Understanding the storage potential of two blueberry cultivars grown in New Zealand

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Abstract

Novel blueberry cultivars such as Eureka (EU) and First Blush (FB) are usually picked, packed, handled, coolstored and commercially sold in the same punnet. However, these cultivars possess different fruit physiological and morphological attributes and consequently may behave differently as influenced by postharvest storage conditions. Two independent experiments were designed to provide a better understanding of the modes of failure (water loss, softening, and rots) as influenced by storage conditions. It is expected that these investigations will facilitate the blueberry industry to decide if these cultivars should be picked, packed and handled differently in the commercial supply chain.

Experiment 1 was setup with four storage temperatures (0.5°C, 1.5°C, 2.5°C, and 3.5°C) as treatments using EU and FB cultivars. Response variables of weight loss (WL), firmness and rots were monitored at weekly intervals over 28 days. WL increased over time with highest WL (3.8-6.1%) at 3.5°C and the lowest WL (1.8-4.3%) at 0.5°C. Cultivar differences were observed with higher WL in the EU cultivar than the FB cultivar. Firmness decreased in both cultivars over time when stored at 2.5-3.5°C. When compared to at-harvest firmness, values after 28 days in storage did not decrease significantly at 0.5-1.5°C. Rot incidence was numerically higher at 3.5°C than 0.5-1.5°C for both cultivars, but could not be statistically verified.

Experiment 2 investigated the effect of storage extension technologies, such as, modified atmosphere packaging (MAP) and sulphur dioxide (SO_2) pads on both cultivars and WL, firmness and rots monitored at weekly intervals when stored at 2°C over 42 days. Blueberries stored in MAP+SO_2 showed sevenfold less WL (1.5%) than control fruit (6.9-10.9%). In addition, MAP+SO_2 treatment had significantly less rots than the control. Cultivar differences were also observed with higher number of rots in the EU cultivar. However, MAP+SO_2 treatment caused SO_2 bleaching to both cultivars observed at all storage timings and hence the use of SO_2 pads is not recommended in these cultivars.

Commercially, the blueberry punnets are packed with approximately 1-2% extra weight at-packing to account for the WL during storage and handling. Results from these experiments demonstrate that both cultivars should be packed separately due to propensity of the EU cultivar to lose more moisture affecting the quantity of extra weight.

The use of storage extension technologies (MAP and SO_2) did not show a significant commercial benefit. Future studies should focus on comparing the efficacy of using MAP vs a standard polyliner and/or creating SO_2 pads with a slower release rate.

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Glossary

RH	Relative humidity (%)
MAP	Modified atmosphere packaging
FB	First Blush
EU	Eureka
SO_2	Sulphur dioxide
SE	Standard error of the mean
SSC	Soluble solids concentration
TA	Titrable acidity
TSS	Total soluble solids
WL	Weight loss

Chapter 1 Introduction

1.1 General Introduction

In recent years, blueberries (*Vaccinium* sp.) have gained significant importance due to its health benefits such as higher antioxidants and vitamins. The New Zealand exports of blueberries has increased from $6.8M in 2000 to $44.4M in 2020 (FreshFacts, 2020).

Blueberries require temperate climate, and acidic soil conditions both of which are ideally present in several parts of New Zealand. Highbush and Rabbiteye are the two main types of blueberries grown in New Zealand. Blueberries are a short storage-life product and need to be carefully managed after harvest. A better understanding of the physiology of the available cultivars is important to optimise the coolchain to guarantee optimal marketability and consumer satisfaction.

Different cultivars can have varied physiology and consequently differ in rates of growth and development, maturity, potentially moisture loss properties, storage requirements and storage potential (Allan-Wojtas et al., 2001; Forney et al., 1998). This implies that a combined harvest across the cultivars available may impact on fruit performance and storage, and that if these differences between cultivars are large, then alternative strategies to harness these differences may need to be designed and employed.

Compared to conventional cultivars, novel blueberry cultivars such as Eureka (EU) and First Blush (FB), are bigger, high quality, and flavourful that are crisp, firm and round. These improvised cultivars, achieved through cross pollination, have become available to growers. For these cultivars, there is a significant knowledge gap in terms of their physiology (that governs most of the on-orchard agronomic practices such as nutrient requirements, chill hours, TSS accumulation). Information on the differences in storability occurring due to skin characteristics, stem scar, resistance to rot pathogens, is also not available for these cultivars.

The current marketing approach has all cultivars being picked and packed together and commercially sold as one product offering. Once more pre- and post-harvest information becomes available, there is a possibility that the cultivars may need to be picked at different timings and/or packed separately.

Two experiments were conducted with a primary focus on moisture loss, storage defects, and firmness loss as the key fruit quality parameters during storage.

1.2 Objectives

- Assess the effect of four storage temperatures on moisture loss and firmness during storage (Chapter 3).
- Assessing the effect of packaging technologies (MAP and SO_2 pads) alongside standard packaging in standard storage conditions on moisture loss and firmness during storage (Chapter 4).

It is expected that these investigations will help the blueberry industry to decide if these cultivars should be handled and packed differently to attain more commercial benefits.

Chapter 2 Literature Review

2.1 Introduction

Most traditional blueberry cultivars (e.g., Duke, Brigitta) are deciduous in nature known as northern highbush (*Vaccinium corymbosum*) that require between 800-1200 chilling hours, but more recent cultivars are mainly interspecific hybrids that require lower (250-550) chilling hours (e.g., Opi, Eureka, First Blush). Consequently, these low chilling requirement cultivars generally remain evergreen most time of the year in growing conditions such as the Waikato and Hawkes Bay regions of New Zealand (Retamales and Hancock, 2018).

New Zealand blueberry production ranks in the top 10 countries worldwide, however, it faces accelerating competition from other blueberry growing countries such as Peru and Chile contending for the international markets of Europe, North America, and China. Because of the large distance to key international markets, New Zealand grown blueberries are needed to combat the major points of postharvest quality failure caused by moisture loss and rots. As a consequence, New Zealand has invested significantly in cultivar breeding programs in the last decade which is well recognised worldwide.

The main focus of the breeding traits has been to develop cultivars that have lower chilling requirement, and are more resistant to frosts and thrive in soils with higher pH. Factors such as early bloom and shorter ripening intervals can expand the harvest season and cater to offseason overseas markets. In terms of berry development and agronomic traits, firmer, long storing fruit, short-heighted spread-out bush architecture, easy detachment of mature berries vs. immature berries, thicker bloom levels, loose fruit clusters, and a smaller stem scar, have been identified as the key breeding goals for new blueberry cultivars.

In recent years, blueberries have gained significant attention due to its health benefits such as higher antioxidants and vitamins such as improved brain activity and memory (Bowtell et al., 2017; Whyte et al., 2016), reduced risk cardiovascular diseases, diabetes, and neurological decline (Manganaris et al., 2014) and improved muscle recovery (McLeay et al., 2012). These health benefits have been identified by the consumers worldwide that has driven global demand for this superfood. In addition, consumers prefer bigger, high tasting, fresh, crisp berries that has a long shelf life.

2.2 Production in New Zealand

New Zealand horticultural produce exports in 2021 were $6.68 billion to 127 countries (FreshFacts, 2021). The New Zealand exports of blueberries (mainly to Australia) has increased from $6.8M in 2000 to $44.4M in 2020 (FreshFacts, 2020) registering an increase of 275% in revenue. Currently the New Zealand blueberry industry spans across 650 ha in production (with 750 ha of planted area), out of which 27% is grown under protected structures (i.e. tunnel houses) and 23% in hydroponics (Brazelton et al., 2022). The total production in 2022 across New Zealand was 9400 metric tons with an average yield of 14,462 kg/ha gaining eighth rank among top 10 highbush cultivar yields by country globally (Brazelton et al., 2022). The blueberry industry is a relatively smaller contributor (0.6%) to

the overall revenues of $6.7 billion of the total horticultural production that mainly comprises of kiwifruit, apples, and wine.

