approximately 6% after 35 d storage in 90 – 95% RH at 0 ºC has shown a decrease in desirable texture characteristics of blueberry fruit (Chiabrando *et al.,* 2009). Whereas storage in low RH conditions at 4 ºC resulted in 15.06% weight loss and extensive berry softening (Paniagua *et al.,* 2013). High RH postharvest conditions help to prevent fruit moisture loss and maintain texture characteristics at a suitable consumer acceptability. Variable texture responses obtained under different conditions of blueberry moisture loss implies a question of causality that still needs to be addressed.

The postharvest softening of blueberries is not very well understood. Forney *et al.* (1998) proposed reduced turgor as a result of blueberry moisture loss was related to the softening of fresh fruit, however no experimental evidence has been reported in literature to support this. Increased firming of blueberries has been observed at low weight loss levels 1- 2% (Miller *et al.,* 1993; Forney *et al.,* 1998; Duarte *et al.,* 2009; Chiabrando and Giacalone, 2011; Paniagua *et al.,* 2013) and is thought to be the result of microscopically observed thickening of parenchyma cell walls (Allan-Wojtas *et al.,* 2001) and corrugation of epidermal cell walls (Bunemann *et al.,* 1957). Although the thickening of parenchyma cell walls was not observed in a following blueberry season (Allan-Wojtas *et al.,* 2001). The underlying mechanisms responsible for decreased texture responses throughout storage need to be addressed.

The main emphasis of this research is to evaluate the possible interactions between moisture loss during blueberry postharvest and the storage atmosphere, in terms of blueberry texture parameters. The influence of RH storage conditions on blueberry texture and moisture loss are assessed in this work with a focus on the mechanisms responsible for structural changes resulting from moisture loss. The literature review provides details, focusing on blueberry postharvest physiology and anatomy, and blueberry textural responses in regards to storage conditions. Optical coherence tomography (OCT) is reviewed in its horticultural application for the suitability to visualise blueberry cellular changes resulting from different storage atmospheres. The literature review provides necessary background for further experimental investigation, in order to identify further research questions.

1.1 Experimental objectives

The main objectives defined for the present study are as follows:

1) To investigate the relationship between blueberry moisture loss and postharvest texture parameters. Moisture loss conditions were created in this present work by the creation of a manifold enabling the regulation of dry air creating differing humidity storage conditions.

2) To determine microstructural changes responsible for variations in texture resulting from moisture loss during postharvest, and how these changes evolve during storage and affect final blueberry quality. Microscopy was used to visualise these changes.

3) To investigate the feasibility of OCT to measure structural changes in blueberries during postharvest in different storage conditions.

The objectives have been investigated through three research chapters in this study. The relationship between weight loss and texture during postharvest storage is reported in Chapter 4. The microstructural characteristics of blueberries during postharvest storage is investigated in Chapter 5 and blueberry cellular structural changes were characterised by using OCT in Chapter 6.

Chapter 2.
Literature Review

This chapter gives a brief overview on how quality progresses during blueberry fruit development and postharvest storage. It also summarises the importance of postharvest storage conditions within industry and tabulates some of the studies undertaken in the past to determine texture responses resulting from different storage conditions and periods. A review of methods employed by researchers to monitor and determine blueberry texture through texture analysis is also presented. The cellular mechanisms likely responsible for changes in texture, proposed by previous studies are also discussed. Finally this chapter summarises some of the non-destructive imaging techniques which show promise and have been adopted by the horticulture industry.

2.1 Blueberry fruit anatomy

Blueberry fruit is comprised of an outer epidermis and is covered with a cuticle about 5 μm thick with a waxy bloom (Gough, 1993). The amount of waxy bloom present is dependent on the cultivar and stage of maturity. This is a thick lipid layer which functions in pathogen resistance and water regulation (Fava et al., 2006) consisting of epicuticular waxes, which vary in form from short narrow rods to unstructured in nature and are responsible for the powdery blue colour of blueberries (Gough, 1993; Fava et al., 2006). The epidermis contains specialised epidermal cells which contain thickened primary cell walls which form the fruits skin. Beneath the epidermal layer is the hypodermal layer or the sub-epidermis, pigment from anthocyanins can be present in these layers as well as the epidermis depending on the variety of blueberry (Figure 2.1). According to Gough (1993) the cortex is delineated from both the epidermal and hypodermal layer by a ring of vascular bundles. The flesh of the blueberry known as the mesocarp contains parenchyma cells. These cells are the most numerous in the blueberry flesh and are mostly water filled vacuole and thin, separated by a non-lignified cell wall which contains a pectin rich middle lamella (Harker *et al.,* 1997).

The mesocarp is located between the epidermal layers and the endocarp. The endocarp consists of carpels containing five highly lignified placentae up to which 65 seeds are attached. Extending into the mesocarp are approximately ten locules which are surrounded by a stony endocarp (Figure 2.1) (Gough, 1994). Within the mesocarp, xylem and sclerenchyma cells have thick and lignified secondary cell walls occurring as fibres and sclereid cells. These cells

are associated with vascular bundles and stone cells in blueberry flesh (Gough, 1994; Harker *et al.,* 1997). Stone cells can be found 460 – 920 µm beneath the epidermis (Gough, 1983), where the number of stone cells present varies by genotype (Blaker and Olmstead, 2014). Stone cells bind to neighbouring parenchyma cells and serve to strengthen the flesh tissue (Gough, 1983). These lignified cells can occur single, doubly or in clusters (Gough, 1983; Blaker and Olmstead, 2014). It has been proposed that stone cells are responsible for the grainy texture of some blueberries, however significant differences in the number of stone cells beneath the surface of the epidermis do not correspond to standard or crisp texture (Blaker and Olmstead, 2014).

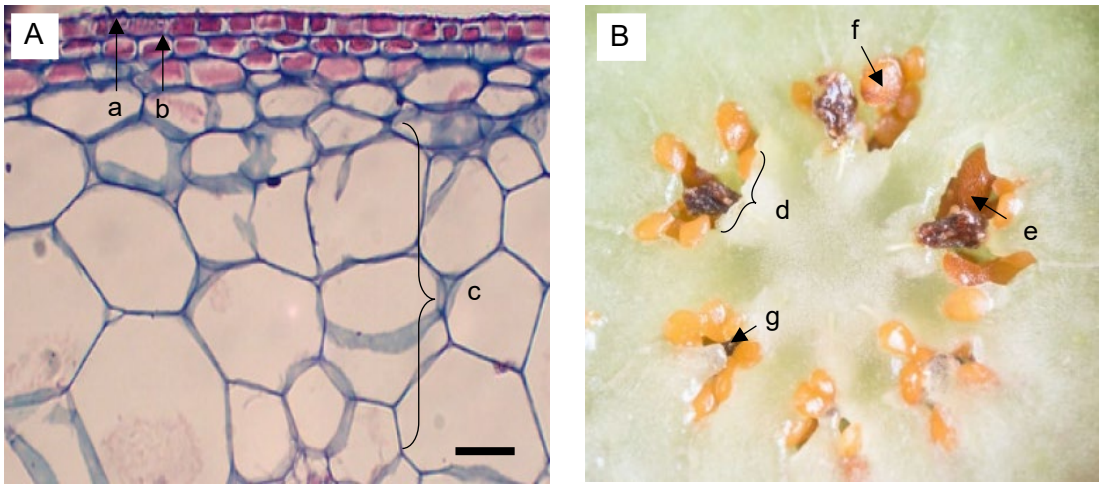

Figure 2.1. (A) Microscopy image of blueberry fruit of 'Sweetcrisp' consisting of epidermis (a), hypodermis (b) and mesocarp (c). Section stained with Safranin O and Aniline Blue. Scale = 100 µm. (B) Typical blueberry endocarp consisting of 5 carpels (d), 10 locules (e), approximately 50 seeds (f) and 5 placentae (g).

2.2 Blueberry fruit development

Blueberries undergo important structural and biochemical changes during ripening, which has an impact on final berry quality at harvest and storage. This can be visualised in the transition of external berry colour from green to dark blue and is considered the first step of senescence mostly comprised of degradation steps (Wills *et al.,* 2007). The respiration rate can accelerate the rate of ripening modifications in some blueberry species, whereas regulation of ripening related changes are often controlled by the plant hormone ethylene (Wills *et al.,* 2007). The presence of an autocatalytic phase of ethylene production and a respiration peak are indicative of climacteric fruit. Fruit can be classified as climacteric or non-climacteric based on this definition. Blueberries have been described as a climacteric fruit (Ismail, 1969; Windus *et al.,* 1976; Shimura *et al.,* 1986; Perkins-Veazie, 2004; Mitcham *et al.,* 2011), where the development of the respiratory peak varies between blueberry species and cultivars (Ismail,

1969). Major modifications occurring during blueberry fruit development include variations in chemical composition, skin colour development and changes in textural attributes (Rhodes, 1970). Understanding of the physiological behaviour during fruit development is critical for further analysis present in this thesis for the investigation of postharvest texture and structural characteristics of 'Centurion' blueberries.

2.2.1 Ethylene and respiration

Early studies demonstrated inconsistent findings in detecting a climacteric rise of respiration rate during blueberry ripening (Forsyth and Hall, 1969; Frenkel, 1972). A climacteric rise of respiration can be described as a stage of fruit ripening associated with an increased production of ethylene (Alexander and Grierson, 2002). Initial attempts to describe the respiration pattern of blueberry reported a climacteric peak (Bergman, 1929). It was observed the greatest production of carbon dioxide occurred during the period of colour development from the first pink colouration to a red ripe stage. After this stage the rate of carbon dioxide production decreased rapidly as ripening proceeded to the mature blue stage when blueberries are harvested (Bergman, 1929). Later research failed to detect a burst in respiration at ripening, generating uncertainty regarding the respiratory behaviour (Forsyth and Hall, 1969; Frenkel, 1972). Further research has demonstrated consistency providing strong evidence of the presence of a respiratory peak during the ripening of blueberry fruit (Ismail, 1969; Windus *et al.,* 1976; Shimura *et al.,* 1986; Perkins-Veazie, 2004; Mitcham *et al.,* 2011) (Figure 2.2). Furthermore a respiratory peak has been observed for highbush (Windus *et al.,* 1976), lowbush (Ismail, 1969) and rabbiteye (Shimura *et al.,* 1986) blueberry species.

The development of the respiratory peak varies between blueberry species and cultivars. Forsyth and Hall (1969), concluded colour is the best criterion of ripening in blueberry instead of increased respiration. Between the green pink colour development stage a consistent climacteric rise has been recorded in highbush (Windus *et al.,* 1976; Shimura *et al.,* 1986), in lowbush blueberries at the pink red stage (Ismail, 1969), and at the green fruit development stage in rabbiteye berries (Shimura *et al.,* 1986). Highlighting variation of fruit development between blueberry species.

A sharp increase in ethylene production during blueberry ripening occurs simultaneously with an increase in respiration (Windus *et al.,* 1976; Lipe, 1978; Shimura *et al.,* 1986; Suzuki *et al.,* 1997) (Figure 2.3). Therefore supporting classification of blueberry as a climacteric fruit. A small initial concentration of ethylene results in the production of larger quantities of ethylene

in climacteric fruit until a peak concentration is reached (Barry and Giovannoni, 2007). In some cases an additional secondary peak has been reported in mature blueberries (El-Agamy, 1982; Shimura *et al.,* 1986). The production rate of ethylene varies between species. In highbush blueberries the production rate ranges between 6.11 and 24.43 ρmol kg^{-1} s^{-1} (Suzuki *et al.,* 1997) and is much higher (122.15 ρmol kg^{-1} s^{-1}) in rabbiteye blueberries (El-Agamy, 1982). Supporting evidence of distinct differences in the stage of ripening climacteric responses occur among blueberry species.

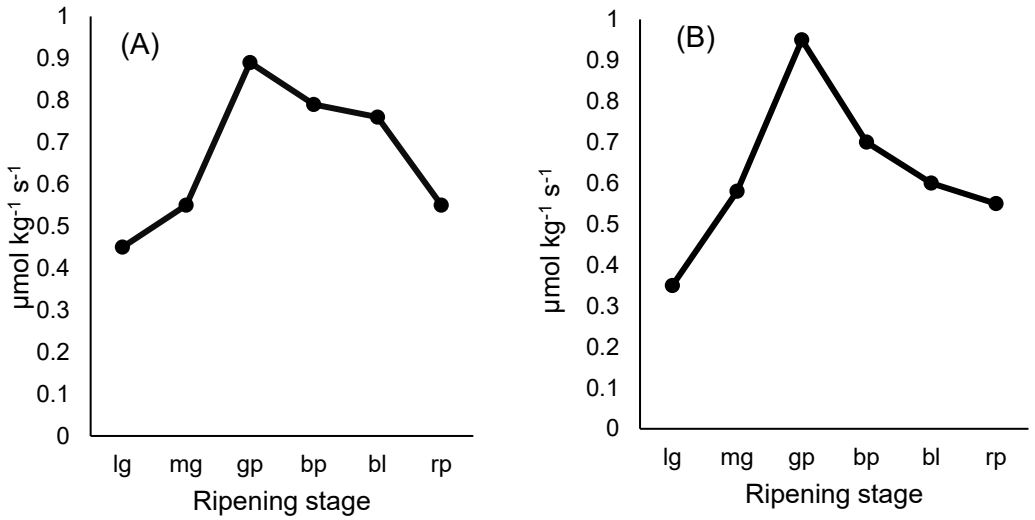

Figure 2.2. The respiration rate of two highbush blueberry fruit cultivars 'Lateblue' (A) and 'Bluecrop' (B) at different ripening stages measured as the rate of CO_2 production at 23 ºC. Ripening stages are described by skin colouration light blue (lg), mature green (mg), green pink (gp), blue pink (bp), blue (bl) and blue ripe (rp). Adapted from Windus et al. (1976).

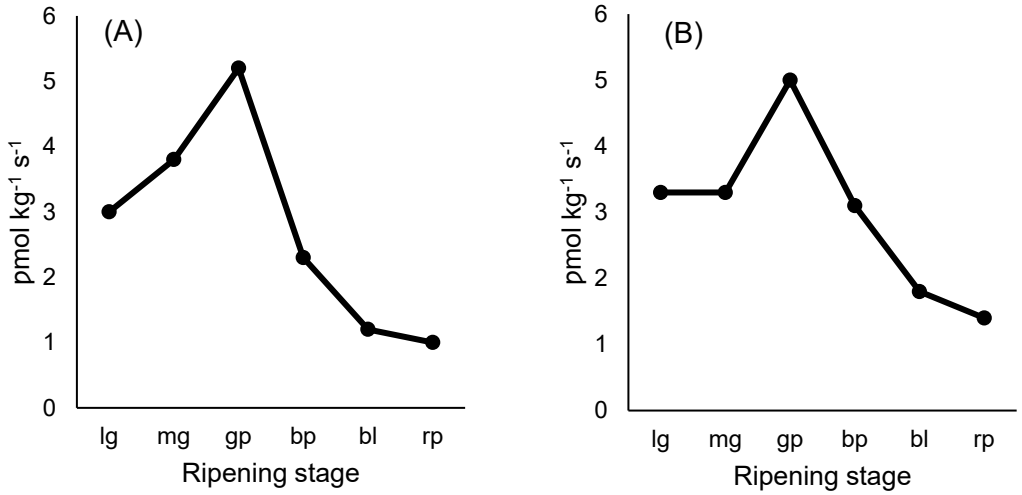

Figure 2.3. The rate of ethylene production of two highbush blueberry fruit cultivars 'Lateblue' (A) and 'Bluecrop' (B) at different ripening stages measured as the rate of CO_2 production at 23 ºC. Ripening stages are described by skin colouration light blue (lg), mature green (mg), green pink (gp), blue pink (bp), blue (bl) and blue ripe (rp). Adapted from Windus et al. (1976).

2.2.2 Changes in chemical composition

In ripe blueberries the most abundant sugars are glucose and fructose being present in an equal concentration of approximately 70 mg g^{-1} in some highbush and lowbush blueberries such as *V. corymbosum* and *V. angustifolium* respectively (Hirvi and Honkanen, 1993; Kader *et al.,* 1993; Darnell *et al.,* 1994; Ayaz *et al.,* 2001). Trace amounts of sucrose below 10 mg g^{-1} are found as sucrose is degraded into glucose and fructose. Invertase is a sucrose degrading enzyme and is responsible for the conversion of sucrose to glucose and fructose (Kader *et al.,* 1993). During ripening increased invertase activity is responsible for a decrease in sucrose concentration at fruit maturity (Kader *et al.,* 1993). The total sugar content is measured as total soluble solids or sugar content. During fruit ripening, as in most fruits the total soluble solids content of blueberries increases (Castrejón *et al.,* 2008). In ripe blue blueberries the total sugar content (mg g^{-1} FW) averages between 1.5 and 3.5 fold greater than in turning blue and green fruit respectively (Forney *et al.,* 2012). Interestingly the total soluble solids content of ripe blueberries stored at a low temperature (0 – 5 ºC) for up to three weeks remains fairly stagnant and does not vary (Prange *et al.,* 1995; Schotsmans, Molan and MacKay, 2007; Chiabrando and Giacalone, 2009; Paniagua, 2012).

Organic acids such as citric, malic, quinic and ascorbic acids accumulate during certain stages of fruit development. In blueberries the bulk of the organic acid content is accounted for by citric acid comprising of 77 – 87% of the total acid content (Forney *et al.,* 2012), and to a lesser extent malic acid (Hulme, 1971). During ripening the concentration of these organic acids does not vary much. However the concentration of citric acid does decrease strongly throughout ripening decreasing 64% from green to blue fruit (Forney *et al.,* 2012). A decline in citric acid throughout ripening has also been reported by Kushman and Ballinger (1968) to produce an overall decline in total fruit acidity. Fruit titratable acidity is another measure of the total fruit acidity indicating the content of organic acids in fruits. In blueberries as fruit ripens the titratable acidity decreases (Kushman and Ballinger, 1968; Castrejón *et al.,* 2008). However, after harvest fully ripe blueberries show an increasing trend in total titratable acidity during cold storage (Prange *et al.,* 1995; Schotsmans, Molan and MacKay, 2007; Chiabrando and Giacalone, 2009; Paniagua, 2012). It is thought that water loss during the postharvest period provokes the total titratable acidity of blueberries as a result of fruit dehydration (Chiabrando and Giacalone, 2009). Therefore lower levels of organic acid concentration are found in blueberries just after harvest.

2.2.3 Evolution of skin colour

The skin colour of blueberries evolves during ripening from green to blue (Figure 2.4). There is an increase in anthocyanin content which correlates with an increased total soluble solids and acidity ratio (Ballinger and Kushman, 1970). Also responsible for the blue colouring of blueberries are epicuticular waxes (Albrigo *et al.,* 1980; Sapers *et al.,* 1984). Blueberry skin colour is a highly complex attribute that is affected by various physical and chemical factors. Anthocyanins are responsible for generating blue and purple hues throughout ripening (Ballinger *et al.,* 1970). The content of anthocyanins increases throughout ripening accumulating in epidermal and hypodermal cells. These cells make up the skin of the blueberry and are synthesized from the calyx end of the fruit towards to the stem (Ballinger *et al.,* 1972).

The light blue shade of blueberries is determined by the amount of epicuticular waxes, also known as 'bloom'. The surface wax refracts and reflects light causing a light blue appearance on the surface of the blueberry (Retamales and Hancock, 2018). Vicente *et al.* (2007) describes the colour stages throughout blueberry fruit development as green, 25% blue, 75% blue, 100% blue and blue ripe. In industry, berry colour is commonly used as a harvest maturity indicator, with 100% blue surface colouration indicating maturity (Kalt, McRae and Hamilton, 1995; Mitcham, 2007).

Figure 2.4. Visualisation of colour changes during the development of 'Rubel' blueberry fruit. Republished with permission of American Society of Plant Biologists, from Gene Expression and Metabolite Profiling of Developing Highbush Blueberry Fruit Indicates Transcriptional Regulation of Flavonoid Metabolism and Activation of Abscisic Acid Metabolism, Zifkin, M., Jin, A., Ozga, J. A., Zaharia, L. I., Schernthaner, J. P., Gesell, A., & Constabel, C. P., Volume 158, Edition 1, 2012; permission conveyed through Copyright Clearance Center, Inc.

Anthocyanins are prevalent in the skin of blueberries and are responsible for the purple and blue hues seen during ripening. The total anthocyanin content increases during ripening with

the highest anthocyanin levels found at high maturity stages (Ribera-Fonseca, Noferini and Rombola, 2016). It has been found the total anthocyanin content of whole blueberries increases linearly with the progression of fruit ripening. In fact, green blueberries contain no anthocyanins, while intermediate fruit stages have very low anthocyanin content (Ribera-Fonseca, Noferini and Rombola, 2016). The total anthocyanin content of ripe blueberry fruits has been reported in a wide range of studies. Where the maximum total anthocyanin content in ripe blueberries varies depending on the genotype of the blueberry (Stevenson and Scalzo, 2012) (Table 2.1). Interestingly, rabbiteye (*V. virgatum*) varieties were found to have a higher average anthocyanin content of 215.94 mg 100 g^{-1} compared to northern highbush and southern highbush (*V. corymbosum)* varieties of 153.97 and 145.69 mg 100 g^{-1} fruit respectively (Stevenson and Scalzo, 2012).

Anthocyanins contributing to blueberry skin colour continue to be produced after harvest. After 21 days of storage Mitcham (2007) reported an increase of 55% in anthocyanin content at 5 °C storage. Therefore blueberries can be harvested when they are not fully blue, allowing colour development to progress during storage (El-Agamy *et al.,* 1982; Connor *et al.,* 2002). Although blueberries are harvested at 100% blue colouration in industry.

Table 2.1. Total anthocyanin content in blueberry fruit of selected genotypes developed in New Zealand by Plant & Food Research (Stevenson & Scalzo, 2012).

Cultivar	Species	Anthocyanins (mg 100 g^{-1} fruit)
'Centurion'	Rabbiteye (*V. virgatum*)	275.5
'Maru'	Rabbiteye (*V. virgatum*)	263.8
'Rubel'	Northern highbush (*V. corymbosum*)	290.0
'Jersey'	Northern highbush (*V. corymbosum*)	184.5
'Brigitta'	Northern highbush (*V. corymbosum*)	101.8
'Island Blue'	Southern highbush (*V. corymbosum hybrids*)	249.6
'Blue Bayou'	Southern highbush (*V. corymbosum hybrids*)	139.5

Epicuticular wax is a thin coating of wax covering the outer surface of blueberry fruits, often forming a whitish bloom on the fruit. These waxes are present in the outer tangential epidermal cell walls, and have been described as thin, discontinuous and electron-lucent (Fava *et al.,* 2006). Once ripe, the epicuticular waxes of blueberry give the normally dark pigmented fruit its powdery blue colour and have been described to vary in form from amorphous to that of short, narrow rods (Gough, 1994; Fava *et al*., 2006). Blue and light blue cultivars result from high amounts of wax structures deposited as upright platelets, which are classified as β-diketones and rodlets (Albrigo *et al.,* 1980; Sapers *et al.,* 1984). The light scattering properties of upright platelets are responsible for the lighter blue cultivars. Whereas, in dark blue and black cultivars the predominant wax structures are flat platelets and highly annealed patches of wax (Sapers *et al.,* 1984). As well as the structure of epicuticular waxes the quantity of the wax plays a role in generating different blue colour intensities. Blueberry

epicuticular waxes undergo structural modifications during ripening, with a high proportion of upright rodlet structures degrading in relation to flat platelets (Freeman *et al.,* 1979; Albrigo *et al.,* 1980). It is possible that the arrangement of platelets could affect texture during storage and imaging techniques.

Consequently the characteristic tones of blueberry cultivars are only expressed in ripe blueberries (100% blue colouration), when epicuticular wax structures, such as upright platelets and flat platelets have been developed. At this stage the epidermal layer is completely covered with anthocyanins, providing cultivars with their specific and unique colouring.

2.2.4 Cellular level changes

The cell size of blueberries varies according to cell type during ripening. During ripening from green to fully ripe blue the epidermis and cell layers following show the same trend of smaller cells in the epidermis to larger cells in the parenchyma (Cano-Medrano and Darnell, 1997; Johnson *et al.,* 2011; Blaker and Olmstead, 2014). The cell area increases in each successive cell layer from the epidermis. The successive cell layers are the epidermis; hypodermis which is located directly beneath the epidermis; and the mesocarp consisting of large parenchyma cells responsible for the fleshiness of blueberry fruit (Gough, 1993).

During fruit expansion, cell division occurs over a longer period of time in the mesocarp resulting in larger cells. As blueberries ripen from mature green to fully ripe the epidermal layer does not show a dramatic increase of cell area. In fact in mature green fruits the average cell area in the epidermal cell layer ranged from 436 – 718 μm^2, and from 429 – 712 μm^2 in the epidermal layer of fully ripe fruit (Blaker and Olmstead, 2014). Cells present in the mesocarp cease in division and increase only in size during the later stages of ripening (Table 2.2) (Harker *et al.,* 1997; Johnson *et al.,* 2011). The average cell area of the mesocarp layer in mature green fruit ranged from 747 – 1149 μm^2 compared to 1126 – 1708 μm^2 in the mesocarp layer of fully ripe fruit (Blaker and Olmstead, 2014). Cano-Medrano and Darnell (1997), reported pigment development and increased cell size in the mesocarp at ripening 72 d after bloom, compared to undeveloped fruit 24 d after bloom (Figure 2.5). Between mature green and fully ripe fruit the difference in average cell area is indicative of cell expansion in the mesocarp and parenchyma (Blaker and Olmstead, 2014). Therefore during ripening it is clear the cells in the mesocarp increase in size, whereas epidermal cells remain fairly constant in size.

Table 2.2. Cell number and cell area at bloom and in ripe blue mature fruit in four rabbiteye blueberry genotypes. In ripe blue mature fruit cells in the mesocarp were measured (n=8). Reproduced from Johnson et al. (2011).

Variety	Bloom		Ripe Blue	
	Cell number ($\times 10^3$)	*Cell area* ($\times 10^3\ \mu m^2$)	*Cell number* ($\times 10^3$)	*Cell area* ($\times 10^3\ \mu m^2$)
'Powderblue'	~8	<1	~6	~16
'Brightwell'	~5	<1	~8	~13
T-959	~8	<1	~14	~12
T-960	~6	<1	~11	~17

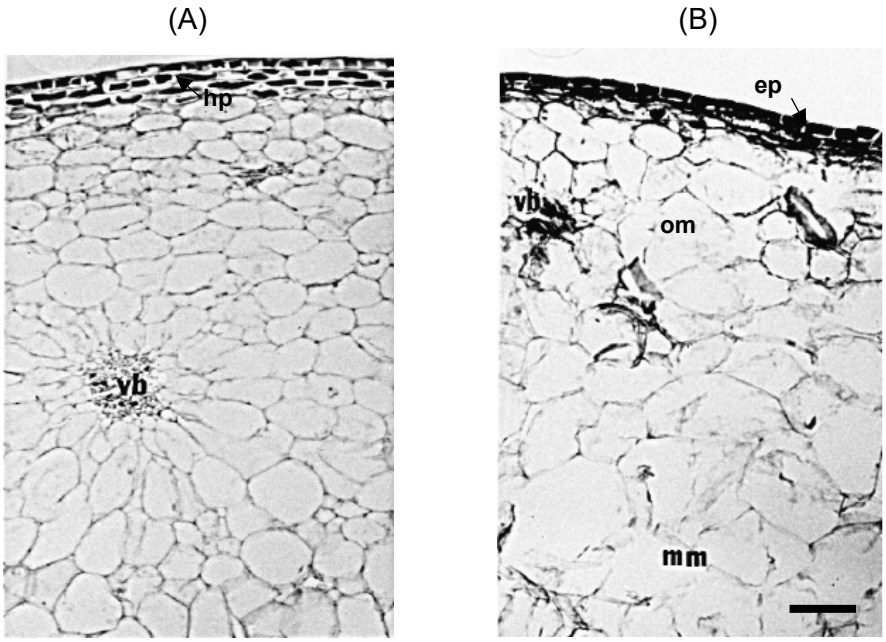

Figure 2.5. Median cross section of pollinated 'Beckyblue' blueberry fruit 24 days (A) and 72 days after flower bloom (fruit ripening) (B). Epidermis (ep); hypodermis (hp); outer mesocarp (om); middle mesocarp (mm) and vascular bundle (vb). Bar = 150 µm. Reprinted with permission from Copyright Clearance Center: Oxford University Press, Annals of Botany, Cell Number and Cell Size in Parthenocarpic vs. Pollinated Blueberry (Vaccinium ashei) Fruits. Cano-Medrano, R., & Darnell, R. L., 80(4), 1997. Copyright (1997).

Short sclereid cells are present in blueberries (Fava *et al.,* 2006). Sclereids are a reduced form of sclerenchyma cells also known as stone cells. Stone cells are dead cells with thick lignified secondary cell walls (Vicente *et al.,* 2007). Stone cells are often found throughout the mesocarp of blueberry fruits between 460 – 920 µm below the epidermis (Gough, 1993) and may contribute to blueberry fruit firmness. They bind neighbouring parenchyma cells strengthening surround cells, occurring singly, doubly or in clusters (Gough, 1993; Allan-Wojtas *et al.,* 2001; Fava *et al.,* 2006). During the ripening of blueberries the number of stone cells present in the mesocarp below the epidermis is not related to ripening of the fruit. The mean number of stone cells per green fruit ranged from 12 – 67 cells. Whereas for fully ripe blue fruit the mean number of stone cells varied from 4 – 23 cells (Blaker and Olmstead, 2014) (Table 2.3). According to Blaker and Olmstead (2014) there is no significant difference

between green and fully ripe blueberries with a standard texture. Indicating the production of stone cells is not related to blueberry ripening. However after harvest, during storage the number of stone cells in the parenchyma appears to increase (Allan-Wojtas *et al.,* 2001).

Table 2.3. Mean number of stone cells per fruit at the mature green and ripe blue stages of maturity for southern highbush blueberry genotypes with standard fruit texture (Blaker and Olmstead, 2014).

Genotype	Stone cells (no.)	
	Mature green	*Ripe blue*
FL06-245	67	22
'Raven'	51	23
'Windsor'	17	4
'Springhigh'	12	13

2.2.5 Cell wall degradation

During fruit ripening a coordinated series of modifications weaken the polysaccharide components of the primary cell wall and middle lamella. The primary cell wall and the middle lamella are responsible for intercellular adhesion and providing the cell with rigidity. An increase in cell separation, softening and swelling of the primary cell wall observed during fruit ripening is thought to be the result of polysaccharide degradation, and changes to the bonding between polymers in the cell wall (Brummell, 2006). This combined with a reduction in turgor pressure lead to a reduction of fruit texture which occurs with fruit ripening. Throughout ripening from the green to blue maturity stage an increase in the solubilisation of arabinose from pectin and hemicellulose has found to be a major cause of fruit softening (Proctor & Peng, 1989; Vicente *et al.,* 2007).

The middle lamella can be described as a pectin layer which cements plant cell walls together (Brett & Waldron, 1996). Throughout the ripening of many fruits the pectin present in the middle lamella has been found to undergo depolymerisation and solubilisation. Generally the content of soluble pectin increases over time with ripening. Indicating the solubilisation of pectin plays a role in the ripening and softening of fruits. Pectin solubilisation has been found to occur in blueberries from an unripe stage towards near blueberry fruit maturity. Soluble pectin can become insoluble due to crosslinking with divalent cations such as magnesium or calcium (Eskin 1979; Huber, 1983). This suggests that in a developing blueberry the pectin content reduces most likely due to this crosslinking mechanism (Proctor & Peng, 1989). During the ripening of 'Jersey' blueberries the pectin content was found to decrease on a dry weight basis, but a simultaneous increase in pectinmethlyesterase activity was also recorded (Woodruff *et al.,* 1960). Pectinmethlyesterase and polygalacturonase are enzymes that are both involved in the degradation of pectin. Even when no evidence of pectin depolymerisation

was detected, the activities of these enzymes peaked when blueberry fruit was at a mature red and blue colour stage. Indicating that pectin depolymerisation may also occur at an overripe and mature fruit development stage (Proctor & Miesle, 1991).

However more specifically with cell wall modifications occurring during ripening, blueberry fruit firmness has been shown to decrease consistently up to the blue ripe stage of fruit development (Vicente *et al.,* 2007). Therefore agreeing with previous research indicating the firmness of blueberries during ripening decreases between the green to blue development stage, with little subsequent change from this point onward to the over ripe purple maturity stage (Ballinger *et al.,* 1973, Proctor & Miesle, 1991). This indicates that fruit softening is associated with substantial arabinose loss, and hemicellulose depolymerisation during ripening. Also to a lesser extent at an overripe stage pectin depolymerisation. This is interesting as these changes associated with the blueberry cell wall during ripening are quite different than those that have been reported for other berries (Vicente *et al.,* 2007). Indeed fruit softening can be explained by a weakening of the pectin network in the cell wall due to the loss of arabinose. Whereas the structure of the cell wall is directly affected by hemicellulose and pectin depolymerisation, decreasing the mechanical resistance of cells (Brummell, 2006). Therefore both the weakening of the pectin network and a decrease in mechanical resistance are likely responsible for the decrease in texture characteristics of blueberry fruits.

2.2.5.1 Model for cell wall changes

A model for cell wall changes accompanying blueberry ripening and softening outlines five developmental stages measuring the colour of blueberries during ripening, the first being green and subsequently 25% blue, 75% blue, 100% blue and blue ripe was proposed by Vicente *et al.* (2007) (Figure 2.6). Both cell wall yield, and fruit firmness were found to decrease throughout ripening. Where the largest amount of fruit softening occurred between the green and 25% blue stages. It is thought that during this period fruit expansion and ripening are occurring concurrently. For cell wall changes accompanying a decrease in firmness, the model proposes that hemicellulose depolymerisation, hemicellulose solubilisation, arabinose solubilisation, pectin solubilisation as well as pectin depolymerisation are responsible for the majority of cell wall changes. Throughout the first four developmental stages hemicellulose depolymerisation and arabinose solubilisation are proposed to be occurring concurrently, whilst pectin solubilisation occurs in the first three developmental stages. Hemicellulose solubilisation is proposed to occur in immature green fruit, as well as mature blue ripe fruit. The proposed model also suggests that pectin depolymerisation occurs in overripe blue

blueberries. Arabinose loss, and depolymerisation of pectin and hemicellulose have been previously associated with fruit softening in tomatoes (Brummell, 2006) and peaches (Brummell *et al.*, 2004).

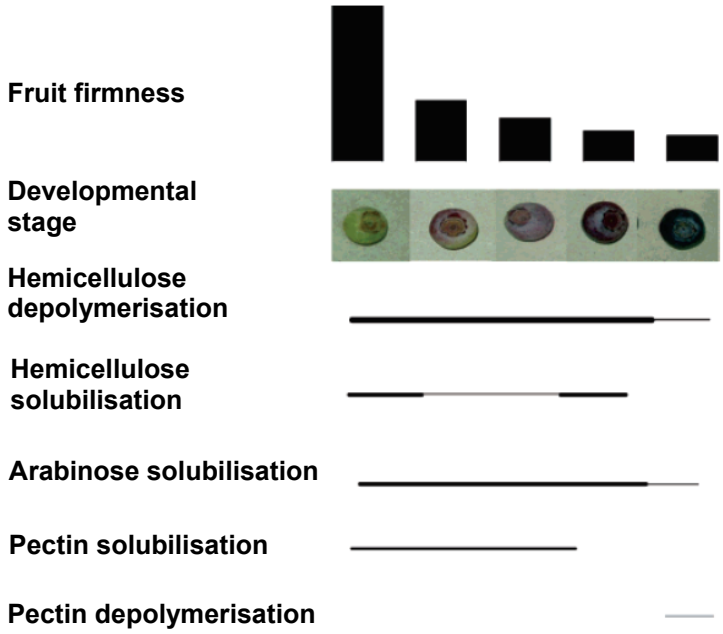

Figure 2.6. Cell modifications at different levels of firmness during blueberry development and ripening. Reprinted with permission from Vicente, A. R., Ortugno, C., Rosli, H., Powell, A. L., Greve, L. C., & Labavitch, J. M. (2007). Temporal sequence of cell wall disassembly events in developing fruits. 2. Analysis of blueberry (Vaccinium species). Journal of Agricultural and Food Chemistry, 55(10), 4125-4130. Copyright 2007 American Chemical Society.

2.2.6 Texture modifications

Blueberry fruit texture is related to cell wall structure and microstructural changes occurring during development and ripening (Giongo *et al.,* 2013). Degradation of the cell wall and middle lamella in addition to a decrease in total water soluble pectin is responsible for a loss in firmness during ripening (Jackman and Stanley, 1995; Chiabrando *et al.,* 2009). Texture depends on cell size and tissue layers during development, which often results in different parameters such as firmness, gumminess and juiciness being reported (Jackman and Stanley, 1995; Chiabrando *et al.,* 2009; Kilma-Johnson *et al.,* 2011; Giongo *et al.,* 2013). One of the most common parameters describing blueberry fruit texture during development is firmness. This parameter is affected by mostly cell size, connections between cells and the strength of cell walls (Vicente *et al.,* 2007).

Blueberries are a soft fruit, in which texture plays an important role in marketability. In general fruit texture is strongly associated with the concept of fruit quality and freshness (Saftner *et al.,* 2008). Texture often evolves from firm to soft throughout fruit development (Harker *et al.,*

1997). In ‘Brigitta Blue’ fruit development three major classes of texture variation was detected by Giongo *et al.* (2013). The first class of blueberries were the firmest (25.37 – 31.10 N) consisting of green blueberries. The second class contained slightly pink blueberries in which texture was measured as intermediate between the first class and third class of blueberries (18.21 – 25.11 N). Finally, the third class consisted of the softest stages of blueberry development (3.33 – 6.49 N) showing a reduction of ~ 90% in firmness compared to the previous stage. The third class consisted of blueberries from 50% blue colouration to blue ripe (Giongo *et al.,* 2013). This indicates during blueberry development the texture profile evolves from a hard fruit, to a soft fruit at maturity.

2.3 Fruit anatomy affecting moisture loss

Moisture loss can generate blueberry shrivelling and dehydration which may affect appearance, texture and consumer acceptability within the commercial supply chain (Ku *et al.,* 2000; Wills *et al.,* 2007). Up to 80 – 90% of fresh produce is water, therefore dehydration and shrivel are clear problems affecting shelf life and marketability (Wills *et al.,* 2007). Within literature, moisture loss from fresh produce in particular blueberries is normally quantified as weight loss (Paniagua, 2012). A major pathway for moisture loss in blueberries is through the stem scar, calyx, stomata and cuticle during postharvest storage (Figure 2.7). Minimisation of weight loss through these pathways is beneficial in increasing saleable weight, as just 5 – 8% moisture loss leads to excessive blueberry shrivelling within the market place (Sanford *et al.,* 1991; Forney *et al.,* 1998). The main anatomical features of blueberries affecting moisture loss are discussed in this section.

Blueberries are a simple fleshy fruit formed from the ovary of one flower, with seeds embedded in the flesh and are true berries, varying in size of up to 25 mm equatorial diameter (Gough, 1993). Blueberries vary in shape from simple round to oblate shape. Typically blueberries have a single layer epidermis with 27 – 91 stomata per mm^2 concentrated in the calyx end of the berry (Vega *et al.,* 1991). The surface of the epidermis is covered with a cuticle about 5 µm thick, which develops a waxy bloom during fruit ripening. Dependent on the stage of maturity and cultivar the amount of bloom varies. Pigments are found in the epidermal and hypodermal layers which are detached by a ring of vascular bundles from the rest of the cortex (Gough, 1993). Majority of blueberry flesh is white, where the fleshy mesocarp is predominantly homogenous parenchyma. Blueberries are harvested without their peduncle, therefore at the stem end of the fruit there is a stem scar (Gough, 1993; Ehlenfeldt *et al.,* 2002). A calyx scar is present at the blossom end of blueberries due to the abscission of the style and corolla resulting in a ring shaped scar (Ehlenfeldt *et al.*, 2002). Additionally, the area

to volume ratio of blueberries is an important characteristic affecting moisture loss, and correlates positively with increased weight loss (Vega *et al.,* 1991; Makus and Morris, 1993). The stem scar size is genetically controlled to a large extent. Therefore a small, and dry stem scar is desired to prevent moisture loss and infection. The stem scar is one of the points of entry for microorganisms and is associated with up to 90% of fruit decay (Gough, 1983). A stem scar can be cured in postharvest storage by a period of delayed cooling, reportedly decreasing decay in the fruit. Where a period of delayed cooling of 8 h at 18 ºC has been shown to stimulate stem scar curing, but the effect on moisture loss was not recorded (Mainland, 1995). The exposure of the stem scar allows water evaporation and is the primary pathway for moisture loss from blueberries during postharvest storage and handling (Perkins-Veazie *et al.,* 1995). According to Perkins-Veazie *et al.* (1995) the diameter of the stem scar correlates positively with weight loss in rabbiteye blueberries. Interestingly, a large blueberry fruit size does not necessarily mean a large stem scar size. A ratio of berry weight to stem scar size was adopted by (Sanford *et al.,* 1991), with a large value favourable for a berry with a high weight and small stem scar. However Ballinger *et al.* (1984) reported no relationship between stem scar and fruit size among various *Vaccinium* species. The diameter of the stem scar varies between species and cultivars. Stem scar diameter varied as much as 50% among southern highbush varieties ranging from 1.46 mm to 2.20 mm, and approximately 0.7 mm to 1.2 mm in rabbiteye blueberries (Perkins-Veazie *et al.,* 1995).

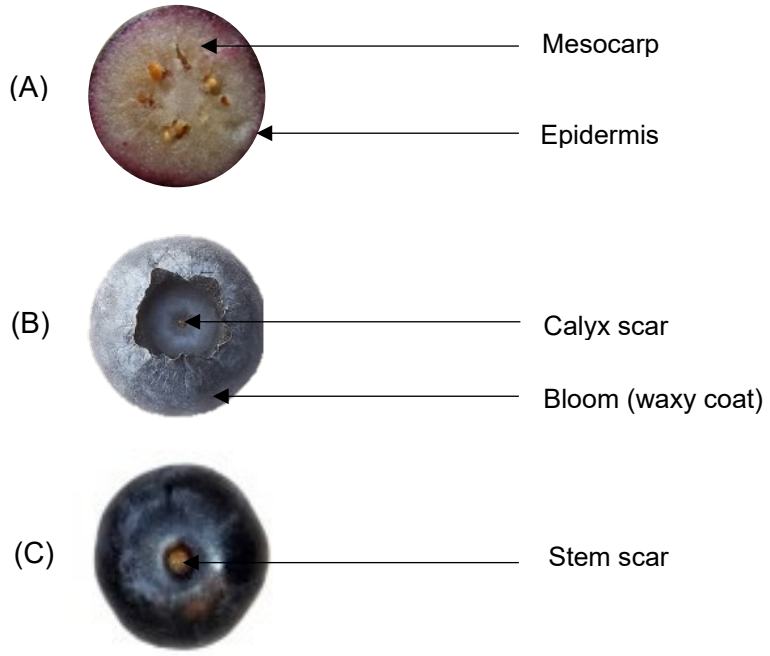

Figure 2.7. Typical ripe blueberry fruit anatomy shown in a transverse section (A), and in an intact berry (B,C). Adapted from Gough (1983) and Paniagua (2012).

The cuticle covers the surface of the epidermis and forms a continuous film composed of cutin in association with epicuticular waxes (Riederer and Schreiber, 2001). The main wax types are β-diketones representing 62% of total wax components. Other major components are triterpenoids and primary alcohols (Freeman *et al.,* 1979). This is the main physical barrier regulating the transpiration of blueberries (Wills *et al.,* 2007). During this process water is lost through the cuticle, water first diffuses as across the cuticle matrix as a liquid and is evaporated as a gaseous phase (Kerstiens, 1996). Epicuticular wax plays an important role in the control of moisture loss from the cuticle (Abrigio *et al.,* 1980; Sapers *et al.,* 1984; Vega *et al.,* 1991). Higher weight loss is observed in overripe blueberries resulting from the degradation of β-diketones which have a reduced portion of upright structures which tend to lose increasing amounts of water (Albrigo *et al.,* 1980). However Vega *et al.* (1991) reported no relationship between wax structural features in ripe blueberries and weight loss for multiple highbush cultivars during storage (Vega *et al.,* 1991).

2.4 Postharvest blueberry texture

Texture is the most important quality attribute influencing marketing and consumer acceptability of fresh blueberries (NeSmith *et al.,* 2000). Harvesting and handling practises can affect final blueberry quality in the marketplace (Appendix A). Blueberries which are hand harvested are firmer and retain quality characteristics during postharvest compared to other harvesting practises (Lobos *et al.,* 2014). During the postharvest chain, blueberries normally soften which compromises texture and quality in the marketplace (Ehlenfeldt and Martin, 2002). The quality of fresh fruit is affected by water content, biochemical constituents and cell wall composition. Changes in the primary cell wall components cellulose, pectin and hemicellulose occur during growth and development and are responsible for changes in texture (Jackman and Stanley, 1995). The postharvest life of fruit has been traditionally defined by firmness (Table 2.4), which may increase, decrease or remain unchanged during storage. The variation in texture may be attributed to cultivar differences or the interaction between berries and postharvest storage conditions (Forney *et al.,* 2003).

Evaluation of texture provides information on the storability and postharvest quality of the produce and is mostly used for research purposes (Chiabrando *et al.,* 2009). Chiabrando *et al.* (2009) suggests instrumental methods of evaluation are preferred to reduce variation among measurements and are precise, compared to sensory evaluations due to uncontrollable human factors. Most instrumental methods measure the force needed to compress, puncture or penetrate the fruit (Chiabrando *et al.,* 2009). However blueberry texture

is not easy to define as there is no standardised method. Therefore various techniques have been utilised in depth to determine postharvest blueberry quality.

2.4.1 Methodology to measure texture

The evaluation of blueberry postharvest texture has been described in terms of firmness, hardness, cohesiveness, gumminess, chewiness, springiness and resilience. Traditionally firmness has been measured by sensory evaluation where berries were individually rolled between thumb and index finger and then rated (Sanford *et al.,* 1991). Typically a 0 to 5 scale, or a hedonic scale has been used to measure berry firmness, with lower values indicating how depressed the berry surface was after touch, and higher values indicating berry firmness (Table 2.4) (Sanford *et al.,* 1991; Donahue and Work, 1998; Donahue *et al.,* 1998; Silva *et al.,* 2005). Instrumental compression methods such as the Instron (Instron Corporation, USA) FirmTech (BioWorks Incorporated, USA) and TA-XT (Stable Micro Systems Ltd, UK) instrumentation have been adopted by literature to measure the firmness of blueberries (Table 2.4). Instrumental methods provide accuracy and precision across multiple measurements. Using a 10 mm diameter cylindrical probe and a crosshead and chart speed of 50 mm min^{-1} and 5 N, the force required for the berry to release juice was measured by Silva *et al.* (2005) using an Instron machine to measure the firmness of blueberries during postharvest storage.

More popular instruments are the Firmtech I and FirmTech II which are commercially available instruments for fruit firmness testing, according to Li *et al.* (2011) Firmtech II is the *de facto* standard instrument for blueberry firmness in the industry. This instrument uses compression to measure fruit firmness by compressing each berry with a load cell, where force-deformation values (g mm^{-1}) are used to determine the firmness of each berry (Li *et al.,* 2011). Minimum and maximum force thresholds are determined before testing as well as cell velocity, where firmness values are reported as the force (N) to deflect the surface of the fruit 1 mm (Saftner *et al.,* 2008). There seems to be no standard minimum and maximum force thresholds used, where Li *et al.* (2011) used a 50 g (minimum) and 350 g (maximum) force of the load cell and Moggia *et al.* (2017) used a 15 g (minimum) and 200 g (maximum) force threshold to assess the harvest effects on blueberry texture during storage and postharvest softening in mechanically damaged and non-damaged highbush blueberries respectively.

Table 2.4. Selected instrumentation and methodology used in literature to measure the texture of blueberries.