New Zealand is now approaching a 52-week supply scenario for its blueberry industry (although with a dip in June-July). Although the global market for New Zealand blueberry is remarkably prevalent for its superior quality as well as distinctive taste, its local demand in the market is still lagging and gridlocked.

2.3 Agronomic requirements for growing blueberries

The suitability of growing different cultivars of blueberries in various growing regions is dependent on its chilling requirement and winter cold hardiness (Retamales and Hancock, 2018). Blueberries are woody perennials and require well-drained, acid soils (4.2-5.5 pH), low cation exchange capacity and low base saturation. The growing medium requires optimally irrigated to keep moist. Most growers use automated fertigation systems to tend to these plant requirements, but foliar nutrition is not uncommon. The lower plant height and the higher density of the bush is one of the most striking differences of the modern-day cultivars.

Most traditional blueberry cultivars are deciduous but modern cultivars require lower chilling and hence remain evergreen most times of the year. The root system is generally shallow (ranging from 30-60 cm), do not have root hairs and are inhabited by endotrophic mycorrhiza (Jacobs et al., 1982). Over 80% of the root biomass (dry weight basis) is typically found in the top 36 cm (Retamales and Hancock, 2018).

2.3.1 Fruit development

The blueberry fruit is a ‘true’ berry with numerous seeds and the seed numbers govern the final fruit size (Retamales and Hancock, 2018). Blueberry fruit growth, in general, follows a double-sigmoidal growth curve (Darnell, 2006) and takes 42 to 135 days after full bloom to fully develop, depending on species and cultivar. This happens over three distinct phases (Figure 2.1). Phase I consists of cell division (~25-30 days), endosperm development (~20 days) and increase in dry matter. Seed development occurs in phase II with nominal gain in fruit size. Phase III consists of rapid cell expansion causing a significant increase in fruit size (over ~30 days) and fruit ripening.

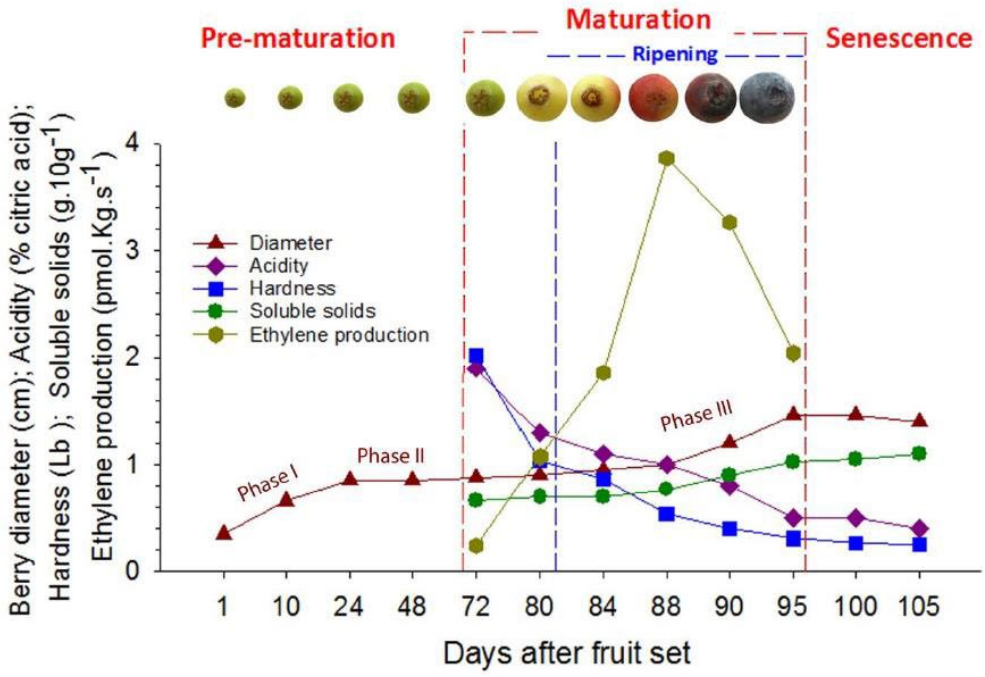

Figure 2.1. Visual appearance, berry diameter, hardness, total soluble solids, acidity and ethylene production during blueberry development (reproduced with permission from the author, Sebastian Rivera).

During fruit ripening, anthocyanin accumulation occurs, leading to a colour change and an increase in sugars (fructose and sucrose) and the accumulation of volatiles (Du et al., 2011); the latter governing the flavour profile of the berries that is considered the most important quality attribute for consumers (Saftner et al., 2008). Sugar accumulation stops when berries are detached during harvest.

Anthocyanins located in the epidermal and hypodermal cells, accumulate during ripening thereby governing skin colouration. Anthocyanin accumulation starts from the calyx end and increase towards the stem end of the berries (Chung et al., 2016). The skin colour changes from green to pink, then to light blue to 100% blue – this last stage is targeted during manual picking; however, it remains the subjective decision of the picker which may have a negative impact in overall quality and storability in the case of untrained pickers.

The blue colouration is also contributed by the development of epicuticular waxes, i.e., the 'bloom'. The bloom has waxes deposited as rodlets and upright platelets that scatters incident light (Sapers et al., 1984), providing a visually appealing berry, highly accepted by the consumer.

The fruit firmness during ripening decreases rapidly especially between the green to blue development stage (Figure 2.1). The firmness changes are associated with solubilisation of arabinose from pectin and hemicellulose depolymerisation happening during the green to blue ripening stages. Solubilisation of pectin, however, occurs mainly during the green stage until 75% blue stage (Figure 2.1). The integrity of the cell wall structure is governed by degrading enzymes and continue to be directly affected by hemicellulose and pectin

depolymerisation, consequently decreasing the texture and mechanical resistance of cells (Vicente et al., 2007).

During ripening, a simultaneous decrease in organic acids also occurs, which provides the balanced sweet flavour (i.e., the TSS: acid ratio). During ripening, softening of fruit tissue (i.e., decrease in berry firmness), occurs due to depolymerisation and solubilisation of cell wall components, pectin, cellulose and hemicelluloses (Darnell, 2006; Vicente et al., 2007). During the ripening process, a typical peak in respiration (initiated by a climacteric ethylene burst) is also observed, just prior to the start of blue colouration (between green and pink-red stage) in highbush cultivars (Farneti et al., 2022; Wang et al., 2022). A generalised role of ethylene in blueberry ripening and storability is not attainable because of the high variability between genotypes (Farneti et al., 2022).

The chemical composition of a ripe blueberry fruit ranges from 83-84% water, 0.6-0.7% protein, 0.4-0.5% fat, 3.0-3.5% dietary fibre and 9.7-15.3% carbohydrate (Hancock et al., 2003; Hancock et al., 2007). The total sugars in blueberries can account for >10% of the fresh weight, with glucose, galactose and fructose representing approximately 2.4%. These sugars co-exist as moieties with nearly five different types of anthocyanins (Michalska and Łysiak, 2015). The chemical composition can vary significantly due to cultivars, agronomic practices and postharvest storage conditions.

2.4 Blueberry cultivars in New Zealand

Blueberries are classified into three major commercial types according to their stature:

- Rabbiteye blueberry, *Vaccinium virgatum* is the tallest (1.8-4.0 m);
- Highbush blueberry, *V. corymbosum*, is intermediate size, and the most common; it includes southern highbush and northern highbush (2.0-3.5 m); and
- Lowbush blueberry, *V. angustifolium*, the smallest in size (0.10-0.15 m).

In 2021, the Crown Research Institute, Plant and Food Research, released 11 cultivars to New Zealand growers - three from the organisation's own breeding programme and eight licensed from Fall Creek Farm and Nursery in the USA (PFR, 2021). Among these 11 cultivars, three are southern highbush, seven are northern highbush and one is rabbiteye. The varieties produce large fruit with good flavour, with a range of early, mid- and late-maturity cultivars. Other companies such as BerryCo, Gourmet Blueberries, Miro Berries, and Darling Group are also involved in breeding programs with various organisations around the world and import their 'own cultivars' with most desirable characteristics, and often grown under licence arrangements.

In New Zealand, blueberries are mainly grown in the Waikato region in the early years due to its acidic soils ideal for plant growth, with production now also spanning across Hawkes Bay and the Bay of Plenty (FreshFacts, 2020). These regions experience a temperate climate with mild to moderate winters, higher sunshine hours (ideal for TSS accumulation and colour development of the berries), and regular rainfall. For instance, the Waikato region registered 1123 mm rainfall, 2059 sunshine hours and 42.2 frost days in 2020 while the Bay of Plenty region registered 1220 mm rainfall, 2348 sunshine hours and 10 frost days. These regions,

however, are also prone to high rainfall and severe frost events, and hence most blueberry farms have established modern tunnel houses with potted plants growing in inert media and equipped with fertigation.

2.4.1 Highbush cultivars

These blueberries originate in north-eastern regions of the United States and consist of majority of the early-planted cultivars in New Zealand. Highbush cultivars are early-maturing, and with the addition of New Zealand-bred cultivars, these can start fruiting from mid-November (southern highbush) and continue through to mid-February (northern highbush), ensuring an expanded supply season.

Cross-pollination of highbush berries require a mix of cultivars. Southern highbush cultivars require a moderate-to-low (150-700 h) amount of winter chilling, and northern highbush cultivars require a high winter chilling (800-1200 h). Highbush cultivars are more suited for the fresh produce market.

Two modern southern highbush cultivars 'Eureka' and 'First Blush' were used in this project offered by BerryCo; a Bay of Plenty-based berryfruit company, that has imported a range of cultivars bred by Mountain Blue Orchards, Australia. A 100-fruit sample of each cultivar was collected in November 2021 from Yieldia packhouse (Te Puke, New Zealand) to characterise key attributes of commercially picked and packed berries (Table 2.1).

'Eureka' is an early to mid-season, evergreen cultivar with a chilling requirement of 200–250 h. The bush is round, upright and high yielding (Figure 2.2). The fruit are jumbo-sized (~22 mm diameter, ~3 grams), spheroid-shaped berries with crisp flesh and best balance of sweet and 'tangy' (Table 2.1). Berries grow in loose clusters, and once ripe, berries are easier to pick than First Blush (personal observations). It is not very self-fertile and needs a polliniser (Retamales and Hancock, 2018).

'First Blush' is also an early to mid-season, evergreen, upright, vigorous, denser bush with a chilling requirement of about 250 h. Its berries are jumbo-sized (~21 mm diameter, ~3 grams; Table 2.1), oblate spheroid (flatter compared to Eureka) berries, relatively less flavourous ('bland') than Eureka and higher bloom levels (personal observations). Berries occur in tight clusters making it difficult to pick the ripe berries amongst the less-ripe berries (Figure 2.3).

Cultivar	**Weight** (g)	**Diameter** (mm)	**Firmness** (g/mm)	**TSS** (%)	**TA** (%)
Eureka	2.9 ± 0.2	22.4 ± 0.3	228.0 ± 3.3	11.8 ± 0.4	0.91 ± 0.3
First Blush	2.8 ± 0.3	21.3 ± 0.3	210.9 ± 3.2	11.1 ± 0.4	0.90 ± 0.2

Table 2.1. Key maturity attributes of the two cultivars used in this study (n = 100 for weight, diameter and firmness and n = 10 for TSS and TA). Data shown are mean ± SE.

Figure 2.2. ‘Eureka blueberry’. Potted blueberry bush (left); loose clusters (right).

Figure 2.3. ‘First Blush blueberry’. Potted blueberry bush (left); tight clusters (right).

2.4.2 Rabbiteye cultivars

These cultivars are mainly grown in the south-eastern regions of the United States. The rabbiteye bush differs in several ways from the highbush: extremely vigorous, tall, upright stature, late-maturing with relatively higher yields compared to highbush cultivars. Rabbiteye cultivars contribute to the majority of late season fruit, starting production in early January and continuing until mid-April. Rabbiteye cultivars require a moderate amount of chilling (400–700 h). Some of the cultivar released from the Plant and Food Research blueberry breeding programme (such as Rahi, Nui) also require moderate to lower chilling.

Rabbiteye production in New Zealand has expanded in recent years, mainly due to its late maturity (hence, filling the market demand), but also because it can be grown across more regions and a wider temperature range from February to early April. Rabbiteye plants require cross-pollination, so two or more cultivars are required.

Compared to the highbush cultivars, rabbiteye berries are smaller in size, with higher seed numbers (hence higher dry matter and fibre content), a thicker epidermis and relatively larger stem scars. Larger stem scars make these cultivars more prone to WL and faster softening rates after harvest (Makus and Morris, 1993). Hence, rabbiteye cultivars are more suited for the processed food industry.

2.5 Blueberry modes of failure and postharvest storage

Firmer, crisp berries are one of the primary drivers of consumer acceptance and hence, soft fruit arriving to the customer is an indication of softening occurring during cold storage and shipment. Blueberries are climacteric fruit that softens during ripening. During fruit softening, the decreasing firmness is associated with the steady reduction in total water-soluble pectin, increased enzymatic activities and the degradation of middle lamella structures (Chen et al., 2015; Giongo et al., 2013). Hemicellulose depolymerisation and arabinose loss govern the cell wall modifications (Vicente et al., 2007). The mechanisms leading to blueberry softening are not fully understood, however, a recent review (Moggia et al., 2022b; Rivera et al., 2022a) showed that the cultivar differences (including skin characteristics, size of the stem scar, presence of stone cells), berry maturity at harvest, and postharvest storage conditions were the most crucial governing factors.

Moisture loss (i.e., weight loss, WL) is a physical process that coincides with berry softening dynamics that affects visual appearance (shrivel) and decreases firmness retention. WL of 5 to 8% has been shown to cause excessive shrivelling affecting the texture, taste and consumer acceptability consequently affecting its saleability (Forney et al., 1998). In addition, even a WL higher than 2% may incur a financial penalty due to decrease in saleable weight (Wills et al., 2007). The harvesting technique (manual picking vs mechanical harvesting) can also affect WL values. For instance, Wills et al. (2007) reported that rabbiteye cultivar harvested mechanically had double the rate of WL than manual picking when stored at 3°C.

Softening and bruising are among the most common defects causing shipment rejections (Moggia et al., 2022a). Bruising and loss of bloom levels, especially during manual picking is

one of the main modes of failure that affects both storability and consumer acceptance. If the damage caused by mechanical harvesting is significant, then the affected berries can become prone to rots and loss firmness rapidly (Moggia et al., 2017b).

Rots are a significant factor in consumer acceptance. Rots can occur due to poor temperature management, mechanical damage due to poor picking practices, preharvest management, the cultivar's resistance to rot pathogens, and poor hygiene, that can cause the transfer of inoculum (Ayala-Zavala et al., 2008; Hosking, 2023).

2.6 Factors affecting moisture loss, firmness and rots

Cultivar, cuticle characteristics, maturity stage, and the use of a moisture barrier (in the form of an artificial intervention), are the key factors governing moisture loss (Moggia et al., 2017a). Depending on the cultivar, the berries' stem scar area, stomata/lenticels, the calyx, and the bloom characteristics (type, thickness) [(Lara et al., 2014; Martin and Rose, 2014)], are the main factors affecting moisture loss. The stem scar is the main pathway for moisture loss during postharvest handling and storage (Albrigo et al., 1980; Moggia et al., 2017a). While the stem scar is an important pathway to maintain internal turgor by losing slight moisture, its diameter is positively correlated with WL (Perkins-Veazie, 2016). WL has been shown to affect the berry texture due to loss in turgor, cell wall modifications and other microstructural changes in the outer cell layers of the fruit, thereby decreasing the firmness as a consequence (Allan-Wojtas et al., 2001).