Instrumentation	Manufacturer	Analysis	Parameters	References
FirmTech I	BioWorks Incorporated	Compression	Firmness	Forney *et al.* (2003) Donahue and Work (1998) Ehlenfeldt and Martin (2002)
FirmTech II	BioWorks Incorporated	Compression	Firmness	Li *et al.* (2011) Saftner *et al.* (2008) Moggia *et al.* (2017) NeSmith *et al.* (2000)
TA-XT2i	Stable Micro Systems	TPA	Hardness Cohesiveness Gumminess Chewiness Springiness Resilience	Chiabrando *et al.* (2009)
		Compression	Firmness	Schotsmans *et al.* (2007)
TA-XT *Plus*	Stable Micro Systems	TPA	Max force Max force strain Final force Area Force linear distance Gradient	Giongo *et al.* (2013) Hu *et al.* (2015)
		TPA	Hardness Cohesiveness Gumminess Chewiness Springiness	Zielinska *et al.* (2015)
		Compression	Firmness	Chen *et al.* (2015) Paniagua *et al.* (2013)
		Compression	Hardness	Jia *et al.* (2016)
TA-XT2	Stable Micro Systems	Compression	Firmness	Angeletti *et al.* (2010)
Instron Universal Materials Testing Machine	Instron Corporation	Compression	Firmness	Silva *et al.* (2005) Donahue and Work (1998)
Sensory Assessment	–	0 – 5 scale	Firmness	Sanford *et al.* (1991)
		9-point hedonic scale	Toughness Texture Texture	Silva *et al.* (2005) Donahue and Work (1998) Donahue *et al.* (1999)

2.4.1.1 Texture analyser

The TA-TX2i and TA-TX *Plus* are commonly used texture analyser machines, widely reported in literature for the measurement of blueberry texture. These texture analyser instruments can be used to measure firmness using a compression test, or to conduct a texture profile analysis (TPA), using a double compression cycle. Paniagua *et al.* (2013) used a TA-TX *Plus* machine to measure the firmness of 'Centurion' blueberries. A pre-test speed (0.8 mm s^{-1}), test speed

(1.6 mm s^{-1}) and trigger force (0.5 g) were chosen, and the peak force (N) necessary to compress the berry 1 mm was recorded, following similar methodology used in literature (Schotsmans *et al.,* 2007; Saftner *et al.,* 2008; Chiabrando *et al.,* 2009). Used in the measurement of firmness is a cylindrical aluminium probe varying in diameter from 5 mm (Chen *et al.,* 2015) to 25 mm (Paniagua *et al.,* 2013) and either a 2.5 kg (Schotsmans *et al.,* 2007) or a 5 kg (Paniagua *et al.,* 2013) load cell. However this is not widely reported in literature. Although this method for measuring blueberry firmness is fairly popular in research, there is no standardised commonly used method adopted by scientists and industry using the TA-TX instrumentation.

Another common analysis conducted using the TA-TX2i and TA-TX *plus* is a texture profile analysis (TPA). This is a popular double compression test for determining the textural properties of foods. During a TPA test the sample is compressed twice using the texture analyser to provide insight into how samples behaved when chewed (Texture technologies, 2018). TPA can quantify multiple textural parameters in just one experiment. Commonly reported parameters in the measurement of postharvest blueberry texture include, hardness, gumminess, cohesiveness, chewiness, springiness and resilience (Chiabrando *et al.,* 2009; Hu *et al.,* 2015; Zielinska *et al.,* 2015). Typical TPA settings previously used in literature to measure blueberry texture are presented in Table 2.5.

Table 2.5. TPA settings for texture analysis of blueberries reported in literature.

Machine	TPA Settings							References
	Pre-test speed (mm s^{-1})	*Test speed (mm s^{-1})*	*Post-test speed (mm s^{-1})*	*Strain*	*Time between cycles (s)*	*Load cell (kg)*	*Probe (mm diameter, flat and cylindrical)*	
TA-XT2i	–	0.08	–	30 %	10	5	35	Chiabrando *et al.* (2009)
TA-XT *Plus*	–	1.7	5	90%	–	5	4	Giongo *et al.* (2013)
	1.6	0.8	2	30%	10	50	50	Hu *et al.* (2015)
	0.33	0.33	0.33	40%	1	50	75	Zielinska *et al.* (2015)

A force-time curve is generated from the TPA (Figure 2.8), where the desired texture parameters can be calculated. Hardness is the peak force during the first compression and is typically the point of deepest compression. Fracturability is the force at the first peak and is

not characteristically seen in blueberries, where cohesiveness is the area of work during the second compression divided the area of work in the first compression and is how well a product withstands a second deformation. Springiness is recorded as a percentage of the berries original height and describes how well the berry springs back after it has been deformed. Gumminess is calculated from hardness and cohesiveness which is only applicable to semi-solid products. Whereas chewiness is only applicable to solid products. Resilience is recorded in blueberries and is how well the berry fights to regain its original position and can be measured in the first compression, but the test-speed of the compression and withdrawal must be the same (Table 2.6) (Breene, 1975; Chiabrando *et al.,* 2009; Rosenthal, 2010; Alvarez *et al.,* 2012; Chen and Stokes, 2012).

Table 2.6. Sensory and instrumental definition of texture parameters from texture profile analysis (TPA).

Parameter	Sensory Definition	Measured on Force-time Curve
Hardness	Maximum force required to compress sample between molars.	Maximum force of first compression (P_1).
Springiness	Height recovered by sample during the time between the first and second bite.	Distance 1 (D_1) / Distance 2 (D_2)
Cohesiveness	Represents the strength of internal bonds that comprise sample.	(A2 + A2W)/(A1 +A1W)
Gumminess	Force necessary to chew a semisolid food until ready for swallowing.	Hardness x Cohesiveness
Chewiness	Energy necessary to chew a semisolid food until ready for swallowing.	Hardness x Cohesiveness x Springiness
Resilience	Represents how well a sample succeeds in gaining its original position.	A_{1W}/A1
Fracturability	Force in which the sample fractures.	Peak Force of Fracture
Adhesiveness	Ability of food to stick to teeth when chewed.	Area when force is <0 g

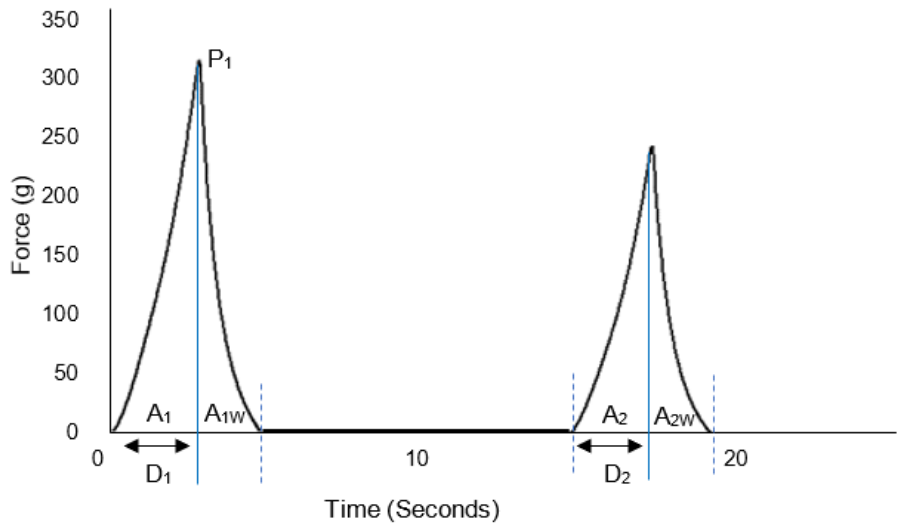

Figure 2.8. Typical force-time curve generated from TPA using a texture analyser.

2.4.2 Moisture loss and texture

The loss of water from fruit and vegetables affects postharvest texture characteristics as a result of decreased quality and firmness due to a loss of turgor (Paull, 1999; Ku *et al* 2000; Wills *et al.,* 2007). Water is attracted into cells producing a turgor pressure within the plasma membrane which is contained by the cell wall, providing physical support to fruit tissues (Taiz and Zeiger, 2010). Postharvest blueberry softening during storage is thought to be induced by turgor loss, however this particular parameter has not been directly evaluated (Forney *et al.,* 1998; Allan-Wojtas *et al.,* 2001, Chiabrando and Giacalone, 2011; Paniagua *et al.,* 2013).

Weight loss during storage has shown interesting correlations with texture parameters (Table 2.7). Chiabrando *et al.* (2009) found that weight loss of >6% in 'Bluecrop' and 'Coville' blueberries correlated with a 19.7% and 54.1% increase in hardness and a 12.4% and 37.4% increase in gumminess respectively after 35 days storage (0 ºC). Where a 24.1% and 18.9% decrease was seen in springiness for 'Bluecrop' and 'Coville' berries respectively (Chiabrando *et al.,* 2009). Interestingly, at >6% weight loss level a decrease of 13.8%, and an increase of 11.7% in chewiness was observed for 'Bluecrop' and 'Coville' blueberries, indicating that cultivar differences could be responsible for different textural parameters obtained after 35 d storage at 0 ºC (Chiabrando *et al.,* 2009). Resilience, springiness and cohesiveness showed negligible differences when weight loss was at >6%. Increased weight loss correlations with texture parameters in blueberries from TPA have not previously been recorded in literature.

Table 2.7. Weight loss of selected blueberries stored in different atmosphere conditions in literature.

Methodology	Cultivar	Time (days)	Temperature (ºC)	Atmosphere conditions	Weight loss (%)	Reference
$(W_i - W_f)/W_i \times 100$	'Bluecrop' 'Coville'	35	0	3.5 g H_2O kg^{-1} air	6.68 6.20	Chiabrando *et al.* (2009)
	'Centurion' 'Maru'	42	1.5	20.1 kPa O_2 + 0.03 kPa CO_2	>15 >15	Schotsmans *et al.* (2007)
	'Centurion' 'Maru'	42	1.5	2.5 kPa O_2 +15 kPa CO_2)	>20 >20	
	'O'Neal' 'Bluecrop'	21	2	–	>6 >5	Angeletti *et al.* (2010)
	'Berkeley'	50	0	–	6 – 13	Jia *et al.* (2016)
	'Centurion'	21	4	0 mL min^{-1} 15 mL min^{-1} 30 mL min^{-1} 60 mL min^{-1}	>1 >7 >9 >15	Paniagua *et al.* (2013)
	'Lateblue'	25	0	90 – 95 % RH	3.49	Chiabrando and Giacalone (2011)

Weight loss has been found to correlate with blueberry firmness. Ferraz *et al.* (2001) found that weight loss between 4 – 7% in rabbiteye blueberries correlated with decreasing firmness from 1.28 – 0.65 N after four weeks storage at 1 ºC, whereas weight loss between 1 – 9% correlated with greater firmness loss from 3 – 33% in rabbiteye blueberries during 4 d storage at different temperatures (Tetteh *et al.,* 2004). Very low levels of moisture loss have been found to generate increased blueberry firming rather than softening. Paniagua *et al.* (2013) observed an increase in blueberry firming with low levels of weight loss (0.22 – 1.34%), whereas extensive softening was obtained with higher weight loss (3.47 – 15.06%). Similarly, Miller *et al.* (1993) showed an increase in sensory firmness of ripe rabbiteye and highbush blueberries when weight loss <1% after 3 weeks storage at 1 ºC, whereas berry softening correlated with 4 – 5% weight loss. In agreement with Paniagua *et al.* (2013) and Miller *et al.* (1993), a weight loss of 1 -2% correlated with 50% and 80% berry firming in highbush blueberries after 3 and 9 weeks storage respectively at 3 ºC, where fruit softening increased when weight loss was between 4 – 14% (Forney *et al.,* 1998). It is likely weight loss ≤1.34% generates localised dehydration in the outer cell layers of blueberries leading to an observed increase in firmness (Paniagua *et al.,* 2013).

Increased fruit shrivel is an expected outcome of increased fruit moisture loss. The quality of fresh fruit can be affected by just 5% moisture loss generating a shrivel (Wills *et al.,* 2007). Accordingly Paniagua *et al.* (2013) demonstrated blueberry shrivel occurred with berry moisture loss ≥8.7% and was not observed with moisture loss ≤6.9% indicating the point at which shrivelling occurs in ‘Centurion’ blueberries is between these two moisture loss values. In agreement the shrivelling of ‘Centurion’ and ‘Maru’ blueberries has been shown to occur with weight loss levels approximately 8% and higher (Schotsmans *et al.,* 2007). In ‘Burlington’ blueberries Forney *et al.* (1998) correlated shrivel with weight loss of 5% and higher. Likewise, with weight loss levels ≥8% wild lowbush blueberries are known to shrivel (Sanford *et al.,* 1991).The relationship between blueberry moisture loss and postharvest texture is an important factor in determining blueberry quality, where further research could provide enhanced information for the postharvest management of blueberries.

2.4.3 Influence of microstructure on postharvest texture

The blueberry skin, comprises of a single layer of cells known as the epidermis which forms a tough tissue, providing resistance against mechanical damage (Allan-Wojtas *et al.,* 2001; Fava *et al.,* 2006). According to Jackman and Stanley (1995), the thickness and toughness of the epidermis affects overall fruit texture. When different fruit flesh texture attributes are assessed, the epidermis may produce similar firmness outputs (Jackman and Stanley, 1995).

For up to twelve different highbush and rabbiteye cultivars, Saftner *et al.* (2008) evaluated skin toughness by a sensory panel and determined skin toughness did not correlate with firmness in ripe blueberries. In contrast, the skin toughness of ripe highbush blueberries correlated with berry firmness after 6 weeks storage at 5 ºC (Bunemann, 1957). Similarly, Silva *et al.* (2005) determined skin toughness by puncture tests, and firmness by a compression test indicating rabbiteye blueberries have a tougher skin than highbush blueberries in agreement with Makus and Morris (1993).

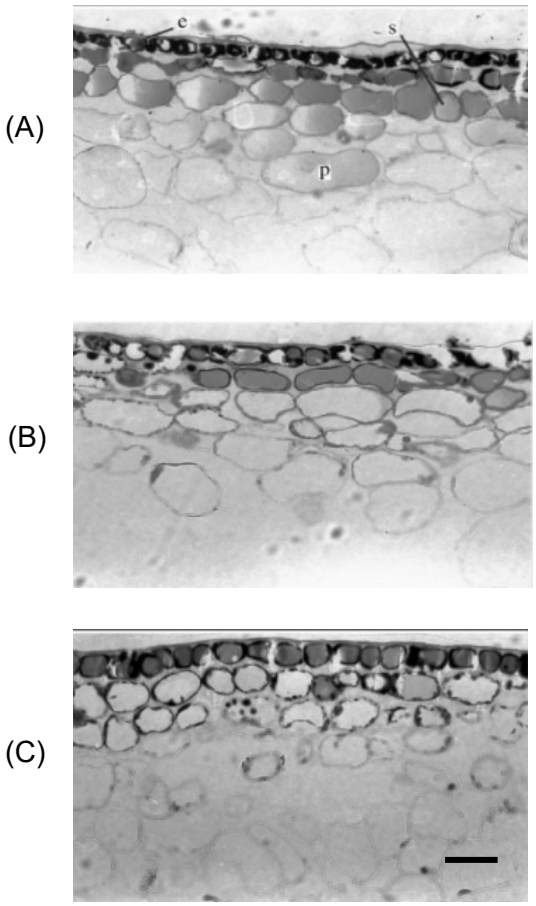

Figure 2.9. The microstructure of 'Burlington' blueberries. (A) Fresh berry (no storage) pigment is present in two epidermal layers and one layer of the sub epidermis. Cells are closely associated with each other and are elliptical in shape. (B) Berry stored in air for 3 weeks at 0 ºC. Pigment is present in only one epidermal layer and one sub-epidermal layer. Increased cellular space between cells, less contact with neighbouring cells. Irregularly shaped cells with breaks in walls. (C) Berry stored in air for 6 weeks at 0 ºC. Pigment is present in only one epidermal layer. Epidermal cells have changed in shape from rectangular to circular. Sub-epidermal and parenchymal cells are round maintaining close cell contact. Epidermis (e); parenchyma (p); sub-epidermis(s). Bar = 100 µm. Reprinted from LWT – Food Science and Technology, 34(1), P.M. Allan-Wojtas,C.F. Forney,S.E. Carbyn,K.U.K.G. Nicholas, Microstructural Indicators of Quality-related Characteristics of Blueberries – An integrated Approach, Page 28, Copyright (2001), with permission from Elsevier.

Postharvest firming and textural changes during postharvest storage have been associated with cell wall modifications. The thickening of the parenchyma cell wall during storage of 'Burlington' blueberries was microscopically observed by Allan-Wojtas *et al.* (2001) after 6

week storage at 0 ºC and was associated with the firming of blueberries during postharvest storage. Although these results have not been repeatable under similar conditions. Corrugation of epidermal and hypodermal cell walls was observed in blueberries during 8 weeks storage in air at 10 ºC and was related to an increase in firmness detected by a sensory panel (Bunemann *et al.,* 1957). Whereas the reduced water content of the epidermal area could be related to an increased firmness and may be associated with the corrugation and thickening of epidermal (Bunemann *et al.,* 1957) and parenchyma cells (Allan-Wojtas *et al.,* 2001). The microstructure of 'Burlington' blueberries after 3 and 6 weeks storage in air at 0 ºC was reported by Allan-Wojtas *et al.* (2001), where a decrease in pigment, increase of cellular space and a change in the shape of epidermal cells from rectangular to round was observed (Figure 2.9). Other cellular characteristics such as the collapse of parenchyma cells, and loss in cell to cell adhesion within the mesocarp, and visible signs of dehydration have been microscopically observed in 'Burlington', 'Coville' and 'Elliot' blueberries stored in air for 6 weeks which may help to explain a decrease in observed firmness throughout postharvest storage.

2.5 Blueberry storage conditions and texture

The relationship between air and water vapour based on the thermodynamic and physical properties of moist air is described by psychrometrics (Thompson, 2002) and can be explained by analysis of the processes involving air humidity gradients, and parameters such as temperature, RH, dew-point temperature and wet-point temperature. Psychrometrics are important in understanding moisture loss and management conditions generated within the horticultural supply chain (Appendix B) (Grierson and Wardowski, 1975). According to Talbot and Baird (1991) the application of these concepts in the storage of fresh produce is often insufficient, leading to limitations in the efficacy of produce storage and quality. This is seen by significant losses of fresh produce in the marketplace.

Controlled atmosphere (CA) is widely used to manipulate the postharvest environment of fresh produce to increase storability throughout the postharvest chain. Used in conjunction with refrigerated conditions, it can be utilised in storage facilities and throughout freight transportation (Wills *et al.,* 2007). The gas composition of CA is manipulated by increasing CO_2 and decreasing O_2 concentrations creating atmospheric conditions to inhibit ethylene production and to inhibit decay. According to Forney (2009) when combined with optimal temperatures CA can increase a berries shelf life by double or triple. CA is a widely adopted practice in horticultural and is beneficial to prolonging the shelf life of blueberries.

It is recommended blueberries are stored at low temperatures (0 ºC), and at 90 – 95% RH in order to reduce moisture losses (Perkins-Veazie, 2004; Mitcham *et al.,* 2011). These conditions result in less wilting, increased storage life and greater turgor (Wills *et al.,* 2007). As low RH conditions can to lead to excessive fruit dehydration and shrivelling. However high RH conditions are ideal for pathogen development which is an important consideration for degradation of quality. In this section psychrometric concepts are outlined to provide understanding behind the moisture of fresh blueberries, as well as standard storage and temperature conditions.

2.5.1 Relative humidity (RH)

To prevent weight loss and shrivelling in blueberries it is recommended to keep RH within the range of 90 – 95% during transportation and storage, in conjunction with low storage temperatures close to 0 ºC (Perkins-Veazie, 2004; Mitcham *et al.,* 2011). High RH conditions are optimal growing conditions for infection and spore germination of the main postharvest blueberry pathogens (Snowdon, 1990; Ramsdell, 1995). Therefore it is important the fruit is stored at low temperatures. The major reason for fruit losses along blueberry supply chains is inadequate management of humidity and temperature conditions, leading to microbial spoilage (Gough, 1994). Consequently, appropriate management of humidity conditions during the postharvest chain of fresh blueberries is imperative to maintain blueberry quality and texture. Special attention must be paid to the regulation and control of RH when comparing different studies in terms of moisture and texture changes.

The manipulation of RH within a closed flow through system was devised by Paniagua *et al.* (2013) whilst maintaining all other conditions (temperature, oxygen and carbon dioxide concentration), by adjusting the rate of air through the system. The faster the airflow the larger the driving force for water loss (lower humidity) and hence a faster water loss from blueberries was observed, compared to a slower airflow where the driving force for water loss (higher humidity) was slower where minimal moisture loss was recorded from blueberries subjected to these conditions (Paniagua *et al.,* 2013). Blueberries were stored for 21 d at 4 ºC, with weight loss of ~15% and ~10% recorded for 60 and 30 mL min^{-1} (low humidity conditions) treatments respectively. Whereas in high humidity conditions, weight loss of ~6% and ~1% was observed for blueberries stored in 15 and 0 mL min^{-1} conditions (Paniagua *et al.,* 2013). Similarly, in high humidity conditions (90 – 95% RH) minimal weight loss 3.49% was reported by Chiabrando and Giacalone (2011) in 'Lateblue' blueberries stored at 0 ºC for 25 d. In literature a lack of low humidity storage conditions exploring the effects on blueberries has

been investigated. While, closed airflow systems altering the RH atmosphere of blueberries have not previously been reported in literature.

According to Ehlenfeldt (2002) and Forney (2009), along the blueberry supply chain fluctuations in temperature and RH management favour condensation, which may enhance the growth of pathogens on the surface of blueberry skin. Different conditions reported in literature such as pathogen species inoculated, cultivar resistance and incubation period may be responsible for differences in the growth of pathogens. For example Cline (1997) reported greater development of a pathogen on wet berries than dry berries after 7 d storage at 21 ºC, whereas Cappellini *et al.* (1983) reported condensation having no effect on blueberry decay after removing berries from storage at 2 ºC to 21 ºC for four days. Therefore, it is unclear whether condensation on the skin of blueberries favours the consistent growth of pathogens and may be the result of cultivar differences.

'Bluecrop' and 'Coville' blueberries were stored under regular atmosphere conditions, with a RH of 90 – 95% for 35 d at 0 ºC (Chiabrando *et al.,* 2009). A weight loss of 6.68% and 6.2% for 'Bluecrop' and 'Coville' blueberries was recorded respectively, where an increase in hardness and gumminess, and a decrease in cohesiveness and chewiness was observed after 35 d storage. The texture of blueberries stored in different RH conditions is under reported in literature, with humidity storage conditions not regulated.

2.5.2 Temperature

Temperature is the main factor limiting the lifespan of fresh blueberries, and influences decay and the growth rate of postharvest pathogens, as well as influencing the concentration of antimicrobial compounds in fresh produce (Barkai-Golan, 2001). To prevent deterioration and to increase the lifespan of blueberries, refrigerated storage is recommended at 0 – 5 ºC to maintain optimal commercial quality (Kader, 2003; Perkins-Veazie, 2004; Mitcham *et al.,* 2011). In highbush blueberries this temperature can maintain acceptable commercial quality for up to two weeks, whereas in rabbiteye blueberries quality can be maintained for up to four weeks. The rate of respiration, ethylene production and enzymatic reactions responsible for major quality attributes such as texture and pigmentation is regulated by temperature. Low temperature storage conditions delay physiological spoilage and enhance the shelf life of fresh produce, whereas increased storage temperatures increase spoilage and result in large quantities of produce neglected in the market due to decay (Cappellini and Ceponis, 1977; Ehlenfeldt, 2002; Forney, 2009). Temperature influences the moisture gradient between the produce and external atmosphere, determining the capacity of air to retain water and governs

the rate of moisture loss from fresh produce (Taiz and Zeiger, 2010). Therefore to extend postharvest shelf life of blueberries and fresh produce refrigerated storage is a primary requirement.

Storage temperature can highly influence blueberry respiration rate. Where Angeletti *et al.* (2010) reported the respiration rate of 'Bluecrop' and 'O'Neal' blueberries as 3.0 mmol CO_2 kg^{-1} h^{-1} after 23 d storage at 2 ºC. Where CO_2 production (0.06 and 0.21 µmol kg^{-1} s^{-1}) was higher at increased temperatures 10 and 20 ºC respectively (Mitcham *et al.,* 2011), and decreased even further to 0.02 µmol kg^{-1} s^{-1} at 0 ºC. Therefore, to reduce respiration and slow down ripening, low storage temperatures are required. Boyette (1993) discovered blueberries produce a considerable amount of heat during storage, which may lead to an increase in storage temperature and increased metabolic ripening reactions. Therefore temperature management of blueberries throughout the supply chain is very important to increasing the shelf life and quality of blueberries.

2.5.2.1 Temperature effects on moisture loss

Higher storage temperatures increase the rate of moisture loss from fresh blueberries and produce, compared to lower storage temperatures (Talbot and Baird, 1991). Moisture loss is a physically driven phenomenon and follows a linear increase during storage when temperature and RH are kept constant (Nunes *et al.,* 2004). Variations of temperature at low storage temperatures (0 – 4 ºC) do not seem to generate large differences in moisture loss (Borecka and Pliszka, 1985; Forney *et al.,* 1998), whereas large variations in temperature (4 – 32 ºC) tend to produce increased rates of blueberry fruit moisture loss (Tetteh *et al.,* 1994; NeSmith *et al.,* 2005). Paniagua (2012) suggests RH conditions such as regulation and control need to be carefully considered when comparing literature in terms of moisture loss and temperature influences.

When RH is kept constant between 95 – 100% a narrower range of weight loss is seen in blueberries which are held at similar temperatures, where weight loss only increased 5.9% between blueberries stored at 15 ºC and 20 ºC after 12 d storage (Nunes *et al.,* 2004). Similarly, Tetteh *et al.* (2004) obtained a weight loss of 4% and 9% in rabbiteye blueberries in fixed humidity conditions (95% RH) after 3 d storage at 4 ºC and 21 ºC respectively. A progressive weight loss increase was observed by Sanford *et al.* (1991) where weight loss between 5.3 – 17.1% was recorded in blueberries stored for 14 d at temperatures between 0 – 20 ºC.

Table 2.8 Effect of storage conditions on blueberry weight loss.

Cultivar	Time (days)	Temperature (ºC)	Weight loss (%)	Atmosphere conditions	Reference
'Herbert' (Highbush)	22	0 2	0.3 1.4	RH not reported. Fruit covered with plastic bag.	Borecka and Pliszka (1985)
Wild genotype (Lowbush)	14	0 5 10 20	5.3 7.6 12.8 17.1	RH varied from 80% – 50% at 0 and 20 ºC.	Sanford *et al.* (1991)
'Burlington' (Highbush)	21	0 3	*NS*	95 – 85% RH fruit placed inside clamshell and plastic bag.	Forney *et al.* (1998)
'Tifblue' (Rabbiteye)	3	4 21	4.0 9.0	95% RH controlled by computer.	Tetteh *et al.* (2004)
'Patriot' (Highbush)	12	0 5 10 15 20	1.8 2.4 2.5 3.4 3.6	95 – 100% RH fruit placed inside clamshell and plastic bag.	Nunes *et al.* (2004)
'Climax' (Rabbiteye)	7	1 12 22 32	1.5 3.4 5.9 15.1	>90% RH fruit placed inside clamshell and plastic bag	NeSmith *et al.* (2005)
'Tifblue' (Rabbiteye)	7	1 12 22 32	0.3 0.8 4.7 8.4		
'Brightwell' (Rabbiteye)	7	1 12 22 32	0.2 1.2 3.0 10.1		
'Powderblue' (Rabbiteye)	7	1 12 22 32	1.3 2.9 4.7 20.2		
'Bluecrop' (Highbush)	21 28 35	0	4.1 5.4 6.7	90 – 95% RH fruit placed inside clamshells.	Chiabrando *et al.* (2009)
'Coville' (Highbush)	21 28 35	0	4.0 5.2 6.2		
'Bluecrop' (Highbush)	7 14 21	2	≈1.5 ≈5.0 ≈5.5	RH not regulated.	Angeletti *et al.* (2010)
'O'Neal' (Highbush)	7 14 21	2	≈1.5 ≈3.5 ≈5.5		
'Lateblue' (Highbush)	7 14 21 28 35	0	0.9 1.56 2.8 2.7 3.5	90 – 95% RH fruit placed inside clamshells.	Chiabrando and Giacalone (2011)
'Centurion' (Rabbiteye)	7 14 21	4	≈0.1 ≈0.1 ≈1.0	0 mL min^{-1} Exact RH values not recorded.	Paniagua *et al.* (2013)
	7 14 21	4	≈1.0 ≈3.0 ≈7.0	15 mL min^{-1} Exact RH values not recorded.	
	7 14 21	4	≈1.0 ≈4.0 ≈10.0	30 mL min^{-1} Exact RH values not recorded.	
	7 14 21	4	≈3.5 ≈9.0 ≈15.0	60 mL min^{-1} Exact RH values not recorded.	
'Berkeley' (Highbush)	50	0	12.7	RH not regulated	Jia *et al.* (2016)

NS: non-significant

NeSmith *et al.* (2005) also reported an increase in progressive weight loss from 1 – 32 ºC with weight loss values between 0.2 – 20.2% for rabbiteye blueberries after 7 d storage. Interestingly differences between rabbiteye cultivars were thought to be responsible for differences in weight loss values across the temperature range (NeSmith *et al.,* 2005) (Table 2.8) The highly variable weight loss reported across literature at different temperature conditions can be explained by cultivar differences and storage conditions.

2.5.2.2 Temperature effects on texture parameters

The texture of fresh produce is affected by turgor, cell wall composition, biochemical constituents and cellular organelles which can have a profound effect on changes in texture during postharvest storage (Jackman and Stanley, 1995). The water content of blueberry fruit seems to be a significant factor affecting postharvest texture evolution. The relationship between postharvest texture and weight loss is influenced by storage temperature conditions, with increased storage temperatures leading to increased berry degradation compared to decreased storage temperatures (Sanford *et al.,* 1991; NeSmith *et al.,* 2005). NeSmith *et al.* (2005) reported firmness losses ranging from 3.4 – 63.8% after 7 d storage between 1 – 32 ºC in rabbiteye blueberries. A progressive decrease of berry firmness as temperature increases, accompanied by a simultaneous increase in weight loss was seen by Sanford *et al.* (1991). Compression (N) of wild lowbush blueberries decreased 62% after storage at 20 ºC compared to 0 ºC for 7 d, with a subsequent increase in weight loss from 5.3% at 0 ºC to 17.1% at 20 ºC (Sanford *et al.,* 1991). Therefore an increase in blueberry weight loss, has been found to correlate with a reduction in firmness and blueberry texture.

In addition, a loss of 20% and 33% firmness of rabbiteye blueberries after 4 and 21 ºC storage respectively was obtained by Tetteh *et al.* (2004) and correlated with an increase of weight loss. Forney *et al.* (1998) did not report any differences in texture and weight loss when ‘Burlington’ blueberries were stored at 0 ºC and 3 ºC for 21 d under constant RH conditions (95 – 100% RH) however decayed fruit was not removed before compression assessment, and these results may reflect differences in decay rather than the firmness of non-decayed fruit. In later research Forney (2009) detected slight variations in the firmness of ‘Bluecrop’ blueberries after storage between 0 – 30 ºC after 8 d storage in constant RH conditions. In addition, Chiabrando *et al.* (2009) reported an increase in hardness and gumminess after 35 d storage at 0 ºC in 90 – 95% RH. Whereas Giongo *et al.* (2013) indicated a wide variation in the texture parameters on 49 blueberry cultivars and selections after 60 d storage at 4 ºC with 85% RH. The direct comparison of texture parameters gained from TPA stored at different

temperatures over the same storage period and conditions have not yet been evaluated in blueberries.

2.6 Non-destructive cellular level imaging (OCT)

Optical coherence tomography (OCT) is an optical, non-destructive, contactless, high resolution imaging method that performs high-resolution, cross sectional, subsurface tomographic imaging of the microstructure in materials and biological systems by measuring back scattered infrared light (Testoni, 2007). First introduced by Huang *et al.* (1991) OCT imaging has numerous features that make it attractive for a wide range of applications. OCT is an emerging non-destructive imaging technique that can be used on the same sample throughout postharvest storage and along the supply chain to track cellular level changes, that may be related to textural changes.

According to Testoni (2007) the physical principal of OCT is similar to that of B-mode ultrasound imaging. Instead of sound waves being measured, echo time delay and the intensity of back-scattered infrared light is measured. Compared to available high frequency ultrasound imaging, OCT imaging can yield an axial and lateral spatial resolution which is 10- to 25-fold better (Testoni, 2007). With the advantage of high resolution, OCT imaging is capable of revealing delicate biological structures where ultrasound imaging is not. Additionally, the depth of penetration of OCT imaging is approximately 1 – 3 mm, depending on the probe used and its depth of focus, tissue structure and the degree of pressure applied to the surface of the tissue (Testoni, 2007). The OCT penetration depth in combination with resolution provides similar results to those of standard histopathology and excisional biopsy, without the need to remove and destroy tissue specimens. In recent years, OCT technology has evolved to a new diagnostic modality in medical practice with a wide spectrum of clinical applications including optometry, gastrointestinal tract and pancreaticobiliary ductal system. Although, more recently investigation into the viability of OCT used in horticulture settings to predict the quality of fresh produce has yielded interesting results.

2.6.1 Principles of operation

The heart of a classic OCT system is based on the fibre optic Michelson interferometer, which is illuminated by a low coherence length light source (750 – 1300 nm wavelength) from a super luminescent diode (SLD) (Huang *et al.,* 1991). The wavelength range used for OCT is dependent on the specimen and is selected for high penetration into the sample. In the interferometer, the beam from the light source is focused onto a beam splitter that divides the

light into a reference and sample arm. The tissue specimen is placed on the 'sample arm' where the interferometer illuminates the tissue and collects the back-scattered light. The light reflected or back-scattered from inside the specimen is measured by comparing with light that has travelled a known reference path. This is known as the 'reference arm' of the interferometer where light travels a fixed distance and is scanned as a function of time. The light reflected from the specimen and the reference beam are combined at a detector where the interference between the two beams is measured (Testoni, 2007). Interference of the optical fields only occurs when the coherence length of the light source is matched to both beam lengths of the reference and samples arms (Fiakkou, 2015). As determined by the reference path lengths the interference signals provide information about the specimen at a particular depth.

At different transverse positions performing multiple axial measurement of back-scattered light by scanning the optical beam, cross-sectional two-dimensional (2D) images of specimen microstructure is constructed (Testoni, 2007). For 2D images, the light beam is scanned laterally in one dimension, but for 3D measurements 2D scanning across both lateral directions is required (Li *et al.,* 2015). A- and B- scans can be classified as single depth scans and cross-sections, respectively. To allow display, processing and quantification of OCT images, sophisticated image processing software such as ImageJ (Schindelin *et al.,* 2012), Aviso® (Visualisation Sciences Group, France), or Matlab® (Mathworks Inc., USA) are normally required to extract information. The resulting OCT datasets represent the displayed image as either a grey-scale or false-colour image (Testoni, 2007).

2.6.2 Resolution

The axial or depth resolution determines how detailed structures can be resolved in the depth direction. The axial resolution is determined by the coherence length of the light source which is inversely proportional to the spectral bandwidth. Therefore the resolution is only limited by the coherence of the light source and can maintain a high depth resolution (Huang *et al.,* 1991). The axial resolution, half of the coherence length (l_c), can be defined by the full width half maximum (FWHM, $\Delta\lambda$) and the centre wavelength (λ_0) of the optical source (Eq. 2.1) (Fercher, 1996; Fercher *et al.,* 2003; Lee *et al.,* 2009). A better in-depth resolution can be achieved from a shorter coherence length of the light source. Subsequently the image penetration depth is determined by the specimen surface absorption and scattering properties (Testoni, 2007).

$$l_c = \frac{2\ln 2}{\pi} \times \frac{{\lambda_0}^2}{\Delta\lambda} \quad \text{(Eq. 2.1)}$$

The transverse or lateral resolution is determined by the spatial width of the scanning beam (Testoni, 2007), and makes possible 2D imaging (Huang *et al.,* 1991). In others words the transverse resolution is determined by the ability to focus the beam and how light is directed and collected from the specimen (Brezinski, 2006). The same methodology is used in conventional microscopy (Fujimoto *et al.,* 2000). Therefore transverse resolution (Δx) can be defined where *f* is the focal length, and *d* is the spatial width of the scanning beam (Eq. 2.2).

$$\Delta x = \frac{4\lambda}{\pi} \times \frac{f}{d} \quad \text{(Eq. 2.2)}$$

A high transverse resolution is desirable and can be obtained by focusing the scanning beam to a small spot size, and by using a large numerical aperture (Fujimoto *et al.,* 2000). In addition, the transverse resolution is also related to the depth of focus. By increasing the transverse resolution a decrease in the depth of focus can be achieved. Importantly, the signal to noise ratio can be calculated (Eq. 2.3). The reflected or back scattered power divided by the noise equivalent of the bandwidth directly compares with the signal to noise ratio (SNR) and can be calculated from optical communications theory (Fujimoto *et al.,* 2000).

$$SNR = 10Log(\eta P/2h\nu NEB) \quad \text{(Eq. 2.3)}$$

Where η represents the detector quantum efficiency*,* P the detector power, hv the photon energy and NEB the noise equivalent bandwidth of detection (Fujimoto et al., 2000). Therefore in order to generate high image resolutions, higher optical powers are required. The same applies for higher image acquisition speeds for a given signal to noise ratio (Fujimoto et al., 2000).

2.6.3 OCT imaging systems

Time-domain optical coherence tomography (TD-OCT) is well established, with an increasing field of applications in medical diagnostics (Leitgeb *et al.,* 2003). The first generation of OCT systems was first demonstrated by Huang *et al.* (1991) for 2D cross sectional images of human retina and coronary artery. Since then, OCT has rapidly developed as a non-invasive diagnostic tool within the medical field with a wide range of applications and has recently found

success within the horticulture industry. Information regarding TD-OCT is presented in Appendix C.

High speed OCT imaging was achieved by introducing the Fourier domain concept, which is also known as “coherence radar”, “spectral radar” (Hausler *et al.,* 1998; Walther *et al.,* 2011) or “spectral interferometry” (Fercher *et al.,* 1995). FD-OCT enables 3D-OCT imaging in vivo (Fujimoto *et al.,* 2000; Brezinski, 2006) with imaging speed only limited by the laser tuning velocity or the readout rate of the line-scan camera (Walther *et al.,* 2011). Providing improvements in the speed of optical delay lines and in detection sensitivity compared to TD-OCT systems (Brezinski, 2006). These systems will be discussed in detail in this section.

2.6.3.1 FD-OCT

The Fourier transformation of the spectrum obtains the backscattering amplitude as a function of depth. It is based on the principle that even if the reference and sample arms have different lengths, each wavelength of light in the Michelson interferometer will interfere. Therefore the interfering light will oscillate with a frequency as a function of wave number which is representative of the length difference for a fixed difference in length between both the sample and reference arm (Walther *et al.,* 2011). The interference spectrum can be acquired two ways, firstly by using a spectrometer to analyse the interfering signal known as spectral domain OCT (SD-OT), or secondly by sweeping the wavelength of light directed to the interferometer as a function of time (Walther *et al.,* 2011). The latter is referred to as swept source OCT (SS-OCT) or optical frequency domain imaging (OFDI).

2.6.3.2 Spectral domain optical coherence tomography (SD-OCT)

In SD-OCT a similar setup to TD-OCT is utilised, with the main differences being a spectrometer is located on the fourth arm of the interferometer and the length of the reference arm is kept constant (Walther *et al.,* 2011) (Figure 2.10). The spectrometer resolves the interference pattern of backscattered light from the specimen. A fiber based principle of configuration enables high flexibility for the insertion of additional components in the reference arm for phase shifting (Wang *et al.,* 2007) and dispersion compensation (Wojtkowski *et al.,* 2004). Leaving the second arm of the fiber coupler open and having the interferometer in the other arm is superior (Walther *et al.,* 2011). Common path OCT systems are where the sample and reference beam travel through the same fiber to the specimen (Vakhtin *et al.,* 2003, Kang *et al.,* 2010; Walther *et al.,* 2011). This configuration is favourable in medical diagnostic applications where a flexible view of the specimen is desired. Therefore the scanner head

containing the interferometer can be placed in any position relative to the specimen (Walther *et al.,* 2011).

In order to achieve desirable spectrometer resolution in the detection arm, the beam must be expanded wide enough on the diffraction grating (Walther *et al.,* 2011). The dispersed beam is focused on the line detector of the spectrometer. Fast fourier transformation of linearized data results in the backscattering amplitude of the specimen as a function of depth. More simply the spectrometer measures the back scattered light amplitude from the specimen, where a back reflection profile as a function of depth is obtained by inverse Fourier transformation. The signal is recorded in the Fourier domain and a scattering profile in the spatial domain is observed by Fourier transformation. The location of the peak at a particular frequency corresponds to the scatter location (Brezinski, 2006).

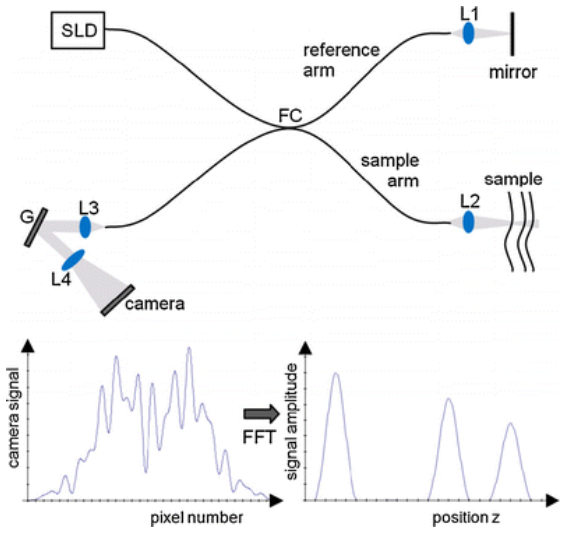

Figure 2.10. Schematic diagram of a SD-OCT system. In the reference arm the mirror is stationary. The grating (G) spectrally resolves interfering light from the fiber coupler (FC) and is focused on the camera. The line-scan camera detects the interference spectrum. After Fourier transformation the signal which is depth dependent is recovered. L1 – L4 are different lenses. Reprinted with permission from Copyright Clearance Center: Springer, Analytical and Bioanalytical Chemistry, Optical Coherence Tomography in Biomedical Research, Walther, J., Gaertner, M., Cimalla, P., Burkhardt, A., Kirsten, L., Meissner, S., & Koch, E, 40(9), 2011. Copyright (2011).

The axial range is limited by the resolution of the spectrometer and is dependent on the shape and width of the acquired interference spectrum (Walther *et al.,* 2011) but offers a higher axial resolution (1 – 3 μm) than TD-OCT and offers 3D imaging (Michalewska *et al.,* 2013). SD-OCT has the advantage of high imaging speed due to higher sensitivity, although the sensitivity is degraded at the high spatial frequencies as a result of the width of the detector elements and finite resolution of spectrometer optics. Therefore at the end of the measurement range sensitivity drops off between 10 to 20 dB (Bajraszewski *et al.,* 2008).

2.6.3.3 Swept source optical coherence tomography (SS-OCT)

Swept source OCT (SS-OCT) is an alternative approach that uses a frequency swept laser light source and a photo detector to measure the interference spectrum (Brezinski, 2006) (Figure 2.11). This approach is based on a tunable light source over a sufficient wavelength range that is comparable in time with the readout rate of SD-OCT line scan cameras (Walther *et al.,* 2011). The resulting interference spectrum is time-resolved when compared to SD-OCT, as the spectral resolution is moved to the entrance of the interferometer from the end (Walther *et al.,* 2011). SS-OCT has improved image penetration depth, reduced optical scattering and importantly can perform imaging at longer wavelengths (1000 – 1300 nm). As well as SD-OCT, SS-OCT enables 3D imaging (Ali and Parlapalli, 2010; Walther *et al.,* 2011; Chen *et al.,* 2016).

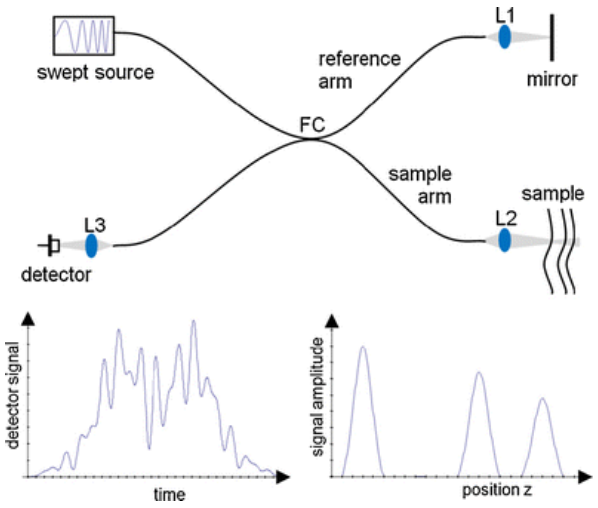

Figure 2.11. Schematic diagram of a SS-OCT system. The fiber coupler (FC) splits light from a swept source (fast tunable light source) into the reference and sample arm. At the FC back reflected light interferes and is sent to a single detector element, this is captured as a function of time. A Fourier transformation is applied to the time-dependent data to resolve the depth signal. L1 – L3 are different lenses. Reprinted with permission from Copyright Clearance Center: Springer, Analytical and Bioanalytical Chemistry, Optical Coherence Tomography in Biomedical Research, Walther, J., Gaertner, M., Cimalla, P., Burkhardt, A., Kirsten, L., Meissner, S., & Koch, E, 40(9), 2011. Copyright (2011).

To achieve wavelength tuning a grating or prism in combination with a laser scanner is inserted into the laser resonator (Chong *et al.,* 2008; Walther *et al.,* 2011). The signal from the second arm of the interferometer is coupled to the second detector or a part of the laser power to reduce the relative intensity noise (RIN) effect of the laser source, increasing the SNR (Walther *et al.,* 2011). Similar to SD-OCT, the reference arm is fixed, and for axial scanning no moving parts are required significantly increasing the speed of scanning (Walther *et al.,* 2011; Chen *et al.,* 2016). An advantage of SS-OCT is the usable dynamic range of detection that is enhanced by the use of a single photodetector, by high-pass filtering of the photodetector

signal to eliminate unwanted DC intensities (Walther *et al.,* 2011; Chen *et al.,* 2016). SS-OCT and SD-OCT provide similar rates of high-speed data acquisition, however SS-OCT does not have the drawbacks of spectral limitations.

2.6.4 Application of OCT imaging

OCT has evolved from an experimental laboratory imaging tool (Huang *et al.,* 1991) to a new diagnostic imaging tool with a wide range of clinical applications. OCT was initially applied for ophthalmology imaging and has since evolved to a large spectrum of medical practises such as cardiology, dermatology, dentistry, gastroenterology, and oncology among others (Chen *et al.,* 2016). Medical applications continue to dominate the use of OCT technology, however additional advantages have made it possible for OCT to be used in a wide range of applications. OCT has found an increasing number of applications within the horticultural industry as a result of the non-destructive nature of imaging.

2.6.4.1 Medical diagnostics

OCT has found success as an ophthalmic diagnostic tool in the areas of retinal disease and glaucoma. Part of this reason is the relatively transparent nature of the human eye which enables easy access to the eye fundus. Notably the near optical quality of many ophthalmological structures fit quite well within the interferometric precision and sensitivity of OCT (Fercher *et al.,* 2003). More importantly OCT enables non-invasive cross sectional visualisation of retinal microstructure, that cannot be obtained by any other non-invasive imaging method (Drexler and Fujimoto, 2008). Interestingly OCT technology has been expanded to endoscopic imaging.

Endoscopic OCT (EOCT) allows for micrometre subsurface in vivo imaging of biological structures within organs and cavities. Most systems operate in the 1300 nm wavelength (Walther *et al.,* 2011). Side imaging probes have been developed for the examination of the gastrointestinal (GI) tract, respiratory and vascular system. For these systems, light is emitted and collected by a prism on the side of the endoscope (Tearney *et al.,* 1996). Side imaging EOCT probes provide 2D depth-resolved imaging by rotating the optical system to enable imaging of sub-surface tissue. Back and forth translation of the probe and catheter simultaneously allow the possibility of 3D imaging (Suter *et al.,* 2008; Bezerra *et al.,* 2009). Forward imaging EOCT is preferred during surgery and for image guided biopsy (Boppart *et al.,* 1997), where light is emitted and collected from the front of the device.

2.6.4.2 Horticulture applications

The application of OCT to assess the internal quality and structure of horticulture products is a recently emerging trend within the horticultural industry (Table 2.9). OCT can be utilised to visualise plant tissues and boundaries, and can detect internal defects and the microstructure of non-transparent scattering media (Hrebesh *et al.,* 2009; Meglinski *et al.,* 2010; Magwaza *et al.,* 2013). OCT enables the non-destructive, contactless visualisation of internal plant structures that could not previously be imaged. A penetration depth of up to 2 mm from the surface of the skin has been reported in onions (Meglinski *et al.,* 2010) and apples (Verboven *et al.,* 2013), with a 5 – 20 μm resolution in fruit (Magwaza *et al.,* 2013). OCT is an emerging technology and provides the opportunity for non-destructive quality assessment of horticultural products across the supply chain.

Table 2.9. Selected horticulture applications of OCT imaging in literature.

Produce	Wavelength (nm)	Axial resolution (μm)	Penetration depth (mm)	OCT system	Reference
Asian pear	840	4.8	0.25	SD-OCT	Wijesinghe *et al.* (2016)
Apple	1310	12	3	SS-OCT	Srivastava *et al. (*2018)
Apple peel	800	2	0.1 – 0.2	TD-OCT	Verboven *et al.* (2013)
	860	2	0.1 – 0.2	SD-OCT	
	1325	7.5	0.5	SD-OCT	
Kiwifruit	1325	5.9	0.68	SD-OCT	Li *et al*. (2015)
Loquat	1300	3.48	1	SD-OCT	Zhou *et al.* (2018)
Mandarin	930	7.0	1.1	SD-OCT	Magwaza *et al.* (2013)
Onion	1325	1.0	~2	SS-OCT	Meglinski *et al.* (2010)
Onion	930	7.0	0.3	SD-OCT	Landhal *et al.* (2012)
Kiwifruit Orange Red-leaf lettuce Cranberries	1300	16	~1.4	TD-OCT	Loeb and Barton (2003)

A feasibility study conducted by Loeb and Barton (2003) successfully imaged the microstructure of kiwifruit, orange, read-leaf lettuce and cranberries using a TD-OCT system. Parenchyma cell vacuoles in the outer pericarp of kiwifruit were successfully visualised (Loeb and Barton, 2003), where more recently the microstructure of large parenchyma cells immediately underlying kiwifruit skin has been quantified (Li *et al.,* 2015). In onions a feasibility studying using a SS-OCT was conducted to differentiate between onion bulbs with variable internal defects (Meglinski *et al.,* 2010). Histological changes were detected between normal onion tissue and onion tissues with possible bruise defects, demonstrating the potential of OCT to visualise temporal and spatial microstructural changes during onion storage. The monitoring of tissue cell changes during disease development in onions using OCT allowed the visualisation of internal structures with a high resolution (Landhal *et al.,* 2012).