A recent study of Hosking (2023) performed in New Zealand conditions, identified *Botrytis cinerea* (botrytis rot/grey mould), *Colletotrichum acutatum* species complex (anthracnose fruit rot/ripe rot), and *Pestalotiopsis* spp. as the major pathogen causing rots in blueberries. Cappellini and Ceponis (1977); Cappellini et al. (1972) reported that pathogens such as *Alternaria* spp. and *Colletotrichum* spp. are more prevalent when storage temperatures exceed 2°C. Hosking (2023) reported that low temperature management is the most important factor governing postharvest rots because of its significant effect in reducing the berry respiration rates. Because respiration rate are governed by enzymatic reactions, slowing its rate reduces the rate of cell wall degradation (Nunes et al., 2004). For instance, Paniagua et al. (2014) observed a significantly higher incidence of rots at 4°C than 0°C after five weeks of storage. Lower temperatures also reduce the rate of pathogen metabolism and hence decrease the incidence of rots (Abdelfattah et al., 2020).

Humidity is defined as the concentration of water vapour present in air. Lower RH at higher temperatures can drive the evaporation of water from within the fruit into the surrounding atmosphere via the stem scar and cuticle, quickly causing weight loss and shrivelling. Maintaining high relative humidity (RH) during cold storage is crucial in preserving the quality of stored fresh produce – by reducing moisture loss and maintaining higher internal turgor. Forney (2009) reported that higher RH can play a key role in the increase of rots in blueberries. This increase is due to higher RH creating optimal conditions for conidia germination and infection (Eckert and Sommer, 1967). Improper temperature management creating frequent temperature fluctuations can cause condensation on the berries in storage

(Paull, 1999) thus creating optimal conditions for pathogens such as *Botrytis* sp. and *Colletotrichum* sp. The rot pathogens generally remain dormant during the fruit growing stage, however, become active during fruit ripening when the berries have reduced chemical and mechanical resistance (Snowdon, 2006).

In addition to the above factors, environmental conditions during the key growth stages (see section 2.3) of blueberries can also negatively affect fruit metabolism, resulting in smaller and softer fruit, with waxes more prone to be removed by rubbing of the leaves or by postharvest handling and storage (Moggia and Lobos, 2023).

2.6.1 Cultivar differences

The stem scar represents approximately 1% of the berry's surface, but can be responsible for 40–60% of the total moisture loss of the fruit during postharvest handling and storage (Moggia et al., 2017a). The stem scar allows the evaporation of water that the berry regulates to maintain the internal turgor pressure, however, is dependent on external conditions of exposure. The diameter of the stem scar varies between cultivars and species ranging from 1.3-2.2 mm in highbush cultivars and 0.7-1.2 mm in rabbiteye cultivars, which can ultimately affect the extent of moisture loss (Moggia et al., 2017b).

Different cultivars can have varied quantities of stone cells i.e., sclereids. These cells are present in the mesocarp of the blueberry flesh with densities varying from 1.5-19.5 per μm^2 (Gough, 1983). The quantities of stone cells can have a role in berry firmness, but more work is required to confirm this.

Another pathway of moisture loss is the thickness of skin cuticle (Wills et al., 2007) and the proportion/type of epicuticular waxes forming the bloom layer (Sapers et al., 1984); both parameters can vary between different cultivars. The transpirational moisture loss via the cuticle is mainly controlled by this bloom layer. For instance, Albrigo et al. (1980) showed that blueberries with 43% more total waxes could reduce WL by 68% when placed at ambient temperatures for 48 h.

The bloom layer of blueberries is composed of triterpenoids (mainly oleanolic acid, ursolic acid, α- and β-amyrin), β-diketones and trace quantities of fatty acids, primary alcohols, alkanes, aldehydes, and esters (Yan and Castellarin, 2022). The triterpenoids can make up to 35-50% of the total waxes in the bloom layer (Moggia et al., 2016). The bloom is hydrophobic in nature and can impart partial impermeability to gases and water vapour. The composition and quantities of the bloom layer can vary due to the maturity stage, cultivar and storage conditions. Moggia et al. (2016) quantified the triterpenoid content of the southern highbush cultivars, 'Duke' and 'Brigitta'. They found that 'Brigitta' had higher α-amyrin and oleanolic acid and 2-4 times lower ursolic acid content than 'Duke' and indicated that these characteristics were directly related to water loss and softening rates. Yan and Castellarin (2022) compared the effect of the bloom layer and stem scar size of four highbush cultivars, 'Calypso', 'Draper', 'Duke', and 'Elliott', and found that 60% of the total water loss could be attributed to the bloom layer. They also found that the cuticular wax contents differed due to cultivars and increased by an average of 49.5% during the four-week postharvest storage period at 0.5°C and 95% RH.

Different cultivars also vary in their tolerance against pathogen attacks depending on the constitutive defences and induced defences (Hosking, 2023).

2.6.2 Storage temperature and humidity

As discussed above, firmness retention is directly related to the extent of moisture loss caused due to changes in internal turgor pressure, texture and temperature and RH during postharvest handling and storage. Studies have shown that blueberries can maintain acceptable quality for up to 2-4 weeks by storing them at 0°C and 90-95% RH (Kader, 2003; Perkins-Veazie, 2016) and fluctuation around these parameters generally have deleterious consequences on the storage life. For instance, Paniagua et al. (2013) conducted an experiment to demonstrate the effect of delayed cooling for prolonged intervals on rabbiteye blueberries. The berries were exposed to a grid of 2 delay times at 20°C and 3 delay times at 10°C. A 20 h delay at 10°C resulted in substantial loss of firmness and consequential WL and concluded that firmness loss occurs when moisture loss is higher than 8%.

An increase in blueberry firmness known as 'firming effect' has been observed in numerous studies, especially at lower levels of WL (0.2-4.0%) in combination with lower (<2°C) storage temperatures (Franklin, 2019). Miller et al. (1993) observed a firming effect in ripe blueberries when WL was lower than 1% after 3 weeks of storage at 1°C, while firmness decreased at 4-5% WL. Weight loss of 1-2% in highbush blueberries accounted form 50-80% of fruit firming after 3–9 weeks in storage at 3°C, whereas firmness decreased at WL values of 4-14% (Forney et al., 1998). Ballinger et al. (1978) demonstrated the detrimental effects of fresh blueberries stored above 10°C and concluded that blueberries destined to the fresh produce market should be stored at or near recommended temperature of 0-1°C.

Sanford et al. (1991) investigated the effect of various storage temperatures (0, 5, 10 and 20°C) for 14 days and found that temperatures exceeding 0°C resulted in deteriorating effect on the qualitative characteristics including reduced firmness and appearance as well as increased leakage due to epidermis breakage. They observed a progressive increase in WL from 5.3 to 17.1% for blueberries stored for 14 days at various temperatures ranging from 0-20°C. At storage temperatures of 4 and 21°C for 3 days, Tetteh et al. (2004) observed WL values of 4 and 9%, respectively, at a constant RH of 95%. Nunes et al. (2004) stored blueberries at five different storage temperatures and reported WL of ~2% at 0°C over a 14-day storage period.

In terms of ideal RH for storage, the most significant effect is on the pathogenicity of decay causing microorganisms present on the berries; generally higher rot incidence has been observed at >95-98% RH (Forney, 2009). Higher RH alongside storage temperatures and condensation can cause optimal conditions for conidia germination and infection (Ayala-Zavala et al., 2008; Eckert and Sommer, 1967). A study using 100% RH and 25°C storage for five days observed up to 29% decay incidence in their berries (Smith et al., 1996). Barrau et al. (2006) also found that rot incidence of six blueberries cultivars after 10 days at 25°C and 100% RH was 10.9-16.7% while the major causal organisms being Anthracnose fruit rot, Alternaria rot, Botrytis rot, and *Monilinia vaccinii-corymbosi* (mummy berry) amongst the cultivars investigated. Another aspect of higher RH is that it can favour pathogen infection due to the presence of surface water. Paull (1999) reported that surface water can form

because of condensation on fruit surfaces due to fluctuations in air temperature at higher RH. Therefore, storage at RH levels >95% is not recommended if the pathogen load is high because it creates ideal conditions for Botrytis infection (Rivera et al., 2013b).