Demonstrating OCT can successfully be applied for screening horticultural products for quality and during disease development.

The peel structure properties of apples have been determined non-destructively using OCT (Verboven *et al.,* 2013). Depth scans revealed the cellular structure of the fruit which was superior to confocal microscopy imaging. Cuticle, epidermis and hypodermis cells were resolved, however the sharpness of the images provided by OCT was limited for quantitative analysis of the cellular structure. Enface OCT images allow the visualisation of surface topology, which show surface roughness, cracks and lenticels (Verboven *et al.,* 2013). OCT provides a range of images which allow the visualisation of internal plant structures quickly and non-destructively compared to already established imaging techniques.

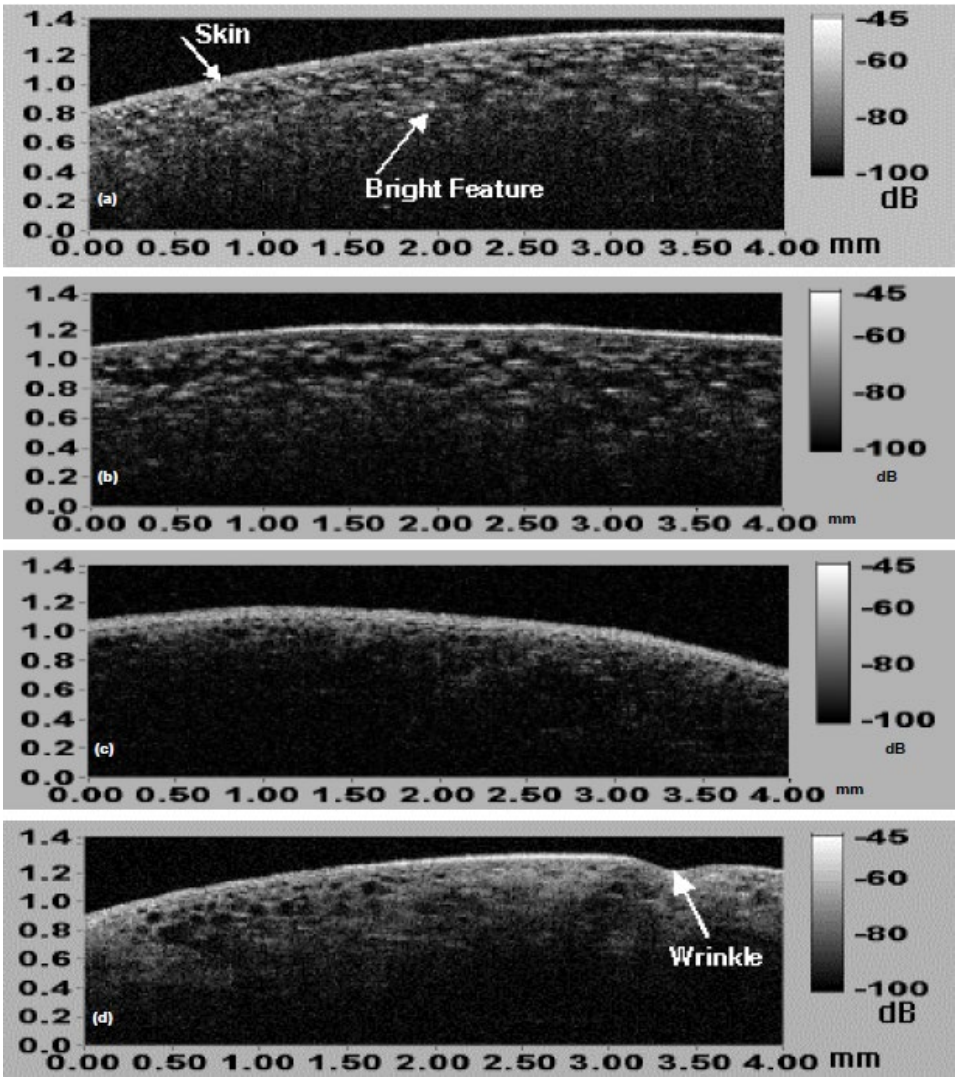

Figure 2.12. Previous OCT imaging of cranberries. (a) – (d) show images of cranberries increasing in ripeness. Bright features are marked with an arrow in (a), and a wrinkle is marked with an arrow in (d). Cranberry skin is shown at the top of the image. From Loeb and Barton (2003). Used with permission.

OCT imaging and quantification has not previously been reported in blueberries. The only study related to berries was conducted by Loeb and Barton (2003), in which the increasing ripeness is imaged, as well as a cranberry that has black rot (Figure 2.12). In an unripe cranberry with white flesh many bright dash like features are observed by OCT probably arising from the boundaries of small vacuoles. Where larger regions of reflectivity are observed deeper within ripe cranberries. Thickened surface reflection was seen in overripe cranberries. Increasing surface reflection appeared throughout OCT imaging and may be due to increased skin thickening. However, quantitative information on the sub-surface cellular structures of cranberries is still lacking.

2.7 Other cellular level imaging techniques

Other cellular level imaging techniques such as Magnetic Resonance Imaging (MRI), hyperspectral imaging, and electron microscopy are powerful imaging tools, which have previously been used to research near cellular level structures and changes occurring in blueberries during storage. Paniagua *et al.* (2013) successfully visualised the water distribution in 'Centurion' blueberries over a three week storage period in different air flow treatments (0, 15, 30, 60 mL min^{-1}) using MRI. The internal quality of blueberries has been predicted using non-destructive hyperspectral imaging (Leiva-Valenzuela, Lu and Aguilera, 2014). Where, structure and function relationships associated with blueberry texture and quality have previously been imaged using electron microscopy (Allan-Wojtas *et al.,* 1999; Allan-Wojtas *et al.,* 2001; Fava, Alzamore and Castro, 2006). Further descriptions of these techniques and previous research on blueberries is presented in Appendix D.

2.8 Conclusions

Fresh blueberries deteriorate rapidly after harvest from softening, and shrivelling. The progression of fruit quality from harvest to the marketplace is determined by physical and physiological processes. Since blueberries are harvested when they are fully ripe, developmental and ripening changes have a minor impact on postharvest quality losses. Moisture loss is responsible for the deterioration of fruit quality along the supply chain, where inconsistencies in postharvest storage are major pathways for moisture loss. Acceptable storage conditions to minimise moisture loss include storage at low temperatures and high humidity conditions.

Postharvest softening of fresh blueberries is a major problem for industry. Moisture loss greater than 3.47% (Paniagua *et al.,* 2013), has been found to correlate with blueberry softening, where the effects of other texture characteristics are limited in literature. The mechanisms for softening are not completely understood. The majority of cell wall modifications occur during ripening before harvest. Although it has been reported cellular level changes such as the collapse of parenchyma cells, loss of cell to cell adhesion and increase in cellular space could be responsible for decreased quality. Improving the current understanding of moisture loss on blueberry texture through microscopy and OCT could provide useful information to improve the quality of fresh blueberries in the marketplace.

Chapter 3.
Materials and Methodology

3.1 Introduction

This chapter details specific materials and methods common to the experiments conducted in this thesis. Some shared issues such as fruit origin, storage system and statistical analysis are detailed here once as a way to avoid repetition and to highlight the most important aspects of each experiment. Specifically, this chapter details the methodology for devising a storage system to allow the manipulation of RH in a closed environment whilst maintaining all other conditions for the duration of the experiment. Texture, microscopy and OCT assessment methodologies are detailed in their respective chapters.

3.2 Fruit source

A late season rabbiteye cultivar ‘Centurion’ (*Vaccinium ashei* Reade) was used in this study. ‘Centurion’ blueberries are a traditional rabbiteye cultivar released by the Department of Agriculture of the United States (USDA) and the North Carolina Agricultural Experiment Station in 1978, with parentage W4×Callaway (Schotsmans *et al.,* 2007). The ‘Centurion’ berries are characteristically dark and firm.

Two kilograms of ‘Centurion’ rabbiteye blueberries were obtained from Omaha Organic Blueberries located near Matakana, Auckland, New Zealand and collected from a single block with homogenous plant conditions and common orchard management (i.e. irrigation, nutrition, pest control and pruning). Fully mature berries were hand harvested on 21/02/2018 by farm pickers according to commercial practises and normal harvest index (i.e. 100% colouration). After picking, berries were moved into the packhouse and stored at 10 °C then subsequently packed into 2 L plastic containers, placed in a 25 L chilly bin with cool gel packs and ice at 12 °C and transported on the same day to the Food Laboratory at Massey Institute of Food Science and Technology (MIFST), Massey University, Albany. An iButton (DS1923-F5 Hygrochron Temperature & Humidity iButton, Maximus Technologies, New Zealand) was inserted onto the wall of the chilly bin to provide temperature and humidity readings during transportation (Figure 3.1).

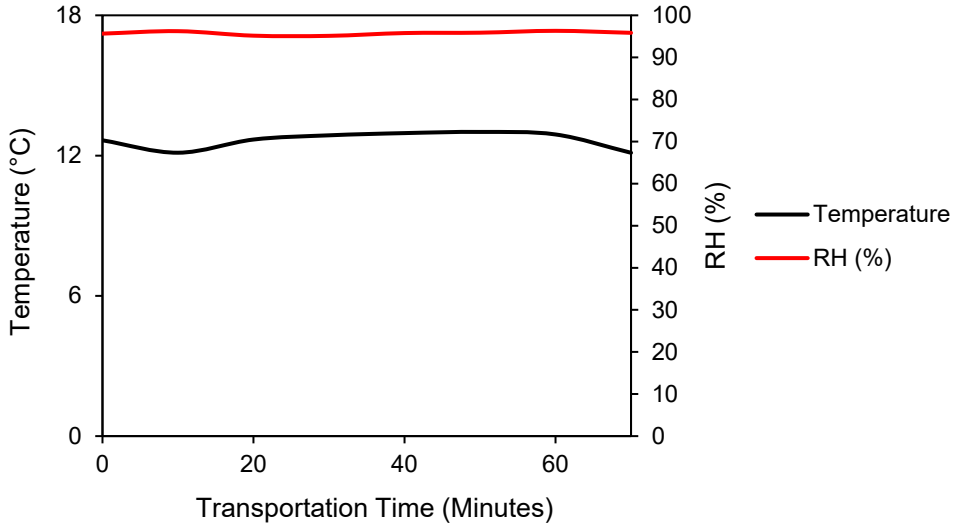

Figure 3.1. Graph of temperature (°C) and RH (%) inside the chilly bin containing fresh blueberries during transportation from Omaha Organic Blueberries, Matakana to Massey University, Albany.

3.3 Manipulation of RH storage conditions

3.3.1 Introduction

The rate of weight loss from a fruit product is influenced by the RH directly surrounding the product, where the partial pressure difference (the driving force) for water loss is defined (Maguire *et al.,* 2001; Paniagua *et al.,* 2013). In order to investigate the role of water loss on texture and microstructure changes in blueberries, an experiment based on the previous work by Paniagua *et al.* (2013) was devised to allow the manipulation of RH within a closed environment whilst maintaining all other conditions (temperature, carbon dioxide and oxygen concentration) consistent for the duration of the experiment (Paniagua *et al.,* 2013). Accordingly, the objective for this experiment was to establish an airflow system that resulted in different humidity conditions, enabling the manipulation of moisture loss to induce blueberry softening and texture responses during postharvest cool storage.

3.3.2 Sample configuration

Fruit were standardised on size (10 – 14 mm equatorial diameter) and quality in laboratory conditions by hand grading at room temperature (20 °C). Fruit that was spilt or degraded was omitted. Forty eight samples were randomly established, each one consisting of 20 blueberries randomly placed inside previously weighed individual mesh bags (10 cm × 10 cm). Glass jars

of 0.578 L contained three weighed and labelled samples each as outlined by Paniagua (2012). Approximately 80 – 85 g of fruit were in each jar.

3.3.3 Experimental design

The experiment was conducted in a flow through system that provided continuous fresh air to the blueberries, therefore removing any chance of gas contamination and modification of carbon dioxide and oxygen conditions between treatments. As described by Paniagua *et al.* (2013) a simple water balance around the jar of a flow through system can be used to determine the steady state humidity (*H,* kg (water) kg^{-1} (air)) established within the jar (Figure 3.2). Inside the jars water addition to the air volume comes from inflowing air (W_i, kg (water) s^{-1}), and the water lost (W_f, kg (water) s^{-1}) from the blueberries, while the outgoing airflow removes water from the system (W_0, kg (water) s^{-1}). The flow rate of air through the system results in an equal flow for both incoming and outgoing air (*F,* kg (air) s^{-1}). Hence the steady state humidity established in this experiment can be related to the flow rate (Eq. 3.1)

$$H = \frac{W_0}{F} \qquad \text{(Eq. 3.1)}$$

According to Paniagua *et al.* (2013) the rate of water lost from the fruit product is governed by the humidity gradient between the blueberries (H_f, kg (water) kg^{-1} (air)) and the surrounding air (H), the resistance of water to flow through the skin (often referred to the water vapour permeance [*P',* kg s^{-1} m^{-2}] which is the inverse of the resistance), and the surface area of the blueberry available for water to transfer through (A_f, m^2). Eq. (3.2) can be modified by substituting Eq. (3.1) resulting in Eq. (3.3).

$$W_f = P' A_f \left(H_f - H\right) \qquad \text{(Eq. 3.2)}$$

$$W_f = P' A_f \left(H_f - \frac{W_O}{F}\right) \qquad \text{(Eq. 3.3)}$$

Given Eq. (3.3) it is clear that manipulation of the rate of blueberry water loss (W_f) is achievable through the manipulation of the gas airflow rate (*F),* as the permeance (*P'),* fruit surface area (A_f) and internal fruit humidity (H_f) can all be assumed to remain constant (Paniagua *et al.,* 2013). The lower the rate of air flow, the smaller the driving force for water loss (higher humidity, *H)* is established, therefore slower water loss will be observed from the blueberries, compared to higher air flow rates. This design enables differences in rates of weight loss to

be established between treatments, while concurrently not influencing other storage parameters that may influence product quality such as temperature, carbon dioxide and oxygen.

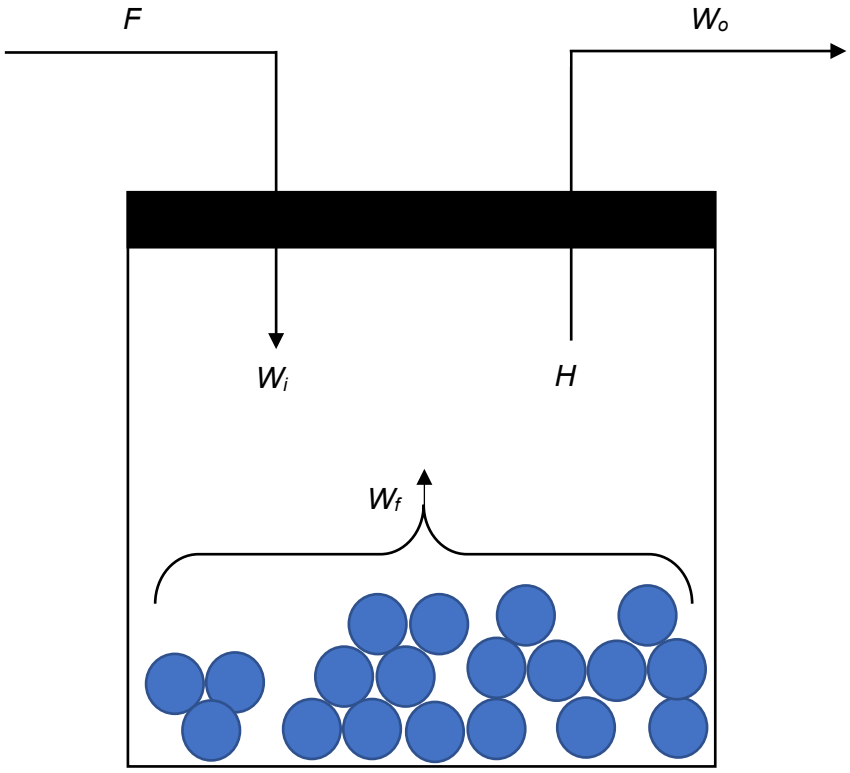

Figure 3.2. Simplistic experimental set-up establishing a flow through system, determining the factors influencing the humidity within glass jars, and the rate of water loss from blueberries. Diagram adapted from Paniagua et al. (2013). F = flow rate of air through the system (kg (air) s^{-1}), W_i = water addition from the inflowing air (kg (water) s^{-1}), W_f = water lost from blueberries (kg (water) s^{-1}), W_o = water removed from the system by the outflowing air flow (kg (water) s^{-1}), H = steady state humidity (kg (water) kg^{-1} (air)) within system.

Using the knowledge provided by Paniagua *et al.* (2013), controlled weight loss conditions were established by supplying dry air to 0.578 L glass jars containing blueberry samples at four different flow rates (0 [control], 15, 30 and 60 mL min^{-1}), establishing four different humidity conditions within the glass jars (Figure 3.3). All gas flow treatments were conducted in triplicate at 5.5°C in fridges located at MIFST Albany for a period of three weeks.

Each glass jar containing blueberry samples was closed using a modified air tight lid containing two rubber septa to create a controllable environment system. One septa acted as an air input, and the other as an air output. Needle valves located in a manifold were used to regulate the rate of air flow supplied to the glass jars (Figure 3.3). Dry air was supplied to the jars by tubing through one septa, the other septa remained open to ensure air renewal within the glass jar and to avoid atmosphere modification (Paniagua, 2012). For the control (0 mL min^{-1}) both the air inlet and outlet were left open, allowing air flow by diffusion. Replication of

each treatment was regulated by an independent needle valve, and each replicate was located in a different jar. Following Paniagua (2012), every two days the flow rate was checked at the outlet and adjusted accordingly as needed using a portable gas flowmeter (ADM 2000, Agilent Technologies, Delaware, USA). A diagrammatic representation can be seen in Figure 3.4.

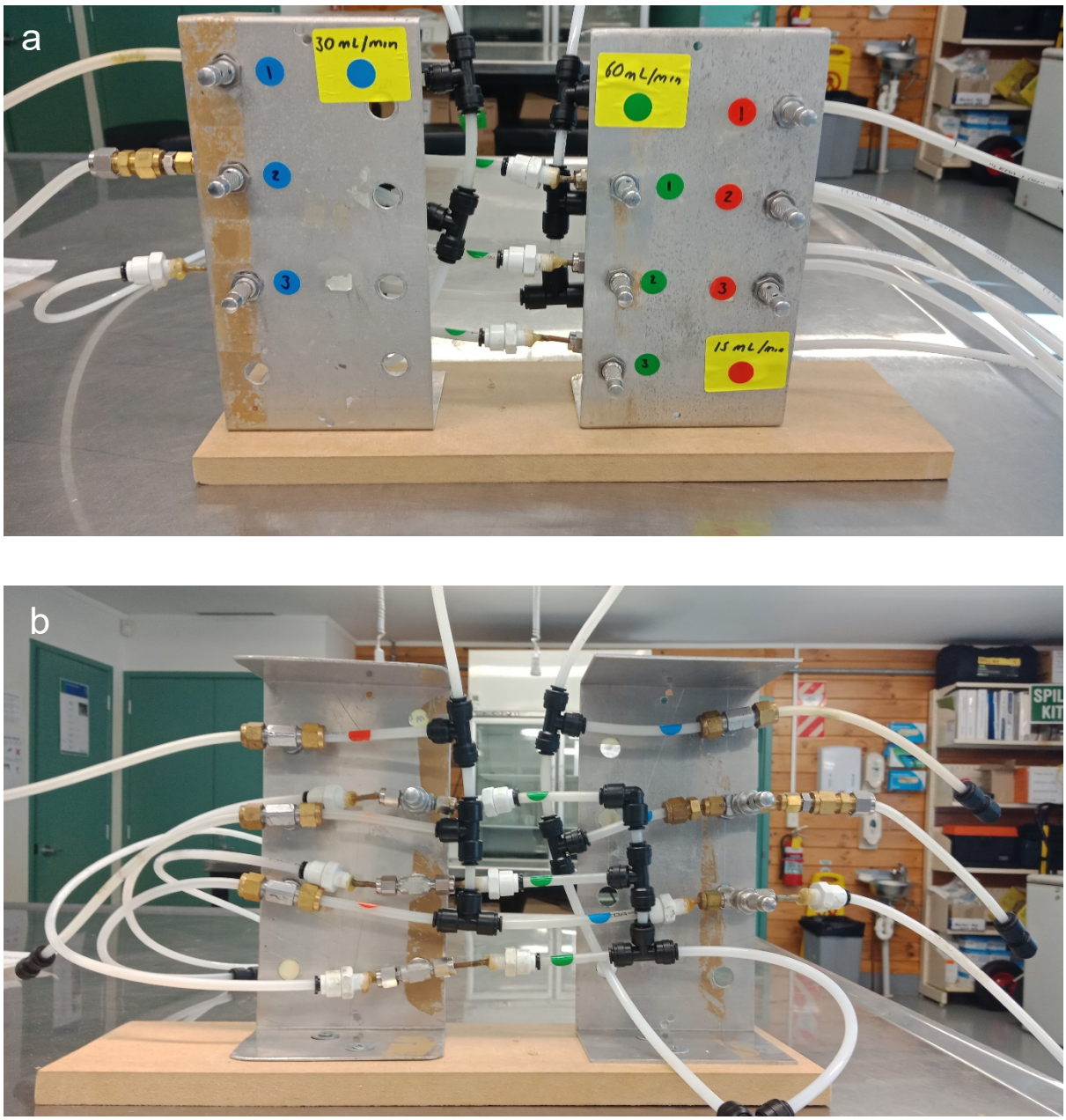

Figure 3.3. Manifold with needle valves used to control the supply and flow rate of dry air into the glass jars. (a) Front view of manifold showing needle valves, (b) back view of manifold showing piping used for the distribution of dry air within the glass jars.

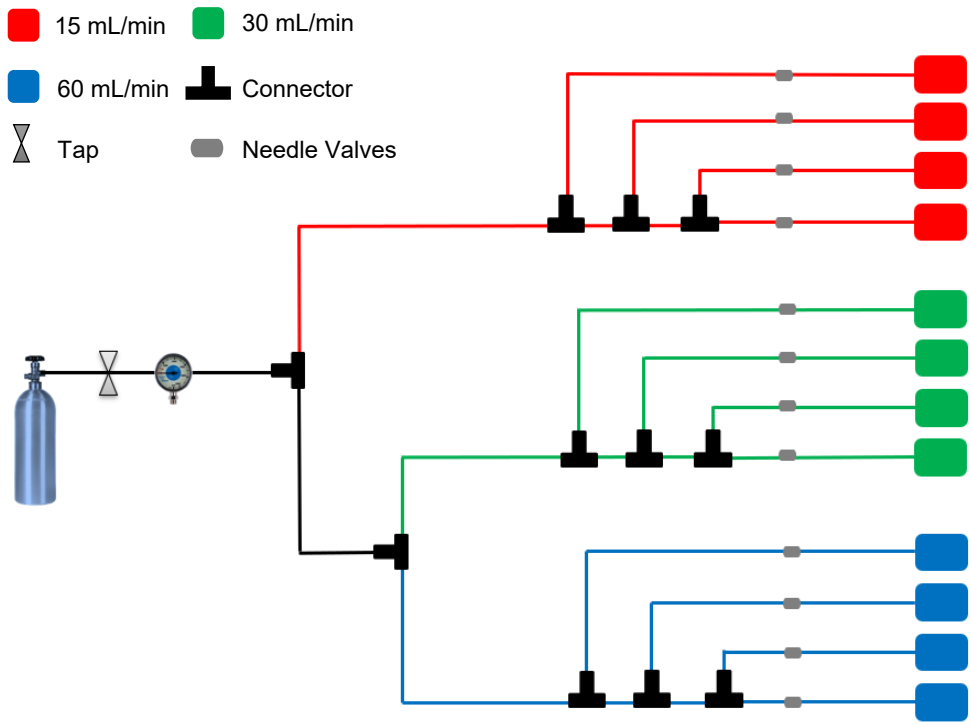

Figure 3.4. Diagrammatic representation of manifold used to control and supply the flow rate of dry air into the glass jars.

3.3.4 Temperature and humidity

A temperature and RH logger (DS1923-F5 Hygrochron Temperature & Humidity iButton, Maximus Technologies, New Zealand), with a diameter and weight of 17.35 mm and 5.0 g respectively, was fixed halfway inside each jar by double sided tape to monitor the temperature and humidity of each flow rate treatment. A three point calibration check was conducted to ensure RH loggers were accurate for the three week duration of the experiment. This is an easy and inexpensive method of checking new loggers to ensure they are functioning properly before they are first used (Alderson and Arenstein, 2011). Three calibration salt chambers were prepared to assess the RH accuracy of the loggers over a six day period. As different salts hold different RH levels when in a saturated solution (Greenspan, 1976), three different saturated salt solutions were used for the purpose of calibration (Table 3.1).

Table 3.1. Fixed RH of saturated salt solutions at 20 ºC (Greenspan, 1976) and average calibration values of RH-Temperature loggers over six days (n= 13).

Salt Solution	Relative Humidity (%RH)	Average Calibration Values (%RH)
Lithium Chloride	11.31 ± 0.31	11.68
Magnesium Chloride	33.07 ± 0.18	34.01
Sodium Chloride	75.47 ± 0.14	75.21

Each salt chamber consisted of a large well sealed plastic container, with saturated salt solutions placed in a small sealed plastic container fitted with a gortex window, allowing for gas exchange and the prevention of salt migration and liquid spills (Alderson and Arenstein, 2011). Once all salt solutions and chambers were prepared, they were stored at 20 ºC in a climate controlled room at MIFST Albany to prevent RH fluctuations, as at a given temperature RH is fixed. The loggers were set to take readings every five minutes using the OneWireViewer software (version 0.3.19.47, Maxim Integrated Products Inc., California, USA). The loggers were placed in the lithium chloride salt chamber first, moving from low to high RH sequentially after every two days (Table 3.1). At the end of the calibration period the RH data from the loggers was download, all loggers reported acceptable accuracy.

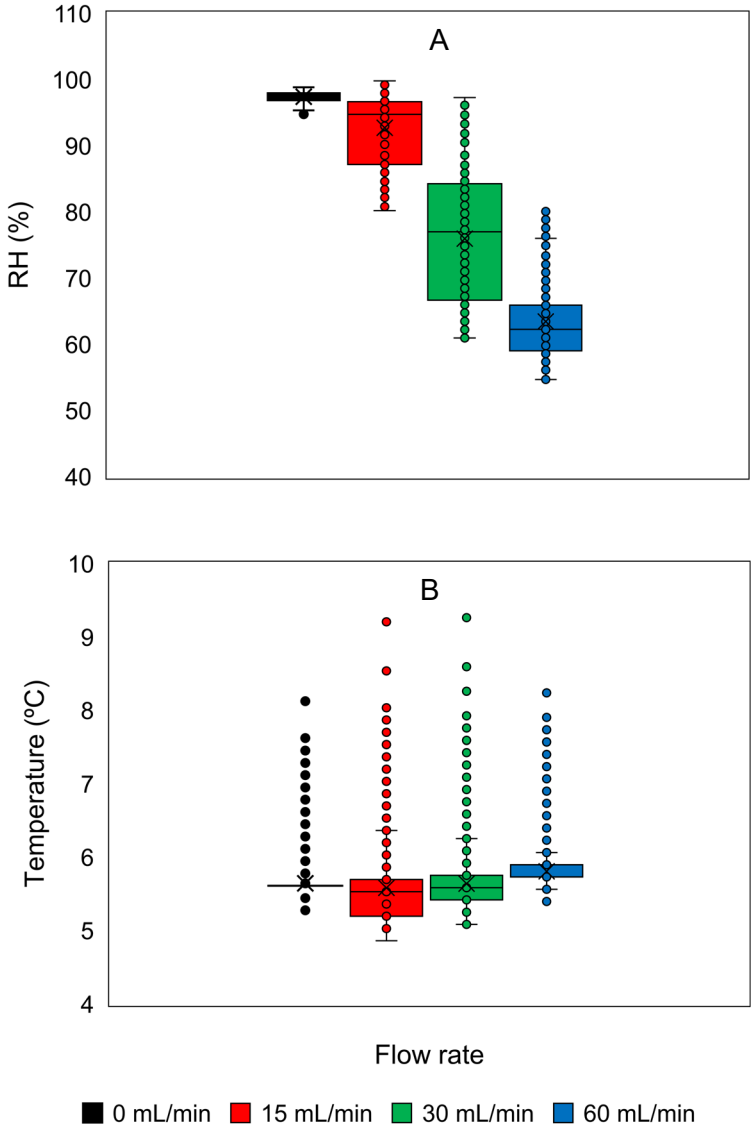

Figure 3.5. (A) Different RH (%) conditions established in glass jars during airflow treatments of the population of measured data. Four different RH environments were created from four different airflow treatments. (B) Temperature (°C) measurements of each airflow treatment throughout three weeks cold storage of the population of the measured data. Temperature conditions remained stable across all airflow treatments. RH-temperature loggers were placed inside each glass jar during storage.

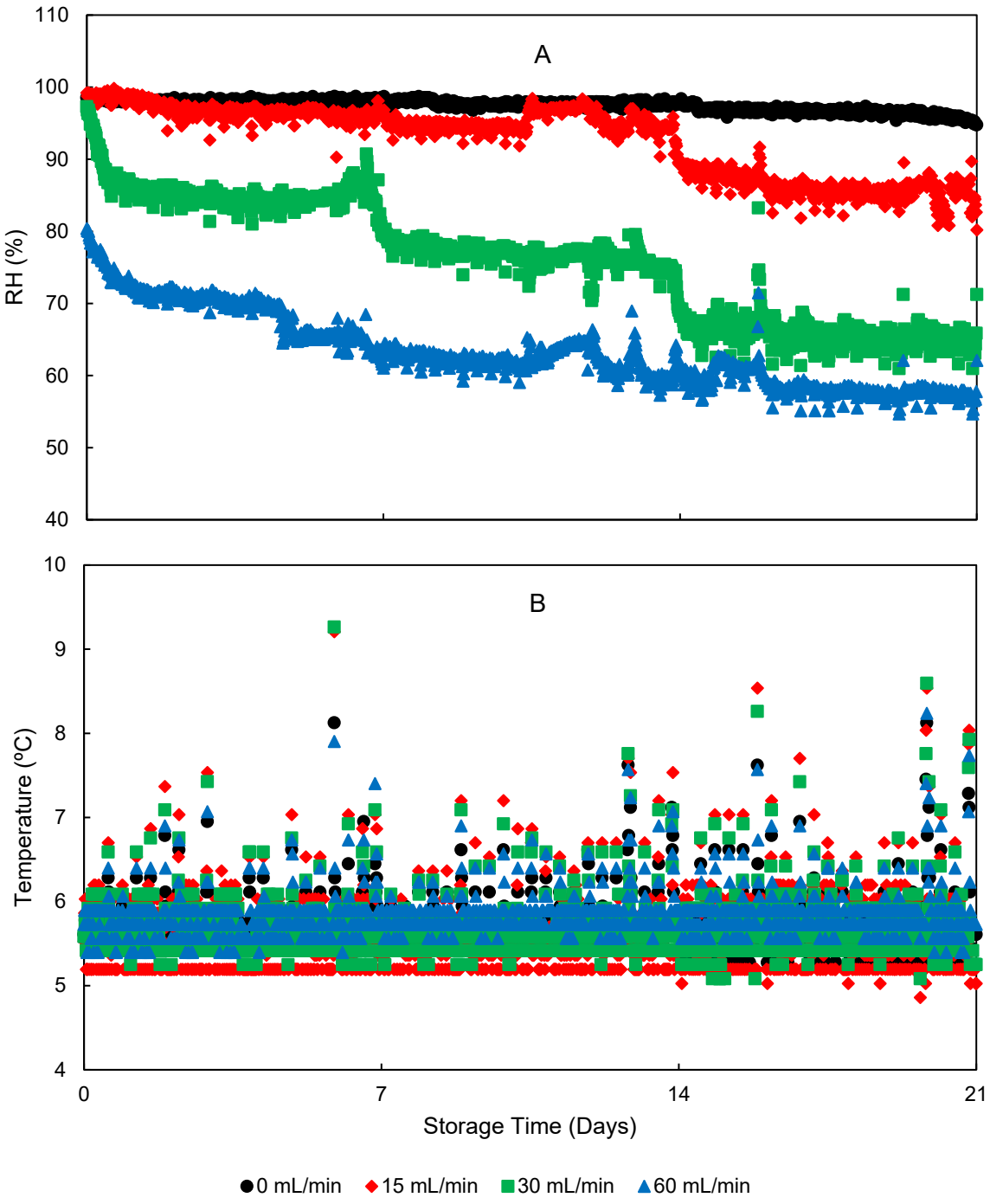

Figure 3.6. (A) Average RH (%) in glass jars during gas flow treatment. (B) Constant temperature (°C) maintained within glass jars during gas flow treatments. Each flow rate represents the data average of three RH-temperature loggers measured in 20 minute intervals during a three week storage period. An average temperature of 5.5 °C was calculated from the data collected by all temperature loggers throughout the duration of the experiment

For the fruit weight loss experiments, the loggers were programmed to take measurements every 20 minutes from the start to the end of the experiment. Data from each logger was downloaded after each measurement period using the OneWireViewer software. The resulting average RH inside the jars was 98%, 93%, 76%, and 62% for 0, 15, 30 and 60 mL min^{-1}

respectively (Figure 3.5a). Condensation was viewed inside the jars for the control group, resulting in a high RH value. At each airflow rate samples were stored and maintained at 5.5 °C throughout the storage period for three weeks (Figure 3.5b).

Overtime the RH declined for all treatments (Figure 3.6a) as the RH is a function of the mass (represented by area A_f, Eq. 3.3) of the fruit, therefore it was expected that the RH would drop as one sample is removed at each measurement period from within the jar (Paniagua *et al.,* 2013). All jars were stored and maintained at 5.5 °C throughout the storage period for 3 weeks (Figure 3.6b). Variability in temperature over time seen in Figure 3.6b could be the result of the cyclic nature of the refrigerator, or due to the opening and closing of the refrigerator door to adjust flowrates, or at measurement days.

3.3.5 Fruit sampling for evaluation

An initial evaluation on 12 remaining prepared samples of fresh fruit was conducted (21/2/18). These samples were then eliminated from the study. For a period of three weeks, treatments were evaluated weekly, by removing one sample for evaluation from each jar (28/2/18, 5/3/18 and 12/3/18). Samples were randomly chosen from each jar and conditioned for 1 hour at room temperature (20 °C) before evaluation (Paniagua, 2012). After evaluation the samples were then subsequently eliminated. The modified lid was closed immediately after the removal of a sample, ensuring minimal effect to the humidity conditions in the glass jar. The parameters assessed in each evaluation included weight loss, texture, and near skin histology where microscopy and OCT imaging methods are detailed in each appropriate chapter.

3.4 Data analysis

Minitab version 18.1.0 (Minitab Inc., Pennsylvania, USA) was used to conduct all statistical analysis. Analysis of variance (ANOVA) was performed by using the General Linear Model (GLM) command after checking assumptions of homogeneity of variance and normal distribution. All data remained untransformed. Correlations were analysed using Pearson correlation test, and differences between means were checked by using Tukey HSD (Honest significant difference) test. Significant differences were considered at the 5% level. All figures presented in this thesis were designed by using Microsoft Excel (2016) and Minitab version 18.1.0 (Minitab Inc., Pennsylvania, USA) whereas tables were created by using Microsoft Excel and Microsoft Word (2016).

Chapter 4.
Weight Loss and Texture Traits of Blueberries during Postharvest Storage

4.1 Introduction

Blueberries (*Vaccinium* spp.) belong to a large group of soft fruit, where fruit texture is a critical factor in determining the economic success of crops (Giongo *et al.,* 2013). The year round global market for fresh blueberries, relies on the storability of fresh berries (Giongo *et al.,* 2013). Often large volumes of blueberries are rejected at the final marketplace due to inadequate quality resulting from poor transportation and storage conditions (Prussia *et al.,* 2006). During postharvest storage decay development is mainly inhibited by low storage temperature (Ceponis and Cappellini,1982), whereas increasing postharvest storage and handling temperatures increases fruit losses through flesh softening and berry degradation (Miller *et al.,* 1984; NeSmith *et al.,* 2000). Therefore innovative research orientated to improve texture retention of blueberries during postharvest has a great potential value for the blueberry industry.

The postharvest softening of blueberries is not very well understood, however turgor is thought to play an important role in fruit softening (Thomas *et al.,* 2008). Turgor interacts with the cell wall, such that when the internal turgor pressure is low, disruption of cell-to-cell adhesion is more likely to occur when an external force is applied to tissues. However when an external pressure is applied to tissues with high turgor pressure the cells are likely to burst, as the cell wall is more taut and stiff (Harker *et al.,* 1997). Therefore cells that burst open due to a high turgor have a crisper texture compared to cells that remain intact where a soft texture has been described (Harker *et al.,* 1997).

The softening of harvested blueberries has been related to a reduced turgor (Forney *et al.,* 1998). Paniagua *et al.* (2013) demonstrated 'Centurion' blueberries stored at lower RH decreased in firmness, as weight loss increased over a 3 week storage period, suggesting that water loss has a major influence on berry firmness (Paniagua *et al.,* 2013). Interestingly, previous research is in agreement with (Paniagua *et al.,* 2013), as moisture loss has correlated to a decrease in firmness in various other experiments (Miller *et al*., 1984; Tetteh *et al.,* 2004; Chiabrando *et al.,* 2009; Angeletti *et al.,* 2010; Cantin *et al.,* 2012). However, as mentioned by Paniagua *et al.* (2013) in these cases the observations are accompanied by differences in

storage time and/or conditions, potentially influencing cell wall modifications. It still remains unclear whether changes in turgor pressure have a profound effect on changes of blueberry texture during storage.

Texture profile analysis (TPA) allows for the prediction of food texture. Texture profiles are curves that record the temporal or spatial characteristics of samples during texture measurements (Chen and Opara, 2013). Analysis of texture profiles indicates the textural properties of samples. The TPA test is based on a double compression cycle imitating chewing and mastication. The typical texture profile of a TPA test can assess a range of fresh food texture characteristics. The texture parameters are calculated from compression force versus time, using software developed and supplied by manufactures of texture equipment (Farahnaky et al., 2012). TPA has been successfully used for the assessment of texture in blueberry varieties 'Bluecrop' and 'Coville' (Chiabrando *et al.,* 2009). A texture analyser equipped with a 5 kg load cell and HDP/90 platform was utilised by Chiabrando *et al.* (2009). The berries were deformed to 30% of the original height using a crosshead speed of 0.8 mm s^{-1} and a 35 mm diameter cylinder probe (Chiabrando *et al.,* 2009), resulting in the successful measurement of hardness, cohesiveness, gumminess, springiness, chewiness and resilience. Similarly, TPA has successfully measured texture responses to blueberries which have undergone extensive cooling and thawing processes (Zielinska *et al.,* 2016; Cao *et al.,* 2018).

Moisture loss appears to influence blueberry texture parameters differently depending on the extent of fruit dehydration (Chiabrando *et al.,* 2009). A firming behaviour in blueberries has been reported in conjunction with total weight loss below 1 – 2% (Miller *et al*., 1984; Forney *et al.,* 1998; Chiabrando *et al.,* 2009; Duarte *et al.,* 2009; Paniagua *et al.,* 2013).The corrugation of epidermal cell walls (Bunemann *et al.,* 1957), and microscopically observed thickening of parenchyma cell walls (Allan-Wojtas *et al.,* 2001), has been associated with an increase in firming of blueberries during storage. An increase in hardness, gumminess, and a decrease in cohesiveness and springiness of highbush blueberries was reported to occur simultaneously with an increase in weight loss during storage (Chiabrando *et al.,* 2009). A softer and more elastic berry structure, due to water leakage is thought to be responsible for an increase in gumminess when weight loss values are between 5 – 7% (Chiabrando *et al.,* 2009).

Fruit softness is thought to affect texture parameters, where moisture loss due to a high humidity leads to degradation of the middle lamella, and disintegration of the primary cell wall (Deng *et al.,* 2005). Therefore moisture loss rates decrease in high humidity environments,

which may help preserve blueberry fruit texture (Chiabrando *et al.,* 2009). Limited description of texture and moisture loss relationships in previous research, and whether variable texture responses obtained under different moisture loss conditions imply a causal relationship between texture and moisture loss still needs to be addressed. The present experiment induces different textural outcomes in 'Centurion' blueberries by altering humidity, to create differential moisture loss conditions during cold storage.

4.1.1 Objectives and aim

The objectives defined for this experiment are as follows:

1. Establish an airflow system resulting in different humidity conditions, enabling the manipulation of moisture loss to induce blueberry softening and texture responses.
2. Confirmation of the potential causal relationship between moisture loss and texture responses for blueberries during storage.
3. Interpret the relationship between texture responses as assessed by textural profile analysis (TPA) for blueberries during storage.

Accordingly, the aim of this experiment was to determine texture outcomes as influenced by both time in storage and moisture loss during storage for blueberries. The results of this experiment will contribute to a better understanding of the postharvest softening and texture responses for rabbiteye blueberry and could be used to potentially adjust postharvest practises to improve blueberry quality in the marketplace.

4.2 Materials and methodology

To address the objectives of this experiment, differing RH conditions were established using a closed flow through system and monitored for the duration of the experiment. Full details of the materials and methodology used to create this system including fruit source, sample configuration and experimental design are provided in Section 3.2 and Section 3.4. Weight loss and texture analysis was conducted in this experiment to assess textural changes in 'Centurion' rabbiteye blueberries throughout three weeks storage in the modified humidity conditions. The relationship between blueberry moisture loss and texture parameters was investigated. Weight loss and texture analysis methods are detailed in this chapter.

4.2.1 Fruit material and preparation

Fifteen 'Centurion' blueberries (Section 3.2) were randomly chosen from each sample for evaluation as detailed previously (Section 3.3.5). Collapsed blueberries or blueberries presenting fungal mycelium were discarded from weight loss and texture evaluation. Experimental dates were determined based on the harvest day, and each subsequent weekly storage evaluation for a period of three weeks. Texture analysis and weight loss measurements were conducted on the 21st and 28th February and the 5th and 12th March 2018 within the Food Laboratory at the Massey institute of Food Science and Technology (MIFST), Massey University, Albany with weight loss measurements occurring first, followed by destructive texture analysis.

A total of fifteen blueberries from each sample, at each measurement day were used for weight loss and texture analysis. At the end of each evaluation day a total of 45 berries per humidity condition were measured. Blueberries were discarded after measurement due to the destructive nature of the experiment. Parameters such as individual berry weight (g), equatorial and polar diameter (mm) were measured manually using a digital calliper with a 0.01 mm accuracy and recorded before texture analysis for each berry (Table 4.1).

Table 4.1. Average weight (g), equatorial and polar diameter (mm) (n=3) of individual blueberries as influenced by humidity as a result of manipulating airflow through a closed system. Measurements taken immediately before texture analysis.

Storage time	RH	Weight (g)	Equatorial diameter (mm)	Polar diameter (mm)
Fresh	98%	1.10	12.65	11.10
	93%	1.19	13.18	11.66
	76%	1.10	12.56	11.20
	62%	1.21	12.74	11.66
Week 1	98%	1.16	12.43	11.11
	93%	1.18	12.67	11.18
	76%	1.16	12.61	10.97
	62%	1.14	12.45	10.95
Week 2	98%	1.22	12.68	11.36
	93%	1.24	12.22	11.01
	76%	1.20	12.24	10.73
	62%	1.08	11.86	10.35
Week 3	98%	1.15	12.49	11.10
	93%	1.23	12.56	10.75
	76%	1.07	11.67	10.19
	62%	1.05	11.58	10.17

4.2.2 Weight loss

Weight loss was obtained for each blueberry sample following Paniagua (2012), as the difference between the initial and final weight of the mesh bag containing berries, after the

subtraction of the mesh bag weight. The final result was expressed as a percentage of weight loss (Eq. 4.1). For a total period of three weeks humidity treatments were evaluated weekly by removing one sample from each jar for evaluation and then subsequent elimination. All samples were initially weighed (initial weight) at the beginning and then placed into glass jars for the duration of the experiment. At each measurement day the sample for evaluation was conditioned at room temperature (20 ºC) for 1 hour before recording the final weight to allow for condensation removal. A digital balance (S-4002, Denver Instruments, Colorado, USA) with a precision of 0.001 g was used to measure weight.

$$\underset{\text{loss}}{\%\ \text{weight}} = \left(\frac{(\text{initial weight} - \text{bag weight}) - (\text{final weight} - \text{bag weight})}{(\text{initial weight} - \text{bag weight})}\right)(100) \quad \text{(Eq. 4.1)}$$

4.2.3 Texture analysis

Fifteen blueberries were randomly chosen from each sub-sample and then evaluated for texture. A non-destructive compression test, mimicking a very gentle squeeze with fingers was used to assess the texture of the fruit. Texture was individually measured for each blueberry. A TPA using a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd, UK) equipped with a 5 kg load cell (Chiabrando *et al.,* 2009; Paniagua *et al.,* 2013), a cylindrical 25 mm flat aluminium probe and a heavy duty platform (ASAE, 2003) was used (Figure. 4.1). Before the start of each measurement day a force calibration was completed by placing a 5 kg weight on the calibration platform, and then removed once calibration was completed to maintain accuracy.

A flat metal ring of 10.65 mm internal diameter, 25 mm external diameter and 1 mm height was fixed above the centre of the platform (Paniagua, 2012), providing support to the blueberries before the compression test. Berries were orientated in the same position over the ring, with the equatorial axis perpendicular to the surface of the probe. Each berry was subjected to a two-cycle compression test with 10 seconds between cycles (Chiabrando *et al.,* 2009). The berries were deformed 15% of the original height using a test speed of 0.08 mm s^{-1}, a pre-test speed of 1.6 mm s^{-1}, and a trigger force of 4.0 N (Table 4.2). Data was collected using Exponent version 6.1.15.0 (Stable Micro Systems Ltd, UK). A resulting force-time curve was generated, from which texture parameters were calculated (Figure 4.2).

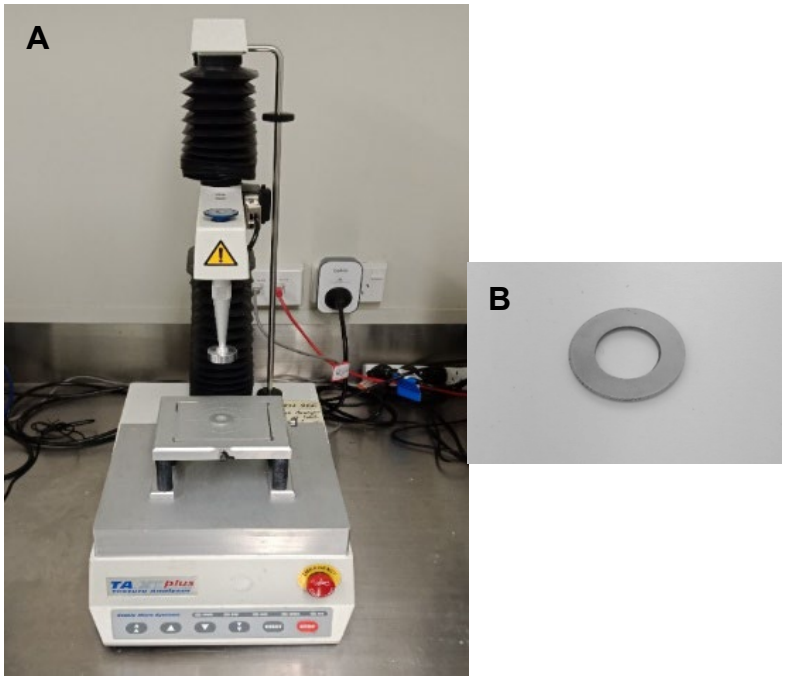

Figure 4.1. (A) TA-TX Plus Texture Analyser equipped with a heavy duty platform, 5 kg load cell, and a cylindrical 25 mm flat aluminium probe utilised to measure the texture of blueberries. (B) Flat metal ring used to support blueberries during measurement.

Table 4.2. Texture analyser settings for the double compression test.

Settings	Values
Pre-test speed	1.6 mm s^{-1}
Test speed	0.8 mm s^{-1}
Post-test speed	0.8 mm s^{-1}
Target mode	Strain
Strain	15%
Time between cycles	10 seconds
Trigger force	4.0 N

From the resulting force-time curve (Figure 4.2), the textural parameters are automatically calculated by Exponent. Since this was a double compression test, the hardness is calculated as the first force peak on the TPA curve (P1). The ratio of the positive force area during the second compression to that of the first compression, represents the strength of the internal bonds comprising the berries, which is known as cohesiveness ((A2 + A2W)/(A1 +A1W)). Springiness is calculated as the height the berry recovers during the time elapsed between the end of the first compression and the start of the second (D2/D1). Adhesiveness is the negative area between the point at which the first curve reaches a zero force value at the start of the second curve, after the first compression (Alvarez *et al.,* 2012). Gumminess is calculated as hardness × cohesiveness and is defined as the force necessary to chew a semisolid food until ready for swallowing (Chen and Stokes, 2012). Chewiness is calculated as hardness ×

cohesiveness × springiness. Resilience is calculated from how well a product regains its original position after the first compression (A_{1W}/A1). The software also records fracturability, as the force value corresponding to the first peak, only when there are three peaks. As this was a double compression test, this parameter was not recorded.

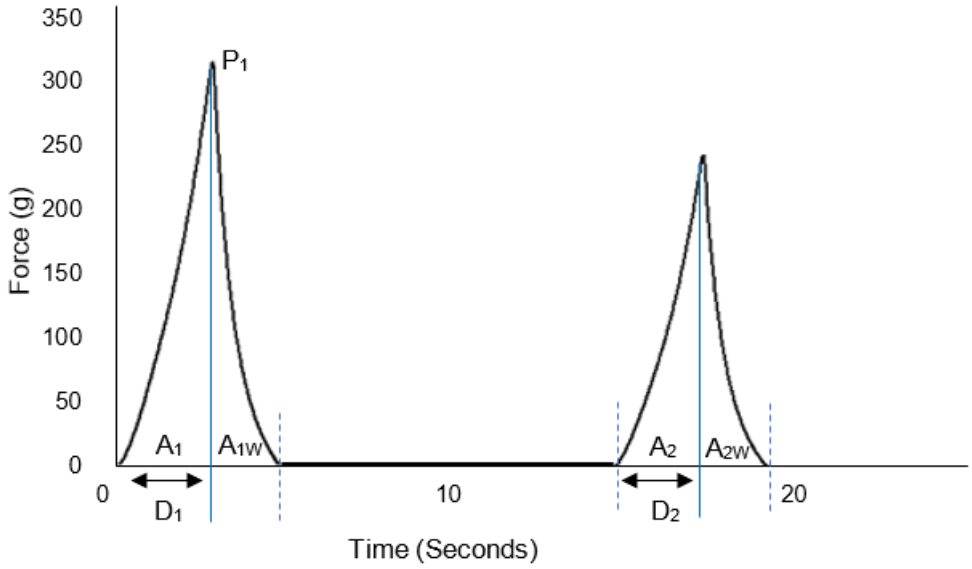

Figure 4.2. Texture profile analysis (force-time curve) representative of analysis conducted on 'Centurion' blueberries in this experiment. Texture parameters calculated from the texture profile. All blueberries analysed have individual texture profiles. P_1 = hardness, D_2 / D_1 = springiness, (A2 + A2W)/(A1 +A1W) = cohesiveness.

4.2.4 Data analysis

Data was analysed using Minitab version 18.1.0 (Minitab Inc., Pennsylvania, USA). The effect of storage period and humidity conditions on texture parameters was analysed using a general linear model (GLM) in ANOVA to measure differences among means. Factors considered in the model were RH (%) and evaluation period (weekly). The data remained untransformed. Correlations between weight loss and texture parameters were analysed using Pearson's correlation test. Differences between means were checked by using Tukey HSD (Honest Significant Difference) test. Significant differences were considered at the 5% level.

4.3 Results

4.3.1 Weight loss

Weight loss increased during storage for all treatments, with increased air flow rates resulting in increased rates of weight loss as expected from the experimental design (Section 3.2.3).

The cumulated values of weight loss after 3 weeks storage were 0%, 6.01%, 11.14%, and 14.67% for 98%, 93%, 76% and 62% RH (0, 15, 30 and 60 mL min^{-1}) conditions respectively (Figure 4.3). According to Paniagua *et al.* (2013) these values are not dissimilar to values commonly observed by industry during blueberry export (5 – 7% after 3 weeks containerised marine transport). Similarly, Sanford *et al*. (1991) reported weight loss of 5.3% and 7.6%, in blueberries stored at 0 ºC and 5 ºC for 14 d, although there was no RH regulation reported. On the contrary, low weight loss of 0.53% over 24 d at 0 ºC when stored in air has previously been reported (Duarte *et al.,* 2009). Paniagua *et al.* (2013) recorded weight loss values between 1 – 15% after 3 weeks storage using the same air flow treatments (0, 15, 30 and 60 mL min^{-1}). Accordingly, weight loss values generated in this experiment represent the range of weight loss values representative of previous research and postharvest storage systems, which validates further comparison.

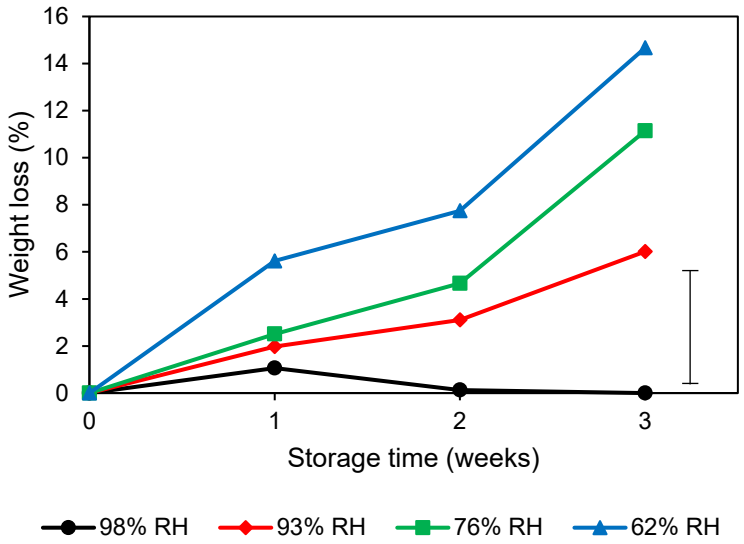

Figure 4.3. Weight loss (n=45) evolution of blueberries during 3 weeks storage, influenced by different RH conditions. Bar represents Honest significant different (HSD) at 0.05 level for each variable across the storage period as determined by Tukey's test.

4.3.2 Texture parameters

4.3.2.1 Firmness

The progression of firmness during storage was dissimilar between air flow treatments (Table 4.3). For 98% RH (0 mL min^{-1}) treatment, after one week's storage berry firmness increased (approx. 8.5%) in comparison to initial fresh firmness (2.07 N), reaching a maximum value of 2.41 N three weeks after harvest, approximately 19.3% firmer. A numerical increase in firmness was seen after one week's storage for 93% RH (15 mL min^{-1}) and 76% RH (30 mL min^{-1}), however these differences were not statistically significant from fresh firmness values.

Later, after three weeks storage blueberries were 47.4% and 53.8% softer in comparison to the initial firmness value for 76% and 62% RH (60 mL min^{-1}) respectively. For 93% RH treatment blueberries were 8.9% softer than compared to the initial firmness value after three weeks storage. For 62% RH after one week's storage firmness decreased by 13.6% in comparison to initial firmness, and continued to significantly soften each week, reaching a minimum firmness of 0.94 N after three weeks storage.

Blueberry firmness has been reported to either increase (Forney *et al.,* 1998; Chiabrando *et al.,* 2009; Duarte *et al.,* 2009; Paniagua *et al.,* 2013) or decrease (Miller *et al.,* 1984; Angeletti *et al.,* 2010; Cantin *et al.,* 2012) during storage. Paniagua *et al.* (2013) reported dissimilar firmness changes between air flow treatments (0, 15, 30 and 60 mL min^{-1}), that are representative of our firmness range. The variable firmness behaviour previously observed in blueberries provides validity to the responses obtained in this experiment, leading to either blueberry firming or softening. This could be the result of weight loss, where low levels of weight loss (0.22 – 1.34%) have shown increased firmness, and softening with high weight loss (3.47 – 15.06%) (Paniagua *et al.,* 2013).

4.3.2.2 Hardness

The evolution of hardness during storage was different between air flow treatments (Table 4.3), similar to the progression of firmness. For 98% RH treatment, fruit hardness increased (approx. 6.9%) after one week's storage in comparison to initial hardness (2.33 N) and reached maximum hardness 2.81 N, approximately 17.1% harder than initial hardness three weeks after harvest. Similar to firmness, 93% and 76% RH treatments showed a numerical increase in hardness after one week storage. Later, blueberries decreased in hardness by 3.4% and 48.2% for 93% and 76% RH treatments in comparison to initial hardness values after three weeks storage. For the 62% RH treatment hardness decreased by 12.77% after one week storage in comparison to initial hardness, and continued to decrease, reaching only 1.06 N after three week's storage, 32.0% softer than at harvest.

Hardness of blueberries is not widely reported in literature but has been reported to either increase (Chiabrando *et al.,* 2009) or decrease (Hu *et al.,* 2015) during storage under experimental conditions. Chiabrando *et al.* (2009) recorded hardness values between 5.70 – 8.90 N 35 d after harvest in blueberries stored at 0 ºC. Conversely, low hardness values of 0.08 N 9 d after harvest at 4 ºC has previously been reported (Hu *et al.,* 2015). Accordingly, previous research in blueberries validates the hardness values obtained in this experiment.

Table 4.3. TPA parameters of 'Centurion' blueberry fruit stored in air for 3 weeks, influenced by storage relative humidity (RH) atmosphere. Results are presented individually for blueberries stored in 98% RH (A), 93% RH (B), 76% RH (C) and 62% RH (D) conditions at 5.5 ºC. Means in rows with different letters are different with statistical significance at 0.05 level for storage period (letter 'a' represents the largest value, while letter 'c' represents the smallest value) (n=45).

A

RH	Texture parameters	Week			
		0	1	2	3
98%	Firmness (N)	1.942^{c}	2.125^{bc}	2.304^{ab}	2.410^{a}
	Hardness (N)	2.325^{c}	2.498^{bc}	2.694^{ab}	2.806^{a}
	Cohesiveness (-)	0.547	0.563	0.549	0.551
	Springiness (mm)	0.823	0.826	0.809	0.810
	Gumminess (N)	1.272^{c}	1.406^{bc}	1.479^{ab}	1.546^{a}
	Chewiness (mJ)	1.047^{b}	1.162^{ab}	1.197^{a}	1.252^{a}
	Resilience (-)	0.322^{a}	0.317^{ab}	0.307^{ab}	0.304^{b}

B

RH	Texture parameters	Week			
		0	1	2	3
93%	Firmness (N)	2.138^{a}	2.145^{a}	2.152^{a}	1.949^{b}
	Hardness (N)	2.386	2.486	2.447	2.303
	Cohesiveness (-)	0.558	0.559	0.579	0.575
	Springiness (mm)	0.822^{a}	0.824^{ab}	0.844^{b}	0.839^{ab}
	Gumminess (N)	1.330	1.390	1.417	1.324
	Chewiness (mJ)	1.094	1.145	1.196	1.111
	Resilience (-)	0.317	0.309	0.312	0.315

C

RH	Texture parameters	Week			
		0	1	2	3
76%	Firmness (N)	2.034^{a}	2.080^{a}	1.866^{a}	1.095^{b}
	Hardness (N)	2.384^{ab}	2.449^{a}	2.167^{b}	1.234^{c}
	Cohesiveness (-)	0.561^{b}	0.571^{b}	0.583^{b}	0.614^{a}
	Springiness (mm)	0.828^{b}	0.840^{ab}	0.857^{ab}	0.879^{a}
	Gumminess (N)	1.337^{a}	1.398^{a}	1.263^{a}	0.758^{b}
	Chewiness (mJ)	1.108^{a}	1.174^{a}	1.083^{a}	0.666^{b}
	Resilience (-)	0.321	0.319	0.315	0.308

D

RH	Texture parameters	Week			
		0	1	2	3
62%	Firmness (N)	2.163^{a}	1.868^{b}	1.414^{c}	0.942^{d}
	Hardness (N)	2.358^{a}	2.050^{b}	1.598^{c}	1.034^{d}
	Cohesiveness (-)	0.557^{b}	0.579^{b}	0.618^{a}	0.633^{a}
	Springiness (mm)	0.821	0.857	0.966	0.872
	Gumminess (N)	1.314^{a}	1.187^{a}	0.987^{b}	0.655^{c}
	Chewiness (mJ)	1.079^{a}	1.017^{a}	0.954^{a}	0.571^{b}
	Resilience (-)	0.317^{a}	0.329^{ab}	0.317^{ab}	0.309^{b}

4.3.2.3 Springiness

The change in springiness during storage was different between all air flow treatments (Table 4.3). For 98% RH, berry springiness did not significantly change for the duration of the experiment. Springiness also showed a numerical increase after three weeks storage for 93% RH treatment, although this difference was not statistically significant. Later, blueberries were 6.1% more springier in comparison to the initial springiness value (0.82 mm) and reached a maximum value of 0.88 mm after three weeks storage at 76% RH treatments. For 62% RH treatment, springiness increased by 4.4% after one week's storage in comparison to initial springiness, and continued to increase reaching 0.97 mm after only two week's storage, 17.7% springier than at harvest. After three weeks storage springiness decreased 9.7% in comparison to week two springiness value reaching 0.87 mm for the 62% RH treatment.

Blueberry springiness has been reported to increase (Deng *et al.,* 2005), and decrease (Chiabrando *et al.,* 2009; Li *et al.,* 2011) during storage under experimental conditions. According to Chiabrando *et al.* (2009) springiness of both 'Bluecrop' and 'Coville' varieties decreased 24.1% and 18.9% respectively after storage at 0 ºC, 35 d after harvest. Similarly, Li *et al.* (2011) reported decreased springiness after two week's storage in 4 ºC at 80 – 85% RH. Although after 3 weeks storage an increase in springiness was observed under the same conditions (Li *et al.,* 2011). Conversely, increased springiness values (20 – 27%) of blueberries stored between -20 – -80 ºC for 3 d in comparison to fresh fruit have been reported (Deng *et al.,* 2005). The behaviour previously observed, validates the springiness responses obtained in this experiment.

4.2.3.4 Gumminess

The change in gumminess throughout storage was dissimilar between airflow treatments (Table 4.3) but resembled a similar trend to hardness values. For 98% RH, berry gumminess increased significantly (16.2%) after two week's storage in comparison to initial gumminess (1.27 N) and reached a maximum value of 1.55 N, approximately 21.5% gummier than initial gumminess three weeks after harvest. Gumminess also showed a numerical increase after one week's storage for 93% and 76% RH treatments. Later, fruit stored at 76% RH were significantly less gummy (43.31%) in comparison to initial gumminess after three weeks storage. For 62% RH (60 mL min^{-1}) treatment, gumminess decreased by 9.66%, 24.84%, and by 50.16% after one, two and three week's storage respectively in comparison to initial gumminess.

Similarly Chiabrando *et al.* (2009) reported an increase in gumminess of 12.5% and 37.5% in 'Bluecrop' and 'Coville' varieties stored at 0 ºC for 35 d, under 90 – 95% RH. On the other hand, a decrease in gumminess of 65.0% and 75.0% at -20 and -40 ºC respectively was observed after 3 d (Cao *et al.,* 2018). Likewise Zielinska *et al.* (2016) reported a decrease in gumminess after freezing/thawing berries. Accordingly, the gumminess values obtained in this experiment are representative of the range of values obtained in previous research.

4.2.3.5 Cohesiveness

The change in cohesiveness was different between all airflow treatments during storage, similar to the progression of springiness (Table 4.3). For 98% and 93% RH cohesiveness did not change throughout storage in comparison to initial cohesiveness value (0.56). Later, blueberries were 9.4% and 13.7% more cohesive in comparison to initial cohesiveness value after three week's storage for 76% RH and 62% RH treatments, respectively. For the 62% RH treatment cohesiveness increased by 4.0% after one week storage in comparison to initial cohesiveness and continued to increase, reaching 0.63 after three week's storage. Chiabrando *et al.* (2009) reported both a 12.5% increase, and a 9.7% decrease in 'Bluecrop' and 'Coville' varieties respectively. Conversely, cohesiveness increased 63.0% when stored for 3 d at -80 ºC in comparison to fresh blueberries (Cao *et al.,* 2018). The behaviour previously observed, validates the cohesiveness values obtained in this experiment.

4.2.3.6 Chewiness

The progression of chewiness during storage was dissimilar between airflow treatments (Table 4.3). For 98% RH, fruit chewiness increased (approx. 7.4%) after one week storage in comparison to initial chewiness (1.05 mJ) and reached a maximum value of 1.25 mJ, approximately 19.6% chewier than initial chewiness three weeks after harvest. Chewiness also showed at numerical increase for 93% and 76% RH treatments after one week's storage. Later, blueberries were 39.9% less chewy in comparison to initial chewiness after three week's storage for the 76% RH treatment. For the 62% RH treatment, chewiness decreased 5.73% after one week storage in comparison to initial chewiness, and continued to decrease reaching 0.57 mJ, 47.1% less chewy than at harvest.

It was expected that with increased weight loss, the chewiness would decrease throughout storage as a result of the cell wall degradation of near-surface structures. Chewiness of blueberries has been reported to decrease (Chiabrando *et al.,* 2009; Zielinska *et al.,* 2015; Cao *et al.,* 2018) under different storage conditions. Chiabrando *et al.* (2009) reported a 17.6%

and 13.8% decrease in chewiness of ‘Bluecrop’ and ‘Coville’ varieties stored 0 ºC for 35 d, similar to the results obtained in this experiment.

4.2.3.7 Resilience

The evolution of resilience during storage was dissimilar between airflow treatments (Table 4.3). At 98% RH, fruit resilience did not significantly change after one week’s storage in comparison to initial resilience value (0.32) reaching a minimum value of 0.30, approximately 5.6% lower in resilience three weeks after harvest. Resilience showed a 3.2% decrease after one week storage for 93% RH treatment reaching a value of 0.32 after three week’s storage, showing no change compared to initial resilience. Later, blueberries were 3.1% and 6.3% less resilient in comparison to the initial resilience value after three week’s storage for 76% and 62% RH treatments respectively. Resilience has been reported to decrease 17.3% at 0 ºC storage for 35 d (Chiabrando *et al.,* 2009). The resilience values generated in this experiment are similar to the values obtained by Chiabrando *et al.* (2009), which validates further comparison. Although there is limited research based on the resilience of blueberries.

4.4 Discussion

4.4.1 Weight loss and texture response relationships

Weight loss was found to have a causal effect on texture during blueberry storage. Opposing texture outcomes were obtained in different weight loss ranges, regardless of the airflow rate and storage time. The experimental design eliminates all other obvious causes of texture change (temperature, carbon dioxide and oxygen concentration) suggesting postharvest water loss is the main influencing factor on changes to texture for ‘Centurion’ blueberries (Paniagua *et al.,* 2013). Blueberry firmness, hardness, gumminess and chewiness increased consistently at low levels of weight loss (0.13 – 3.11%), whereas a decrease in these texture parameters was observed with high weight loss (4.66 – 15.96%). At all levels of weight loss cohesiveness increased. Although weight loss was not found to have a causal effect on springiness or resilience parameters. Firmness, hardness, chewiness and gumminess were found to correlate negatively ($r<-0.8$). Whereas cohesiveness correlated positively with weight loss ($r>0.8$). These high correlations agree with previous research (Chiabrando *et al.,* 2009; Paniagua *et al.,* 2013)

The results of this work are in agreement with Paniagua *et al.* (2013), who obtained blueberry firming with low levels of weight loss (0.22 – 1.34%), whereas weight loss higher than 3.47% correlated with softening in the same storage experiment. Similarly, Jia *et al.* (2014) reported minimal firmness evolution of 'Berkeley' blueberries simultaneously with approx. 4% weight loss, but softening when weight loss was approx. 13%. Furthermore, firming of 'Burlington' blueberries occurred simultaneously with 1 – 2% weight loss, with fruit softening when weight loss was between 4 – 14% (Forney *et al.,* 1998). In these studies possible explanations were not provided.

The firming of blueberries observed for 98% and 93% RH treatments at low weight loss, is in agreement with a number of previous studies (Forney *et al.,* 1998; Duarte *et al.,* 2009; Chiabrando and Giacalone, 2011; Paniagua *et al.,* 2013), where softening has been reported to occur together with ≥2% weight loss (Miller *et al.,* 1993; Ferraz *et al.,* 2001; Tetteh *et al.,* 2004; Almenear *et al*., 2010; Angeletti *et al.,* 2010; Cantin *et al.,* 2012; Jia *et al.,* 2014). Importantly these studies used different methodology and materials to measure firmness; such as FirmTech1 (Bioworks, Stillwater, Oklahoma, USA) (Forney *et al.,* 1998; Allan-Wojtas *et al.,* 2001), Effegi penetrometer (Effegi, Italy) (Duarte *et al.,* 2009) and TA.XT Texture Analyser (Stable Micro Systems Ltd., UK) (Paniagua *et al.,* 2013) indicating the increase in texture responses is not due to a methodology error.

Nevertheless, the possible causes leading to this increased firmness have not been fully addressed. Paniagua *et al.* (2013) reported reduced water content of the epidermal area of 0 mL min^{-1} treatments could be related to increased firmness. This may be associated with the thickening and corrugation of parenchyma (Forney *et al.,* 1998) and epidermal cell walls (Allan-Wojtas *et al.,* 2001). Microstructural changes occurring in the outer cell layers as a result of dehydration in low moisture conditions have been proposed as an explanation for increasing firmness by Paniagua *et al.,* (2013). Although this conflicts with the increased firmness observed for 98% RH treatments (high humidity) in the present experiment. Previous MRI assessment obtained for 15, 30 and 60 mL min^{-1} treatments suggested that moisture was lost from not only the epidermal area but also from within parenchyma cells (Paniagua *et al.,* 2013), which supports the possibility of a reduction in berry turgor as the main cause of decreased berry firmness in this experiment.

The increase in hardness, chewiness and gumminess observed for 98% and 93% RH treatments at low weight loss is in agreement with firmness values obtained in the present experiment, sharing a similar trend (Figure 4.4).

Hardness of blueberries is rarely reported in literature and is often interchangeable with firmness. Chiabrando *et al.* (2009) obtained significantly higher hardness values for 'Coville' blueberries with the advancement of storage period when weight loss was 6.68%. This is in agreement with the results obtained in this experiment, where an increase in hardness for 98% and 93% RH treatments was observed under low weight loss conditions. Factors causing an increase in postharvest hardness are not yet fully understood. Although similar mechanisms behind an increase in firmness are likely responsible. Disintegration of the primary cell wall and middle lamella are the main factors responsible for fruit softening (Deng *et al.,* 2005).

The chewiness results obtained in this experiment for 76% and 62% RH treatments are in agreement with Chiabrando *et al.* (2009), who obtained 13.8 and 7.6% decrease in the chewiness of 'Bluecrop' and 'Coville' blueberries 35 d after harvest, indicating a less elastic berry structure. Similarly, a decrease in chewiness has been reported in other soft fruits such as strawberry (Caner and Aday, 2009) and bayberry (Yang *et al.,* 2007). Similar to previous discussion, degradation in the cell wall of the outer layers has been proposed as the likely mechanism for decreasing chewiness during storage. Moreover, an increase in chewiness was reported by Cao *et al.* (2018) in berries stored at -20 ºC. Indicating temperature, and moisture loss may slow the decline of chewiness, in agreement with 98% and 93% RH treatments. Therefore prevention of enzymatic activity may prevent cell wall degradation, preserving texture.

The increase in gumminess for 98% and 93% RH treatments at low weight loss, agrees with Chiabrando *et al*. (2009) who reported a 12.6 and 37.5% increase in Bluecrop' and 'Coville' blueberries respectively 35 d after harvest. Microstructural changes and degradation of the cell wall resulting from increase enzymatic activity (Chiabrando *et al.,* 2009) may have a minor role in the observed increase in gumminess. Conversely, a decrease in gumminess occurred under high flow rate (76% and 62% RH) treatments. Zielinska *et al.* (2016) reported decreased gumminess after a freeze/thaw process. Similarly, a decrease of 70% was observed in blueberries stored at -40 ºC (Cao *et al.,* 2018). In conjunction with previous research and the results obtained in this experiment, freezing and humidity conditions during storage may affect the gumminess of berries.

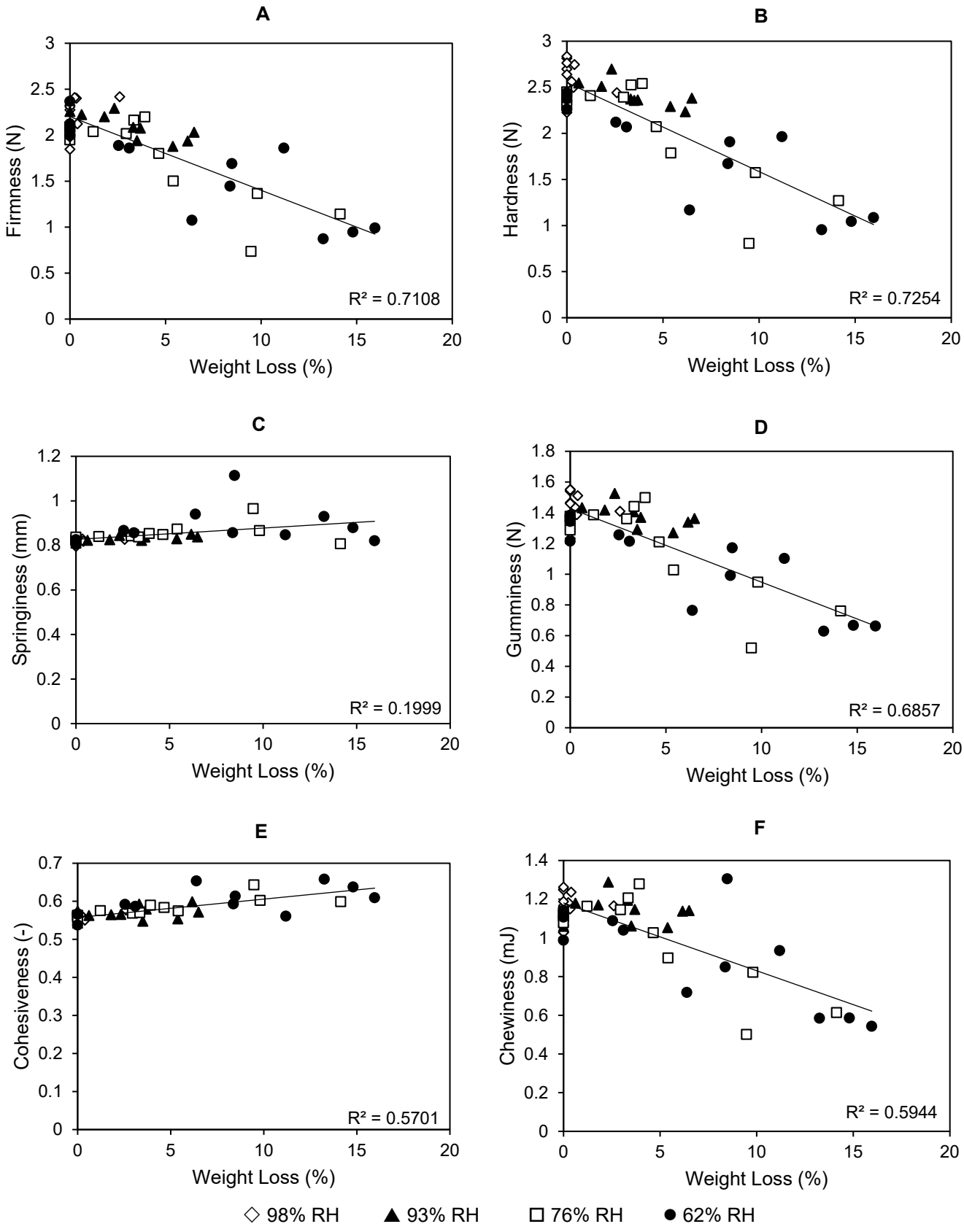

Figure 4.4. Correlation between texture characteristics and weight loss means. Each symbol represents weight loss and firmness (A), hardness (B), springiness (C), gumminess (D), cohesiveness (E) and chewiness (F) after a storage period at 5.5 ºC subjected to humidity treatments (n=15).

Weight loss was found to have a causal effect on cohesiveness change of blueberries during storage. Opposing cohesiveness outcomes were obtained in different weight loss ranges. Higher cohesiveness values consistently occurred at higher levels of weight loss. Cohesiveness tended to be higher for 76% and 62% RH reaching 0.6 where weight loss was the greatest (15.96%). Cohesiveness was found to correlate positively with berry weight loss during storage, with a high Pearson's coefficient ($r>0.8$) obtained for weight loss and cohesiveness (Figure 4.4). This high correlation agrees with previous research (Zielinska *et al.,* 2015; Cao *et al.*, 2018). The results of this work are in agreement with the work of Cao *et al.* (2018) who reported a 60% increase in cohesiveness between fresh and frozen/thawed blueberries, although weight loss was not reported. A possible explanation for the increase in cohesiveness for 93%, 76% and 62% RH treatments is the strengthening of the internal bonds of the berry during dehydration. It is highly likely changes to berry microstructure resulting from moisture loss are responsible for this increase.

Chiabrando *et al.* (2009) reported weight loss of 6.68% simultaneously with a 9.75% decrease in cohesiveness 35 d after harvest which is in agreement for 98% RH treatments. The solubilisation and depolymerisation of cell wall constituents including pectin have been suggested as a potential mechanism for decreasing cohesiveness of Chinese Bayberry fruit (Yang *et al.,* 2007). Both cohesiveness responses previously reported were successfully induced in this experiment. The results of this experiment indicate variable cohesiveness responses across humidity conditions, additional research to elucidate the exact mechanism for this relationship is necessary.

4.4.2 Industry applications

The causal relationship between blueberry moisture loss and texture responses could have an important impact on the postharvest management of this crop within industry. According to this data, maintaining weight loss levels below 8 – 12% during the postharvest chain and storage would minimise textural degradation of blueberries. Paniagua *et al.* (2013) has suggested the use of palletised modified atmosphere bags or less vented clamshell designs in order to retain firmness throughout the commercial supply chain. Considering these technologies and materials that limit weight loss, texture characteristics can be maintained throughout the postharvest chain. Postharvest texture responses could be monitored by detecting loses in weight, allowing for easier control mechanisms potentially avoiding undesirable texture characteristics below commercial standards. This would allow for the opportunity for correction of atmospheres throughout the commercial supply chain, or to redirect fruit batches to less distant markets.

Furthermore monitoring of the internal storage humidity and controlling the humidity environment in the postharvest chain could improve texture characteristics by minimising weight loss in parts of the supply chain which enhance moisture loss such as cooling delays. This will allow for extended shelf life of fresh berries, enabling export to distant market previously unreachable. Controlling the humidity environment in this experiment indicates texture characteristics can be enhanced during storage between 93 – 98% RH environments. Further investigations into the texture responses of different blueberry cultivars will determine the optimum humidity environment throughout the commercial supply chain to retain and optimise blueberry texture attributes. The results of this experiment suggest texture responses may be predictable given an accurate measure of weight loss due to the strong relationship between texture response and weight loss during storage.

The evidence provided by this work indicates a causal relationship between moisture loss and texture characteristics for ‘Centurion’ blueberries. To confirm the potential mechanism of this relationship the evaluation of turgor, microstructural changes, and cellular resistance should be investigated. Moreover the evaluation of more cultivars to determine optimum humidity conditions, and the consistency of texture responses across all varieties should be carried out.

4.5 Conclusion

The results of this experiment indicate variable softening and texture responses can be successfully induced during storage by the manipulation of blueberry weight loss resulting from different humidity conditions from the alteration of air flow rates. At a given temperature, humidity or atmosphere condition the manipulation of the air flow rate can be utilised to induce variable textural responses of stored blueberries. These experimental settings could present an effective option to increase desirable blueberry texture characteristics throughout cold storage and transportation of this crop. This may well be a simple, cost effective option to preserve fresh blueberry texture characteristics after storage, or transportation to international markets. The settings used in this experiment may be used in future research on other varieties focused on texture responses and moisture loss to confirm the responses seen in this experiment.

The hypothesis of a causal relationship between moisture loss and texture responses is supported by the results of this experiment. The correlation found between firmness, hardness, springiness, gumminess, cohesiveness and weight loss, suggests berry moisture loss plays an important role in determining texture responses of blueberries during postharvest

storage. It is likely differing water loss patterns, cell wall modifications, increased skin toughness and turgor are responsible for the mechanism between moisture loss and texture responses. Although additional research to elucidate the exact mechanisms responsible for this relationship in blueberries is necessary. The reduction of moisture loss during postharvest storage and transportation is a way to improve the quality status of blueberries in the market place. There is potential for the blueberry industry to benefit from the results of this experiment, providing superior blueberries to consumers.

The interaction between moisture loss and postharvest texture responses could be used to evaluate blueberry texture characteristics along the postharvest chain. By monitoring weight losses of blueberries throughout storage and transportation, quality texture characteristics could easily be monitored and predicted. To make this a more comprehensive technique the texture characteristics in relation to moisture loss for other blueberry cultivars need to be evaluated. Monitoring of blueberry texture characteristics could provide industry with the opportunity to take correction measures, by determining whether a fruit batch will have desirable textural characteristics when it reaches its international market. Therefore, only blueberry varieties that retain desirable texture characteristics weeks after harvest would be exported to distant markets. Thus, improving the integrity of the blueberry industry and maintaining consumer satisfaction. The prediction of blueberry texture characteristics on arrival in retail conditions, in addition to quality control systems, allows for the improvement and maintenance of blueberry quality. Therefore, the evidence provided by this experiment indicates it is possible to ensure acceptable blueberry texture standards at the market place.

Chapter 5.
Microstructural Characteristics of Blueberries Throughout Storage in different Humidity Conditions

5.1 Introduction

The year round global demand for fresh blueberries, relies on the storage ability of fresh blueberries which is a fundamental requirement for export to global markets (Giongo *et al.,* 2013). Texture is an important indicator of fruit quality and is dependent on the underlying microstructural characteristics of the fruit. Often resulting from poor transportation and storage conditions, large volumes of blueberries are rejected at the market place due to inadequate quality and texture (Prussia *et al.,* 2006). High storage humidity conditions maintain blueberry texture characteristics, and in some instances have resulted in increased texture attributes (Paniagua *et al.,* 2013). Therefore innovative research orientated to determine microstructure changes during postharvest storage has great potential for the blueberry industry in determining which blueberry cultivars are resistant to microstructural degradation during storage.

Several microstructural components such as cell type, cell size, cell to cell adhesion, cell wall thickness, extracellular space, packing and shape contribute to overall fruit texture (Harker *et al.,* 1997). Where changes in fruit texture often result from underlying microstructural changes (Bunemann *et al.,* 1957; Sapers *et al.,* 1984; Sapers *et al.,* 1985; Allan-Wojtas *et al.,* 2001). Differences of several anatomical features such as tissue layers and cell size result in the expression of different phenotypes such as firmness, gumminess, juiciness and mealiness (Jackman and Stanley, 1995; Chiabrando *et al.,* 2009; Johnson *et al.,* 2011; Giongo *et al.,* 2013). Blueberry parenchyma cells have thin, non-lignified cell walls with large water filled vacuoles, being the most numerous type of cell in blueberry flesh (Harker *et al.,* 1997). The epidermis is composed of specialised parenchyma cells called epidermal cells that have thickened cell walls covered by a cuticle consisting of cutin and epicuticular waxes (Esau, 1997). Other elements such as collenchyma cells and phloem structures provide tensile strength to surrounding tissues through thickened primary cell walls.

The size of cells between the epidermis, sub-epidermis and flesh of fresh blueberries varies throughout storage, with each successive layer of cells from the epidermis increasing in size

(Cano-Medrano and Darnell, 1997; Allan-Wojtas *et al.,* 2001; Blaker and Olmstead, 2014). Interestingly, the amount of contact and space between neighbouring cells is influenced by the shape and packing of cells (Harker *et al.,* 1997). This may influence the texture of fruit, where Batisse *et al.* (1996) observed large intercellular spaces in crisp textured cherries compared to soft textured cherries. It still remains unclear whether humidity storage conditions have a profound effect on blueberry microstructure during storage.

It is important to consider blueberry fruit anatomy when searching for a possible explanation for microstructural changes occurring throughout storage in different humidity conditions. The epidermis of the blueberry originates from the flowers calyx and is associated with epicuticular waxes which contribute to the fruits dark pigmented blue colour (Gough, 1994). The production of anthocyanins within the epidermis and sub-epidermis are responsible for pigmentation and purple colour seen in these layers. The flesh of the blueberry is located below the sub-epidermis layer, and contains mostly parenchyma cells, along with sclerified stone cells, and vascular bundles. Stone cells can be found 460 to 920 μm below the epidermis (Gough, 1983), although for highbush varieties they have been found approximately 100 μm below the surface of the epidermis (Blaker and Olmstead, 2014). Blaker and Olmstead (2014) demonstrated the cross sectional area of epidermal cells varied between 429 μm^2 and 712 μm^2 in ripe blueberries, whereas the average epidermal cell area of 'Beckyblue' blueberries was 1175 μm^2 (Cano-Medrano and Darnell, 1997). Interestingly the parenchyma cell area of 20 rabbiteye blueberry genotypes ranged between 11580 μm^2 and 18840 μm^2 in mature fruit (Johnson *et al.,* 2011). A large difference between the cell area of cells present in different microstructural layers may indicate genotype differences, and differences in textural characteristics

Postharvest modification of blueberry microstructure is not very well understood, however it is thought turgor plays an important role in fruit softening (Thomas *et al.,* 2008) and the accompanying microstructural changes that occur throughout storage. Turgor interacts with the cell wall, such that when the internal turgor pressure of the cell is low, disruption of cell to cell adhesion is likely to occur. This is related to the degradation of the middle lamella and has been proposed as a mechanism for the increasing air space between cortical cells during cold storage of apples (Harker and Hallett, 1992). Paniagua *et al.* (2013) demonstrated rabbiteye blueberries stored in lower humidity conditions displayed high weight loss values over a three week storage period although changes in the microstructure of blueberries stored in different humidity conditions have not been fully investigated.

Microstructural changes have been tracked in blueberries stored in air for six weeks (Allan-Wojtas *et al.,* 2001), where epidermal cells changed from a square shape to a round shape after six weeks, and sub-epidermal and parenchyma cells remained in close contact with each other but became rounded (Allan-Wojtas *et al.,* 2001). The rounding of cells and subsequent increase in cell area during storage is in agreement with Harker and Hallett (1992), where cortical cells of 'Braeburn' apples appeared rounded after cold storage. A reduction of cell to cell adhesion enabling the cell wall to expand slightly, was proposed as the likely mechanism responsible for the visible rounding of cells.

Limited description of microstructure changes during blueberry storage in previous research, and whether different storage humidity conditions result in significant changes in blueberry microstructure still needs to be addressed. The present experiment induces different microstructural responses in 'Centurion' blueberries by altering humidity conditions, creating different moisture loss conditions during cold storage.

5.1.1 Experimental objectives

The objectives for this experiment are as follows:

1. Perform a histological analysis of cell type, cell area and structure of the outermost layers of 'Centurion' blueberries.
2. Characterise postharvest microstructural changes in blueberries as a result of storage period and humidity conditions.
3. Determine the optimum humidity condition that preserves the microstructure of blueberries during storage.

The outputs of this experiment will provide insight into the microstructure of blueberries throughout storage in different humidity conditions, providing relevance to the blueberry industry to determine the optimum microstructure characteristics desirable in blueberries for export. The results of this research could be used in conjunction with texture results to adjust postharvest practises in order to improve the internal quality of blueberries in the final market place.

5.2 Materials and methods

To address the objectives of this experiment, different RH conditions were created using a closed airflow system and monitored throughout the experiment. Full details of the materials and methods to create this system including fruit source, sample configuration and experimental design are provided in Section 3.2 and 3.4.

5.2.1 Fruit material

Light microscopy was used to assess microstructural changes throughout storage of 'Centurion' rabbiteye blueberries at different humidity conditions (98%, 93%, 76% and 62% RH). Five blueberries were randomly chosen from each treatment every week for 3 weeks for evaluation. Collapsed fruit or fruit presenting fungal mycelium were discarded from microscopic examination as they were not representative of the blueberry sample population.

5.2.2 Blueberry sample preparation

5.2.2.1 Fixation

A standard Formalin, Alcohol, Acetic Acid (FAA) fixative was prepared within a fume hood for the fixation of blueberry samples (Ruzin, 1999). The standard formulation of FAA consisted of 50 mL of 95% drum alcohol, 10 mL formalin (37% formaldehyde, Sigma-Aldrich), 5 mL of glacial acetic acid (Sigma-Aldrich) and deionised water to 100 mL. Approximately 200 mL of fresh fixative was prepared fresh before each evaluation day and 50 mL of fixative was poured into four 75 mL containers, sealed and stored at 4 ºC. At each evaluation day a total of four vials were used, one for blueberries from each humidity treatment. A total of 16 glass vials were used in this experiment.

At each evaluation day a 0.23 mm width steel razor blade was used to remove the stem and calyx end of each blueberry in a wax bottom dish at room temperature within a fume hood, resulting in approximately 5 mm radial sections of blueberry samples. The five cut blueberries from each treatment were placed into the same glass vial containing five to ten times excessive fixative than the sample volume. Vials containing radial blueberry sections and fixative were then labelled, screwed tightly and covered in parafilm before being placed in 2 L plastic containers and stored at 4 ºC for approximately two months.

5.2.2.2 Processing

The fixed blueberry samples were transported by car on 24/4/2018 to the Microscopy and Cell Wall laboratory located at The New Zealand Institute for Plant and Food Research Mt Albert, Auckland, New Zealand to be stored at 4 ºC where further fruit sample preparation and processing occurred under the guidance of Professor Ian Hallett.

Blueberry sections were transferred from glass vials into a wax bottom dish inside a fume hood and cut in half using a 0.23 mm width scalpel blade. One half of the cut blueberry section was placed back into the glass vial containing fixative and stored at 4 ºC. The other half was transferred into an individual embedding cassette (approximately 4 x 3 x 1 cm) and labelled with pencil. The cassette was then placed in a 50% ethanol holding solution. A Leica TP1020 Semi-enclosed Benchtop Tissue Processor (Leica Microscopy Systems Ltd., Heerbrugg, Switzerland) was used for the dehydration and infiltration of blueberry material. Cassettes were loaded into a processing basket and securely attached to a carrier in the processing system. Samples were dehydrated in a standard ethanol sequence of 50%, 70%, 95% and 100% (Table 5.1). Prior to wax infiltration ethanol was replaced by a solvent for paraffin. The most commonly used in plant research is xylene. A 1:1 100% pure ethanol:xylene solution followed by two pure xylene steps was utilised in this experiment. Dehydration and infiltration occurred automatically over two and a half days under constant agitation. After the completion of dehydration and wax infiltration, samples were embedded with wax.

Table 5.1. Automatic dehydration and infiltration process steps.

Bath Number	Contains	Time	Vacuum
1	50% ethanol	15 mins	OFF
2	70% ethanol	3 hours	ON
3	95% ethanol	3 hours	ON
4	100% drum ethanol	3 hours	ON
5	100% pure ethanol	3 hours	ON
6	100% pure ethanol	3 hours	ON
7	1:1 100% pure ethanol:xylene	3 hours	ON
8	Xylene	3 hours	ON
9	Xylene	3 hours	ON
10	Wax-paraplast	12 hours	ON
11	Wax-paraplast	12 hours	ON
12	Wax-paraplast	12 hours	ON

5.2.2.3 Embedding

A Leica EG1160 paraffin dispensing system (Leica Biosystems, Heerbrugg, Switzerland) was used to embed samples with wax. Cassettes containing embedded blueberry sections were removed from the processing basket and transferred into a wax holding bath. Each cassette was then removed individually from the holding bath to a heated preparation area. The lid of the cassette was removed, and specimen placed on heated tray using forceps. A wax mould of 3.0 cm x 2.4 cm was placed under a liquid wax dispenser and filled 1/3 with wax and specimen added. The wax mould containing blueberry sample was gently slid onto a cool plate (≤0 ºC) to cool the specimen and to partially solidify wax. Using forceps the specimen was orientated in the middle with the cut side facing the short edge of the wax mould (Figure 5.1). Subsequently the mould was filled to the surface with wax, the labelled cassette lid was removed from the cassette and placed on the top of the wax mould before the wax hardened to identify the blueberry sample. Moulds were then moved to a cold plate to allow the wax to solidify. After 30 minutes the wax had solidified, and the samples embedded in wax were removed from the moulds and stored at room temperature in a plastic container. Once embedded in wax these samples remain stable and can be stored at room temperature for many years.

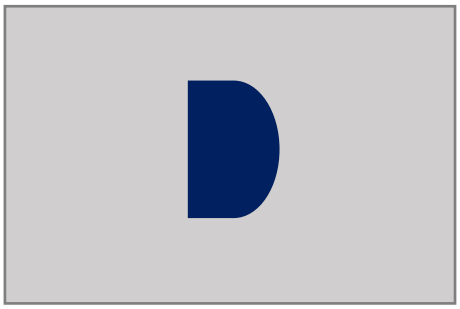

Figure 5.1. Diagram of the orientation of half blueberry sections embedded in wax. Blueberry sections are orientated with the cut face facing the short sides of the embedding cassettes.

5.2.2.4 Sectioning

Embedded wax samples were sectioned with a Leitz 1512 rotary microtome (Leitz, Germany), using a 0.25 mm disposable microtome steel blade. Prior to sectioning embedded wax samples were trimmed by hand using a 0.23 mm steel blade into a triangular shape, desirable for creating a ribbon of thin blueberry sections (Figure 5.2). Blueberry samples of 15 µm were sectioned and placed in a 42 ºC water bath (Leica HI1210, Leica Biosystems, Heerbrugg, Switzerland), where they were collected onto the middle surface of a positively charged, pre-cleaned 75 x 25 mm microscope slide. Slides were labelled and placed on a drying rack

overnight at 40 ºC, to drive off solvent and water from the sample, and so the wax section could adhere to the glass microscopy slide.

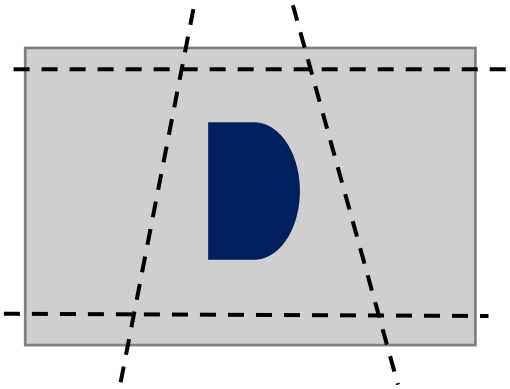

Figure 5.2. Example of how embedded wax sample were trimmed. Samples were cut along the dotted lines resulting in a triangular section. Wax outside of this section was cut approximately 5 mm deep leaving a trapezoid raised section of the sample and wax.

5.2.2.5 Staining

Dry sections were stained with toluidine blue using Sakai's toluidine blue method (Sakai, 1973). The primary use of toluidine blue is to detect pectin and lignin (O'Brien *et al.,* 1964; Mori and Bellani, 1996). An advantage of using toluidine blue is that many elements of the cell can be visualised (Mitra and Logue, 2014). Differentiation between cell types and tissue structures is determined by the colour developed by toluidine blue (Table 5.2). In order for the success of this staining method, the sections must not be allowed to melt, as toluidine blue stain is aqueous is not able to penetrate the relatively blueberry tissue. The sections should be allowed to dry completely before deparaffinization and alcohol must be avoided as it destroys metachromatic staining of the samples (Sakai, 1973).

Toluidine blue was an appropriate stain for the visualisation of blueberry cells in this experiment. Dry sections on slides were stained five at a time in a five slide Coplin staining jar using a 0.05% aqueous solution of toluidine blue for 5 minutes. Sections were rinsed in water and left to dry for 30 minutes on a drying rack at 40 ºC before deparaffinisation. To deparaffinise samples, slides were taken through xylene 1 to 4 for 15 minutes each in Coplin staining jars, then air dried and permanently mounted with a 24 x 50 mm glass cover slip using TBS SHUR/Mount™ liquid mounting medium (Triangle Biomedical Science, North Carolina, United States). Mounted slides were left in a fume hood overnight at room temperature before microscopic evaluation.

Table 5.2. Differentiation between cell types and tissue structures observed using toluidine blue stain. Adapted from Parker et al. (1982).

Cell Type or Tissue Element and Structure	Colour Developed by Toluidine Blue
Callose and starch	Unstained
Collenchyma	Red-Purple
Parenchyma	Red-Purple
Phloem	Red
Sclerenchyma	Blue-Green
Xylem	Green or Blue-Green

5.2.3 Microscopy

An Olympus light microscope (Vanox AHT3, Olympus Optical Co. Ltd., Tokyo, Japan) was used to visualise blueberry samples using the 10x objective lens. Images were captured with a colour camera (DP74, Olympus Optical Co. Ltd., Tokyo, Japan). Blueberry sections were visualised using cellSens standard digital imaging software version 1.7 (Olympus Optical Co. Ltd., Tokyo, Japan).

5.2.4 Image analysis

ImageJ-Fiji software (Schindelin *et al.,* 2012) was the primary image analysis software used in this experiment. ImageJ was calibrated to the appropriate scale before use each time.