2.7 Technologies to enhance storage potential

From the above sections, it is clear that blueberries are extremely sensitive to fluctuations in temperature and humidity during postharvest handling and storage. However, there are promising techniques that have been proven to significantly prolong storage period without compromising quality. These include but are not limited to techniques such as low-temperature storage (coolstorage), controlled atmosphere (CA) (Schotsmans et al., 2007), calcium dips (Hanson et al., 1993), UV irradiation (Perkins-Veazie et al., 2008), ozonation (Piechowiak et al., 2020), hot water (Fan et al., 2008), plant-originated antimicrobial agents (Wang et al., 2008; Wang et al., 2010), and edible coatings (Duan et al., 2011).

2.7.1 Controlled atmosphere

The CA approach involves modification of ambient gas concentrations (reduced O_2 availability and increased CO_2 accumulation) during cold storage, consequently lowering the fruit's respiratory metabolism rate, decrease pathogen respiration, and decrease ethylene production, thereby accomplishing extended storage life (Falagán et al., 2020; Yahia, 2009). The desired CA levels are attained by a static or dynamic method. In the static method, the horticultural produce to be stored, respires thereby generating higher CO_2 while desired levels are maintained by ventilation and scrubbing. In the dynamic method, CA is achieved by first flushing a hermetically-sealed, cold storage room with nitrogen gas (or liquid nitrogen) to attain the desired O_2 concentration (2-3%), and then injecting CO_2 using a gas generator to raise the CO_2 concentrations up to 12-15%. In the dynamic method, the optimum CA levels can also be maintained by external burners, gas separator systems and using hypobaric storage (Escobedo-Avellaneda and Welti-Chanes, 2016).

CO_2 affects the pathogens directly by acidification of the internal environment and while maintaining an O_2-deficit environment, inhibits fruit decay by reducing O_2 required for fungal spore germination, while fruit firmness and sensory quality are maintained (Beaudry, 1999).

Vicente et al. (2007) reported that CA has the most significant benefit in controlling rots albeit prolonging blueberry quality, however, with an optimal CO_2/O_2 ratio, quality benefits may also be achieved (Rodriguez and Zoffoli, 2016). CA benefits in terms of prolonging postharvest quality are minimal because blueberries are harvested at full maturity. At 100% blue stage, hemicellulose and pectin depolymerisation has already degraded the cell walls, and hence, suppression of ethylene production may no longer influence the postharvest softening of berries (DeLong et al., 2003). Ethylene production during ripening has been a topic of debate in previous studies.

Forney (2009) reported that the optimum combination of storage temperatures and CA can extend the storage life of blueberries by 2-3 times. Recommended CO_2 concentrations for

blueberries range from 10-12%, with higher levels causing negative effects on firmness, fruit splitting, flavour profile, flesh discolouration and titrable acidity (Forney et al., 2022; Harb and Streif, 2004). O_2 concentrations <2% can lead to fermentation and hypoxia (Retamales and Hancock, 2018). Rodriguez and Zoffoli (2016) reported lower firmness for blueberries stored at 1% O_2 compared to 15% O_2, in combination with equivalent CO_2 levels (5–7%).

Previous studies (Duarte et al., 2009; Schotsmans et al., 2007) did not show any effect on moisture loss due to the use of CA. Schotsmans et al. (2007) conducted an experiment to test the effect of CA (2.5% O_2, 15% CO_2) vs regular atmosphere, on two rabbiteye cultivars, 'Maru' and 'Centurion' stored at 1.5°C. The authors showed that CA could extend the storability of both cultivars by up to 42 days and a significantly lower rot incidence while regular atmosphere could store up to 28 days with much higher number of rots. However, a higher antioxidant activity and total phenolic content and firmer berries was observed in regular atmosphere storage.

It is important to note that the drastic changes to O_2 and CO_2 proportions can pose a high risk to the storability of the berries due to berries experiencing a 'metabolic shock'. In order to prove the concept of metabolic shock, Falagán et al. (2020) subjected 'Duke' blueberries to four different storage conditions; control (air storage), standard CA (immediate exposure to 5% O_2; 10% CO_2), gradual CA (GCA) reaching 5% O_2 and 10% CO_2 in 3 or 7 days stored for 28 days at 0°C. The authors found that the GCA method had 27% berries firmer than that at normal CA, reduced rot incidence, and maintained individual sugars and ascorbic acid contents.

Rivera et al. (2022b) investigated the effect of three CA combinations (4% O_2 and 5, 10, 20% CO_2) on the mechanical properties of Nui and Rahi cultivars stored for 42 days at 4.6°C and found that the 5% CO_2 concentration induced a beneficial effect on the mechanical properties than the 10% concentration.

Forney et al. (2022) investigated the effect of CA ranging from 10-20% CO_2 and 10% O_2 in five highbush cultivars stored at 1°C for 6 weeks and found that these levels were sufficient in inhibiting fruit decay. Exposure to higher CO_2 levels for prolonged periods caused fermentation that increased ethanol, acetaldehyde and decreased monoterpenoids, all of which resulted in off flavoured fruit, decreased firmness and flesh discoloration. The authors also found that the tolerance to CO_2 exposure was dependent on the cultivar and may also depend on the fruit maturity at harvest alongside growing environment.

2.7.2 Modified atmosphere packaging

MAP works on similar principles as used for CA; the main difference is that the fresh produce in question, reaches the respiratory equilibrium at a much slower rate in a MAP system while in CA, the desired levels are achieved relatively quickly, especially the O_2 levels achieved by flushing the storage room using nitrogen gas (Lee et al., 2016). The desired atmosphere in MAP is a combination of three aspects – fruit respiration consuming the majority of O_2 within the sealed environment (reduction in O_2 concentrations), consequently causing accumulation of CO_2, and thirdly, fruit transpiration increasing the vapour pressure while the laser micro-perforations of the MAP material allowing certain

permeability to the external environment. The efficacy of MAP can greatly be influenced by the cultivar, the quantity of fruit within the designed MAP (which affects the headspace, i.e., free volume), storage temperature; and the MAP material itself (permeability of the film, material's thickness) [(Koort et al., 2018; Paniagua et al., 2014)]. Paniagua et al. (2014) indicated that cultivar differences such as respiration rate and cuticular waxes can influence the diffusion of respiratory gases thereby affecting the internal gas concentration and its response to the exterior environment, such as in modified atmospheres.

Using MAP is specifically more advantageous than CA because it can maintain higher RH (>95%) during the whole supply chain and can reach the consumer in a desired size and packaging. CA requires sealed environment for its optimum use, hence can only be beneficial during storage and/or transportation. Conversely, using CA can especially be beneficial in controlling rots due to the possibility of maintaining higher (10-20%) CO_2 concentrations, however, MAP cannot maintain CO_2 concentrations higher than 6-8%.

Koort et al. (2018) investigated the effect of storing two blueberry cultivars (a lowbush and a half-highbush) in regular atmosphere, low-density polyethylene (LDPE) modified atmosphere bag and Xtend® modified atmosphere blueberry bag at 3°C for 6 weeks. The authors found that berries stored in regular atmosphere stored for 22 days while the MAP treatments could extend it up to 37 days. At the end of the trial period, they found that O_2 concentrations ranged between 13.0-14.0% while CO_2 concentrations ranged between 8.5-9.4% in the Xtend treatment. The Xtend treatment also maintained higher blueberry firmness over the storage period when compared to storage in regular atmosphere. The authors observed significant cultivar differences under Xtend treatment with respect to rot incidence with 7% rots in the half-highbush cultivar compared to 0.1% rots in the lowbush cultivar. The overall reduction in rots in the Xtend treatment was attributed to lower CO_2 accumulation that is responsible for reducing fungal rots.