5.2.4.1 Cell area

Blueberry cell area was calculated using ImageJ by segmentation of individual cells in each image using the analyse and measure function. The epidermis and sub-epidermis were split into the outer three cell layers of the half section of ripe fruit. The average cross sectional area of cells in each of these three layers was calculated. In the outermost cell layer (epidermis) the area was determined for 20 random cells, whereas in layer 2 and layer 3 (sub epidermis) 10 random cells were used to determine the average area of a cell in these layers. Finally, layer 4 included all layers in the parenchyma section to a depth of approximately 0.5 mm of the fruit, where 10 random cells were used to determine the average area of a cell in this region. The average cell area of a cell in each of these four layers is presented in this experiment.

5.2.5 Microstructural indicators

During light microscopy analysis, sections of fresh blueberry samples at harvest were observed first to more easily determine microstructural changes throughout storage at each humidity treatment.

5.2.6 Data analysis

The data set was analysed by testing the effect of each factor and their interactions on the average cell area in each layer, using ANOVA on untransformed data. Differences between means were assessed by using Tukey HSD test. Factors considered in the model were humidity conditions and storage time (i.e. week). Cell layers were analysed independently. Significant differences were considered at a 5% level. Data was analysed using Minitab version 18.1.0 (Minitab Inc., Pennsylvania, USA).

5.3 Results

Blueberry cell walls and pigment were preserved when berry samples were prepared by the method described. Selected samples were chosen to study microstructural differences among blueberries stored at different humidity conditions and to determine changes during storage. Any changes observed are assumed to reflect microstructural changes throughout storage and humidity treatments.

5.3.1 Fresh fruit storage (98% RH)

Figure 5.3 shows microstructural differences between blueberries subjected to high RH (98%), and the effect of storage period on the structure of ‘Centurion’ blueberries. Storage differences were observed in the relative size and arrangements of cells, integrity and turgidity of cells, which is related to blueberry texture. These cellular characteristics as well as the distribution of pigment is related to the susceptibility of bleeding, that is the number of cells containing pigment.

Fresh ‘Centurion’ blueberries (Figure. 5.3a) consisted of an epidermis of one exterior layer of small, square, pigment containing cells which were in close contact with each other. The epidermal cells were tightly packed, turgid cells, and maintained good cell to cell contact. Two interior layers of similar size, rectangular cells containing large deposits of pigment were tightly packed together consisting of subepidermal cells. In comparison, after one week’s storage in

air (Figure. 5.3b) the epidermis consisted of one small exterior layer of pigment containing cells. Maintaining close contact with each other, these cells were less square in shape, tightly packed and turgid. Two interior layers of pigment containing cells consisted of the sub-epidermis were larger than epidermal cells and swelled to a circular shape. The sub-epidermal layers were beginning to lose shape and develop a random structure.

After two weeks storage in air (Figure. 5.3c), differences were observed in the relative size and arrangement of cells, as well as the integrity of blueberry cells. The exterior epidermal layer consisted of rounded and elongated cells, maintaining good cell to cell contact. Pigment deposits in epidermal cells were present in extracellular spaces and migrated from the centre to the outer of cells. The interior layers of the sub-epidermis lost integrity, characteristic of a loss in turgidity. A lack of structure, irregular shape, and increasing extracellular space are indicative of blueberry moisture loss and degradation resulting in increased fruit softening.

In blueberries stored in air for three weeks (Figure. 5.3d), epidermal cells containing pigment maintained a tightly packed structure consisting of elongated, round cells. The pigment contained in the external epidermal layer was larger in shape and consumed most of the volume of these cells. Lacking structure, larger, rounded subepidermal cells were observed in an unorganised arrangement. A loss of integrity, as well as pigment deposits present in extracellular spaces are an indication of a breakdown in the microstructure of ‘Centurion’ blueberries.

Storage period effects were also observed in the berry flesh in regards to the degree of cell to cell contact, interfaces between parenchyma cells and subepidermal cells, and the shape of parenchyma cells. The flesh of fresh ‘Centurion’ blueberries consisted of large, round cells in close contact with each other (Figure 5.3a). These cells were observed to have a high turgidity. While flesh of blueberries stored for one week in air were large in size, but irregular shaped indicating a potential loss in turgidity (Figure 5.3b). Pigment bleeding was seen in the first layer of parenchyma cells. Diminishing parenchyma cell to cell contact below the sub-epidermal layers, and the appearance of extracellular space were characteristics observed in the flesh of blueberries stored in air for one week. After two weeks storage in air total collapse of irregular parenchyma cells was concentrated below the sub-epidermis (Figure 5.3c). Increased cell separation and extracellular space was observed up to 0.5 mm below the sub-epidermis in the flesh after three weeks storage in air (Figure 5.3d). Extensive cell collapse was observed below the sub-epidermis. Pigment bleeding can be seen deep into the flesh.

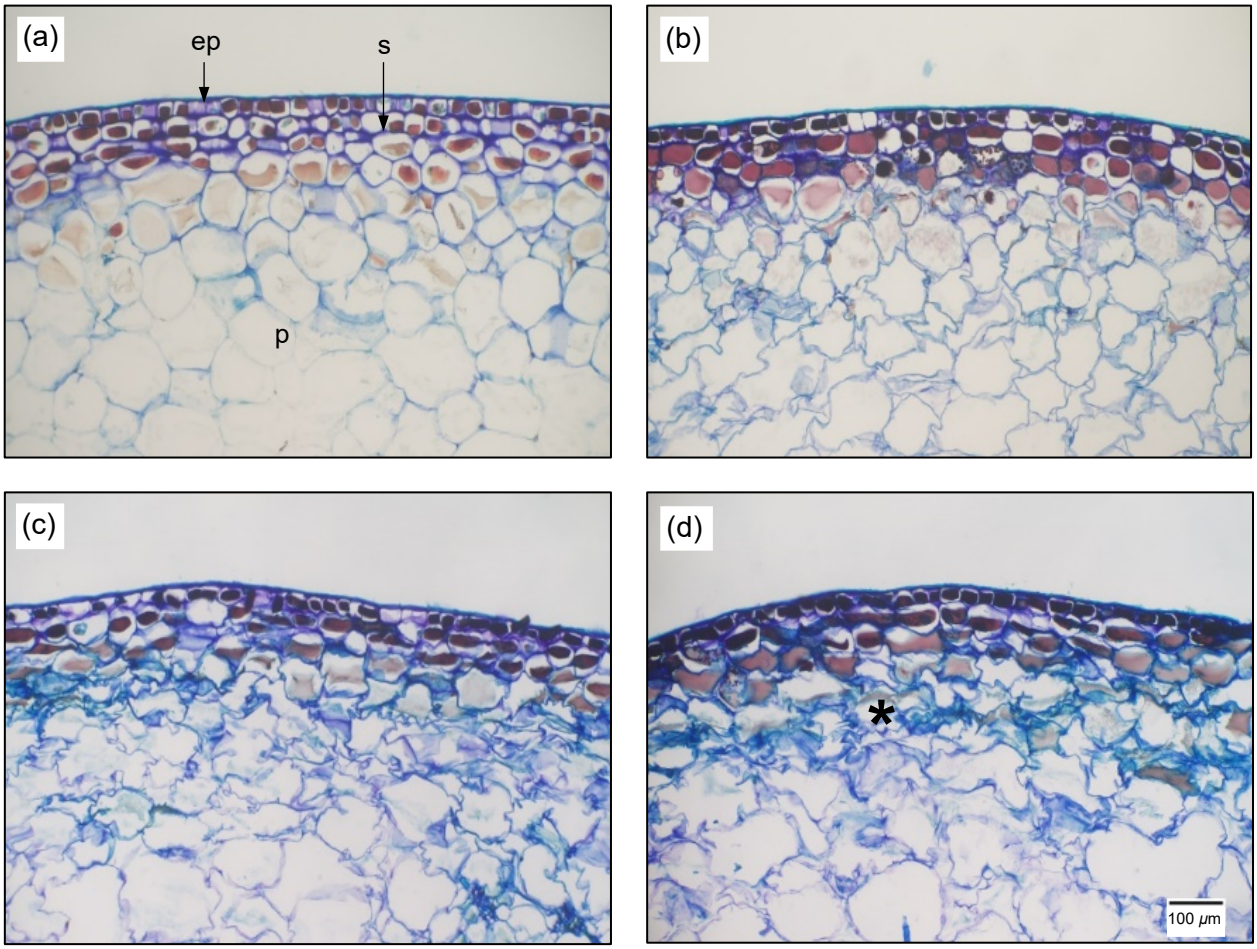

*Figure 5.3. Light microscopy showing the effects on the microstructure of 'Centurion' blueberries after storage in 98% RH. (a) Fresh berry (no storage). Berry stored for 1 (b), 2 (c) and 3 (d) weeks in air. All micrographs are of light microscopy 15 µm sections stained with toluidine blue. Abbreviations used: (ep = epidermis, p = parenchyma (flesh), s = sub epidermis, * = cell collapse) (Bar is 100 µm).*

5.3.2 One week's storage (93%, 76% and 62% RH)

Differences in microstructure during storage were amplified after air storage in different humidity environments. In the epidermis, these changes appeared as thinning or crushing of the cells, rounding up of epidermal cells, and breaks in the arrangement of cells within the epidermal layer. After a week's air storage at 93%, 76% and 62% RH 'Centurion' blueberries shared similar microstructural characteristics (Figure 5.4b; Figure 5.5b; Figure 5.6b). The epidermis consisted of one layer of small, thin rectangular pigment containing cells that were in close contact with each other. Epidermal layers became compressed and retained original pigment. Two interior sub-epidermal layers were tightly packed, with good cell to cell contact, and similar in size to cells in the epidermis after storage at 93% RH (Figure 5.4b). Whereas in the sub-epidermal layers of blueberries stored in 76% and 62% RH environments a reduction of cell to cell contact, rounding and enlargement of sub-epidermal cells was observed. Tightly

concentrated intense pigment was present and retained within the sub-epidermal region (Figure 5.4b; Figure 5.5b).

Storage period effects were observed in the flesh of blueberries stored in 93%, 76% and 62% RH after one week storage. Pigment leakage into the first layer of parenchyma cells was observed. Medium to large sized, rounded, tightly packed, turgid parenchyma cells were present in the flesh of blueberries stored in 76% RH (Figure 5.5b). Whereas the flesh of blueberries stored in 76% and 62% RH, consisted of irregular shaped cells characteristic of a loss in turgidity, increased intercellular gaps and decreased cell to cell adhesion. A thinning and partial loss of cell integrity in the parenchyma was observed approximately 0.2 mm below the surface of blueberries subjected to 93% RH. Blueberries stored in 76% and 62% RH environments consisted of decreasing cell to cell contact and formation of intercellular gaps within the berry flesh.

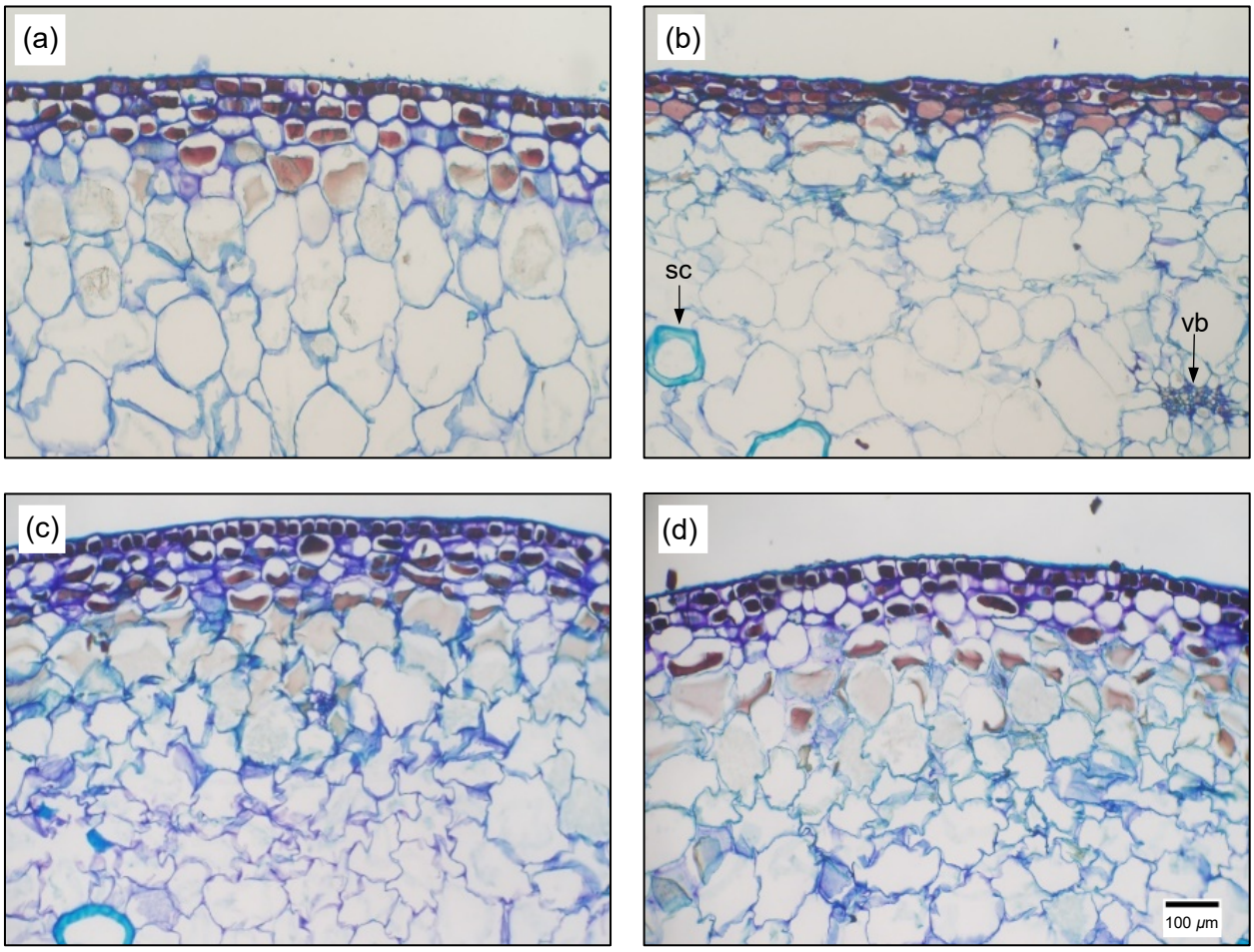

Figure 5.4. Light microscopy showing the effects on the microstructure of 'Centurion' blueberries after storage in 93% RH. (a) Fresh berry (no storage). Berry stored for 1 (b), 2 (c) and 3 (d) weeks in air. All micrographs are of light microscopy 15 µm sections stained with toluidine blue. Abbreviations used: (sc = stone cell, vb = vascular bundle) (Bar is 100 µm).

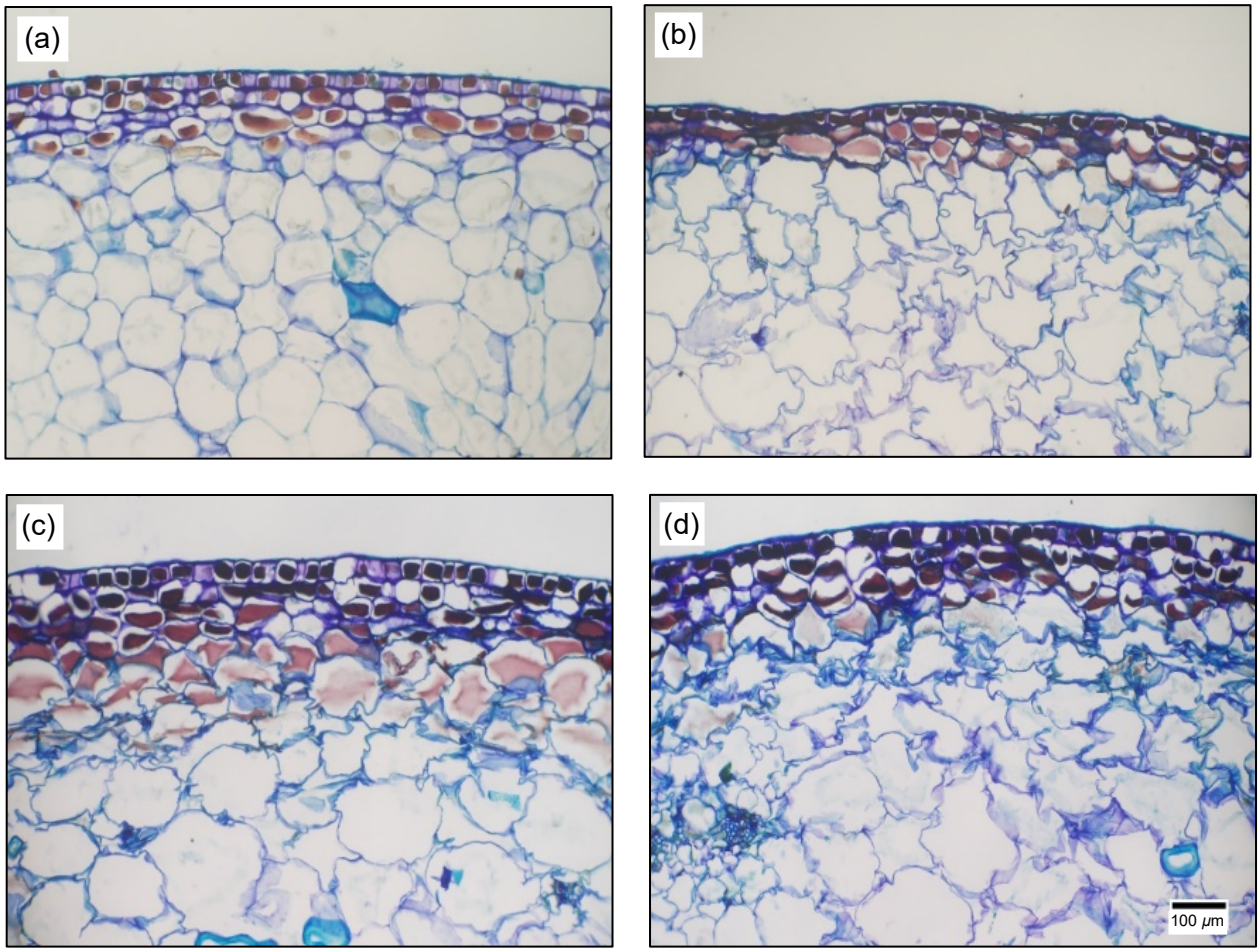

Figure 5.5. Light microscopy showing the effects on the microstructure of 'Centurion' blueberries after storage in 76% RH. (a) Fresh berry (no storage). (Berry stored for 1 (b), 2 (c) and 3 (d) weeks in air. All micrographs are of light microscopy 15 µm sections stained with toluidine blue. (Bar is 100 µm).

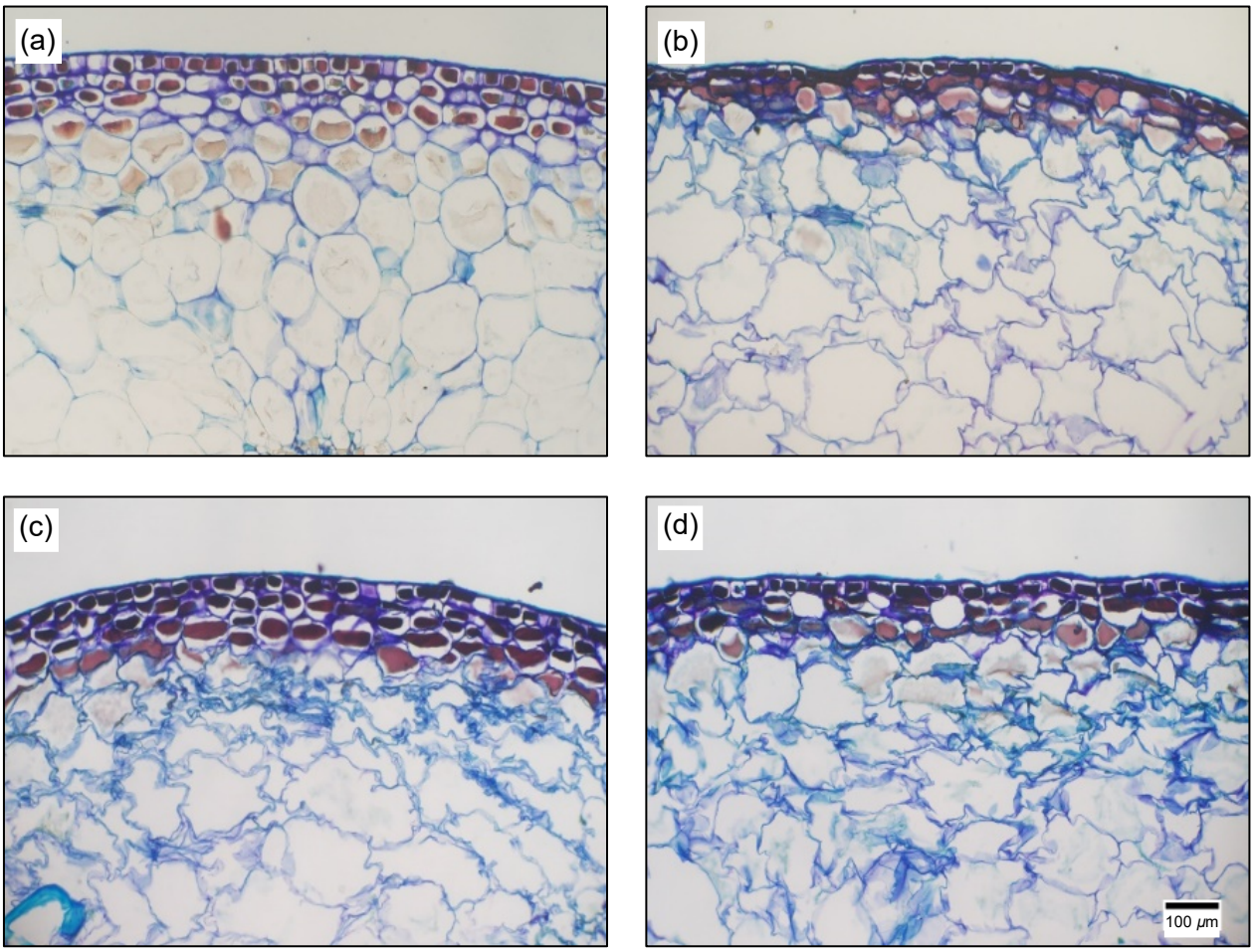

Figure 5.6. Light microscopy showing the effects on the microstructure of 'Centurion' blueberries after storage in 62% RH. (a) Fresh berry (no storage). Berry stored for 1 (b), 2 (c) and 3 (d) weeks in air. All micrographs are of light microscopy 15 µm sections stained with toluidine blue. (Bar is 100 µm).

5.3.3 Two and three week's storage (93%, 76% and 62% RH)

After two and three weeks storage in air 'Centurion' blueberries subjected to 93%, 76% and 62% RH shared similar microstructural characteristics, with an increased degradation in the microstructure of blueberries after three weeks storage at 76% RH (Figure 5.4c; Figure 5.4d; Figure 5.5c; Figure 5.5d; Figure 5.6c; Figure 5.6d). In the epidermis and sub-epidermis after two weeks storage changes consisted of the rounding of cells, breaks in the arrangement of cells within the sub-epidermal layer and a reduction of pigment containing cells. These changes were amplified after three weeks storage in air. Interestingly, epidermal cells of blueberries exposed to 93% RH after two weeks storage were extensively rounded and retained original pigment, after three weeks storage original pigment loss was observed (Figure 5.4c; Figure 5.4d). Pigment was present in intercellular spaces and disappeared throughout storage and decreased humidity conditions.

Two interior sub-epidermal layers were tightly packed, contained medium sized cells, maintained good cell to cell contact with breaks in the arrangement of cells. However after three weeks storage the sub-epidermal layers showed no particular arrangement. Increased breaks in the arrangement of sub-epidermal layers were observed in blueberries stored in 93% and 76% RH after two weeks storage (Figure 5.4c; Figure 5.5c). After three weeks storage small sub-epidermal cells were interspersed with larger sub-epidermal cells after storage in 76% and 62% RH conditions (Figure 5.5d; Figure 5.6d), whereas sub-epidermal cells were rounder in size and larger than epidermal cells after storage at 93% RH (Figure 5.4d). Cell breakage and increased intercellular gaps were characteristics of berries stored in low humidity environments (62% RH) after three weeks storage (Figure 5.6d). Distinguishing between the subepidermal interface and parenchyma after three weeks storage was not apparent due to pigment present in the sub-epidermal region.

Different storage period effects were observed in the flesh of 'Centurion' blueberries stored in 93%, 76% and 62% RH environments after two and three weeks storage. In flesh pronounced microstructural differences were observed in the relative size and shape of cells, cell to cell adhesion, increase in intercellular space, and interfaces between the sub-epidermis and flesh. These differences were more profound in berries stored at lower humidity conditions (62% RH) after two and three weeks storage respectively. Medium to large size parenchyma cells were present in berries stored at 76% and 62% RH. Whereas berries stored at 62% RH contained predominately medium sized parenchyma cells after two weeks storage (Figure 5.6d). After three weeks storage increased irregular shaped parenchyma cells were observed for all humidity conditions indicative of a loss in turgor, although this effect was greater at lower humidity conditions.

Extensive pigment leakage into berry flesh up to 0.2 mm below the sub-epidermis was apparent for blueberries stored in 93% and 76% RH after two weeks storage and for blueberries subjected to 76% RH after three weeks storage (Figure 5.4c: Figure 5.5c; Figure 5.5d). At low humidity (62% RH) blueberries presented irregular shaped cells, cell breakage, a loss in cell to cell adhesion and an increase in extracellular gaps after two and three weeks storage (Figure 5.6c; Figure 5.6d). A thinning, and total cell collapse in the parenchyma was observed at the sub-epidermis and flesh interface and approximately 0.1 mm below the sub-epidermal layers in blueberries stored at 62% RH after two weeks indicating a loss in turgidity. Whereas visible cell collapse was seen in a section of the parenchyma 0.2 mm below the sub-epidermal layer. Cell collapse was increasingly common the longer the storage period. After three weeks storage no particular arrangement of parenchyma cells was apparent for blueberries stored in 93%, 76% and 62% RH environments.

5.3.5 Cell area

The average cross sectional area of a cell in the epidermal layer ranged from 464 μm^2 in fresh fruit after harvest to 658 μm^2 after a storage period of three weeks, indicating an increase in cell size. In the first sub-epidermal layer below the epidermis the average area of a sub-epidermal cell ranged between 798 μm^2 to 1185 μm^2 in fresh fruit and after three weeks storage at 5.5 ºC. The average cross sectional area of a cell in the second sub-epidermal layer below the epidermis reached a minimum of 1116 μm^2 after one week storage in 76% RH, and a maximum of 1619 μm^2 after two weeks storage in 62% RH conditions. There were no obvious differences between average cell area and storage period and humidity conditions. The average cross sectional cell area of parenchyma cells varied between 6706 and 9342 μm^2. For all humidity conditions and storage periods cell area increased with each successive layer from the epidermis.

In mature fresh blueberries there was no significant difference ($P > 0.05$) in average cross sectional cell area based on storage environment humidity (RH). However, there were significant differences ($P < 0.05$) in average cell area for the blueberry storage period. At the epidermis, differences in average cell area were significant between one week and two weeks storage ($P < 0.05$), and one week and three weeks storage ($P < 0.05$). Differences between the area of epidermal cells of fresh fruit and fruit stored for one week were not significant ($P > 0.05$). In the first layer of the sub-epidermis the average cell area was significantly different between berries at each storage period ($P < 0.05$). In the second subepidermal layer above the parenchyma of the blueberry average differences in cell area between each storage period were the same as the first sub-epidermal layer. Medium and large sized parenchyma cells were numerous in blueberry flesh. However, no difference in average parenchyma cell area between each storage period was observed ($P > 0.05$) (Figure 5.7).

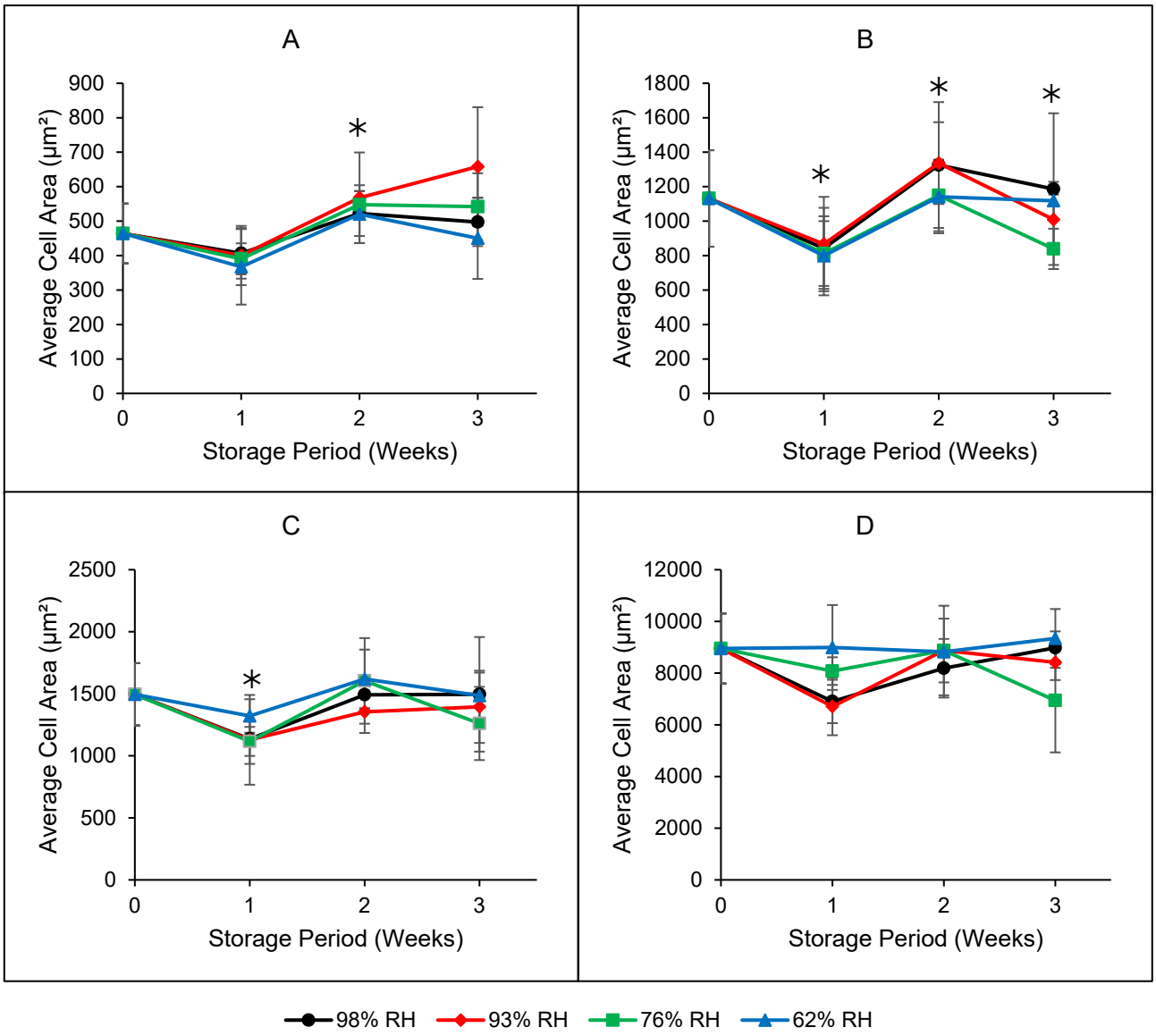

Figure 5.7. Changes in average cross sectional cell area in each successive cell layer from the epidermis to the flesh of 'Centurion' blueberries stored in differing humidity conditions. Cell layer 1 (A), 2 (B), 3 (C) and 4 (D) from the epidermis. Values displayed are averages (n =20). Asterisks indicate average cell area is significantly different (P < 0.05) between storage periods within a given cell layer. Error bars represent standard deviation.

5.3.6 Summary

Microstructural differences for 'Centurion' blueberries treated at 93%, 76% and 62% RH were observed at each storage period. These microstructural differences were distinctive between blueberries stored at 62% and 93% RH. The epidermis of fresh blueberries consisted of one layer of small, square, tightly packed pigment containing cells. Where the epidermis of berries after three weeks storage in air consisted of one layer of small, rounded epidermal cells of inconsistent size and shape. The change in epidermis during storage was increasingly apparent in blueberries stored at 62% RH compared to berries stored in 93% RH. Two interior layers of similar size, rectangular cells containing large deposits of pigment, tightly packed

consisted of the sub-epidermal region in fresh blueberries. After three weeks storage the sub-epidermal region consisted of cells in no particular arrangement, and a loss of pigment into intercellular gaps. Following the same trend of enhanced differences in blueberries stored at a lower RH of 62% throughout the storage period.

Importantly changes in the flesh of blueberries were observed throughout storage that were indicative of changes in the texture responses of blueberries. Parenchyma cells changed from medium to large sized, rounded and turgid to irregular in shape and size, increased cell breakage and extracellular gaps after three weeks storage in air. These changes were enhanced in blueberries treated at lower humidity rates (62% RH), which was expected as berries treated with a lower relative humidity have a higher rate of moisture loss. Pigment, which was enclosed in epidermal and subepidermal cells and in the first layer of flesh cells in fresh blueberries, gradually bled into the flesh layers during storage, but was retained in the epidermis and sub-epidermis.

5.4 Discussion

5.4.1 Humidity effects on microstructural characteristics

The difference in microstructure between ‘Centurion’ blueberries treated at different humidity conditions is indicative of moisture loss during storage. While blueberries at each humidity treatment showed similar trends, we observed a layer of collapsed parenchyma cells concentrated below the sub-epidermis extending 0.1 mm into the parenchyma tissue of blueberries stored for three weeks in 98% RH with no external flow rate (Figure 5.3d). By contrast, a collapse of parenchyma cells was not present in blueberries stored in 93%, 76% and 62% RH conditions after three weeks storage, suggesting lower humidity rates preserve parenchyma cellular structure during storage of ‘Centurion’ blueberries. While this is not conclusive, a possible explanation for the collapse of these cells during storage in a high humidity environment could be associated to the structure and chemical composition of cell walls and turgor differences. Previous research indicates blueberries stored in lower humidity conditions had a greater rate of moisture loss than blueberries stored in higher humidity conditions (Section 4.3.1).

It is possible that a greater water content of parenchyma cells could lead to softening of the cell wall causing structural damage, and subsequently cell collapse during the fixation process. An increase in internal turgor pressure as supported by an increase in firmness (Section 4.3.2)

may lead to osmotic stress, resulting in the softening of the parenchyma cell wall, however these mechanisms are not yet fully understood. It is possible that fixation induced the collapse of these cells, as they could have been structurally different to the surrounding parenchyma cells in a certain capacity resulting from differences in the chemical composition of the cell wall. While these observations are not conclusive, they offer a possible explanation of how the water content of these cells could have an impact on cellular structure leading to the collapse of cells during the fixation process and may be worth further exploration.

Another possible explanation is that an increase in water loss of blueberries stored in low humidity conditions, is the result of a greater movement of water outwards of cells which could result in cells that are stronger compared to cells that have minimal water movement through them. This could be a factor in the collapse of parenchyma cells in blueberries stored at 98% RH. Since blueberries stored in higher humidity conditions have a lower rate of water loss compared to those in lower humidity conditions (Section 4.3.1), it is possible the amount of water within cells could change the composition of the cell walls. Interestingly, the conformation and interaction of cell wall polymers is strongly influenced by numerous factors including the degree of hydration, pH and turgor (Rees, 1977; Preston, 1979; Hall, 1981, Huber, 1983). During fruit storage an increase in transpirational water loss was observed in blueberries stored at lower humidity conditions, accompanying water efflux from the cell reducing the water potential of the apoplast resulting in irregular cell walls of parenchyma cells (Vicente *et al.,* 2007). It is possible that the efflux of water from cells in blueberries stored at a lower humidity level changes the synthesis of cell wall metabolites and results in chemical compositional changes of the cell wall. This is also observed in tomato ripening where the apoplastic solutes increases substantially, in conjunction with transpirational water loss from cells (Shackel *et al.,* 1991; Almeida and Huber, 1999).

‘Centurion’ blueberries stored at each humidity treatment showed similar microstructural trends such as increased cellular dehydration and loss of turgor throughout storage, with these trends being enhanced in blueberries stored at lower RH conditions. The rate at which water is lost from fresh produce is governed by the humidity gradient between the product and the surrounding air (Paniagua *et al.,* 2013). The lower humidity gradient creates a larger driving force for water loss (Paniagua *et al.,* 2013). According to Wills *et al.* (2007) just 5% moisture loss can affect the quality of fresh produce.

In the present experiment after three weeks storage it was expected parenchyma cells of berries stored in lower humidity (62% RH) would be characteristic of cells that had lost turgor. Light microscopy images indicated these cells showed shrivelled characteristics in line with a

loss of turgor, where cell walls were irregular and increased intercellular gaps were present compared to the parenchyma of blueberries stored at higher RH (Figure 5.6d). The degree of cell wall separation and intercellular space, as well as irregular cell wall borders was more intense under lower humidity conditions compared to higher humidity conditions, where the rate of moisture loss was much lower. This was an expected consequence of increased fruit moisture loss. It is likely parenchyma cell shrinkage is a microstructural indicator of future physical berry shrinkage. In previous research fruit shrivel occurred with weight loss ≥8.7% in 'Centurion' blueberries (Paniagua *et al.,* 2013), this is not dissimilar to research where shrinkage of 'Centurion' and 'Maru' blueberries occurred with weight loss ≥8% (Schotsmans *et al.,* 2007). It is possible that the microstructural shrinkage of parenchyma cells in blueberries during storage could be used to predict the rate of shrivel in blueberries. This is something to be explored further.

5.4.2 Storage effects on microstructural characteristics

Microstructural differences were found at the epidermal, sub-epidermal and flesh levels throughout storage in cell arrangement, cell size, distribution of pigment, degree of cell to cell contact, cell to cell contact, number of air spaces, and corrugation of the cell wall. Our results indicate changes in microstructure associated with storage are in agreement with those of Allan-Wojtas *et al.* (2001) and Bunemann *et al.* (1957) where a general disintegration of the cell wall occurs, corrugation of the epidermal and sub-epidermal walls, and a heavy concentration of pigment in the epidermal layer can be observed. A loss of arrangement and shape of cells as well as increased space and pigment bleeding was indicative of blueberries stored in air for three weeks. Similar observations were recorded in 'Burlington' blueberries where after three weeks air storage, cells were irregularly shaped, with some cells having breaks in their cell walls. Cells had increased spaces between them and less cell to cell contact with neighbouring cells (Allan-Wojtas *et al.*, 2001).

Blueberry epidermis is a tough tissue that provides resistance to mechanical damage and fruit shrivelling. The epidermal layer is made up of strong thick walled cells that resist damage and retain pigment during microscopy sample preparation. Therefore during storage in air pigment leakage could be observed progressively increasing using light microscopy. Sapers *et al.* (1985) used light microscopy to show highbush blueberry cultivars with tender skins were prone to pigment leakage after freezing and subsequent thawing, unlike the skin of highbush 'Burlington' blueberries which was resistant to pigment leakage during storage. This is in agreement with Allan-Wojtas *et al.* (2001) who found no pigment leakage in 'Burlington' blueberries stored in air for three weeks. In this experiment 'Centurion' rabbiteye blueberries

showed extensive pigment bleeding into the parenchyma after three weeks storage in air, in agreement with research conducted by Sapers *et al.* (1985) for highbush blueberries. The susceptibility of pigment leakage during air storage into the sub-epidermis and flesh of blueberries may be partly due to differences in the structure of blueberry skin including cuticle, mechanical strength, and thickness of the skin. It is thought these differences affect the development of point source leaks after blueberry freezing and thawing (Sapers *et al.,* 1985). Although it is likely a breakdown in the structure and chemical composition of the skin and pigment containing cells is responsible for pigment leakage in blueberries stored in air for up to three weeks leading to increased fruit softening during storage.

The epidermal layer of 'Centurion' blueberries consisted of small, square, tightly packed epidermal cells. Throughout storage these cells evolved into rounded cells which maintained decent cell to cell contact. In other rabbiteye species fresh 'Beckyblue' blueberries exhibited similar square shaped epidermal cells and contained pigment deposits in epidermal and sub-epidermal layers (Cano-Medrano and Darnell, 1997). In comparison, highbush blueberry varieties 'Coville' and 'O'Neal' displayed rectangular, thin epidermal cells with pigment present only in the first layer of epidermal cells (Allan-Wojtas *et al.,* 2001; Fava *et al.,* 2006). In previous research the rounding of square epidermal cells was observed in 'Burlington' blueberries after six weeks storage in air (Allan-Wojtas *et al.*, 2001). The cause of this is likely related to the degradation of the middle lamella and a reduction in cell to cell adhesion, where epidermal cells are able to expand slightly and become more rounded. The rounding of epidermal cells seems to be a turgor driven process, resulting from transpiration during blueberry storage.

Throughout storage parenchyma cells became considerably distorted, yet for the most part remained intact. These cells were increasingly characteristic of hypertonic cells, where shrivelling was observed indicating a loss in turgor resulting from postharvest transpiration and moisture loss. The rate of water loss from produce is governed by the humidity gradient between the product and the surrounding air, the resistance of water to flow through the skin and the surface area available for water to transfer across (Paniagua *et al.,* 2013). Therefore a greater driving force for water loss occurs at lower humidity conditions. Our results are in agreement with Allan-Wojtas *et al.* (2001) who reported irregular shaped cells, increased cell spaces and gaps between cells in 'Burlington' blueberries after three weeks storage in air. Subsequently, after six weeks storage in air parenchyma cells of 'Burlington' blueberries changed to a rounded irregular shape, similar to the flesh microstructure of 'Centurion' blueberries stored for three weeks in the present experiment. Turgor interacts with the cell wall, such that when internal cell pressure is low the cell is less likely to burst, and disruption of cell to cell adhesion is more likely (Harker *et al.,* 1997). This was observed in the

parenchyma of blueberries stored in air for three weeks. It is possible a reduction in turgor due to transpiration throughout storage is responsible for microstructural differences observed in the flesh of ‘Centurion’ blueberries.

The amount of air space within the flesh of blueberries expanded during cool storage and was greater in berries stored in low humidity conditions. It seems possible the cause of this space is related to the degradation of the middle lamella and corresponding reduction in parenchyma cell to cell adhesion. In ‘Braeburn’ apples it has been proposed that this is a likely mechanism for the increasing air space between cortical cells during cold storage (Harker and Hallett, 1992). Although another explanation for the increase in extracellular gaps between parenchyma cells is related to dehydration, and subsequent increase in concentration of solutes causing the shrivelling of the cell and increase in space observed in the flesh of ‘Centurion’ blueberries.

5.4.3 Differences in cell area

The cross sectional cell area increased with each successive layer from the epidermis to the flesh of the blueberry. The average area of a cell in the epidermal layer of fresh ‘Centurion’ blueberries was 464 μm^2. However in ripe highbush blueberries research conducted by Blaker and Olmstead (2014) reported an average cell area of 712 μm^2 in the epidermal cell layer. This difference can be explained in term cultivar differences. After three weeks cold storage the average cell area of ‘Centurion’ blueberries in the epidermal layer increased to 658 μm^2. This indicated there was not a dramatic increase of cell area in the epidermal layer of berries during storage. Previous research suggested that cell division persists throughout ripening in the epidermis, resulting in cells that are smaller than each successive cell layer in the blueberry (Harker *et al.,* 1997; Blaker and Olmstead, 2014).

The largest difference in average cell area was between the subepidermal layers and the blueberry flesh. Parenchyma cells decreased in size after one week’s storage in air and then increased after three weeks cold storage in all humidity conditions. For all humidity conditions and storage periods the average cell area successively increased in the second, third and fourth cell layers from the epidermis. The cause of a change in cell area is probably related to the degradation of the middle lamella, and corresponding reduction in cell to cell adhesion. A loss of cell to cell adhesion enables the cells to expand slightly and become more rounded, resulting in a change of cell area during storage. The change of average cell area in each layer during storage seems to be a turgor driven process. Although between two and three weeks storage for blueberries stored under all humidity conditions there seems to be little

change in cell area. A possible explanation for this is a slowing rate of moisture loss, however an increased loss of cell structure is observed at two and three weeks storage.

5.5 Conclusion

Compared to optimum microstructure conditions (93% RH, 15 mL min^{-1}), subjecting fresh blueberries to 98% RH at 5.5 ºC for three weeks would deteriorate the microstructure leading to a collapse of parenchyma cells directly below the sub-epidermal layers. However, this difference would not have an impact on the external fruit surface quality. Minimal microstructure disruptions and preservation of microstructural quality throughout three weeks storage can be achieved by storing blueberries in 93% RH conditions. This could be achieved by monitoring the flow of dry air into palletised containers containing fresh blueberries for export.

Future work should be focused on elucidating the mechanisms for the differences in microstructure throughout storage, through molecular approaches to enhance our understanding of why some cells collapsed during storage (98% RH, 0 mL min^{-1}) and between differences in the structure of cells at each storage period. These results could help in understanding the structural response of outer tangential epidermal, subepidermal and flesh cells after storage in different humidity conditions. This knowledge will improve the design of blueberry preservation methods that involve humidity storage conditions. Likewise microstructural changes reported could be related to texture properties throughout shipping and transportation.

At present, the blueberry industry lacks standardised methods to measure the quality of blueberries after postharvest treatments during storage and transportation. The development of a standardised method would be useful to predict blueberry quality and shelf life. The collapse and degradation of parenchyma cells after three weeks storage could be an early indicator of berry shrivelling, and subsequent quality. Internal quality is now increasingly important to consumers compared with external blueberry appearance. Correlating microstructural changes with internal quality during different storage conditions, could provide a more informed method to optimise storage conditions to improve quality retention for rabbiteye blueberry cultivars. This could help in the selection of new cultivars to ensure blueberry varieties resistant to cellular collapse during storage are selected for breeding purposes. Additional microstructural research during storage could provide an added dimension to preserving blueberry microstructure, which may be associated with increased blueberry quality.

Chapter 6.
Characterising Cellular Structure Changes in Blueberries during Humidity Storage using OCT

6.1 Introduction

The year round global demand for fresh blueberries relies on the storage ability of fresh blueberries, which is a fundamental requirement for export to global markets (Giongo *et al.,* 2013). Often resulting from poor transportation and storage conditions, large volumes of blueberries are rejected at the market place due to inadequate quality and texture (Prussia *et al.,* 2006*).* Texture is an increasingly important indicator of fruit quality and is dependent on the underlying microstructural characteristics of the fruit. Consumers are becoming increasingly concerned about interior fruit quality.

Blueberries are composed of several near surface structures such as the epidermis containing pigment, the sub-epidermis and the parenchyma tissue (Gough, 1983; Cano-Medrano and Darnell, 1997; Allan-Wojtas *et al.,* 2001; Johnson *et al.,* 2011; Blaker and Olmstead, 2014). Within the parenchyma tissue, sclerified cells (stone cells) which are a similar size to parenchyma cells are found and are responsible for the grainy texture that is present in some blueberry varieties. In 'Centurion' blueberries the epidermis consists of one layer of small, rounded, closely packed cells containing pigment with a maximum depth of approximately 0.05 mm (Chapter 5). The sub-epidermis is located below the epidermal region and consists of two layers of similar sized cells up to 0.06 mm in diameter, tightly packed with pigment (Chapter 5). Underneath, the parenchyma tissue is composed of vascular bundle, stone cells and parenchyma cells. The stone cells are scattered amongst parenchyma cells and have previously been found approximately 0.1 mm below the surface of the epidermis (Blaker and Olmstead, 2014). In blueberries that have been subjected to extended storage periods, round closely packed parenchyma cells become elongated and collapse as a result of increased intercellular spacing and dehydration (Chapter 5). Changes in the cellular structure of blueberries have potential consequences for postharvest fruit quality and storability seen previously in this thesis.

Storage factors such as humidity conditions can affect postharvest fruit quality. The relative humidity directly surrounding the blueberries influences the rate of weight loss from the fruit

by defining the driving force for water loss (Maguire *et al.,* 2001). Manipulation of the RH of blueberries in storage is a promising technique to preserve fresh berry quality characteristics and to extend berry shelf life (Paniagua *et al.,* 2013). The application of modified humidity conditions during storage has shown positive effects by maintaining texture attributes and increasing firmness after periods of cool storage (Paniagua *et al.,* 2013), where 'Centurion' blueberries stored in low humidity conditions displayed higher weight loss values over a three week storage period compared to berries stored under high humidity conditions, although changes in the microstructure of blueberries after storage in different humidity conditions has not yet been fully investigated (Paniagua *et al.,* 2013). Allan-Wojtas *et al.* (2001) reported microstructural changes in blueberries stored in air for six weeks. Epidermal cells changed from a square to rounded shape, and the rounding of sub-epidermal and parenchyma cells was observed (Allan-Wojtas *et al.,* 2001). The rounding and subsequent increase in surface area is in agreement with the work of Harker and Hallett (1992) where the cortical cells of 'Braeburn' apples appeared rounded after cold storage. Previous microscopy analysis (Chapter 5) indicated elongated and collapsed parenchyma cells after three weeks storage in different humidity conditions. A reduction of cell to cell adhesion and shrivelling was proposed as the likely mechanism responsible for the visible rounding and shrivelling of parenchyma cells.

OCT is a non-destructive, contactless, optical high resolution imaging method applicable to semi-transparent media (Drexler and Fujimoto, 2008; Li *et al.,* 2015). OCT has been increasingly used in the assessment of horticultural products. These applications include apples (Verboven *et al.,* 2013), kiwifruit (Li *et al.,* 2015), loquats (Zhou *et al.,* 2018), mandarins (Magwaza *et al.,* 2013), potatoes (Landhal *et al.,* 2017) and onions (Meglinski *et al.,* 2010; Landhal *et al.,* 2012). Near skin cellular structures such as large parenchyma cells were visualised in kiwifruit, demonstrating the potential of OCT to visualise and characterise the microstructure of parenchyma cells immediately underlying kiwifruit skin (Li *et al.,* 2015). In blueberries OCT has not been previously used to visualise the near skin cellular structures. OCT has the advantages of minimal sample preparation and enables repeated measurement on the same sample over a period of time, allowing for the acquisition of three-dimensional (3D) depth resolved images in situ and in real time, with resolution as good as one micrometre (Li *et al.,* 2015). As such, OCT technology provides the potential for fruit products to be monitored in real time for quality throughout storage and transportation, as well as the monitoring of fruit products throughout development.

6.1.1 Experimental objectives

The objectives for this experiment are as follows:

1. Visualise and characterise the sub-surface cellular structures of ‘Centurion’ blueberries non-destructively after storage in different humidity conditions.
2. Determine whether microstructural differences influenced by storage period and humidity conditions are detectable from OCT.
3. Investigate whether OCT shows promise as a non-destructive assessment tool for blueberries, and to evaluate potential applications of this technique to measure the microstructure of blueberries.

The outputs of this experiment will provide insight into the microstructure of blueberries throughout storage in different humidity conditions, whilst evaluating the potential of OCT for applications in postharvest fruit quality management. If OCT shows promise as a non-destructive assessment tool for blueberries, the results of this research could be used in conjunction with results from texture analysis (Chapter 4), and microscopy analysis (Chapter 5) to identify postharvest cultivar differences and the quality of blueberries throughout the supply chain and within the market place.

6.2 Materials and methods

To address the objectives of this experiment, different RH conditions were established using a closed airflow system. These conditions were monitored throughout the experiment. Full details of the materials and methods used to create this closed airflow system, including fruit source, sample configuration and experimental design are outlined in Chapter 3. OCT was used in this present experiment to assess microstructural changes in ‘Centurion’ blueberries throughout storage and to assess the suitability of using OCT for evaluation of blueberry texture and microstructural properties.

6.2.1 OCT fruit sample preparation

Five ‘Centurion’ rabbiteye blueberries were randomly chosen from each humidity treatment every week for three weeks for OCT image evaluation. Collapsed fruit or fruit presenting fungal mycelium were discarded as these berries were not representative of stored blueberries. OCT

imaging was used to visualise microstructural and textural changes throughout the storage of 'Centurion' blueberries at different humidity conditions (98%, 93%, 76% and 62% RH).

Experiment dates were determined based on the harvest day, and each subsequent weekly storage evaluation. Berries were evaluated at harvest and after 1, 2 and 3 weeks storage at different humidity treatments at 5.5 ºC. Images were captured on the 21st and 28th February 2018 and the 5th and 12th March, 2018. At each evaluation day blueberries were placed into four separate labelled 75 mL plastic containers for each of the storage humidity conditions (98%, 93%, 76% and 62% RH). The plastic containers containing blueberry samples were then placed in a small 1 L cooler bag with a cool gel pack at 4 ºC and transported one hour by car from the Food Laboratory at the Massey Institute of Food Science and Technology (MIFST), Massey University, Albany to the Biophotonics Laboratory within The Department of Physics at The University of Auckland, Auckland to gather blueberry OCT images.

A total of five blueberries for each treatment, at each measurement day were used for OCT image capture. Therefore at the end of the experiment a total of 5 x 4 x 4 = 80 images of blueberry flesh were captured. Although OCT image capture is a non-destructive technique, the samples were discarded at the end of image analysis, as they had been exposed to fluctuations in temperature during transportation and image capture which could adversely affect the microstructure of blueberries

6.2.2 OCT instrumentation and image capture

6.2.2.1 SD-OCT setup

A spectral domain OCT (SD-OCT) scanning device located at the Biophotonics Laboratory, operating at 840 nm was assembled for imaging blueberries. The SD-OCT set up (Figure 6.1) was made of two parts, an interferometer and a spectrometer. The interferometer consisted of a light source (SLD, Superlum Broadlighter T840), and a reference and sample arm. The light from the light source was guided to an optical coupler by a fibre and directed through polarisation controllers (PC) to the sample and the reference arms containing focusing lenses (with focal length [f] of reference arm = 50 mm, and focal length of sample arm = 75 mm). The light was collimated by a fibre collimator (f=8.1mm, 60FC-4-M8-10 from Schäfter and Kirchhoff) in the reference arm, and in the sample arm by a collimating lens (f=15 mm). Additionally, a neutral density filter was placed in the reference arm. The spectrophotometer consisted of a collimating lens (f= 50 mm), a focusing lens (f= 100 mm), a diffraction grating

(Volume Phase Holographic Grating; Transmission = 1200 lines/mm) and a CCD-based line-scan camera (Basler ruL2048-10gm).

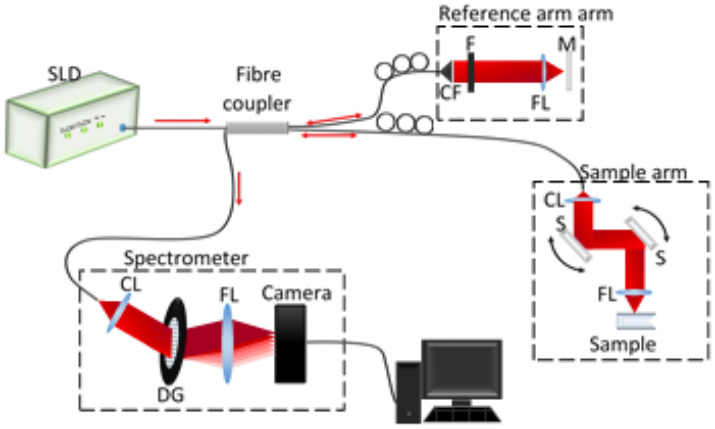

Figure 6.1. Schematic of the SD-OCT setup provided by the Biophotonics Laboratory at the University of Auckland. FL – focusing lens, CL – collimating lens, CF – collimating fibre, DG – diffraction grating, M – mirror, and F- filter, S – scanning mirror.

The axial resolution created was 3.6 µm and the lateral resolution was ≈ 15 µm. The choice of an 840 nm wavelength was driven by the need to penetrate beneath the layers of pigmentation of the blueberry skin while providing good resolution, as OCT systems operating at lower wavelengths offer higher axial resolution than systems operating at higher wavelengths in most cases. No prior sample preparation was required before image capture. As outlined by Li *et al.* (2015) the basic steps to capture an image include: focusing the laser on the surface of the fruit; choosing a suitable surface on the fruit skin and then capturing the raw image.

LabVIEW 2018 (National Instruments, Austin, TX, USA) was used to acquire signals and control the galvanometer. The spectrum was acquired, linearized and the Fast Fourier Transform (FFT) was taken to produce a single depth-wise intensity profile (A-scan). At every increment of the galvanometer this was repeated, and the images were saved to a hard disk. The depths of the raw images were corrected to reflect the sample refractive index. This affects the depth scale of the final estimated values, with a single value being applied across the entire data image (Li *et al.,* 2015). In literature the refractive index of blueberry juice has been reported as 1.35 (Oancea *et al.,* 2012; Castagnini *et al.,* 2015).

6.2.3 Image processing

Image analysis was carried out on 3D data, however results presented in this thesis are presented in 2D format for ease of presentation. Raw OCT images displayed sub-surface

structures such as layers of the epidermis and parenchyma. The aim was to identify and select objects only that were in line with cellular and microstructural measurements from previous microscopy analysis (Chapter 5). A high reflection of the pigmented epidermal and sub-epidermal layers was observed providing difficulty to distinguish between objects in these layers. In this case only large parenchyma cells were identified from the background tissue in order to enable further analysis. Image processing was carried out using ImageJ/Fiji (Schindelin *et al.,* 2012) (Figure 6.2). Raw images were first converted to an 8-bit greyscale image and treated with a smoothing filter to reduce the effects of artefacts. The smoothing filter blurred raw images and replaced each pixel with an average of its 3 x 3 neighbourhood. The MorphoLibJ plugin (Legland *et al.,* 2016) was utilised for image processing.

Similar to the method used by Li *et al.* (2015) and Zhou *et al.* (2018) for selecting large cells, interactive threshold binarisation was applied to select large parenchyma cells beneath the sub-epidermal region in the flesh of 'Centurion' blueberries. In this technique an 8-bit grey scale image was converted into a binary image with only exterior and interior materials enabling the visualisation of objects of interest from the background, allowing segmentation to take place. The lower threshold was set as the lowest grey level value for the image. Whereas the upper threshold was set at a value where there was the best contrast between dark cells and light background tissue (Table 6.1). All objects with a grey level value between these two thresholds were selected for further analysis.

After threshold binarisation, many of the boundaries of selected cells were merged, and not clear. In order to separate the potential cells, morphological segmentation was applied to detect cell boundaries (Table 6.1). Morphological segmentation combines morphological operations such as extended minima and morphological gradient, with watershed algorithms to segment binary images (Legland *et al.,* 2016). The morphological gradient is the difference of a morphological dilation and erosion within the same structuring element, enhancing the edges of the original images (Legland *et al.,* 2016). The structuring element is a matrix identifying the pixel in the image being processed and defines the neighbourhood used in the processing of each pixel (Legland *et al.,* 2016). The watershed algorithm uses different coloured water labels from a series of marker images to stimulate 'flooding'. The efficiency of separation was maximised by the tolerance factor. The tolerance factor is the dynamic of intensity for the search of regional minima. Increasing the tolerance value reduces the number of segments in the final result, while also decreasing its value produces more split objects (Legland *et al.,* 2016). Therefore, sensitive to the input image type.

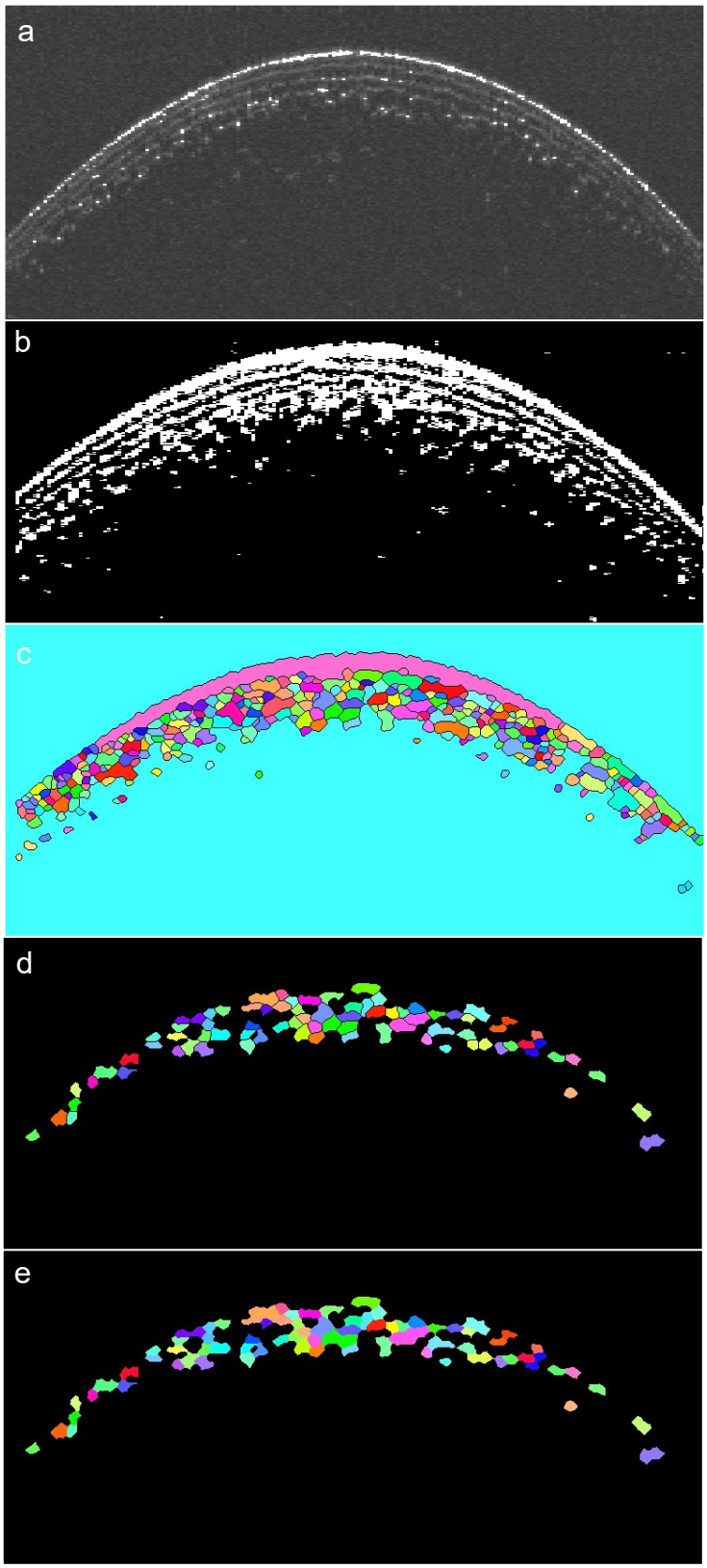

Figure 6.2. Example of OCT image processing protocol presented in 2D cross-sectional images, for the identification of large parenchyma cells of 'Centurion' blueberries during storage at different humidity conditions using ImageJ Fiji for fresh blueberries: (a) smoothing; (b) interactive threshold binarization; (c) morphological segmentation; (d) filtering and (e) closing.

Subsequently, for our binary images a tolerance factor of 10 was appropriate. Separated cells were displayed by selecting 'catchment basins' and displayed using a 16-colour cyclic map to ensure that objects in close proximity were labelled in a different hue (Li *et al.,* 2015). Comparing the labelled objects from the segmented images with raw OCT images, it is clear that objects which had the same grey level values as the dark voids and layers present in the raw OCT image were selected.

Table 6.1. Procedures and settings for OCT image processing using ImageJ Fiji.

Procedure	Parameter	Setting
Smoothing	Area of average	3 × 3 pixel
Threshold binarization	Grey scale	55 – 105
Morphological segmentation	Morphological gradient Watershed segmentation Results	Gradient radius = 2 Tolerance = 10 Display = catchment basins
Filtering	Vertical depth Cell length Cell area	0.15 – 0.6 mm 0.1 – 0.2 mm ≤0.015 mm^2
Closing	Disc size of closing	2 pixels added to 6 neighbouring pixels

The filtering of mislabelled objects of was conducted. This included screening of undesirable objects by using different image processing techniques. Firstly the vertical distance of the object was restricted to 0.15 – 0.6 mm below the surface of the skin for all images. Secondly, the maximum cell length was restricted to 0.1 – 0.2 mm. Microstructural analysis (Chapter 5) indicated parenchyma cells start to appear below the sub-epidermis (approximately 0.15 mm below the surface of the skin), with a maximum length of 0.2 mm, becoming more prevalent in length at 0.15 mm (Chapter 5). Lastly, a maximum average cell area was determined at 0.015 mm^2 based on previous microstructural analysis (Chapter 5). These parameters ensured that all over-sized and under-sized objects other than blueberry parenchyma cells were removed (Table 6.1). The final step of this image processing protocol was to apply a 'closing' of the assessed region (Table 6.1). The closing technique consisted of the dilation of selected objects, immediately followed by an erosion. This helped to close dark structures and holes within the cells and ensured the smoothness of cell boundaries. An essential part of morphological closing is the structuring element. A disk structuring element was used, as this was similar shape to the selected parenchyma cells. The radius of dilation was set as 2 pixels so that separated cells were not reconnected, and additional volume was not added to the larger parenchyma cells (Li *et al.,* 2015). Image processing time using the imaging process described by manually selecting each individual cell was 10 – 15 minutes for each image.

6.2.4 Image analysis

Quantitative image analysis was conducted on the final processed images of all blueberry samples using the method described previously to evaluate the number of cells and to define the characteristics of parenchyma cells below the sub-epidermal region of 'Centurion' blueberries exposed to different storage conditions (Table 6.2). The number of cells that could be identified was not constrained.

Table 6.2. Microstructural parameters and descriptions used to quantify these properties.

Microstructural parameters	Unit	Description
Total surface area	mm^2	Total 2D surface area of 20 random objects in the image.
Average surface area	mm^2	2D cross-sectional surface area of an individual object.
Maximum length	mm	The distance between two outermost tangential lines of the object projected into a plane.
Number of cells	-	Number of objects within the assessed region.

6.2.5 Data analysis

The effect of storage period and RH (%) was analysed using a general linear model (GLM) in ANOVA to examine differences among means. The data remained untransformed. Data was analysed using Minitab version 18.1.0 (Minitab Inc., Pennsylvania, USA).

6.3 Results

6.3.1 Features of raw images

2D slices of the data set are presented in this thesis. Several distinct layers of sub-surface structures were observed in the raw images and were consistent across all storage period's and humidity effects. These structures include a small epidermal layer, a layer of homogenous small cells consisting of the sub-epidermal layer, followed by the parenchyma and black voids (parenchyma cells) located in the sub-surface region. In addition some image artefacts were present in the raw images as a result of direct reflection of light back to the sensor from the surface of the blueberry skin (Figure 6.3). This was observed as white streaks in the vertical direction back to the sensor. This was a common issue for all blueberry samples regardless of storage period or humidity condition. This direct reflection of light is the result of high pigmentation levels within the epidermis and sub-epidermal layers of 'Centurion' blueberries. Details of any cellular structures within the epidermal region were unable to be observed and

extracted from raw OCT images. As a result of the direct reflection of light back to the sensor the vertical depth of the objects was limited to 0.15 – 0.6 mm beneath the surface of the skin.

Visualisation of cells immediately underneath the skin showed that cells are separated into three or four distinct layers for blueberries at all storage periods and humidity conditions, with larger parenchyma cells more prevalent 0.15 – 0.6 mm from the surface of the blueberry. For all humidity treatments parenchyma cells appeared to be more spherical and rounded in fresh blueberries and blueberries stored for one week, whereas parenchyma cells appeared to be flatter and more elongated after two and three weeks storage in all humidity conditions. Details of differences between humidity treatments were unable to be visually observed in raw OCT images.

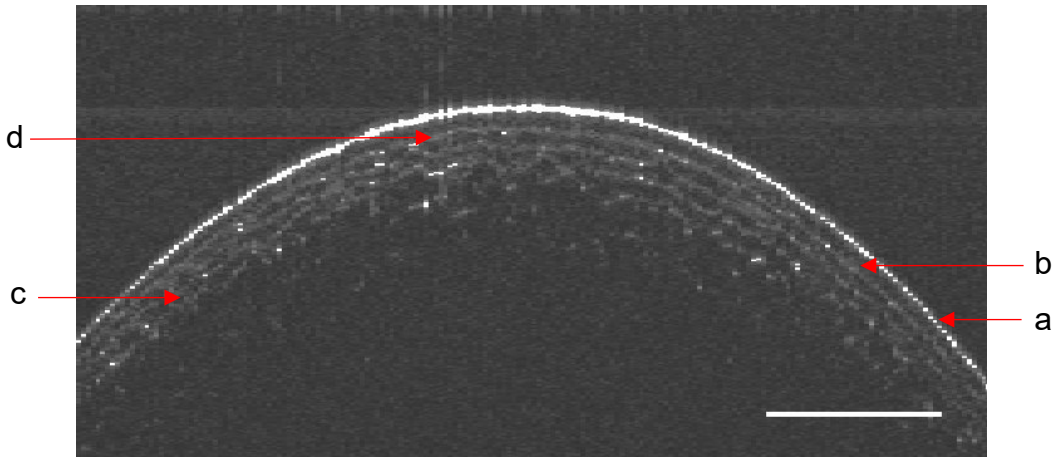

Figure 6.3. Example of a 2D OCT raw image for fresh 'Centurion' blueberries: (a) the epidermis; (b) layers of homogenous small cells; (c) parenchyma; (d) direct reflection of light back to the sensor from the surface of the blueberry skin. Scale bar = 1 mm.

6.3.2 Characterisation of fresh blueberries

Processing of raw OCT images enabled quantification of the number, size and shape of large parenchyma cells observed (Table 6.2). In fresh 'Centurion' blueberries the maximum cell length ranged between 0.1 – 0.6 mm. The large parenchyma cells found using the manual processing method were rounded, regular shaped, in agreement with previous microscopy research (Chapter 5). The average 2D surface area of fresh 'Centurion' blueberries ranged between 0.008 – 0.009 mm^2 once raw OCT images were processed. This was the smallest average surface area recorded during storage across all humidity conditions. However the inability to distinguish between large parenchyma cells and stone cells in OCT analysis could have contributed to the smaller surface area recorded in fresh blueberries.

6.3.3 Differences between storage period for each humidity condition

Analysis of OCT images was able to provide some information regarding differences in microstructure amongst 'Centurion' blueberries treated and stored at four humidity conditions for a period of three weeks. Statistical analysis indicated storage period was statistically significant for mean differences observed in the microstructure of berries treated at all humidity conditions ($p<0.05$). Specifically, total and average cell surface area generally became larger as storage period increased for 93%, 76% and 62% RH.

Table 6.3. Microstructure description and statistics of the cell size distribution of large parenchyma cells present in the flesh of 'Centurion' blueberries at different storage humidity conditions. Values were averaged from five berries per storage condition. Means in columns with different letters are different with statistical significance at 0.05 level for storage period (letter 'a' represents the largest value, while letter 'c' represents the smallest value amongst storage period).

		Microstructural Parameter			
RH	Storage period	Total surface area (mm^2)	Average surface area (mm^2)	Maximum length (mm)	Average number of cells (-)
98%	Fresh	0.1772 ± 0.02	0.00886 ± 0.002	0.126 ± 0.02^{bc}	36 ± 11
	Week 1	0.1769 ± 0.02	0.00884 ± 0.003	0.122 ± 0.02^{c}	50 ± 6
	Week 2	0.1941 ± 0.01	0.0097 ± 0.003	0.134 ± 0.02^{ab}	50 ± 14
	Week 3	0.1889 ± 0.01	0.009445 ± 0.003	0.136 ± 0.03^{a}	35 ± 12
93%	Fresh	0.1681 ± 0.02^{b}	0.008575 ± 0.003^{b}	0.127 ± 0.02	46 ± 15
	Week 1	0.1728 ± 0.02^{ab}	0.008638 ± 0.002^{b}	0.122 ± 0.02	34 ± 12
	Week 2	0.1971 ± 0.02^{a}	0.009855 ± 0.004^{a}	0.13 ± 0.02	48 ± 9
	Week 3	0.1978 ± 0.008^{a}	0.00989 ± 0.003^{a}	0.128 ± 0.02	29 ± 8
76%	Fresh	0.1683 ± 0.01^{b}	0.008414 ± 0.003^{b}	0.129 ± 0.02	44 ± 9
	Week 1	0.1882 ± 0.01^{ab}	0.009412 ± 0.003^{ab}	0.129 ± 0.02	35 ± 10
	Week 2	0.1985 ± 0.02^{a}	0.009926 ± 0.003^{a}	0.133 ± 0.02	43 ± 7
	Week 3	0.2024 ± 0.001^{b}	0.01012 ± 0.003^{a}	0.133 ± 0.02	28 ± 7
62%	Fresh	0.1739 ± 0.02	0.008695 ± 0.003^{b}	0.129 ± 0.03^{a}	59 ± 14
	Week 1	0.1742 ± 0.01	0.008709 ± 0.002^{b}	0.12 ± 0.01^{b}	48 ± 9
	Week 2	0.1962 ± 0.008	0.009808 ± 0.003^{a}	0.131 ± 0.02^{a}	57 ± 17
	Week 3	0.1842 ± 0.001	0.009211 ± 0.003^{ab}	0.133 ± 0.02^{a}	42 ± 21

6.3.3.1 98% RH

Overall for blueberries stored at 98% RH, the average surface area ranged between 0.0088 – 0.0097 mm^2 with the largest 2D surface area recorded in berries after two weeks storage, followed by three weeks storage (Table 6.3). The smallest recorded average surface area was recorded for both fresh and berries stored for one week, indicating there was no difference in average 2D surface area for parenchyma cells after one week storage. The same trend was observed for total 2D surface area where the largest average total surface area was obtained in berries stored for two weeks, followed by storage for three weeks. The mean total 2D surface area ranged between 0.179 – 0.1941 mm^2. Over the storage period the parenchyma cells change from a rounded, regular shape to a more elongated shape (Figure

6.4 – 6.11a). This is also evidenced by berries which had a larger surface area after two and three weeks storage where a higher maximum cell length was observed. The maximum average cell length was 0.136 mm after three weeks storage in comparison to a maximum average cell length 0.122 mm in berries after one week storage. The average number of cells within the assessed region ranged between 35 – 50 cells across the three week storage period (Table 6.3).

6.3.3.2 93% RH

Differences in microstructure across the three week storage period for blueberries stored at 93% RH were similar compared to those stored at 98% RH (Figure 6.11a; Figure 6.11b). Overall the average 2D surface area ranged between 0.0086 – 0.0099 mm^2, where the smallest average 2D surface area was recorded in fresh blueberries and did not significantly change after one week storage (Table 6.3). After two and three weeks storage the average 2D surface area significantly increased. The same trend was observed over three weeks for the total 2D surface area, with fresh berries having the smallest total surface area, with the largest being after three weeks storage. Interestingly the maximum length of parenchyma cells did not follow the same trend. Berries stored for two weeks had the largest maximum cell length. Minimal difference in the maximum length was observed in fresh berries and berries stored for three weeks. Berries stored for one week had the smallest maximum length 0.122 mm (Table 6.3), indicating that a change in cellular shape is occurring, this is supported by previous microscopy research (Chapter 5). A possible explanation for the change in parenchyma shape is the loss of moisture during storage (Chapter 4).

6.3.3.3 76% RH

Overall, berries treated at 76% RH over a three week storage period showed similar microstructure trends to berries stored at different humidity conditions in this experiment. The greatest difference in average 2D surface area and total surface was observed across the three week storage period. The average 2D surface area ranged between 0.0084 – 0.010 mm^2, with berries having the largest 2D surface area after three weeks storage, and fresh berries the smallest. This trend was also observed across the total surface area for these blueberries. Interestingly, the difference in maximum length of parenchyma cells was minimal. For fresh berries and berries stored for one week, the same average maximum length was recorded. Surprisingly the same average maximum length was also recorded for berries after one and two week storage. Across the whole storage period the maximum length ranged 0.129 – 0.133 mm (Table 6.3). The increase in cell area alongside a minimal change in the

average cell length, suggests the shape of parenchyma cells changed from a rounded, regular shape to a more elongated shape (Figure 6.4 – 6.11c). The average number of cells within the assessed region over the whole storage period ranged between 28 – 35 parenchyma cells (Table 6.3).

6.3.3.4 62% RH

Differences in microstructure at 62% RH were very similar to those recorded for berries stored at 98% RH conditions (Figure 6.4 – 6.11a; Figure 6.4 – 6.11d). The average 2D surface area varied between 0.0087 – 0.0098 mm^2 with the largest 2D surface recorded in berries after two weeks storage, followed by three weeks storage (Table 6.3). The smallest average 2D surface area was recorded in fresh berries and berries stored for one week. The same trend was observed for the total 2D surface area. suggesting that over the storage period the parenchyma cells changed from a rounded, regular shape to a more elongated shape after two weeks storage. Interestingly this is also evidenced by a higher maximum length observed in berries after three weeks storage followed by two weeks storage. The maximum parenchyma cell length was 0.133 mm (after three weeks), whereas the smallest was 0.12 mm recorded after one week storage, suggesting smaller and elongated shapes after two weeks storage which was observed in previous microscopy research (Chapter 5). The difference in maximum cell length after two weeks storage could also be the result of image processing and may have contributed to the decreased cell length recorded. Perhaps fitting ellipsoids to the cells would have been more realistic in this instance. The average number of cells within the assessed region ranged between 42 – 59 parenchyma cells across the storage period (Table 6.3).

6.3.4 Effects of humidity condition manipulation and storage period

RH conditions significantly affected fruit quality at different storage periods compared to fresh berries in this experiment. The application of different humidity conditions led to different rates of water loss from berries, either preserving textural attributes or leading to the shrivelling of blueberries (Chapter 4). Despite the macro-scale effects of these treatments observed alongside differences observed in microscopy (Chapter 5), the internal cellular structures of berries from the sub sample showed minimal differences in OCT imaging (Figure 6.4 – 6.11). OCT imaging showed minimal differences between berries that had been treated at different humidity conditions at each storage period, in contrast to microscopy imaging (Chapter 5). However from OCT imaging the storage period was found to have a significant effect on the descriptive parameters, in line with previous microscopy imaging descriptions (Chapter 5).

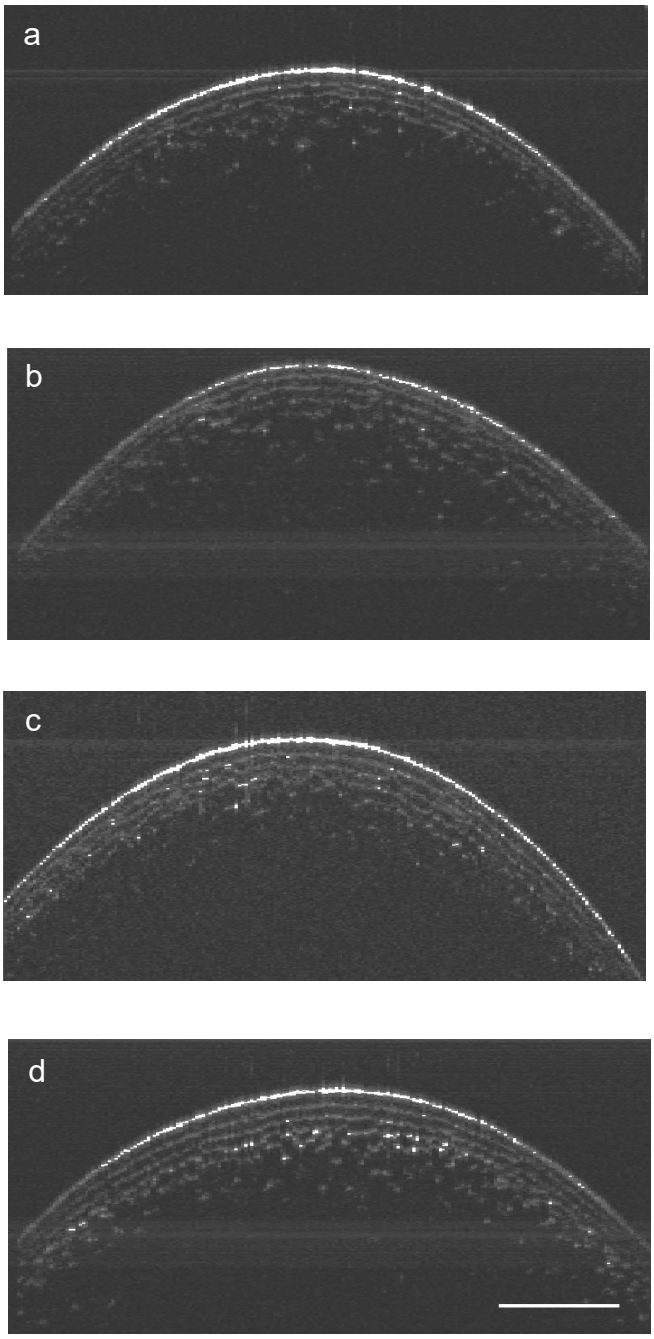

Figure 6.4. Selected representative raw 2D-OCT images showing the effects on the microstructure of fresh 'Centurion' blueberries (control) before humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

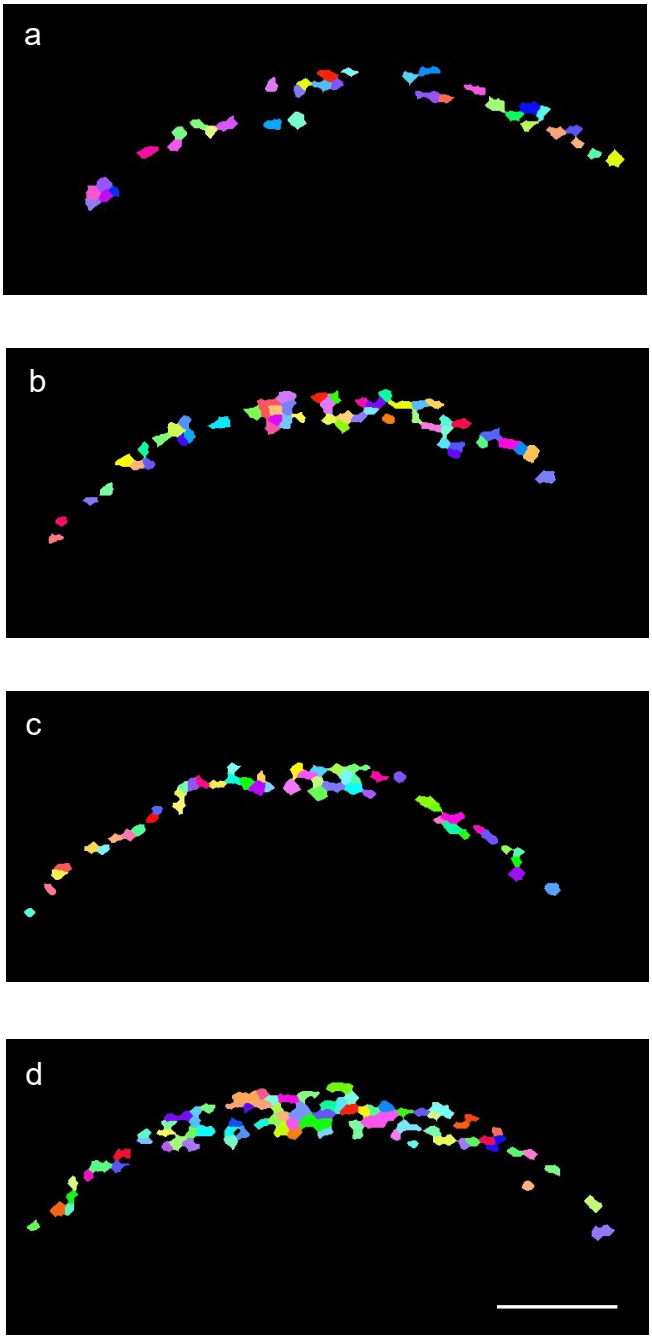

Figure 6.5. Corresponding processed images after manual segmentation showing the effects on the microstructure of fresh 'Centurion' blueberries (control) before humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

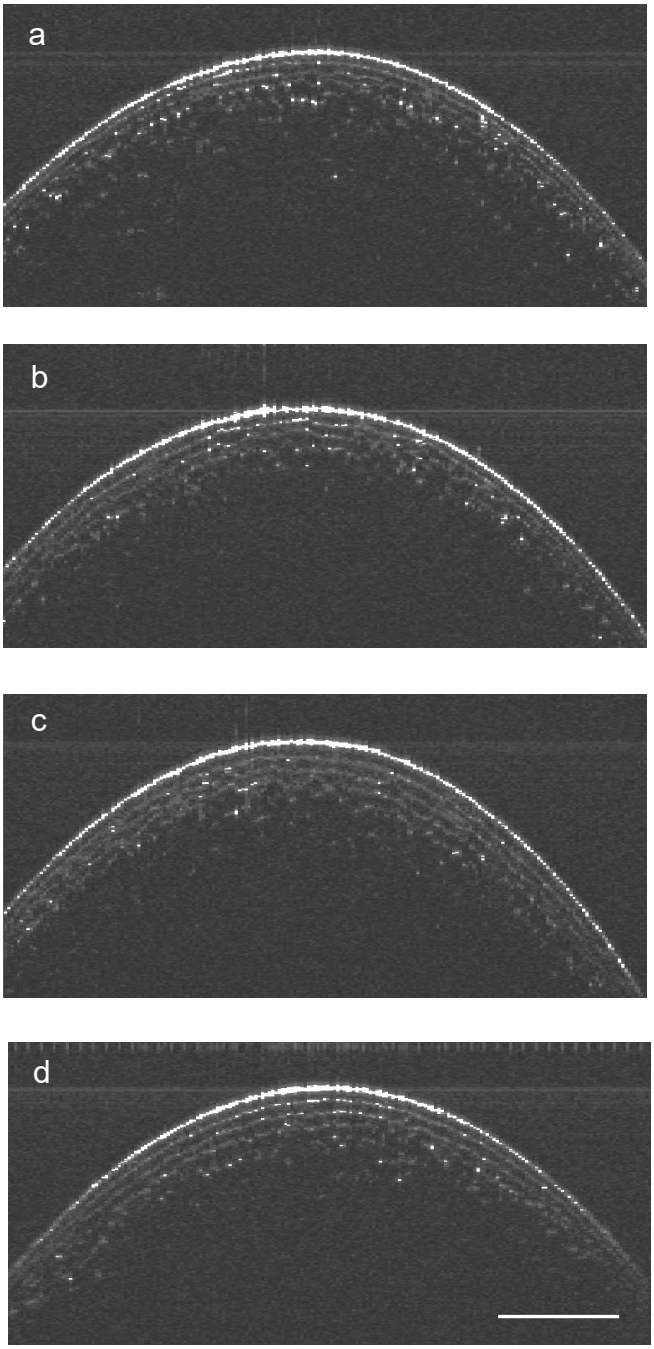

Figure 6.6. Selected representative raw 2D-OCT images showing the effects on the microstructure of 'Centurion' blueberries after one week cold storage at different humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

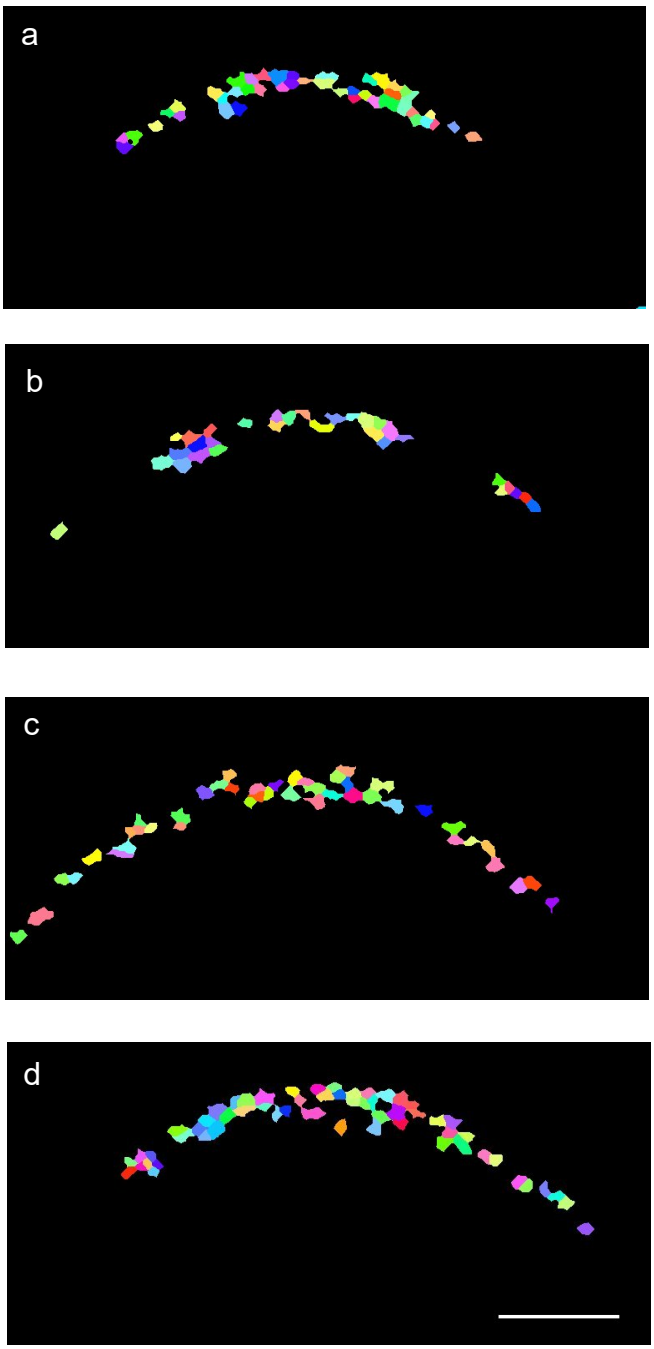

Figure 6.7. Corresponding processed images after manual segmentation showing the effects on the microstructure of 'Centurion' blueberries after one week cold storage after humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

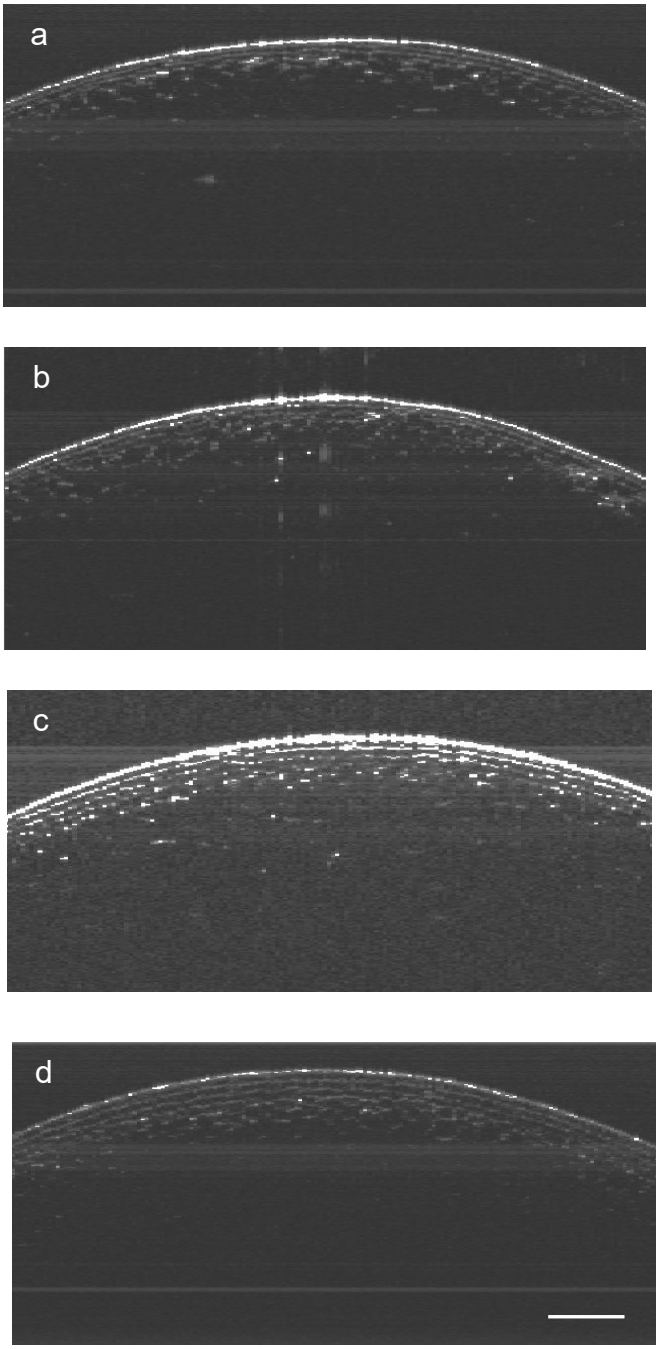

Figure 6.8. Selected representative raw 2D-OCT images showing the effects on the microstructure of 'Centurion' blueberries after two weeks cold storage at different humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

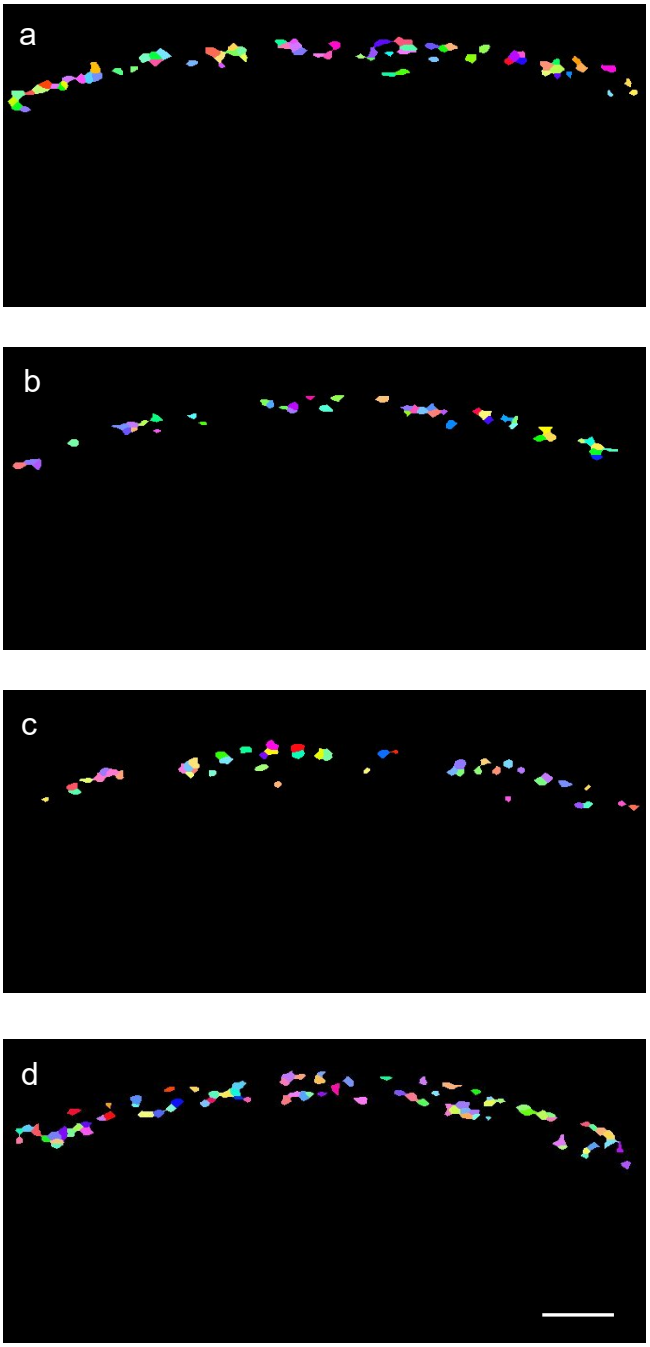

Figure 6.9. Corresponding processed images after manual segmentation showing the effects on the microstructure of 'Centurion' blueberries after two weeks cold storage after humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

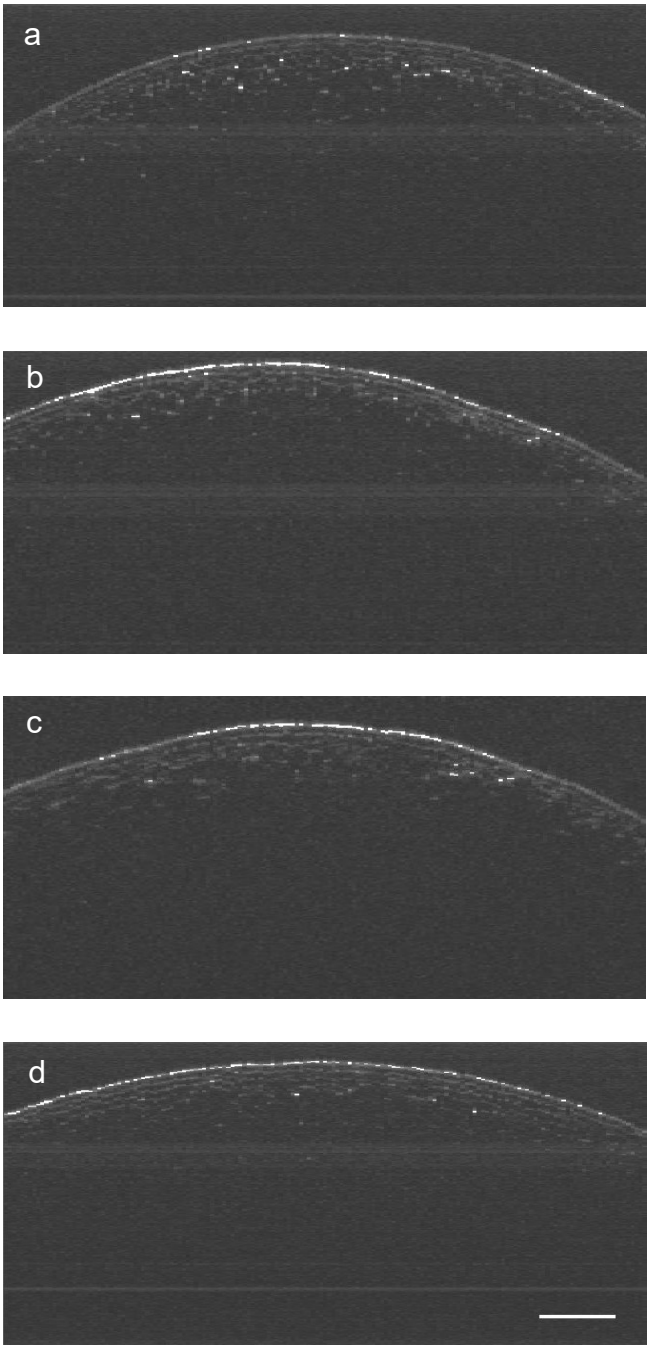

Figure 6.10. Selected representative raw 2D-OCT images showing the effects on the microstructure of 'Centurion' blueberries after three weeks cold storage at different humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

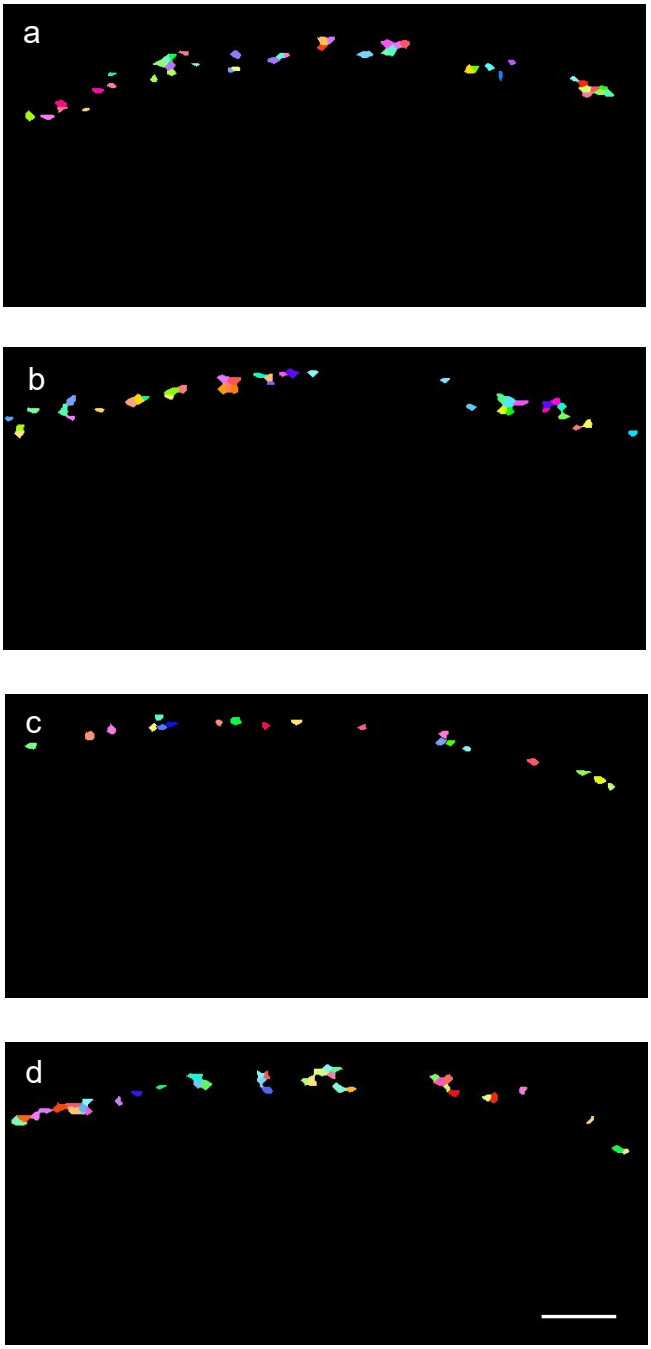

Figure 6.11. Corresponding processed images after manual segmentation showing the effects on the microstructure of 'Centurion' blueberries after three weeks cold storage after humidity treatments: (a) 98% RH, (b) 93% RH, (c) 76% RH and (d) 62% RH conditions. Scale bar = 1 mm.