Another study by Moggia et al. (2014) investigated the effect of MAP vs regular atmosphere storage of 'Brigitta' cultivar at 0°C for 30 and 45 days after storage. The authors found a lower proportion of dehydrated fruit in the MAP treatment (4-10%) compared to that of the control (20-30%) while also recording higher firmness in the MAP-stored berries. The authors did not report the concentrations of O_2 and CO_2 in the MAP bags.

Rodriguez and Zoffoli (2016) investigated the effect of different levels of CA (CO_2%/O_2%, 5/1, 7/15, 15/5) on 'Brigitta' highbush cultivar stored for 30-45 days at 0°C. CO_2 concentrations >8% and O_2 <2% caused fruit softening. The CA ratio of 7 % CO_2/15% O_2 and the regular atmosphere could maintain significantly higher firmness than the remaining CA ratios. Moreover, the CA ratio of 15 % CO_2/5% O_2 had significantly lower rots than the remaining CA ratios and control.

The above studies highlight the fact that the ideal CO_2/O_2 ratio either in CA or MAP is dependent on factors such as the cultivar's proneness to rots, storage potential and WL characteristics.

2.7.3 Sulphur dioxide

Sulphur dioxide (SO_2) is one of the techniques widely used on several fresh produce to prevent decay during storage due to its antimicrobial activity (Carter et al., 2015), anti-browning of the pericarp (in litchi, longan and date fruit) [(Kuijpers et al., 2012)] and can also act as an inhibitor of enzymatic and chemical oxidation (Kuijpers et al., 2013) and inactivate oxidation enzymes.

SO_2 applied during cold storage is well-known to be extremely effective in controlling Botrytis rots in grapes by killing the surface-borne conidia (Junior et al., 2019; Lichter et al., 2008; Palou et al., 2002; Zoffoli et al., 2008), however studies on the efficacy of SO_2 in blueberries are limited.

The SO_2 fumigation process can generally kill conidia and inactivate mycelia. SO_2 can also penetrate into the plant tissue and trigger a cascade of biological effects, including modulation of the antioxidant defence system, osmotic adjustment, cell wall modification, and synthesis of stress proteins, secondary metabolites, and plant hormones (Li et al., 2023). SO_2 pads emit SO_2 at a slower but constant rate causing an inhibitory effect on pathogens (Chen et al., 2020) compared to an eradicative effect caused by fumigation. Apart from controlling certain blueberry rot pathogens, Carter et al. (2015) found that SO_2 fumigation could also reduce and eradicate human pathogens such as *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* on table grapes under cold storage conditions.

The reaction of sulphite salts of sodium or potassium with moisture (arising from fruit respiration and the external storage environment) releases SO_2. Based on the fruit type, cultivar, size of the packaging enclosure, the sodium metabisulfite chemical is carefully embedded within the pads which ultimately reacts with the moisture, partly generated due to slight temperature changes and marginally released due to fruit respiration during storage (Li et al., 2023). There are different salts used as SO_2 source, while its use in food is a collective term for sulphite, hydrogen sulphite (bisulfite), and disulphite (metabisulfite and pyrosulfate) salts of sodium and potassium (Ruiz-Capillas and Jiménez-Colmenero, 2009). The application methods of SO_2 includes fumigation (Jia et al., 2021), controlled-release technology by SO_2-generating pads (Saito et al., 2020), and soaking in sulphur-containing salts solution (Shuai et al., 2018).

The effect of SO_2 fumigation (Rivera et al., 2013a) and use of SO_2 pads (Saito et al., 2020; Saito and Xiao, 2017) in blueberries have been tested in previous studies, and there are several types of MAP and SO_2 products commercially available. SO_2 is released from the pad as function of environmental moisture and hence excellent temperature management is required, otherwise excessive SO_2 is released causing bleaching of the berries.

Rodriguez and Zoffoli (2016) investigated the effect of a perforated bag and MAP fumigated with SO_2 in ‘Legacy’ cultivar stored for 30-45 days at 0°C and found that SO_2 fumigated fruit packed into a perforated bag was a better option than storing in MAP.

Saito and Xiao (2017) investigated the effect of SO_2 generating pads alone or in combination with MAP on ‘Emerald’ blueberry cultivar stored at 1°C for five weeks. The authors found

that the incidence of botrytis rots was significantly reduced due to SO_2 pads compared to the control; and it was further reduced when used in combination with MAP. However, 25% of the berries exhibited bleaching due to SO_2 injuries. The berries in the SO_2-alone and SO_2 with MAP treatments had a lower firmness than that of MAP-alone or the control.

Phytotoxicity is one of the factors that limit the use of SO_2. However, Rivera et al. (2013a) and Cantín et al. (2012) did not find any bleaching effect on the blueberries in their studies but berry softening was observed in both studies. An earlier study (Smilanick and Henson, 1992) found that the SO_2 toxicity against botrytis increased by 3.8-fold when increasing temperatures from 0°C to 20°C. SO_2 fumigation has been shown to kill the surface-borne conidia of botrytis in grapes. Removal of the pedicel during harvest is the natural infection point and Rivera et al. (2013a) found that SO_2 fumigation can reduce this infection.

It is clear that SO_2 applications can be a promising technique in extending blueberry storability by controlling the incidence of rots. However, its concentration, and exposure time, at a specific storage temperature, depends on the cultivar, maturity, and inherent differences in the sensitivity to SO_2.

2.8 Challenges of New Zealand blueberry industry

Blueberries inherently has a relatively short storage life (3-8 weeks) compared to that of other fruit such as kiwifruit and apples (~well over 20-25 weeks in storage). To maintain and possibly extend its storage given New Zealand's geographical distance from key markets, is one of the most significant challenges faced by the New Zealand blueberry industry. Additionally, the higher retail cost causes a deterring effect on the consumers' spending ability in comparison to other competitors such as Peru and Chile since blueberries are primarily channelled via air to Asia, incurring uneconomical transportation costs. Bezuidenhout et al. (2020) suggested that an implementation of supply chain integration can effectively overcome the challenges faced by the New Zealand blueberry industry.

This literature review has shown that although New Zealand has imported new cultivars with much higher quality and productivity, it is important to understand their physiology and if the storability of these cultivars are different, consequently different parameters for postharvest handling and storage may be required.

Chapter 3 Effect of storage temperatures on moisture loss and firmness

3.1 Introduction

Studies have shown that blueberries can be stored up to 2-4 weeks with an acceptable quality by storing the fruit at 0°C and 90-95% RH (Kader, 2003; Perkins-Veazie, 2016). Suggestions in these studies for a better coolchain include, forced air cooling to <10°C, followed by grading, then maintained in refrigeration at 0-3°C within an hour after harvest to remove field heat and extend storage life followed by coolstorage in the aforementioned conditions (Perkins-Veazie, 2016). Disruptions in the temperatures during postharvest handling and storage can cause excessive moisture loss, shrivelling, loss in firmness and increase in rots (Paniagua et al., 2013). Despite the importance of lower temperatures during storage, New Zealand's blueberry coolchain operates as – picking at ambient temperatures (20-25°C) within tunnel houses / greenhouses and stored on-site at 4°C within 30 min after picking (to remove field heat); transported (under controlled temperature conditions) to the packhouse within 12 h, precooled to 4°C, packed at <10°C, and coolstored at 2°C (Alan Mclean, per. comm.). It is therefore important to understand if further refinements to the current coolchain can be employed.