6.4 Discussion

6.4.1 Limitations of OCT technology for horticultural products

A primary limitation of the images captured is that the depth of penetration of the data was estimated to be approximately 0.8 mm underneath the skin, where the epidermis and sub-epidermis are approximately 0.3 mm below the surface of the skin and the parenchyma is >0.3 mm below the surface of the skin in 'Centurion' blueberries (Chapter 5). There is no published evidence as to whether the distribution of large and small cells in the analysed parenchyma region is the same as the majority of the parenchyma >1 mm beneath the surface of the skin. Previously published evidence has indicated that cells increase in size from small to large each successive layer away from the skin of the blueberry (Cano-Medrano and Darnell, 1997; Allan-Wojtas *et al.,* 2001; Blaker and Olmstead, 2014). Processed OCT images were only able to detect large cells in the parenchyma region, therefore the images captured by OCT are unable to confirm this. The data probably represents only the sub-surface transition zone or outer mesocarp of the parenchyma and regions observable in OCT images may not necessarily represent the flesh as a whole.

A barrier preventing increased penetration depth of the data was the high reflectivity of light back to the detector. 'Centurion' blueberries contained dark red pigment in the first three cellular layers beneath the surface of the skin (Chapter 5). It is highly likely the high pigmentation of these berries presents a limitation to the potential of this technology for use on blueberries and other highly pigmented fruit and vegetables. Although this information is limited, in future the use of OCT technology on blueberries containing only one layer of pigmentation such as 'Burlington' (Allan-Wojtas *et al.,* 2001) and 'Sweetcrisp' (Blaker and Olmstead, 2014) may be useful to determine the effects of pigmentation on the use of this technology.

However despite these limitations there is still potential for this technology to provide useful information on near surface cell size and structure of blueberries within the parenchyma non-destructively. For this reason this thesis continues to discuss the differences observed between storage period and RH treatments with known data from previous microscopy analysis (Chapter 5) due to the lack of other quantitative sub-surface data for 'Centurion' blueberries. Previous microscopy imaging (Chapter 5), provides strong evidence that the objects identified from OCT imaging are in fact objects that are representative of parenchyma

cells. Therefore the OCT images gained from image processing are directly comparable to microscopy images from previous research (Chapter 5).

For Northern highbush cultivars stone cells have been previously reported to be scattered among small and large parenchyma cells in the region approximately 1.2 mm beneath the surface of the skin with approximately 56 stone cells observed (Blaker and Olmstead, 2014). Stone cells have been found 0.1 – 0.2 mm below the surface of the skin (Allan-Wojtas *et al.,* 2001), as large parenchyma cells begin to show prevalence and may extend further into the parenchyma tissue for some cultivars. Stone cells were seen in 'Centurion' blueberries scattered amongst parenchyma cells from 0.2 mm beneath the surface of the skin extending deeper into the parenchyma tissue, being a similar size to large parenchyma cells (Chapter 5). The images from OCT processing showed no differentiation between the observed large voids and the stone cells proximity in the assessed image region. This is another weakness of the technique as it is possible that some of the near-surface parenchyma cells observed in 'Centurion' blueberries throughout storage and humidity conditions could have been stone cells. While acknowledging that this technique is unable to differentiate between stone cells and parenchyma cells in the flesh of 'Centurion' blueberries, discussion in this thesis is under the assumption that all large filtered objects observed are parenchyma cells.

The image penetration and resolution was compared with previous studies on other horticultural products. The depth resolution (3.6 μm) and the penetration depth, to which cellular discrimination was possible (0.8 mm), were comparable those in loquats (3.48 μm and 0.5 mm, respectively; Zhou *et al.,* 2018) where a longer wavelength (1300 nm) was used. The depth resolution and penetration depth was higher compared to those in onions (1.0 μm and 0.5 mm, respectively; Meglinski *et al.,* 2010). When operating at 1325 nm wavelength the depth resolution of kiwifruit (5.9 μm) and apple (5.0 μm) was better than that for blueberries, with a similar penetration depth of 0.68 mm and 0.5 mm, respectively for kiwifruit (Li *et al.,* 2015) and apples (Verboven *et al.,* 2013). This was similar when operating at 930 nm for onions where the depth resolution was better, but the penetration depth was lower (7.0 μm and 0.3 mm, respectively; Landahl *et al.,* 2012). Common problems acknowledged across studies on horticultural products identified by Li *et al.* (2015) include the choice between good penetration depth and high resolution, and the compromise in data processing speed between manual and automatic processing for better accuracy whilst processing images.

6.4.2 Storage period and humidity condition effects on large sized cells

OCT imaging of 'Centurion' blueberries enabled the non-destructive measurement of large cells near the surface of the skin below the epidermis and the sub-epidermis of blueberry tissue. Subsequent manual segmentation and analysis described and identified large cells efficiently and accurately. The manual OCT data analysis method used in this paper has benefits of quick processing (approx. 15 – 20 minutes) and allowed for differentiation between cells located in the parenchyma and the epidermal and sub-epidermal layers. Therefore it enabled the successful selection of large cells within the parenchyma of 'Centurion' blueberries. This study investigated the viability of this technique to determine differences between blueberries treated at different humidity conditions stored for different periods up to three weeks. Comparison amongst storage conditions and storage period may determine what the resulting information on large to medium sized parenchyma cells may be useful for. This could be useful for predicting the length of the storage period for 'Centurion' blueberries.

Using OCT imaging, humidity conditions were not found to affect microstructural changes of medium to large sized parenchyma cells in 'Centurion' blueberries throughout a three week storage period, despite shrivelling of blueberries after three weeks storage (Chapter 4), with previous microscopy results indicating differences in the microstructure of berries stored in 93% and 62% RH conditions (Paniagua *et al.,* 2013). The minimal changes observed in medium to large cells after storage in different humidity conditions at each storage period could indicate these differences observed are very small and are difficult to differentiate between using this technique. It is also possible as a result of the limitation that the assessed region only represented layers near the surface and had limited resolution. Consequently any significant changes resulting from differing humidity conditions were not reflected, or only reflected to a limited extent. The same limitation was reported by Li *et al.,* (2015) when describing a lack of significant changes to the bulk of the outer pericarp of kiwifruit after girdling. Where kiwifruit genotypes which have high dry matter contents, also have a higher proportion of small cells (Nardozza, 2008). Therefore, future research relating blueberry parenchyma cell size with dry matter content during storage still needs to be investigated.

Storage period was observed to affect the total surface area, average surface area and maximum cell length of large parenchyma cells, where a longer storage period resulted in an increased total surface area, average surface area and maximum cell length. It has been suggested during storage parenchyma cells change from a rounded regular shape, to irregular and elongated (Allan-Wojtas *et al.,* 2001), characteristic of moisture loss or dehydrated blueberries (Paniagua *et al.,* 2013). Therefore the shape and total volume of large cells within

the parenchymal region may affect the processing of these images. Cells that are too large and do not meet the filter guidelines are eliminated from processing when in fact they have the potential to be large sized cells. In this research the total number of large parenchyma cells was not different between storage conditions and period, ranging between 28 – 59 cells per image.

The shape and total surface area of large size parenchyma cells may affect the overall texture of the berry tissue. After three weeks storage large cells from processed images were possibly a mixture of more flattened and elongated parenchyma cells and stone cells compared to fresh berries and the other storage periods. More elongated and perhaps collapsed larger cells may cause more intercellular gaps as cellular packing may be less dense as a result from increased water loss after three weeks storage (Chapter 4). It might be the case that the elongated large sized cells were stacked less densely against one another, resulting in a softer texture than fresh blueberries (Paniagua *et al.,* 2013). After three weeks storage microscopy analysis of 'Centurion' blueberries indicated collapsed and elongated parenchyma cells, and an increase in intercellular spaces (Chapter 5). It has been suggested that accumulation of sugar in parenchyma cells leads to an increase in water flow due to osmotic pressure, resulting in cell expansion (Ezura and Hiwasa-Tanase, 2010). This helps to explain why we have observed an increase in total surface area, average surface area and maximum length large sized cells in processed OCT images over the storage period.

Processed OCT images of 'Centurion' blueberries after one week storage under all humidity conditions experienced a decrease or no change in maximum cell length compared to fresh blueberries, where all other microstructural parameters observed increased. Previous microscopy imaging on 'Centurion' blueberries harvested from the same growers at the same time found that the average cell area of parenchyma cells to remain the same or decrease across all humidity conditions (Chapter 5). However the same trend in average cell area was not observed in berries after one week storage from processed images, where an increase was found. The observed decrease in maximum length of large parenchyma cells after one week might be the result of water movement within the tissue from a low concentration to a high concentration resulting from differing humidity conditions. The rate of water lost from the product is governed by the humidity gradient between the product and the surrounding air, the resistance of water to flow through the skin and the surface area of the berry available for water to cross (Paniagua *et al.,* 2013). It is also possible manual processing of images allowed human error and bias during selection of large cells.

6.5 Conclusion

This work demonstrates that OCT has the potential as a non-destructive technique to visualise and characterise the microstructure of large parenchyma cells immediately under 'Centurion' blueberry skin, provided microscopy imaging has previously determined the microstructural characteristics of the horticulture product of interest to ensure that the cells of interest have been characterised. However there was no clear differentiation between large parenchyma cells and stone cells present within the parenchyma region. The details of the skin surface, epidermis and sub-epidermis could not be observed from raw or processed images. The data acquired was limited to a penetration depth of 0.8 mm underneath the skin and might not represent the parenchyma flesh as a whole. The developed image processing techniques enabled the identification and characterisation of large parenchyma cells 0.15 – 0.6 mm beneath the surface of the skin in blueberries stored in different humidity conditions for a period of three weeks in an efficient manner. After a storage period of two weeks large cells of blueberries treated at all humidity conditions were found to have a similar or smaller maximum cell length than in fresh 'Centurion' blueberries.

Characterisation of microstructure of large cells in the parenchyma tissue showed an increase of average surface area, and total surface area after each storage period. Humidity did not affect the microstructure of large cells during storage based on OCT imaging and data analysis. Storage period had the greatest effect on the microstructure of 'Centurion' blueberries. The ability to non-destructively characterise the microstructure and distribution of large cells may be useful for cultivar and crop selection. The potential use of OCT technology as a device to determine the postharvest quality of fruit, depends on the relationship between the nature of large parenchyma cells (microstructure) and texture during postharvest storage. However, improvement in penetration depth and data capture is required to provide more comprehensive information and better understanding of the application of this technology.

The use of OCT as a tool for assessing horticultural products is still in its infancy and requires improvements in methodology for it to become widely adapted. Increased depth penetration will be necessary to provide more information for understanding cultivar and microstructural differences. As stated by Li *et al.* (2015) improved resolution and increased signal to noise ratio are necessary to allow for quantification of a wide range of horticultural products. Better understanding of the effects of pigmentation within blueberries and highly pigmented produce requires improvements in order for this technology to be widely accepted. Nonetheless the information obtained in this study suggests that OCT used in conjunction with microscopy

imaging has the potential to provide information on near surface cellular structures in blueberries and possibly highly pigmented produce as a non-destructive tool. Increased potential for this technology lies within less pigmented horticultural products with thin cuticles, to detect microstructural differences at a cell level between different crop varieties and cultivars. This technology may be used to monitor cellular changes in a crop during fruit development, at the time of harvest and during storage. The development of OCT in conjunction with microscopy and texture analysis would be useful in understanding the physiology and texture attributes postharvest in relation to internal microstructural changes. With improved speeds of image capture and data analysis it could be used as a fast screening tool during postharvest for quality, or as a screening tool for plant breeding.

Chapter 7.
Conclusions and Recommendations

The different texture responses obtained under different storage condition regimes confirmed a high correlation between weight loss and texture response parameters, as well as blueberry microstructural differences resulting from water loss observed by microscopy and OCT analysis. This provides evidence that fruit moisture loss plays an important role in determining the texture of blueberries after harvest. The relationship of causality between blueberry moisture loss and texture offers new opportunities to orientate postharvest management to improve blueberry quality during storage and transportation, minimising weight loss and improving blueberry texture in the market place.

7.1 Weight loss impacts on blueberry quality

The potential for industry is clear, minimising weight loss during the postharvest chain primarily during storage and transportation improves the postharvest texture of blueberries in the final market place. Weight loss levels below 8 – 12% would maintain texture characteristics such as firmness, hardness, cohesiveness and chewiness, minimising excessive softening and degradation ensuring high quality blueberries. Moisture retention along the commercial supply chain, could be attained using clamshell designs that are less vented, and modified atmosphere palletised bags for use in transportation along the supply chain, or modified humidity packaging. The relative humidity should be maintained between 95 – 99% RH to reduce the rate of moisture loss during postharvest management. Paniagua (2012) suggested any increase of the RH environment in contact with blueberry fruit surface should be complemented with fungicidal treatments. Therefore an interesting option to replace conventional controlled atmosphere systems used by exporters could be the use of palletised modified atmosphere bags to control the RH of the system used alone or together with fungicidal treatments (i.e. chlorine and sulphur dioxide and ozone and ultraviolet radiation) to reduce the risk of degradation from condensation.

Achieving a low weight loss environment (98 – 99% RH) in a commercial cool store is challenging and often comes with the risk of condensation. However the ability to maintain a low weight loss environment has the commercial benefit of increased firmness and could be an effective application to maintain the quality of blueberries during export to distant markets.

The relationship of causality between moisture loss and postharvest texture responses could be easily observed and predicted, by monitoring weight losses throughout storage and transportation. Blueberries stored in low weight loss environments (93 – 98% RH) preserved textural characteristics after three weeks cold storage (5.5 ºC), whereas extensive degradation of texture and increased softening occurred in blueberries stored in high weight loss conditions (62 – 73% RH) in agreement with findings reported in previous studies.

In order to make this a more comprehensive modelling technique, texture responses to moisture loss in other blueberry cultivars need to be evaluated. Development of a standard weight loss protocol to predict optimal texture outcomes could be used by industry across all facets of postharvest management, allowing correction measures and redirecting fruit batches to local markets to avoid texture characteristics below commercial standards. This will help to prevent fruit batches being rejected by distant markets and increase profitability within industry. In addition, the texture attributes on arrival to retail markets could be estimated using standard weight loss knowledge for blueberries that have come from a particular destination. This knowledge could be used by industry in a retail setting to inform consumers on the quality of blueberries. Moreover, the knowledge gained by this experiment could be used to improve the efficiency and accuracy of existing quality control systems to ensure optimum blueberry texture characteristics by industry at the marketplace.

Low relative humidity storage conditions (62 – 76% RH) result in the disruption of cell to cell adhesion and cell wall collapse which is likely responsible for substandard postharvest texture. Several microstructural characteristics such as cell size, cell to cell adhesion, shape and packing were preserved at low weight loss conditions (95 – 99% RH). For all weight loss storage conditions and periods, cell size increased with each successive layer from the epidermis to the parenchyma. The collapse and degradation of parenchyma cells in low humidity conditions after cold storage could be an early indicator of visible blueberry shrivel and inferior fruit quality.

The use of OCT as a non-destructive technology is still in its infancy, however it has the potential to visualise and characterise the microstructure of medium and large parenchyma cells immediately beneath blueberry skin. OCT was not able to distinguish between small epidermal and subepidermal pigment containing cells in this research. It is likely this occurred as a result of the high reflectively of the pigment. Variability of microstructure was not observed between humidity conditions (62 – 76% RH) using this technology. However an increase in cell size was observed in each successive storage period from fresh blueberries to three weeks after storage, in line with previous microscopy analysis. Microscopy provided further

complete information surrounding cellular level differences responsible for changes in quality characteristics during storage. However OCT provides a non-destructive technique to quickly determine fruit quality during storage. Continued research on the application of handheld devices and improvements in penetration depth and data capture could provide more comprehensive microstructural information, that could be applied to the postharvest supply chain to monitor the internal quality of blueberries. With future improvements, OCT technology could become an increasingly popular method of quality detection within the blueberry industry.

The technique of modifying air flow rate in the present experiment enabled the manipulation of moisture loss in 'Centurion' blueberries, which successfully induced differing texture and microstructural responses over a specific storage period. This technique could be adopted by industry as an effective low cost option to modify the storage atmosphere of blueberries to induce desirable texture and microstructural responses in postharvest management. In order for this technique to be applied by industry, increased storage periods, dissimilar flow rates and different storage temperatures need to be applied to different blueberry cultivars. It is possible that for this technique to work at a larger scale, differing flow rates compared to the flow rates used in this experiment may induce the similar texture responses to the results in the present experiment.

7.2 Future recommendations

At present the blueberry industry lacks standardised methods to detect the quality of berries after postharvest treatments along the commercial supply chain. The development of a standardised method would be helpful in predicting shelf life and final blueberry quality within the marketplace. Continued microstructural research during storage could provide an added dimension to preserving blueberry quality. In addition microstructural analysis could help aid the selection of cultivars resistant to weight loss and cellular collapse during postharvest to be exported to distant markets, as well as selecting new cultivars resistant to cellular degradation to preserve berry quality and to determine whether MYB transcription factors are responsible for different texture characteristics (Appendix E).

Efforts to standardise texture assessments in both industry and research conditions should be made and standard texture protocol applicable to industry still needs to be investigated. Furthermore blueberry texture can also be measured by a human sensory panel, as well as by mechanical instruments. Efforts should be made to correlate instrumental texture

responses with human sensorial responses, although human sensory evaluations are more representative of consumer perception (Li *et al.,* 2011). Instrumentation methods are becoming increasingly popular due to the objectiveness of the method, however these techniques are high cost and not suitable for industry use along the supply chain. Increased uptake of a handheld penetrometer instrument Durofel® (CTIFL Copa Technologie, France) is likely due to the portability, and cost effectiveness of this instrument to measure the hardness of soft fruits (Chiabrando *et al.,* 2009). Therefore validation of techniques to measure blueberry texture, that are low cost and portable to be used along the supply chain are still needed to establish a standard for texture measurements in industry.

The main pathways for blueberry moisture loss are transpiration through epicuticular waxes (Albrigo *et al.,* 1980) and the picking scar (Cappellini and Ceponis, 1977). The thickness of epicuticular waxes and the diameter of the picking scar could influence the rate of moisture loss from the fruit. Therefore it would be useful to determine the extent of moisture loss through each pathway during postharvest. This information could lead to recommendations for growers to consider harvesting with the pedicel attached or a period of stem scar curing after harvest. Anatomical improvements to reduce the rate of moisture loss should be considered in future breeding programs to retain desirable texture characteristics throughout the postharvest chain. For instance, cultivars with smaller picking scar diameters and increased epicuticular waxes thickness could limit the moisture loss in fresh blueberries preserving fresh texture attributes in the market place. The development of blueberries to minimise moisture loss could open up new export destinations that previously were inaccessible to suppliers.

Methodologies such as electron microscopy, near infrared spectroscopy, nuclear magnetic resonance imaging as well as evolving non-destructive OCT techniques could provide useful information about the water distribution within blueberries to discern the mechanism involved in the causal relationship between moisture loss and texture in blueberries.

Microstructural differences have been observed between blueberry cultivars, such as a difference in the number of cell layers in the epidermis, cell size, volume and number which may influence how moisture loss and texture responses are related in blueberry (Allan-Wojtas *et al.,* 2001; Cano-Medrano and Darnell, 1997; Fava *et al.,* 2006; Johnson *et al.,* 2011). Moreover the size and distribution of stone cells and vascular bundles could influence the texture characteristics of blueberry in relation to moisture loss. Therefore it is recommended the high genetically variability between highbush, lowbush and rabbiteye blueberry species needs to be evaluated so it is necessary to validate that weight loss values below 8 – 12%

would minimise blueberry softening and maintain blueberry texture characteristics in different species and cultivars.

The application of OCT to non-destructively characterise the microstructure of blueberries needs continued research for this technology to be adopted by industry. Increased depth penetration will be necessary to provide more information for understanding cultivar and microstructural differences. Microscopy investigation into cultivar differences will provide information to understand pigmentation effects and for which berries OCT is suitable for application. In order to be adopted by industry, handheld, portable, low cost instruments need to be developed that can be utilised along the postharvest supply chain.

References

Abbott, J. A., Lu, R., Upchurch, B. L., & Stroshine, R. L. (1997). Technologies for non-destructive quality evaluation of fruits and vegetables. *Horticultural Reviews*, *20*, 1-120.

Aberg, P., Nicander, I., Hansson, J., Geladi, P., Holmgren, U., & Ollmar, S. (2004). Skin cancer identification using multifrequency electrical impedance – a potential screening tool. *IEEE Transactions on Biomedical Engineering, 51*(12), 2097-2102.

Abragam, A., & Abragam, A. (1961). *The principles of nuclear magnetism* (No. 32). Oxford university press.

Aguayo, J. B., Blackband, S. J., Schoeniger, J., Mattingly, M. A., & Hintermann, M. (1986). Nuclear magnetic resonance imaging of a single cell. *Nature, 322*(6075), 190.

Albrigo, L. G., Lyrene, P. M. & Freeman, B. (1980). Waxes and other surface characteristics of fruit and leaves of native *Vaccinium elliotti* Chapm. *Journal of the American Society for Horticultural Science, 105*(2), 230-235.

Alderson, S., Arenstein, R P. (2011). *Checking the Calibration of Dataloggers with Saturated Salt Solutions.* Retrieved from A. M Art Conservation , LLC website: http://www.connectingtocollections.org/wp-content/uploads/2012/03/Calibration-using-saturated-salt-solutions.pdf

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

Ali, M., & Parlapalli, R. (2010). Signal processing overview of optical coherence tomography systems for medical imaging. *Texas Instruments, SPRABB9–June*.

Allan, A. C., & Espley, R. V. (2018). MYBs drive novel consumer traits in fruits and vegetables. *Trends in Plant Science*.

Allan-Wojtas, P. M., Forney, C. F., Carbyn, S. E., & Nicholas, K. U. K. G. (2001). Microstructural indicators of quality-related characteristics of blueberries — an integrated approach. *LWT-Food Science and Technology*, *34*(1), 23-32.

Allan-Wojtas, P., Goff, H. D., Stark, R., & Carbyn, S. (1999). The effect of freezing method and frozen storage conditions on the microstructure of wild blueberries as observed by cold-stage scanning electron microscopy. *Scanning, 21*(5), 334-347.

Almeida, D. P., & Huber, D. J. (1999). Apoplastic pH and inorganic ion levels in tomato fruit: a potential means for regulation of cell wall metabolism during ripening. *Physiologia Plantarum, 105*(3), 506-512.

Almenar, E., Samsudin, H., Auras, R., & Harte, J. (2010). Consumer acceptance of fresh blueberries in bio-based packages. *Journal of the Science of Food and Agriculture*, *90*(7), 1121-1128.

Alvarez, M., Canet, W., & López, M. (2002). Influence of deformation rate and degree of compression on textural parameters of potato and apple tissues in texture profile analysis. *European Food Research and Technology*, *215*(1), 13-20.

Angeletti, P., Castagnasso, H., Miceli, E., Terminiello, L., Concellón, A., Chaves, A., & Vicente, A. R. (2010). Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*) varieties. *Postharvest Biology and Technology*, *58*(2), 98-103.

Ayaz, F. A., Kadioglu, A., Bertoft, E., Acar, C., & Turna, I. (2001). Effect of fruit maturation on sugar and organic acid composition in two blueberries (*Vaccinium arctostaphylos* and *V. myrtillus*) native to Turkey. *New Zealand Journal of Crop and Horticultural Science, 137 - 141*

Bajraszewski, T., Wojtkowski, M., Szkulmowski, M., Szkulmowska, A., Huber, R., & Kowalczyk, A. (2008). Improved spectral optical coherence tomography using optical frequency comb. *Optics Express*, *16*(6), 4163-4176.

Ballinger, W. E., & Kushman, L. J. (1970). Relationship of stage of ripeness to composition and keeping quality of highbush blueberries. *Journal of the American Society of Horticultural Science*, *95*, 239-42.

Ballinger, W. E., Maness, E. P., Kushman, L. J., & Galletta, G. J. (1972). Anthocyanins of ripe fruit of a 'pink-fruited' hybrid of highbush blueberries, *Vaccinium-corymbosum*-L. *Journal of the American Society for Horticultural Science*, *97*(3), 381-384.

Ballington, J. R., Ballinger, W. E., Mainland, C. M., Swallow, W. H., & Maness, E. P. (1984). Ripening period of *Vaccinium* species in Southeastern North Carolina [Blueberry, breeding for both early-and late-ripening Vaccinium genotypes]. *Journal American Society for Horticultural Science*.

Barry, C. S., & Giovannoni, J. J. (2007). Ethylene and fruit ripening. *Journal of Plant Growth Regulation*, *26*(2), 143.

Basiouny, F. M., & Chen, Y. (1988). Effects of harvest date, maturity and storage intervals on postharvest quality of rabbiteye blueberry (*Vaccinium ashei* Reade). In *Proc Fla State Hortic Soc* (Vol. 101, pp. 281-284).

Batisse, C., Buret, M., & Coulomb, P. J. (1996). Biochemical differences in cell wall of cherry fruit between soft and crisp fruit. *Journal of Agricultural and Food Chemistry*, *44*(2), 453-457.

Bergman, H. F. (1929). Changes in the rate of respiration of the fruits of the cultivated blueberry during ripening. *Science*, *70*(1801), 15-15.

Bezerra, H. G., Costa, M. A., Guagliumi, G., Rollins, A. M., & Simon, D. I. (2009). Intracoronary optical coherence tomography: a comprehensive review: clinical and research applications. *JACC: Cardiovascular Interventions*, *2*(11), 1035-1046.

Blaker, K. M., & Olmstead, J. W. (2014). Stone cell frequency and cell area variation of crisp and standard texture southern highbush blueberry fruit. *Journal of the American Society for Horticultural Science*, *139*(5), 553-557.

Bohringer, H. J., Lankenau, E., Stellmacher, F., Reusche, E., Hüttmann, G., & Giese, A. (2009). Imaging of human brain tumor tissue by near-infrared laser coherence tomography. *Acta Neurochirurgica*, *151*(5), 507-517.

Boppart, S. A., Bouma, B. E., Pitris, C., Tearney, G. J., Fujimoto, J. G., & Brezinski, M. E. (1997). Forward-imaging instruments for optical coherence tomography. *Optics Letters*, *22*(21), 1618-1620.

Boppart, S. A., Luo, W., Marks, D. L., & Singletary, K. W. (2004). Optical coherence tomography: feasibility for basic research and image-guided surgery of breast cancer. *Breast Cancer Research*

Bottomley, P. A., Rogers, H. H., & Foster, T. H. (1986). NMR imaging shows water distribution and transport in plant root systems in situ. *Proceedings of the National Academy of Sciences, 83*(1), 87-89.

Bouma, B. E., Tearney, G. J., Compton, C. C., & Nishioka, N. S. (2000). High-resolution imaging of the human esophagus and stomach in vivo using optical coherence tomography. *Gastrointestinal Endoscopy*, *51*(4), 467-474.

Brazelton, C., & Young. K. (2017). World Blueberry Statistics and Global Market Analysis Spring Preview for GBC. *International Blueberry Organisation.*

Breene, W. M. (1975). Application of texture profile analysis to instrumental food texture evaluation. *Journal of Texture Studies*, *6*(1), 53-82.

Brett, C. T., & Waldron, K. W. (1996). *Physiology and biochemistry of plant cell walls* (Vol. 2). Springer Science & Business Media.

Brezinski, M. E. (2006). *Optical coherence tomography: principles and applications*. Elsevier.

Brown, J. M., Johnson, G. A., & Kramer, P. J. (1986). In vivo magnetic resonance microscopy of changing water content in *Pelargonium hortorum* roots. *Plant Physiology, 82*(4), 1158-1160.

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

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

Bunemann, G. E., Dewey, D. H., & Watson, D. P. (1957). Anatomical changes in the fruit of the Rubel Blueberry during storage in controlled atmospheres. In *Proc. Amer. Soc. Hort. Sci* (Vol. 70, pp. 156-60).

Buran, T. J., Sandhu, A. K., Azeredo, A. M., Bent, A. H., Williamson, J. G., & Gu, L. (2012). Effects of exogenous abscisic acid on fruit quality, antioxidant capacities, and phytochemical contents of southern high bush blueberries. *Food Chemistry*, *132*(3), 1375-1381.

Caner, C., & Aday, M. S. (2009). Maintaining quality of fresh strawberries through various modified atmosphere packaging. *Packaging Technology and Science: An International Journal*, *22*(2), 115-122.

Cano-Medrano, R., & Darnell, R. L. (1997). Cell number and cell size in parthenocarpic vs. pollinated blueberry (*Vaccinium ashei*) fruits. Annals of Botany, 80(4), 419-425.

Cantín, C. M., Minas, I. S., Goulas, V., Jiménez, M., Manganaris, G. A., Michailides, T. J., & Crisosto, C. H. (2012). Sulfur dioxide fumigation alone or in combination with CO2-enriched atmosphere extends the market life of highbush blueberry fruit. *Postharvest Biology and Technology*, *67*, 84-91.

Cao, X., Zhang, F., Zhao, D., Zhu, D., & Li, J. (2018). Effects of freezing conditions on quality changes in blueberries. *Journal of the Science of Food and Agriculture*.

Cappellini, R. A., & Ceponis, M. J. (1977). Vulnerability of stem-end scars of blueberry fruits to postharvest decays. *Phytopathology*, *67*(1), 118-119.

Capitani, D., Sobolev, A. P., Delfini, M., Vista, S., Antiochia, R., Proietti, N., & Mannina, L. (2014). NMR methodologies in the analysis of blueberries. *Electrophoresis, 35*(11), 1615-1626.

Castagnini, J. M., Betoret, N., Betoret, E., & Fito, P. (2015). Vacuum impregnation and air drying temperature effect on individual anthocyanins and antiradical capacity of blueberry juice included into an apple matrix. *LWT-Food Science and Technology*, *64*(2), 1289-1296.

Castrejón, A. D. R., Eichholz, I., Rohn, S., Kroh, L. W., & Huyskens-Keil, S. (2008). Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chemistry*, *109*(3), 564-572.

Ceponis, M. J., & Cappellini, R. A. (1982). Control of postharvest decays of blueberries by carbon dioxide enriched atmospheres. *Plant Disease*, *67*(2).

Chen, J., & Stokes, J. R. (2012). Rheology and tribology: Two distinctive regimes of food texture sensation. *Trends in Food Science & Technology*, *25*(1), 4-12.

Chen, L., & Opara, U. L. (2013). Approaches to analysis and modelling texture in fresh and processed foods – A review. *Journal of Food Engineering*, *119*(3), 497-507.

Chen, Y., Bousi, E., Pitris, C., & Fujimoto, J. G. (2016). Optical Coherence Tomography: Introduction and Theory. In *Handbook of Biomedical Optics* (pp. 275-300). CRC Press.

Chiabrando, V., Giacalone, G., & Rolle, L. (2009). Mechanical behaviour and quality traits of highbush blueberry during postharvest storage. *Journal of the Science of Food and Agriculture*, *89*(6), 989-992

Chiabrando, V., & Giacalone, G. (2011). Shelf-life extension of highbush blueberry using 1-methylcyclopropene stored under air and controlled atmosphere. *Food Chemistry*, *126*(4), 1812-1816.

Chong, C., Morosawa, A., & Sakai, T. (2008). High-speed wavelength-swept laser source with high-linearity sweep for optical coherence tomography. *IEEE Journal of Selected Topics in Quantum Electronics*, *14*(1), 235-242.

Clark, C. J., Hockings, P. D., Joyce, D. C., & Mazucco, R. A. (1997). Application of magnetic resonance imaging to pre and post-harvest studies of fruits and vegetables. *Postharvest Biology and Technology, 11*(1), 1-21.

Connor, A. M., Luby, J. J., Hancock, J. F., Berkheimer, S., & Hanson, E. J. (2002). Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *Journal of Agricultural and Food Chemistry*, *50*(4), 893-898.

Creech, D., & Young, L. (2012). The Texas Blueberry Industry—History, Trends, and Cultural Strategies. *International Journal of Fruit Science*, *12*(1-3), 92-99.

Darnell, R. L., Cano-Medrano, R., Koch, K. E., & Avery, M. L. (1994). Differences in sucrose metabolism relative to accumulation of bird-deterrent sucrose levels in fruits of wild and domestic *Vaccinium* species. *Physiologia Plantarum*, *92*(2), 336-342.

Deng, Y., Wu, Y., & Li, Y. (2005). Effects of high O2 levels on post-harvest quality and shelf life of table grapes during long-term storage. *European Food Research and Technology*, *221*(3-4), 392-397.

Donahue, D. W., & Work, T. M. (1998). Sensory and textural evaluation of Maine wild blueberries for the fresh pack market. *Journal of Texture Studies*, *29*(3), 305-312.

Donahue, D. W., Debruyne, D. A., Fecteau, J. D., Storey, J. A., & Hazen, R. A. (1999). Consumer preference and mechanical property assessment of Maine wild blueberries for the fresh pack market 1. *Journal of Food Quality*, *22*(5), 545-551.

Drexler, W., & Fujimoto, J. G. (Eds.). (2008). *Optical coherence tomography: technology and applications*. Springer Science & Business Media.

Du, X., Plotto, A., Song, M., Olmstead, J., & Rouseff, R. (2011). Volatile composition of four southern highbush blueberry cultivars and effect of growing location and harvest date. *Journal of Agricultural and Food Chemistry*, *59*(15), 8347-8357

Duarte, C., Guerra, M., Daniel, P., Camelo, A. L., & Yommi, A. (2009). Quality changes of highbush blueberries fruit stored in CA with different CO2 levels. *Journal of Food Science*, *74*(4), S154-S159.

Eck, P., Gough, R. E., Hall, I. V., & Spiers, J. M. (1990). Blueberry management. *Small Fruit Crop Management. Prentice-Hall, Inc, Englewood Cliffs, New Jersey*, 273-333.

Ehlenfeldt, M. K., & Martin, R. B. (2002). A survey of fruit firmness in highbush blueberry and species introgressed blueberry cultivars. *HortScience*, *37*(2), 386-389.

El-Agamy, S. Z. A., Aly, M. M., & Biggs, R. H. (1982, January). Fruit maturity as related to ethylene in 'Delite' blueberry. In *Proc. Fla. State Hort. Soc* (Vol. 95, pp. 245-246).

ElMasry, G., & Sun, D. W. (2010). Principles of hyperspectral imaging technology. In *Hyperspectral Imaging for Food Quality Analysis and Control* (pp. 3-43).

Esau, K. (1977). Anatomy of Seed Plants. New York: John Wiley& Sons. *Anatomy of Seed Plants1977*.

Eskin, N. A. M. (1979). The plant cell wall. *Plant Pigments, Flavours and Textures: Textural Components of Food*, 123-138.

Ezura, H., & Hiwasa-Tanase, K. (2010). Fruit development. In *Plant Developmental Biology-Biotechnological Perspectives* (pp. 301-318). Springer, Berlin, Heidelberg.

Farahnaky, A., Azizi, R., & Gavahian, M. (2012). Accelerated texture softening of some root vegetables by ohmic heating. *Journal of Food Engineering*, *113*(2), 275-280.

Fava, J., Alzamora, S. M., & Castro, M. A. (2006). Structure and nanostructure of the outer tangential epidermal cell wall in *Vaccinium corymbosum* L.(Blueberry) fruits by blanching, freezing-thawing and ultrasound. *Food Science and Technology International*, *12*(3), 241-251.

Fercher, A. F. (1996). Optical coherence tomography. *Journal of Biomedical Optics*, *1*(2), 157-174.

Fercher, A. F., Drexler, W., Hitzenberger, C. K., & Lasser, T. (2003). Optical coherence tomography-principles and applications. *Reports on Progress in Physics*, *66*(2), 239.

Fercher, A. F., Hitzenberger, C. K., Kamp, G., & El-Zaiat, S. Y. (1995). Measurement of intraocular distances by backscattering spectral interferometry. *Optics Communications*, *117*(1-2), 43-48.

Ferraz, A. C., Sargent, S. A., & Fox, A. J. (2001). A non-destructive method for measuring firmness of blueberry fruit. In *Proc. Fla. State Hort. Soc* (Vol. 114, pp. 138-142).

Fiakkou, M. (2015). *Digital image processing in optical coherence tomography imaging for the evaluation of watermelon properties* (Doctoral dissertation, University of Cyprus).

Forney, C. F. (2008). Postharvest issues in blueberry and cranberry and methods to improve market life. In *IX International Vaccinium Symposium 810* (pp. 785-798).

Forney, C. F., Jordan, M. A., & Nicholas, K. U. K. G. (2003). Effect of CO2 on physical, chemical, and quality changes in 'Burlington' blueberries. *Acta Horticulturae*.

Forney, C. F., Kalt, W., Jordan, M. A., Vinqvist-Tymchuk, M. R., & Fillmore, S. A. (2012). Blueberry and cranberry fruit composition during development. *Journal of Berry Research*, *2*(3), 169-177.

Forney, C. F., Kumudini, U. K. G. N., & Jordan, M. A. (1998). Effects of postharvest storage conditions on firmness of 'Burlington' blueberry fruit. In *8th North American Research and Extension Workers Conference, Wilmington, North Carolina. Proceedings, Wilmington/NC* (pp. 227-232).

Forsyth, F. R., & Hall, I. V. (1969). Ethylene production with accompanying respiration rates from the time of blossoming to fruit maturity in three Vaccinium species. *Nature Can*.

Freeman, B., Albrigo, L. G., & Biggs, R. H. (1979). Cuticular waxes of developing leaves and fruit of blueberry, *Vaccinium ashei* Reade cv. Bluegem. *J. Amer. Soc. Hort. Sci*, *104*, 398-403.

Frenkel, C. (1972). Involvement of peroxidase and indole-3-acetic acid oxidase isozymes from pear, tomato, and blueberry fruit in ripening. *Plant Physiology*, *49*(5), 757-763.

Fujimoto, J. G., Pitris, C., Boppart, S. A., & Brezinski, M. E. (2000). Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. *Neoplasia (New York, NY)*, *2*(1-2), 9.

Gamble, G. R. (1994). Non-invasive determination of freezing effects in blueberry fruit tissue by magnetic resonance imaging. *Journal of Food Science, 59*(3), 571-573.

Gatley, D. P. (2005). *Understanding psychrometrics*. American Society of Heating, Refrigerating, and Air-Conditioning Engineers.

Giongo, L., Poncetta, P., Loretti, P., & Costa, F. (2013). Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. *Postharvest Biology and Technology*, *76*, 34-39.

Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W., Scott, J. H. J., & Joy, D. C. (2017). *Scanning electron microscopy and X-ray microanalysis*. Springer.

Goodman, B. A., Williamson, B., & Chudek, J. A. (1992). Nuclear magnetic resonance (NMR) microimaging of raspberry fruit: further studies on the origin of the image. *New Phytologist, 122*(3), 529-535.

Gough, R. E. (1993). *The highbush blueberry and its management*. CRC Press.

Gowen, A. A., O'Donnell, C., Cullen, P. J., Downey, G., & Frias, J. M. (2007). Hyperspectral imaging–an emerging process analytical tool for food quality and safety control. *Trends in Food Science & Technology, 18*(12), 590-598.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards*, *81*(1), 89-96.

Grierson, W., & Wardowski, W. F. (1975). Humidity in horticulture. *Hortscience, 10*(4), 356-360.

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

Hall, M. A. (1981). Quality in stored and processed vegetables and fruit. Goodenough, P.W and Atkinr. K. ed.

Hall, I. V., Craig, D. L., & Lawrence, R. A. (1983). A comparison of hand raking and mechanical harvesting of lowbush blueberries. *Canadian Journal of Plant Science*, *63*(4), 951-954.

Hancock, J. F., Erb, W. A., Goulart, B. L., & Scheerens, J. C. (1996). Utilization of wild blueberry germplasm: The legacy of Arlen Draper. *Journal of Small Fruit & Viticulture*, *3*(2-3), 1-16.

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

Harker, F. R., Redgwell, R. J., Hallett, I. C., Murray, S. H., & Carter, G. (1997). Texture of fresh fruit. *Horticultural Reviews*, *20*, 121-224.

Hausler, G., & Lindner, M. W. (1998). " Coherence radar" and" spectral radar" new tools for dermatological diagnosis. *Journal of Biomedical Optics*, *3*(1), 21-31.

Hen-Avivi, S., Lashbrooke, J., Costa, F., & Aharoni, A. (2014). Scratching the surface: genetic regulation of cuticle assembly in fleshy fruit. *Journal of Experimental Botany*, *65*(16), 4653-4664.

Hirvi, T., & Honkanen, E. (1983). The aroma of blueberries. *Journal of the Science of Food and Agriculture*, *34*(9), 992-996.

Hrebesh, M. S., Dabu, R., & Sato, M. (2009). In vivo imaging of dynamic biological specimen by real-time single-shot full-field optical coherence tomography. *Optics Communications*, *282*(4), 674-683.

Hu, M. H., Dong, Q. L., Liu, B. L., Opara, U. L., & Chen, L. (2015). Estimating blueberry mechanical properties based on random frog selected hyperspectral data. *Postharvest Biology and Technology*, *106*, 1-10.

Huang, D., Swanson, E. A., Lin, C. P., Schuman, J. S., Stinson, W. G., Chang, W., & Puliafito, C. A. (1991). Optical coherence tomography. *Science*, *254*(5035), 1178-1181.

Huber, D. J. (1983). The role of cell wall hydrolases in fruit softening. *Horticultural Reviews*, *5*, 169-219.

Hulme, A. C. (1971). The biochemistry of fruits and their products. Vol. 2. *The Biochemistry of Fruits and their Products. Vol. 2.*

Ishida, N., Kobayashi, T., Koizumi, M., & Kano, H. (1989). 1H-NMR imaging of tomato fruits. *Agricultural and Biological Chemistry, 53(*9), 2363-2367.

Ismail, AA (1969). Evidence of a respiratory climacteric in highbush and lowbush blueberry fruit. *HortScience* , *4* , 342-344.

Jackman, R. L., & Stanley, D. W. (1995). Perspectives in the textural evaluation of plant foods. *Trends in Food Science & Technology*, *6*(6), 187-194.

Jesser, C. A., Boppart, S. A., Pitris, C., Stamper, D. L., Nielsen, G. P., Brezinski, M. E., & Fujimoto, J. G. (1999). High resolution imaging of transitional cell carcinoma with optical coherence tomography: feasibility for the evaluation of bladder pathology. *The British Journal of Radiology*, *72*(864), 1170-1176.

Jia, L., Liu, S., Liu, Z., Xie, Y., Sun, Q., Wang, B., & Feng, X. (2014, August). Effect of different packaging and cold storage on quality of blueberry. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1120* (pp. 65-70).

Jiang, Y., Li, C., & Takeda, F. (2016). Non-destructive detection and quantification of blueberry bruising using near-infrared (NIR) hyperspectral reflectance imaging. *Scientific Reports, 6,* 35679.

Johnson, L. K., Malladi, A., & NeSmith, D. S. (2011). Differences in cell number facilitate fruit size variation in rabbiteye blueberry genotypes. *Journal of the American Society for Horticultural Science*, *136*(1), 10-15.

Kader, A. A. (2001). A summary of CA requirements and recommendations for fruits other than apples and pears. In *VIII International Controlled Atmosphere Research Conference 600* (pp. 737-740).

Kader, F., Rovel, B., & Metche, M. (1993). Role of invertase in sugar content in highbush blueberries (*Vaccinium corymbosum*, L.). *LWT-Food Science and Technology*, *26*(6), 593-595.

Kalt, W., McDonald, J. E., Ricker, R. D., & Lu, X. (1999). Anthocyanin content and profile within and among blueberry species. *Canadian Journal of Plant Science*, *79*(4), 617-623.

Kang, J. U., Han, J. H., Liu, X., & Zhang, K. (2010). Common-path optical coherence tomography for biomedical imaging and sensing. *Journal of the Optical Society of Korea*, *14*(1), 1-13.

Kerstiens, G. (1996). Cuticular water permeability and its physiological significance. *Journal of Experimental Botany*, *47*(12), 1813-1832.

Kim, Y. K., & Kim, Y. P. (2010). High-speed time-domain optical coherence tomography with an imaging speed of ten frames per second with 2000 A-scan. *Optical Engineering*, *49*(5), 055601.

Ku, V. V., Wills, R. B., & Leshem, Y. A. Y. (2000). Use of nitric oxide to reduce postharvest water loss from horticultural produce. *The Journal of Horticultural Science and Biotechnology*, *75*(3), 268-270.

Kushman, L. J., & Ballinger, W. E. (1968). Acid and sugar changes during ripening in Wolcott blueberries. In *Proceeding of the American Society for Horticultural Science* (Vol. 2, pp. 290-295).

Landahl, S., Foukaraki, S., McWilliam, S., & Terry, L. (2017, September). Optical coherence tomography imaging of potato skin to understand variability in response to pre-and postharvest factors. In *VII International Conference on Managing Quality in Chains (MQUIC2017) and II International Symposium on Ornamentals in 1201* (pp. 331-338).

Landahl, S., Terry, L. A., & Ford, H. D. (2012). Investigation of diseased onion bulbs using data processing of optical coherence tomography images. In *VI International Symposium on Edible Alliaceae 969* (pp. 261-270).

Lashbrooke, J. G., Adato, A., Lotan, O., Alkan, N., Tsimbalist, T., Rechav, K., & Granell, A. (2015). The tomato MIXTA-like transcription factor coordinates fruit epidermis conical cell development and cuticular lipid biosynthesis and assembly. *Plant Physiology*, pp-01145.

Lee, S. W., Jeong, H. W., Kim, B. M., Ahn, Y. C., Jung, W., & Chen, Z. (2009). Optimization for axial resolution, depth range, and sensitivity of spectral domain optical coherence tomography at 1.3 μm. *The Journal of the Korean Physical Society*, *55*(6), 2354.

Legay, S., Guerriero, G., André, C., Guignard, C., Cocco, E., Charton, S., & Hausman, J. F. (2016). MdMyb93 is a regulator of suberin deposition in russeted apple fruit skins. *New Phytologist*, *212*(4), 977-991.