The mechanisms leading to blueberry softening are not fully understood, however, a review of the current firmness assessment techniques to date (Moggia et al., 2022b; Rivera et al., 2022a) indicate that the cultivar, maturity, calcium management, and postharvest storage conditions are the most crucial governing factors. Chapter 2 (literature review) of this thesis indicates that the main reasons for postharvest softening of blueberries is moisture loss during storage (Paniagua et al., 2013). Moisture loss, expressed as WL, has been shown to affect the berry texture due to loss in turgor, cell wall modifications and other microstructural changes in the outer cell layers of the fruit, thereby decreasing the firmness as a consequence (Allan-Wojtas et al., 2001).

Moisture loss also incurs a financial penalty due to decrease in saleable weight (Wills and Golding, 2016). In addition, WL of 5 to 8% has been shown to cause excessive shrivelling to the point the berries becomes unsaleable (Forney et al., 1998). The thickness of epicuticular waxes (i.e., the 'bloom') and the diameter of the stem scar could influence the rate of moisture loss from the fruit. As a comparison, the stem scar represents 1% of the berry's surface, however, is responsible for 40–60% of the total moisture loss of the fruit during postharvest handling and storage (Moggia et al., 2017a).

Temperature and humidity are the key drivers controlling moisture loss, both during the various blueberry growth stages and during post-harvest storage. Higher summer temperatures (>32°C) during the key growth stages (such as cell division and expansion) can affect the rate of respiration and ethylene production and exert negative effects on fruit metabolism, resulting in smaller and softer fruit, with the 'bloom' being more prone to removal by rubbing of the leaves or by handling during manual harvest (Mainland, 1989).

Temperatures variations during cool storage can affect the taste, texture, and chemical composition of harvested produce (Wills and Golding, 2016). A constant storage temperature and humidity generally results in a linear increase in moisture loss since it is a physically driven process (Nunes et al., 2004). Higher storage temperatures can drive higher moisture loss, which can be even higher when RH is <80-85%. Higher storage temperatures (>10°C) can also increase the respiration rate and ethylene production of the stored blueberries. For instance, Mitcham et al. (2011) reported a respiration rate of 0.02 CO_2 μmol kg^{-1} s^{-1} at 0°C that increased to 0.06 and 0.21 CO_2 μmol kg^{-1} s^{-1} at 10 and 20°C, respectively. Moisture loss has a direct relationship to blueberry firmness. NeSmith et al. (2005) investigated the effect of increasing storage temperatures (1, 12, 22, 32°C) on the firmness of six rabbiteye cultivars and found that firmness decreased progressively alongside WL. The authors could not separate the firmness change associated with ripening vs caused due to moisture loss. However, Forney et al. (1998) found non-significant firmness and WL differences when stored at 0°C and 3°C stored for 21 days.

A RH of 90-95% is ideal for minimising moisture loss during storage. However, previously reported studies have shown that temperature fluctuations of >10°C in the supply chain can cause condensation on the stored blueberries that can result in infection by postharvest pathogens (Bezuidenhout et al., 2020). Similarly, during cool storage, a temperature fluctuation as small as 0.5°C can result in condensation and rots (Paull, 1999). Moggia et al. (2017b) showed that a blueberry in a chamber kept at 20°C and 65% RH can experience up to ten times stronger vapour pressure deficit (~820 Pa) than the one kept at 0°C and 88% RH (~73 Pa) thus indicating that a higher deficit would cause more water loss from the fruit cells to the vacuoles. Consequently, the cuticle and the stem scar are the two points on the fruit surface for this proportion of moisture to escape and cause a loss in internal turgor (Moggia et al., 2017a; Yan and Castellarin, 2022).

It is clear that a coolchain that has consistent temperature with high RH from the field to the point of consumption, is the key to optimum berry quality (Forney, 2009; Ktenioudaki et al., 2021) – and if the coolchain is disrupted then softening and dehydration could account for 10–45% and 10–25%, respectively, of the total defects (Lobos et al., 2018).

3.1.1 Introduction to the cultivars

Both (southern highbush) blueberry cultivars ‘Eureka’ (EU) and ‘First Blush’ (FB) used in this study are considered ‘modern’ cultivars with bigger berry size and high TSS content. To differentiate key attributes of these cultivars from conventionally known cultivars, a 100-fruit sample of each cultivar was acquired from Yieldia packhouse, and tested for size and firmness (FirmTech II, BioWorks, Wamego, US); and TSS and TA (Brix-Acidity meter, Atago Instruments, Tokyo, Japan). Table 3.1 (has same data of Table 2.1) below shows the aforementioned maturity attributes and a comparison with other cultivars.

Cultivar	Type	Weight (g)	Diameter (mm)	Firmness (g/mm)	TSS (%)	TA (%)
Eureka (EU)	Southern highbush	2.9	22.4	228.0	11.8	0.91
First Blush (FB)	Southern highbush	2.8	21.3	210.9	11.1	0.90
Nui[A]	Northern highbush	NA	15.6	NA	10.8	1.4
Rahi[A]	Rabbiteye	NA	16.7	NA	11.1	0.62
Brigitta[B]	Northern highbush	2.5	NA	183.5	11.4	1.0
Legacy[B]	Northern highbush	2.5	NA	171.3	13.7	0.8

Table 3.1. Key maturity attributes of the two cultivars used in the current study. Letter A denotes data acquired from Rivera et al. (2021). Letter B denotes data acquired from Hancock et al. (2008).

It is clear that the attributes of these newer cultivars in this current study are different than the traditionally known cultivars and hence may differ in their storage potential. The following experiment was therefore conducted to investigate, the optimum storage temperature of the EU and FB cultivars.

3.2 Materials and methods

3.2.1 Experimental design

The trial was setup as a randomised block design with four storage temperatures as treatments for both cultivars. This trial was repeated in three experimental sets, each termed as a 'Batch' depicting 'MainPack' (November) and 'late MainPack' (December) season and were conducted in an identical fashion.

A punnet was considered a replicate. Fresh set of punnets were tested at each storage time on days 7, 14, 21, and 28 that were tested for WL, firmness and rots. At each storage time, all berries within a punnet per replicate were destructively analysed. Batch 1 consisted of four replicates in the form of four 'jumbo size' punnets. Batches 2 and 3, had six replicates as 'standard size' punnets that were tested at each storage time.

Dimensions of the 'jumbo' punnets were 185 mm × 110 mm × 30 mm (L × B × H) and had 12 ventilation holes (6.95 mm diameter). Dimensions of the 'standard' punnets were 125 mm × 100 mm × 35 mm (L × B × H) and had 22 ventilation holes (6.95 mm diameter).

An analysis of variance (ANOVA) was performed for WL and firmness data and treatment differences were tested using Tukey's test (95% at confidence interval) using the statistical software Minitab version 19.0 (Minitab, US). All data presented here are mean ± SE (n = 4 or 6). Each datapoint in Batch 1 denoted 320-berries and 450-berries in Batches 2 and 3.

3.2.2 Sample collection and transport

For Batch 1 (collected on 22 November 2021), 16.0 kg berries of each cultivar were acquired from Yieldia packhouse (Te Puke, New Zealand) in the form of commercially packed 200 g 'jumbo' punnets. Each of these punnets contained approximately 60-70 berries. For Batch 1, 4 punnets × 200 g × 5 storage timings × 4 treatments, equated to 16 kg berries of the EU cultivar and likewise for the FB cultivar.

For Batch 2 ('standard' sized 125 g punnets were collected on 1 December 2021) – 6 punnets × 125 g × 5 storage timings × 4 treatments, equated to 15 kg berries of the EU cultivar and 15

kg of the FB cultivar. Same quantity of berries was collected for Batch 3 on 9 December 2021. Each of these 'standard sized' punnets contained approximately 70-80 berries.

The samples were transported in a refrigerated vehicle (maintained at ~2°C) from the packhouse facility (Te Puke) to the premises of Start Afresh Limited (i.e., main coolstorage and research trial site), located in Mount Maunganui that took ~45 min. Prior to transferring the punnets from the packhouse to the storage site, all punnets were weighed for a 'time zero' measurement in the packhouse's coolstore (maintained at 2°C), using a digital balance (SDAB323, Wedderburn Instruments, Mount Maunganui, New Zealand) with a precision of 0.001 g.