Legland, D., Arganda-Carreras, I., & Andrey, P. (2016). MorphoLibJ: integrated library and plugins for mathematical morphology with ImageJ. *Bioinformatics*, *32*(22), 3532-3534.

Leitgeb, R., Hitzenberger, C. K., & Fercher, A. F. (2003). Performance of Fourier domain vs. time domain optical coherence tomography. *Optics Express*, *11*(8), 889-894.

Leiva-Valenzuela, G. A., Lu, R., & Aguilera, J. M. (2013). Prediction of firmness and soluble solids content of blueberries using hyperspectral reflectance imaging. *Journal of Food Engineering, 115*(1), 91-98.

Leiva-Valenzuela, G. A., Lu, R., & Aguilera, J. M. (2014). Assessment of internal quality of blueberries using hyperspectral transmittance and reflectance images with whole spectra or selected wavelengths. *Innovative Food Science & Emerging Technologies, 24*, 2-13.

Li, C., Luo, J., & MacLean, D. (2011). A novel instrument to delineate varietal and harvest effects on blueberry fruit texture during storage. *Journal of the Science of Food and Agriculture*, *91*(9), 1653-1658.

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

Lipe, J. A. (1978). Ethylene in fruits of blackberry and rabbiteye blueberry [Relation to developmental stages]. *Journal American Society for Horticultural Science*.

Lobos, G. A., Callow, P., & Hancock, J. F. (2014). The effect of delaying harvest date on fruit quality and storage of late highbush blueberry cultivars (*Vaccinium corymbosum* L.). *Postharvest Biology and Technology*, *87*, 133-139.

Loeb, G., & Barton, J. K. (2003). Imaging botanical subjects with optical coherence tomography: a feasibility study. *Transactions of the ASAE*, *46*(6), 1751.

Lohachoompol, V., Mulholland, M., Srzednicki, G., & Craske, J. (2008). Determination of anthocyanins in various cultivars of highbush and rabbiteye blueberries. *Food Chemistry*, *111*(1), 249-254.

Lyrene, P. M. (1990). Low-chill highbush blueberries. *Fruit Varieties Journal*, *44*(2), 82-86.

MacFall, J. S., Johnson, G. A., & Kramer, P. J. (1991). Comparative water uptake by roots of different ages in seedlings of loblolly pine (*Pinus taeda* L.). *New Phytologist, 119*(4), 551-560.

Machemer, K., Shaiman, O., Salts, Y., Shabtai, S., Sobolev, I., Belausov, E., & Barg, R. (2011). Interplay of MYB factors in differential cell expansion, and consequences for tomato fruit development. *The Plant Journal*, *68*(2), 337-350.

Maguire, K. M., Banks, N. H., & Opara, L. U. (2001). Factors affecting weight loss of apples. *Horticultural Reviews*, *25*, 197-234.

Magwaza, L. S., Ford, H. D., Cronje, P. J., Opara, U. L., Landahl, S., Tatam, R. P., & Terry, L. A. (2013). Application of optical coherence tomography to non-destructively characterise rind breakdown disorder of 'Nules Clementine' mandarins. *Postharvest Biology and Technology*, *84*, 16-21.

Mainland, C. M. (1989). Harvesting, sorting and packing quality blueberries. *23rd Annual Open House, Southeastern Blueberry Council*.

Mainland, C. M. (1995). Comments on fruit quality and handling. Paper presented at the 30th annual meeting of the North American Blueberry Council, North Carolina.

Makus, D. J., & Morris, J. R. (1993). A comparison of fruit of highbush and rabbiteye blueberry cultivars. *Journal of Food Quality*, *16*(6), 417-428.

Meglinski, I. V., Buranachai, C., & Terry, L. A. (2010). Plant photonics: application of optical coherence tomography to monitor defects and rots in onion. *Laser Physics Letters*, *7*(4), 307.

Michalewska, Z., Michalewski, J., & Nawrocki, J (2013). Swept-source OCT. *Retina Today*, 50-56.

Miller, W. R., McDonald, R. E., & Cracker, T. E. (1993). Quality of two Florida blueberry cultivars after packaging and storage. *HortScience*, *28*(2), 144-147.

Miller, W. R., McDonald, R. E., Melvin, C. F., & Munroe, K. A. (1984). Effect of package type and storage time-temperature on weight loss, firmness, and spoilage of rabbiteye blueberries [Marketing, export, consumer packaging, *Vaccinium ashei*]. *HortScience (USA)*.

Mitcham, E. (2007). Quality of berries associated with preharvest and postharvest conditions. *Food Science and Technology New York Marcel Dekker*, *168*, 207.

Mitcham, E. J., Crisosto, C. H., & Kader, A. A. (2002). Bushberry: blackberry, blueberry, cranberry, raspberry: Recommendations for maintaining postharvest quality. Department of Pomology, University of California, Davis.

Mitra, P. P., & Loqué, D. (2014). Histochemical staining of Arabidopsis thaliana secondary cell wall elements. *Journal of Visualized Experiments: JoVE*, (87).

Moggia, C., Graell, J., Lara, I., González, G., & Lobos, G. A. (2017). Firmness at Harvest Impacts Postharvest Fruit Softening and Internal Browning Development in Mechanically Damaged and Non-damaged Highbush Blueberries (*Vaccinium corymbosum* L.). *Frontiers in plant science*, *8*, 535.

Mori, B., & Bellani, L. M. (1996). Differential staining for cellulosic and modified plant cell walls. *Biotechnic & Histochemistry*, *71*(2), 71-72.

Nardozza, S. (2008). *Genotypic variation in Actinidia deliciosa fruit size and carbohydrate content* (Doctoral dissertation, alma).

NeSmith, D. S., Prussia, S., Tetteh, M., & Krewer, G. (2000). Firmness losses of rabbiteye blueberries (*Vaccinium ashei* Reade) during harvesting and handling. In *VII International Symposium on Vaccinium Culture 574* (pp. 287-293).

NeSmith, D. S., Nunez-Barrios, A., Prussia, S. E., & Aggarwal, D. (2005). Postharvest berry quality of six rabbiteye blueberry cultivars in response to temperature. *Journal of the American Pomological Society*, *59*(1), 13.

Nicolai, B. M., Lötze, E., Peirs, A., Scheerlinck, N., & Theron, K. I. (2006). Non-destructive measurement of bitter pit in apple fruit using NIR hyperspectral imaging. *Postharvest Biology and Technology, 40*(1), 1-6.

Nunes, M. C. N., Emond, J. P., & Brecht, J. K. (2004). Quality curves for highbush blueberries as a function of the storage temperature. *Small Fruits Review*, *3*(3-4), 423-440.

Oancea, S., Stoia, M., & Coman, D. (2012). Effects of extraction conditions on bioactive anthocyanin content of *Vaccinium corymbosum* in the perspective of food applications. *Procedia Engineering*, *42*, 489-495.

O'Brien, T. P., Feder, N., & McCully, M. E. (1964). Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*, *59*(2), 368-373.

Opara, U. L., & Pathare, P. B. (2014). Bruise damage measurement and analysis of fresh horticultural produce—a review. *Postharvest Biology and Technology*, *91*, 9-24.

Paniagua, A. C., East, A. R., Hindmarsh, J. P., & Heyes, J. (2013). Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biology and Technology,* 79, 13-19.

Paniagua, A.C. (2012). *Influence of temperature management deficiencies during postharvest on the quality of sea exported blueberries* (Master thesis, Massey University, Palmerston North, New Zealand). Retrieved from https://mro.massey.ac.nz/bitstream/handle/10179/4112/02_whole.pdf?sequence=3&isAllowed=y

Parker, A. J., Haskins, E. F., & Deyrup-Olsen, I. (1982). Toluidine blue: a simple, effective stain for plant tissues. *The American Biology Teacher*, 487-489.

Paul T. Callaghan. (1993). *Principles of nuclear magnetic resonance microscopy*. Oxford University Press on Demand.

Paull, R. (1999). Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biology and Technology*, *15*(3), 263-277.

Perkins-Veazie, P. (2004). Blueberry. *The commercial storage of fruits, vegetables, and florist and nursery stocks. Edited by KC Gross, CY, Wang, and M. Saltveit. Agricultural Handbook*, *66*.

Pitris, C., Goodman, A., Boppart, S. A., Libus, J. J., Fujimoto, J. G., & Brezinski, M. E. (1999). High-resolution imaging of gynaecologic neoplasms using optical coherence tomography. *Obstetrics & Gynecology*, *93*(1), 135-139.

Prakash, A., Rigelhof, F., & Miller, E. (2001). Medallion Laboratories Analytical Progress. *Antioxidant Activity*, *19*(2), 1-6.

Prange, R. K., DeEll, J. R., Westgarth, A. R., & Asiedu, S. K. (1995). Quality of Fundy and Blomidon lowbush blueberries: Effects of storage atmosphere, duration and fungal inoculation. *Canadian Journal of Plant Science*, *75*(2), 479-483.

Preston, R. D. (1979). Polysaccharide conformation and cell wall function. Annual Review of Plant Physiology, 30(1), 55-78.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. *Journal of Agricultural and Food Chemistry*, *46*(7), 2686-2693.

Prior, R. L., Lazarus, S. A., Cao, G., Muccitelli, H., & Hammerstone, J. F. (2001). Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry*, *49*(3), 1270-1276.

Proctor, A., & Miesle, T. J. (1991). Polygalacturonase and pectinmethylesterase activities in developing highbush blueberries. *HortScience*, *26*(5), 579-581.

Proctor, A., & Peng, L. C. (1989). Pectin transitions during blueberry fruit development and ripening. *Journal of Food Science*, *54*(2), 385-387.

Prussia, S. E., Tetteh, M. K., Verma, B. P., & NeSmith, D. S. (2006). Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. *Transactions of the ASABE*, *49*(1), 113-121.

Rees, D. A. (1977). Polysaccharide shapes: Outline Studies in Botany Series.

Retamales, J. B., & Hancock, J. F. (2018). *Blueberries* (Vol. 28). Cabi.

Rhodes, M. J. C. (1970). The climacteric and ripening of fruits. In A. C. Hulme (Ed.), The Biochemistry of Fruits and their Products (pp. 521 – 533). London: Academic Press.

Ribera-Fonseca, A., Noferini, M., & Rombolá, A. D. (2016). Non-destructive assessment of highbush blueberry fruit maturity parameters and anthocyanins by using a visible/near infrared (vis/NIR) spectroscopy device: A preliminary approach. *Journal of Soil Science and Plant Nutrition*, *16*(1), 174-186.

Riederer, M., & Schreiber, L. (2001). Protecting against water loss: analysis of the barrier properties of plant cuticles. *Journal of Experimental Botany*, *52*(363), 2023-2032.

Rosenthal, A. J. (2010). Texture profile analysis–how important are the parameters? *Journal of Texture Studies*, *41*(5), 672-684.

Ruzin, S. E. (1999). *Plant micro technique and microscopy* (Vol. 198). New York: Oxford University Press.

Saftner, R., Polashock, J., Ehlenfeldt, M., & Vinyard, B. (2008). Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biology and Technology*, *49*(1), 19-26.

Sakai, W. S. (1973). Simple method for differential staining of paraffin embedded plant material using toluidine blue O. *Stain Technology*, *48*(5), 247-249.

Sanford, K. A., Lidster, P. D., McRae, K. B., Jackson, E. D., Lawrence, R. A., Stark, R., & Prange, R. K. (1991). Lowbush blueberry quality changes in response to mechanical damage and storage temperature. *Journal of the American Society for Horticultural Science*, *116*(1), 47-51.

Sapers, G. M., Burgher, A. M., Phillips, J. G., Jones, S. B., & Stone, E. G. (1984). Color and composition of highbush blueberry cultivars. *Journal of the American Society for Horticultural Science*, *109*, 105-11.

Sapers, G. M., Jones, S. B., & Phillips, J. G. (1985). Leakage of anthocyanins from skin of thawed, frozen highbush blueberries (*Vaccinium corymbosum* L.). *Journal of Food Science*, 50(2), 432-436.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., & Tinevez, J. Y. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods*, *9*(7), 676.

Schotsmans, W., Molan, A., & MacKay, B. (2007). Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. *Postharvest Biology and Technology*, 44(3), 277-285.

Shackel, K. A., Greve, C., Labavitch, J. M., & Ahmadi, H. (1991). Cell turgor changes associated with ripening in tomato pericarp tissue. *Plant Physiology*, 97(2), 814-816.

Shimura, I., Kobayashi, M., & Ishikawa, S. (1986). Characteristics of fruit growth and development in highbush and rabbiteye blueberries (*Vaccinium corymbosum* L. and *V. ashei* Reade) and the differences among their cultivars. *Journal of the Japanese Society for Horticultural Science*, *55*(1), 46-50.

Silva, J. L., Marroquin, E., Matta, F. B., Garner Jr, J. O., & Stojanovic, J. (2005). Physicochemical, carbohydrate and sensory characteristics of highbush and rabbiteye blueberry cultivars. *Journal of the Science of Food and Agriculture*, *85*(11), 1815-1821.

Skrede, G., Wrolstad, R. E., & Durst, R. W. (2000). Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *Journal of Food Science*, *65*(2), 357-364.

Srivastava, V., Dalal, D., Kumar, A., Prakash, S., & Dalal, K. (2018). In vivo automated quantification of quality of apples during storage using optical coherence tomography images. *Laser Physics*, *28*(6), 066207.

Stefanaki, E. C. (2008). Electron microscopy: the basics. *Physics of Advanced Materials Winter School*, 1-11.

Stevenson, D., & Scalzo, J. (2012). Anthocyanin composition and content of blueberries from around the world. *Journal of Berry Research*, *2*(4), 179-189.

Strik, B. C. (2007). Berry crops: Worldwide area and production systems. *Berry fruit: Value-added products for health promotion*, 3-51.

Studman, C. (1997, September). Factors affecting the bruise susceptibility of fruit. In *Proceedings of the 2nd International Conference of Plant Biomechanics* (pp. 7-12). Centre for Biomimetics, University of Reading.

Suter, M. J., Vakoc, B. J., Yachimski, P. S., Shishkov, M., Lauwers, G. Y., Mino-Kenudson, M., & Tearney, G. J. (2008). Comprehensive microscopy of the esophagus in human patients with optical frequency domain imaging. *Gastrointestinal Endoscopy*, *68*(4), 745-753.

Suzuki, A., Kikuchi, T., & Aoba, K. (1997). Changes of ethylene evolution, ACC content, ethylene forming enzyme activity and respiration in fruits of highbush blueberry. *Journal of the Japanese Society for Horticultural Science*, *66*(1), 23-27.

Taiz, L., & Zeiger, E. (2010). Plant physiology 5th Ed. *Sunderland, MA: Sinauer Associates*.

Talbot, M. and Baird, C. (1991). Psychrometrics and postharvest operations. Paper presented at the Proceedings of the Florida State Horticultural Society Washington, DC.

Tearney, G. J., Boppart, S. A., Bouma, B. E., Brezinski, M. E., Weissman, N. J., Southern, J. F., & Fujimoto, J. G. (1996). Scanning single-mode fiber optic catheter–endoscope for optical coherence tomography. *Optics letters*, *21*(7), 543-545.

Tearney, G. J., Brezinski, M. E., Bouma, B. E., Boppart, S. A., Pitris, C., Southern, J. F., & Fujimoto, J. G. (1997). In vivo endoscopic optical biopsy with optical coherence tomography. *Science*, *276*(5321), 2037-2039.

Tearney, G. J., Jang, I. K., & Bouma, B. E. (2006). Optical coherence tomography for imaging the vulnerable plaque. *Journal of Biomedical Optics*, *11*(2), 021002.

Testoni, P. A. (2007). Optical coherence tomography. *The Scientific World Journal*, *7*, 87-108.

Tetteh, M. K., Prussia, S. E., Nesmith, D. S., Verma, B. P., & Aggarwal, D. (2004). Modelling blueberry firmness and mass loss during cooling delays and storage. *Transactions of the ASAE*, *47*(4), 1121.

Texture Technologies. (2018). *Overview of Texture Profile Analysis.* Retrieved from http://texturetechnologies.com/resources/texture-profile-analysis#tpa-measurements

The New Zealand Institute for Plant & Food Research Ltd. (2017). *Breeding blueberries to extend the season.* Retrieved from https://www.plantandfood.co.nz/growingfutures/case-studies/breeding-new-blueberries/new-zealand-exports

Thomas, T. R., Shackel, K. A., & Matthews, M. A. (2008). Mesocarp cell turgor in *Vitis vinifera L*. berries throughout development and its relation to firmness, growth, and the onset of ripening. *Planta*, *228*(6), 1067.

Thompson, J. (2002). Psychrometrics and perishable commodities. In A. Kader (Ed.), Postharvest technology of Horticultural Crops (3rd ed., pp. 535). Oakland, University of California.

Vakhtin, A. B., Kane, D. J., Wood, W. R., & Peterson, K. A. (2003). Common-path interferometer for frequency-domain optical coherence tomography. *Applied optics*, *42*(34), 6953-6958.

Vega, A., Luza, J., Espina, S., & Lizana, L. A. (1991). Characterization of the epidermis of three blueberry (*Vaccinium corymbosum* L.) cultivars and water loss at different storage temperatures. *Proc Interamer Soc Tropic Hort*, *35*, 263-274.

Verboven, P., Nemeth, A., Abera, M. K., Bongaers, E., Daelemans, D., Estrade, P., & Verlinden, B. (2013). Optical coherence tomography visualizes microstructure of apple peel. *Postharvest Biology and Technology*, *78*, 123-132.

Vicente, A. R., Ortugno, C., Rosli, H., Powell, A. L., Greve, L. C., & Labavitch, J. M. (2007). Temporal sequence of cell wall disassembly events in developing fruits. 2. Analysis of blueberry (*Vaccinium* species). *Journal of Agricultural and Food Chemistry*, 55(10), 4125-4130.

Walther, J., Gaertner, M., Cimalla, P., Burkhardt, A., Kirsten, L., Meissner, S., & Koch, E. (2011). Optical coherence tomography in biomedical research. *Analytical and Bioanalytical Chemistry*, *400*(9), 2721-2743.

Wang, R. K., Jacques, S. L., Ma, Z., Hurst, S., Hanson, S. R., & Gruber, A. (2007). Three dimensional optical angiography. *Optics Express*, *15*(7), 4083-4097.

Wijesinghe, R., Lee, S. Y., Ravichandran, N. K., Shirazi, M. F., Kim, P., & Jung, H. Y. (2016). Optical screening of *Venturianashicola* caused *Pyruspyrifolia* (Asian pear) scab using optical coherence tomography. *International Journal of Applied Engineering Research*, *11*(12), 7728-7731.

Williams, D. B., & Carter, C. B. (1996). The transmission electron microscope. In *Transmission electron microscopy* (pp. 3-17). Springer, Boston, MA.

Wills, R. B. H., McGlasson, W. B., & Joyce, D. C. (2007). *Postharvest. An introduction to the physiology and handling of fruit and vegetables and ornamentals* (5th ed). Sydney University of new South Wales Press.

Wills, R. H. H., Lee, T. H., Graham, D., McGlasson, W. B., & Hall, E. G. (1981). Postharvest. An Introduction to the Physiology and Handling of Fruit and Vegetables. Granada.

Wills, R., & Golding, J. (2016). *Postharvest: an introduction to the physiology and handling of fruit and vegetables*. UNSW press.

Windus, N. D., Shutak, V. G and Gough, R. E. (1976). CO_2 and C_2H_4 evolution by highbush blueberry fruit. *Hortscience, 11*(5), 515 – 517.

Wojtkowski, M., Srinivasan, V. J., Ko, T. H., Fujimoto, J. G., Kowalczyk, A., & Duker, J. S. (2004). Ultrahigh-resolution, high-speed, Fourier domain optical coherence tomography and methods for dispersion compensation. *Optics Express*, *12*(11), 2404-2422.

Wood, L. A. (1970). The use of dew-point temperature in humidity calculations. *Journal of Research of the National Bureau of Standards–C. Engineering and Instrumentation C*, *74*, 117-122.

Woodruff, R. E., Dewey, D. H., & Sell, H. M. (1960). Chemical changes of Jersey and Rubel blueberry fruit associated with ripening and deterioration. In *Proceedings. American Society for Horticultural Science* (Vol. 75, pp. 387-401).

Xu, R., Takeda, F., Krewer, G., & Li, C. (2015). Measure of mechanical impacts in commercial blueberry packing lines and potential damage to blueberry fruit. *Postharvest Biology and Technology*, *110*, 103-113.

Yang, C., Lee, W. S., & Gader, P. (2014). Hyperspectral band selection for detecting different blueberry fruit maturity stages. *Computers and Electronics in Agriculture, 109*, 23-31.

Yang, V. X., Gordon, M. L., Tang, S. J., Marcon, N. E., Gardiner, G., Qi, B., & Wilson, B. C. (2003). High speed, wide velocity dynamic range Doppler optical coherence tomography (Part I): in vivo endoscopic imaging of blood flow in the rat and human gastrointestinal tracts. *Optics Express*, *11*(19), 2416-2424.

Yang, V. X., Tang, S. J., Gordon, M. L., Qi, B., Gardiner, G., Cirocco, M., & Wilson, B. C. (2005). Endoscopic Doppler optical coherence tomography in the human GI tract: initial experience. *Gastrointestinal Endoscopy*, *61*(7), 879-890.

Yang, W. Q., Harpole, J., Finn, C. E., & Strik, B. C. (2008). Evaluating berry firmness and total soluble solids of newly released highbush blueberry cultivars. In *IX International Vaccinium Symposium 810* (pp. 863-868).

Yang, Z., Zheng, Y., Cao, S., Tang, S., Ma, S., & Li, N. A. (2007). Effects of storage temperature on textural properties of Chinese bayberry fruit. *Journal of Texture Studies*, *38*(1), 166-177.

Yaqoob, Z., Wu, J., McDowell, E. J., Heng, X., & Yang, C. (2006). Methods and application areas of endoscopic optical coherence tomography. *Journal of Biomedical Optics*, *11*(6), 063001.

Zhou, Y., Wu, D., Hui, G., Mao, J., Liu, T., Zhou, W., & Chen, F. (2018). Loquat Bruise Detection Using Optical Coherence Tomography Based on Microstructural Parameters. *Food Analytical Methods*, 1-7.

Zielinska, M., & Michalska, A. (2016). Microwave-assisted drying of blueberry (*Vaccinium corymbosum* L.) fruits: Drying kinetics, polyphenols, anthocyanins, antioxidant capacity, colour and texture. *Food Chemistry*, *212*, 671-680.

Zielinska, M., Sadowski, P., & Błaszczak, W. (2015). Freezing/thawing and microwave-assisted drying of blueberries (*Vaccinium corymbosum* L.). *LWT-Food Science and Technology*, *62*(1), 555-563.

Zifkin, M., Jin, A., Ozga, J. A., Zaharia, L. I., Schernthaner, J. P., Gesell, A., & Constabel, C. P. (2012). Gene expression and metabolite profiling of developing highbush blueberry fruit indicates transcriptional regulation of flavonoid metabolism and activation of abscisic acid metabolism. *Plant Physiology*, *158*(1), 200-224.

Appendices

Appendix A: Harvesting and handling practises

Blueberries are harvested when they are fully mature and may remain on the plant for up to ten days to ensure full ripeness. Creech and Young (2012) suggested when blueberries reach full blue colouration, they may not be fully ripe. Hand harvesting is a common harvesting practise used by growers and is popular with boutique orchards. Hand harvesting results in less damage and bruising of blueberries as berry fruit is susceptible to mechanical damage (Moggia *et al.,* 2017). In damaged blueberry flesh, bruises develop resulting in internal browning from the oxidation of phenolic compounds and subsequent tissue breakdown (Studman, 1997; Opara and Pathare, 2014; Moggia *et al.,* 2017). Consequently hand harvesting techniques produce increases in the total amount of fruit available for export (Moggia *et al.,* 2017).

Rising costs and insufficient labour available to harvest large growing areas has led to an increase in mechanical harvesting practises. Mechanical hand-held shakers enable a person to harvest over 10 kg per hour, whereas an adult hand picker may only harvest up to 4 kg per hour of blueberries (Hall *et al.,* 1983). Mechanical hand-held harvesting devices increase the efficiency and capacity of harvesting, however increased fruit damage and bruising arise due to the falling distance of blueberries into containers (Moggia *et al.,* 2017). Machine harvesting is an increasingly popular, efficient and adopted harvesting technique. In general a reduction in the amount of acceptable fruit for export has been reported resulting from excessive softening and bruising arising from machine harvesting. In order to combat the reduction of marketable blueberries, harvesting machines designed to minimise blueberry losses are being developed. Lobos *et al.* (2014) reported promising results using a particular shaker machine that is currently being trialled during critical harvesting peaks.

After blueberries have been harvested, they are transported from the field into the packing house where they are graded based on quality and size (Xu *et al.,* 2015). Grading commonly occurs by hand, however new technologies such as the Blueberry Vision 2 (Figure A.1; Unitec Technology, Italy) and Kato 260 (BBC Technologies, New Zealand) enable high throughput screening to analyse internal and external quality parameters such as bruising, the presence of stem, peeling on the stalk, scar, decay, dehydration, softness, damage and colour. Therefore, blueberries can be graded on size, colour and external and internal defects. Interestingly after harvest when blueberries are transported to the pack house and during processing blueberries can develop bruising. During grading and sorting a 10 – 15% loss in

overall berry firmness was recorded by NeSmith *et al.* (2000) in 'Brightwell' blueberries indicating a degradation in fruit texture. It is important blueberries are stored in refrigerated and temperature controlled environments to slow down the process of internal browning in order to maintain a quality berry fruit for export.

Figure A.1 The Blueberry Vision 2 for automated blueberry sorting and grading (Unitec technology, Italy)

A.1 Hand harvesting

Hand harvesting is a traditional blueberry harvesting technique but can become considerably expensive for growers requiring intensive quality labour. To harvest blueberries by hand pickers run a hand around a cluster of blueberries and only blueberries that are ripe come off easily (Creech and Young, 2012). Multiple hand devices make harvesting much easier and the use of harness like equipment is worn to support plastic containers. Plastic containers are lined with a plastic bag to make processing simpler. Typically hand harvesting requires 5 – 10 people per acre, and a total of 550 worker hours during harvest time (Creech and Young, 2012) making this a fairly labour intensive process. Techniques such as hand raking and striking branches enables this process to be more efficient but leads to an increase in bruising and loss of texture.

Blueberries which are hand harvested are firmer in texture and retain quality characteristics compared to other harvesting practises (Lobos *et al.,* 2014). Hall *et al.* (1983) indicated that more blueberries were available after and harvesting than mechanical harvesting, as hand harvesting only resulted in 3.2% poor blueberries compared to 6.5% in mechanical harvesting. Hand raking requires an average harvest time of 42 min/plot, where machine harvesting requires 5 min/plot (Hall *et al.,* 1983). Even though hand harvesting produce higher quality blueberries retaining textural characteristics, it is less efficient, with growers turning to mechanical technologies to increase efficiency and yield.

A.2 Mechanical harvesting

Mechanical and machine harvesting is an increasingly popular blueberry harvesting technique arising from the demand for more efficient and less costly labour, growers do not need to rely on labour intensive harvesting. Machine harvesting is more efficient than hand harvesting, completing the same area of harvest in less than half the time (Hall *et al.,* 1983). Over the row, side mount, and rotary head harvesters are some typical machine harvesters. Air jet harvesters are becoming increasingly popular, and example can be seen in Figure A.2. Although mechanical harvesting is efficient, degradation in blueberry texture and quality can be increased during harvest. NeSmith *et al.* (2000) reported a 20 – 30% loss in firmness of 'Brightwell' blueberries harvested with a BEI model LBT (Blueberry Equipment Inc, USA). Similarly a 10 – 30% increase in softness was seen in blueberries harvested by machine, compared with hand harvesting (Mainland *et al.,* 1989; Lobos *et al.,* 2014).

Mechanical damage is referred to as internal bruising that is caused by the height of the berry fall into the container which affects the berries shelf life (Mainland, 1989; Brown *et al.,* 1996) Internal bruising leads to the degradation in the overall fruit texture and quality. Lobos *et al.* (2014) reported 41.5% mechanical damage for machine harvested 'Brigitta' blueberries, compared to 9.0% mechanical damage for hand harvested blueberries after 60 d storage at 1 ºC. As a result of the high texture and quality damage present in machine harvested blueberries during storage, these blueberries often undergo further processing and are not sold as single fresh blueberries. Although machine harvesting requires less labour, machinery needs to be cleared of leaves and debris prior to use which could increase labour costs. While machine harvesting is highly efficient, consumers value berry quality and texture therefore machine harvesting is not often used alone and is used in conjunction with hand harvesting techniques.

Figure A.2. The Kokan 500S (A), is towed behind a harvester and uses pulsating air jets of controlled velocity and frequency to shake the bush and catch the fruit (B) (BSK, Serbia).

Appendix B: Psychrometrics of fresh produce

Water loss of fresh produce is determined by the difference in the moisture content of the surrounding air and the fresh produce (Wills and Golding, 2016). Moisture can be defined as a mixture dry air and any amount of water vapour (Grierson and Wardowski, 1975). The content of moisture in air can be described as humidity. As ambient pressure decreases, and temperature increases the capacity of air to hold moisture increases (Grierson and Wardowski, 1975). The degradation of fresh produce due to enzymatic and microbial reactions rapidly increases at high temperatures, fresh produce is often stored at low temperatures. However humidity is kept high to prevent fruit dehydration and shrivel.

The absolute humidity represents the weight of water contained in an air volume, and the weight of dry air contained in the same volume (Talbot and Baird, 1991). In horticulture management absolute humidity is often expressed as vapour pressure (Thompson, 2002). The gradient of absolute humidity between two air conditions, allows the movement of water vapour from high to low vapour concentrations until an equilibrium is reached. This drives water vapour movement (Taiz and Zeiger, 2010). The driving force for water loss, is the absolute humidity gradient between intracellular spaces of plant tissue and the surrounding air. This defines the rate at which fresh produce loses water and is dehydrated (Wills and Golding, 2016). As the temperature of a product increases, the gradient of absolute humidity between the product and the environment increases, therefore leading to increased moisture loss. Rapidly cooling a product is an effective post-harvest management tool reducing moisture loss from fresh produce (Thompson, 2002).

Relative humidity (RH) is defined as the ratio of water vapour pressure in the air to the saturation pressure possible at the same temperature (Wills and Golding, 2016). This is expressed as a percentage, therefore a RH of 100% indicates saturated air. RH can be measured directly using a hygrometer (Wills *et al.,* 2007). RH is a psychrometric variable, that is the most known and poorly applied. In postharvest environments it is misused to indicate humidity gradient (Talbot and Baird, 1991). Increasing the RH of air within a cool room reduces the amount of moisture lost from fresh produce before the surrounding air is saturated (Wills and Golding, 2016). At high RH, condensation on cool surfaces can result from a small fluctuation in temperature (<0.5°C). Interestingly poor air distribution within a refrigeration system may mean that air from the coil at 0°C, 95% RH, could be in an area at 5°C, 70% RH (Paull, 1999). It is important fresh produce is near air temperature to prevent moisture loss at high RH (Paull, 1999). A very high RH (>95%) can promote the growth of bacteria and fungi,

leading to fast degradation of produce. In a dry atmosphere (80% RH) fungi and bacteria cease to grow. Although in low RH conditions produce dehydrates and is not desirable for market. Therefore when determining desirable humidity conditions it becomes a balancing act between microbial spoilage and water loss. For most produce humidity conditions tend to be between 85 – 95% RH (Wills and Golding, 2016). In order to evaluate the potential moisture loss of fresh produce at a given storage condition, RH must always be given together with the temperature of air (Wills and Golding, 2016).

In addition to RH, dry bulb temperature, wet bulb temperature and dew point temperature are other psychrometric variables used to evaluate the storage conditions of fresh produce (Wills and Golding, 2016). Dry bulb temperature, commonly known as air temperature is the temperature of the air, measured by a thermometer. Whereas wet bulb temperature is measured from a water-soaked bulb of regular thermometer over which air is passed. The dry bulb temperature and wet bulb temperature when combined can be used to calculate the absolute humidity of an air volume (Talbot and Baird, 1991). It represents the minimum temperature to which an air volume can be cooled by increasing its water content. At 100% RH the wet bulb temperature and dry bulb temperature are equal (Talbot and Baird, 1991). As temperature decreases, air decreases its capacity to hold water at a given environment pressure resulting in condensation. The temperature at which air condensates is referred to as the dew point temperature (Grierson and Wardowski, 1978). Consequently the partial saturation vapour pressure (VP) at the dew point temperature is equal to the partial VP at a given temperature (Wood, 1970).

The relationships between psychrometrics variables are represented by a psychrometric chart. In essence psychrometric charts are graphic representations of the psychrometric properties of air. The psychrometric chart is valid for a specific ambient pressure and is normally reported at atmospheric pressure. As long as two psychrometric variables of moistened air are known, the chart can be used to obtain all psychrometric properties. For instance psychrometric charts can be used to calculate the absolute humidity (humidity ratio) from RH and dry bulb temperature, or from the wet and dry bulb temperature (Wills and Golding, 2016). The dry bulb temperature (ºC) is represented by the horizontal axis. Whereas the wet bulb temperature (ºC) is indicated by an axis sloping diagonally upwards from right to left (Gatley, 2005). The right vertical axis is the water vapour pressure (kPa). Dew point temperature is an important psychrometric property and can be plotted on the chart. However it seldom is, as its horizontal isolines could be confused with the horizontal absolute humidity (humidity ratio) isolines (Gatley, 2005). Specific enthalpy and specific volume per kilogram of dry air can be plotted on a psychrometric chart. The application of psychrometric concepts on

postharvest management are a useful tool to minimise water loss of fresh produce in storage ad packhouse facilities.

Appendix C: Time-domain optical coherence tomography (TD-OCT)

TD-OCT is already well established, with an increasing field of applications in medical diagnostics (Leitgeb *et al.,* 2003). The first generation of OCT systems was first demonstrated by Huang *et al.* (1991) for 2D cross sectional images of human retina and coronary artery. Since then, OCT has rapidly developed as a non-invasive diagnostic tool within the medical field with a wide range of applications and has recently found success within the horticulture industry.

A single detector at the output of the interferometer and a broadband light source are characteristic of TD-OCT systems (Figure C.1). Multiple parallel Low Coherence Interferometer (LCI) scans are performed to recorded 2D images. The light back scattered from within the sample is analysed by changing the length of the reference beam in regards to the optical pathway (Walther *et al.,* 2011). A measurement beam backscattered from the specimen may have various optical properties dependent on the different layers within the specimen, resulting in different time delays (Brezinski, 2006). A longitudinal profile of reflectivity in respect to depth can be generated by changing the path length of the reference arm and simultaneously recording the magnitude and intensity of interference fringes (Brezinski, 2006). Consequently the origin of back scattered light from the specimen can be determined accurately, as a fringe signal is only detected when both arms of the interferometer have a difference in length shorter than the coherence length (l_c) of the light source (Walther *et al.,* 2011). By varying the length of the reference arms in a triangular manner a depth scan can be achieved by recording the oscillating signal (Walther *et al.,* 2011). Therefore the location of the internal structures of a specimen can be determined with a suitable resolution by locating the maximum fringe visibility position (Brezinski, 2006; Walther *et al.,* 2011).

At one point of the sample surface the amplitude of the back scattered signal as a function of depth is called an A-scan (Leitgeb *et al.,* 2003; Walther *et al.,* 2011). A single processed plot can show this. Whereas by moving the beam laterally relative to the specimen to obtain a cross sectional view is referred to as a B-scan. Relative to the specimen surface the beam is deflected in two dimensions to provide volumetric information about the specimen. Scanning can be achieved by moving the sample, although for high speed imaging deflection of the beam with a galvanometer is more efficient.

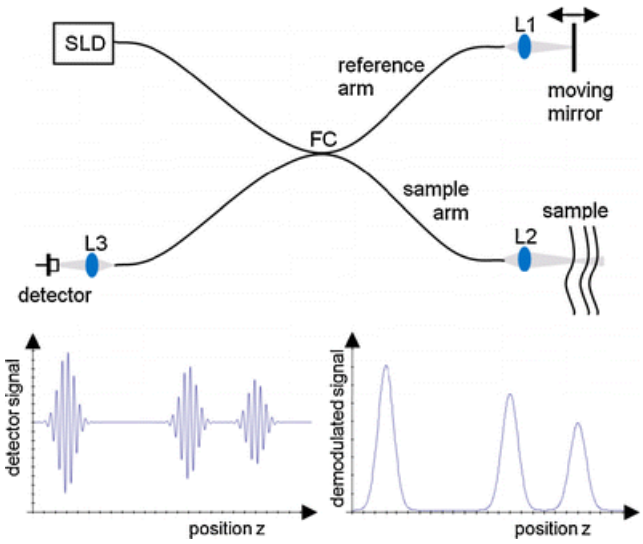

Figure C.1. Schematic diagram of a TD-OCT system. The fiber coupler (FC) splits light from the SLD into the reference and sample arm. The beam is reflected from different structures of the sample, and in the reference arm the light is reflected by a moving mirror. At the detector the interfering light is focused. L1 – L3 are different objectives focusing the beam. At the corresponding time position a burst of signal at the detector is recorded from each reflection from the sample, which is demodulated. Reprinted with permission from Copyright Clearance Center: Springer, Analytical and Bioanalytical Chemistry, Optical Coherence Tomography in Biomedical Research, Walther, J., Gaertner, M., Cimalla, P., Burkhardt, A., Kirsten, L., Meissner, S., & Koch, E, 40(9), 2011. Copyright (2011).

A very high transverse resolution over the total depth in the specimen can be achieved as the focus position of the beam can simultaneously be adjusted with the length of the reference arm, this is a major advantage of TD-OCT systems. Although these systems are limited by the movement of the translation stage, meaning they are restricted in imaging speed (Kim, 2010).

Appendix D: Other cellular level imaging techniques

D.1 MRI/NMR

Magnetic Resonance Imaging (MRI) is based on the principals of NMR (Nuclear Magnetic Resonance) and can achieve high resolution, non-destructive imaging of the internal structure for complex biological materials. It has achieved general acceptance as a powerful diagnostic tool for the assessment of clinical conditions within the medical field (Clark *et al.,* 1997). Through the development of different contrast mechanisms to encode molecular dynamics and the ability to act in a non-destructive manner alternative approaches within the horticultural domain are prevalent in literature. Some of the first applications within horticulture included fruit maturation (Ishida *et al.,* 1989), water transport (Bottomley *et al.,* 1986; Brown *et al.,* 1986; MacFall *et al.,* 1991) and pathogen invasion (Goodman *et al.,* 1992). Subsequently, MRI has been used in blueberry fruit to show water and sugar distribution before and after freeze/thaw (Gamble, 1994), and for the visualisation of water distribution throughout storage (Paniagua *et al.,* 2013; Capitani *et al.,* 2014).

Proton (^{1}H) MRI has been predominantly used to determine mobility and spatial distribution of protonaecous specimens at microscopic resolution (Aguayo, 1986; Callaghan, 1993; Clark *et al.,* 1997). In blueberries there are numerous proton containing species that are capable of absorbing radio frequency (rf) and being stimulated in MRI. As seen in previous studies (Gamble, 1994; Paniagua *et al.,* 2013), the water distribution of blueberries has been imaged, as H atoms associated with water molecules are in a high abundance (commonly greater than 80% of fresh weight sample) and are selectively targeted for imaging (Clark *et al.,* 1997). Shorter imaging times are possible with a large signal to noise ratio, due to the high relative abundance of H atoms. It is possible to image other metabolites such as sugars and lipids where they are localised and occur in high concentrations. The corresponding H atoms make imaging of these metabolites possible, where changes in sugar concentration of freeze/thawed blueberries has been determined (Gamble, 1994).

Water and sugar distribution in single blueberries were recorded before and after freeze/thaw using MRI (Gamble, 1994). A slice selective spin echo pulse sequence, including a T_1 inversion recovery time period was used to obtain images, and allowed for the selection of either the water or sugar signal. Regions of tissue were distinguished based upon different proton relaxation rates. T_1 (spin lattice) and T_2 (spin-spin) relaxation are two time constants that are regularly used to define proton relaxation times (Abragam, 1961). Gamble, (1994)

describes T_1 as the mechanisms which the induced magnetisation decays to equilibrium within the applied field. Where T_2 are the mechanisms where magnetisation induced into the plane perpendicular to the applied field, loses coherence. Both T_1 and T_2 relaxation were used by Gamble, 1994 in blueberries as contrast agents to determine the effects of freezing on water and sugar distribution. Therefore allowing changes in blueberry tissue structure caused by freezing to be mapped.

Paniagua *et al.* (2013) successfully visualised the water distribution in 'Centurion' blueberries over a three week storage period in different air flow treatments (0, 15, 30, 60 mL min^{-1}). Water content was visualised as different greyscale values. Axial slice of proton density weighted images were taken simultaneously through the centre of each fruit. Paniagua *et al.* (2013) obtained final images from multiple scans using parameters established by Gamble, (1994). The degree of berry shrinkage was measured, where areas near to the epidermis, stem and calyx scars decreased in water content throughout storage. Showing the ability of MRI to be used to measure water loss during storage which has potential to be used by industry to predict fruit quality.

The water distribution of fresh blueberries and those left to wither was recorded by Capitani *et al.* (2014) using a portable NMR device. Portable devices are advantageous as they allow whole samples to be placed on the probe, although a low resolution image is often produced. Proton pulsed low-resolution NMR measures the relaxation parameters and amplitudes of NMR signals, detecting proton signal contributed by water molecules (Clark *et al.,* 1997). A similar method to Gamble, (1994) detected a water loss of 11 – 16% and 20 – 34% after three and six days of withering, respectively compared to fresh blueberries (Capitani *et al.,* 2014). Longer T_1 and T_2 relaxation rates measured in withered blueberries indicated potential modification of fruit texture as a result from the occurrence of water loss (Capitani *et al.,* 2014). Therefore, measurement of the water status of whole berries using NMR could be transferred to quality control applications within the supply chain to monitor freshness and shelf-life.

D.2 Hyperspectral imaging

Hyperspectral imaging has emerged as a powerful non-destructive imaging technique for horticultural products. This technique consists of the acquisitions of both spectral and spatial information simultaneously from an object (ElMasry and Sun, 2010). It is advantageous over conventional imaging as each hyperspectral image is represented by a spectral data cube or a hypercube (Geladi *et al.,* 2004; Nicolai *et al.,* 2006; Leiva-Valenzuela, Lu and Aguilera, 2013). Advantages in comparison to other conventional imaging techniques are outlined in

Table D.1 (Gowen *et al.,* 2007). A typical hyperspectral image consists of hundreds if not thousands of spectral or narrow-band images, with each pixel being associated with a spectrum that may include NIR and visible wavelengths (Leiva-Valenzuela, Lu and Aguilera, 2014). Hyperspectral imaging can be implemented in reflectance, interactance and transmittance modes. Blueberry bruising (Jiang, Li and Takeda, 2016), internal quality (Leiva-Valenzuela, Lu and Aguilera, 2014), maturity stage (Yang, Lee and Gader, 2014),) firmness and soluble solids content (Leiva-Valenzuela, Lu and Aguilera, 2013) has been observed using hyperspectral imaging.

Table D.1. Advantages of hyperspectral imaging (HSI) in comparison to RGB imaging, multispectral imaging (MSI) and NIR spectroscopy (NIRS). Reprinted from Trends in Food Science & Technology, 18(12), Hyperspectral imaging- an emerging process analytical tool for food quality and safety control., Page 591., Copyright (2007), with permission from Elsevier.

Feature	RGB Imaging	MSI	NIRS	HSI
Spatial information	✔	✔		✔
Spectral information		Limited	✔	✔
Sensitivity to minor structures		Limited		✔
Multiple component information	Limited	Limited	✔	✔

Also known as chemical or spectroscopic imaging (Gowen *et al.,* 2007), each pixel in hyperspectral imaging contains the spectrum of that specific position of the target specimen. Each hypercube contains 3D blocks of data, allowing for the visualisation of biochemical components, separated into specific areas of the image where regions of the sample which have similar spectral properties have similar chemical compositions (Gowen *et al.,* 2007). Therefore image processing is a critical step in hyperspectral imaging applications. Techniques such as filtering, correction and segmentation to extract specific regions of interest are used in image processing (Leiva-Valenzuela, Lu and Aguilera, 2014). One of the most common methods used to build models with latent variables reoriented along directions of maximal covariance between the response vector and the spectral matric is partial least squares (PLS) regression (Nicolai *et al.,* 2007; Leiva-Valenzuela, Lu and Aguilera, 2014). PLS avoids overtraining issues that are commonly observed with non-linear models, as well as allowing for simpler interpretation and comparison of results compared to non-linear processing techniques (Leiva-Valenzuela, Lu and Aguilera, 2014).

The firmness and soluble solids content of blueberries was predicted in the visible and short wave near-infrared region of 500 – 1000 nm using a pushbroom hyperspectral imaging system (Leiva-Valenzuela, Lu and Aguilera, 2014). Calibration models using PLS were developed to

predict these parameters. Fruit orientation (stem and calyx end) was found to have an insignificant effect on firmness and SSC predictions. Further analysis showed that blueberries could be sorted into two firmness classes, demonstrating the feasibility of this technology for sorting blueberries based on quality (Leiva-Valenzuela, Lu and Aguilera, 2014). Furthermore the internal quality of blueberries was predicted using hyperspectral imaging, where reflectance and transmittance images in the spectral region of 400 – 100 nm were gathered. PLS calibration models and interval partial least squares (iPLS) regression with intervals of nine different wavelengths was used to reduce spectral depth. Reflectance modes gave better results than transmission, demonstrating it is possible to predict the internal quality of blueberries using a few selected wavelengths instead of whole spectral information.

D.3 Electron microscopy

Structure and function relationships associated with blueberry texture and quality have previously been imaged using electron microscopy (Allan-Wojtas *et al.,* 1999; Allan-Wojtas *et al.,* 2001; Fava, Alzamore and Castro, 2006). Electron microscopes use a beam of highly energetic electrons to characterise objects on a fine scale (Stefanaki, 2008). Electron microscopes work similarly to conventional light microscopes, however instead of focusing a beam of light, they use a focused beam of electrons to image the specimen. An electron stream is formed in a vacuum by electron guns and is accelerated towards the specimen. Metal apertures and magnetic lens focus the electron stream into a thin monochromatic beam which irradiates the specimen. Interactions occur inside the irradiated specimen which affects the electron beam, these interactions are detected and are effectively transformed into an image. Electron microscopy can yield information about the surface of samples, composition, morphology and crystallographic information (Stefanaki, 2008).

Scanning electron microscopy (SEM) produces images of a sample by scanning the surface with a focused beam of electrons (Goldstein *et al.,* 2017). This imaging technique has been used to visualise fresh berry interior where the epidermis was pulled away from the surface of the berry and visualised (Allan-Wojtas *et al.,* 2001). Large voids were seen in the centre of berries resulting from tissue shrinking (Allan-Wojtas *et al.,* 2001). Cryo-SEM is a similar imaging method, where sample components including water are stabilised physically by freezing *in situ.* Frozen blueberries held in storage for 5 months were visualised by Allan-Wojtas *et al.* (1999), where berries were fractured both longitudinally and cross sectionally in a -40 ºC freezer, then coated with gold/palladium and viewed using SEM. Indicating microstructural changes observed in frozen blueberries are characteristic of their temperature history. Cryo-SEM has also been used to show endocarp stone cells of blueberries stored in

air (Allan-Wojtas *et al.,* 2001). Therefore cryo-SEM can be a valuable tool in assessing frozen blueberry quality.

Transmission electron microscopy (TEM) is an imaging technique where a beam of electrons is transmitted through a specimen to form an image (Williams and Carter, 1996). The structural changes associated with blanching, freezing and thawing of blueberry fruit reported by Fava, Alzamora and Castro (2006) were observed using TEM. In fresh blueberries the visualisation of the outer tangential epidermal wall showed epicuticular waxes as discontinuous, and thin. The cuticle exhibited an electron dense outer zone and an electron dense inner zone, which was well defined. In addition, this research showed micropores in the upper zone of contact epidermal cells, and electron dense areas which suggested the presence of pectin (Fava, Alzamore and Castro, 2006). TEM can yield information about the internal quality of blueberries on a very fine scale and has had success measuring the quality of blueberries within a research environment.

Appendix E: Future work – MYB transcription factors

The identification of MYB transcription factors responsible for texture characteristics in blueberries after harvest needs to be investigated. Plant MYB transcription factors have diverse roles and are implicated during development, metabolite synthesis and hormone signalling (Allan and Espley, 2018). New research is revealing MYBs control the texture traits of fresh food products (Allan and Espley, 2018; Hen-Avivi *et al.,* 2014; Lashbrooke *et al.,* 2015; Legay *et al.,* 2016; Machemer *et al.,* 2011). Tomato cell expansion is controlled by several MYB related genes functioning as a complex (Machemer *et al.,* 2011), which influences fruit texture. Cuticle biosynthesis appears to be controlled by MYBs, where in tobacco the expression of *MYB93* induces the synthesis and deposition of suberin, and when expressed in apple suberized skin is observed (Legay *et al.,* 2016). Therefore identification of MYB transcription factors in blueberries responsible for texture parameters still needs to be determined. If MYB transcription factors are identified in blueberries, they could be regulated to preserve texture characteristics along the postharvest supply chain