3.2.3 Sample storage conditions

Immediately upon arrival at the research site, the punnets were equally distributed in pre-set refrigerated vessels called 'QPods®'. The QPods are insulated, refrigeration units capable of maintaining temperatures in the range of -2°C to 5°C. Four of these QPods were acquired that were set at storage temperatures (± 0.5°C) of 0.5, 1.5, 2.5 and 3.5°C. The QPods use a static refrigeration system that is not capable of regulating the RH within the units. The QPods were opened (probably affecting the RH within) only briefly while placing or taking out desired number of punnets for experimental analysis. Each QPod consisted of an Xsense Hitag2™ datalogger (model T20, Xsense, Midgal Tefen, Israel) capable of continuously monitoring temperature and RH (every 30 minute) over the storage period. The storage temperatures and RH in the QPods throughout the trial period is shown in Figure 3.1. The fluctuations observed in the graphs indicate the approximate timepoints when the QPods were opened to take out the required number of punnets for analysis. When averaged across the storage duration, RH was 87.8% ± 2.3%, 80.9% ± 0.2%, 76.6% ± 0.6%, and 70.4% ± 2.0% in the 0.5°C ± 0.3°C, 1.5°C ± 0.2°C, 2.5°C ± 0.1°C and 3.5°C ± 0.3°C, respectively (Figure 3.1; Panels A-D).

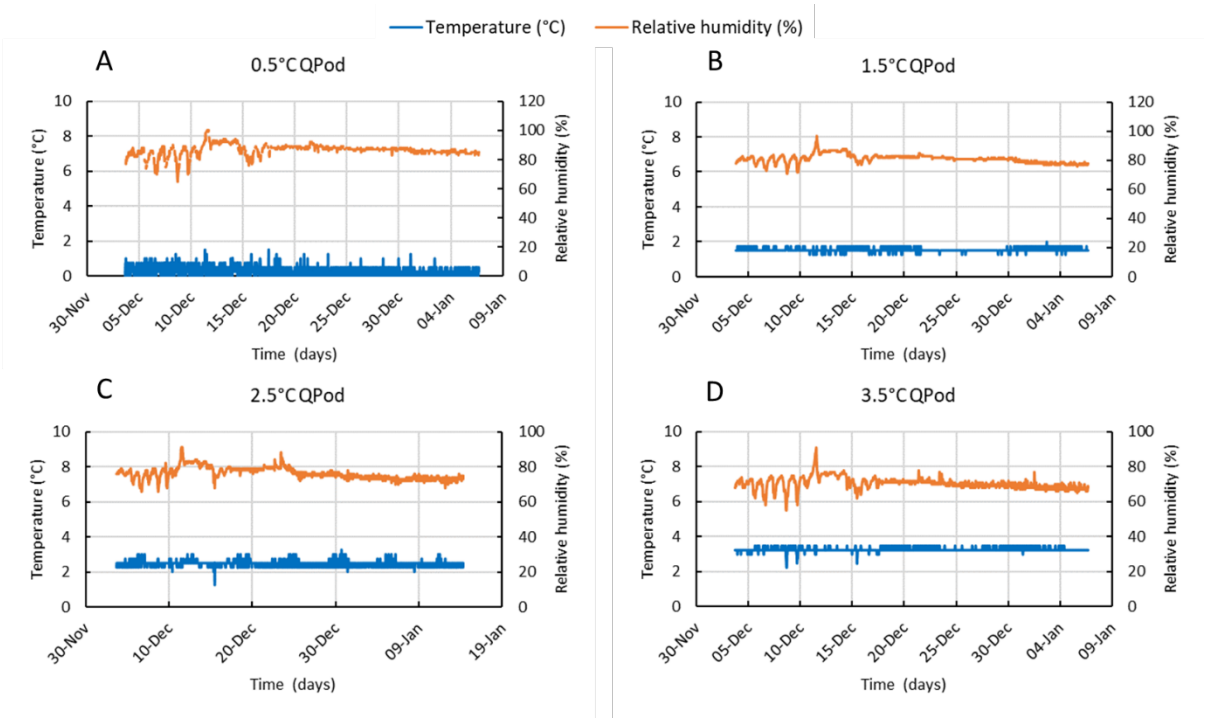

Figure 3.1. Temperature and relative humidity changes during a 28-day storage period of two blueberry cultivars. The fluctuations observed in the graphs indicate the approximate timepoints when the QPods were left open while taking out the required number of punnets for analysis.

3.2.4 Measurements

At each storage time, all berries from within a punnet were measured for WL, berry firmness and rots. Punnets were measured at timepoint 'zero day' at the packhouse, and then separate set of punnets at days 7, 14, 21 and 28 while in storage.

3.2.4.1 Weight loss

To avoid error caused due to condensation on the berries within a punnet, each punnet was weighed in the coolstore environment. WL was then calculated using Equation 1 and expressed as a % mass loss. All measurements were performed using a digital balance (SDAB323, Wedderburn Instruments, Mount Maunganui, New Zealand) with a precision of 0.001 g.

$$Weight\ loss\ \% = \frac{(Initial\ weight - Final\ weight)}{Initial\ weight} \times 100$$

Eq 1

3.2.4.2 Berry firmness

Berry firmness was measured using a FirmTech II (BioWorks, Wamego, US; Figure 3.2) that has been a widely used instrument in firmness evaluation of small fruit such as raspberries,

blackberries, blueberries, cherries, and strawberries (Li et al., 2011; NeSmith et al., 2002). This equipment measures the chord stiffness - the slope of the line drawn between to specific forces (minimum and maximum force threshold). Data is reported as force/displacement (g/mm). After measuring the punnet weight in the coolstore for WL, the respective punnets were conditioned at room temperature (20ºC) for 1 h prior to being processed on the FirmTech.

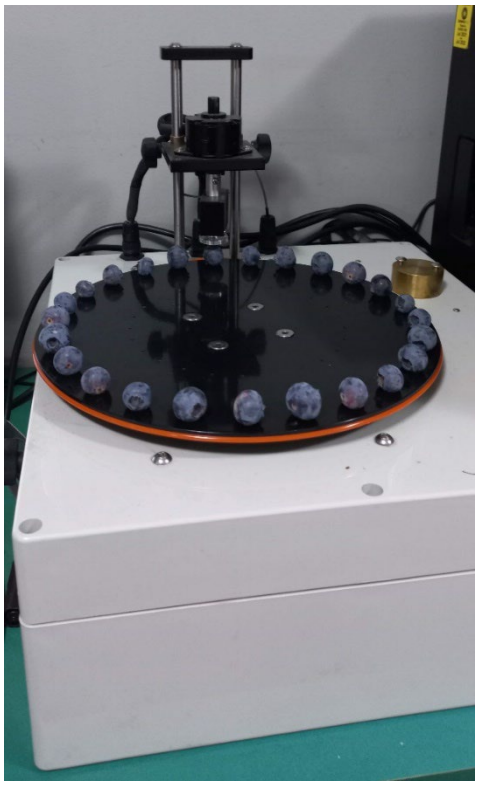

Figure 3.2. FirmTech II instrument used in the trial for measuring berry firmness.

Prior to testing for firmness, at each storage time, the load cell (250 g) of the FirmTech instrument was attached to the moving arm and calibrated. The force thresholds of the device were set between 250 g (maximum) and 50 g (minimum) (Ehlenfeldt and Martin, 2002; Saftner et al., 2008). Load cell and table speed was set to 7 mm/sec and 0.28 rotations/min, respectively. The calibration was also checked using specialised 3D-printed rubber berries of known range of firmness values and diameter (Rivera et al., 2023). Firmness was expressed as g/mm.

3.2.4.3 Number of rots

At each storage time, berries were visually observed for rots per punnet (i.e., replicate) and the total number of affected berries were recorded. A berry with superficial fungal growth that disintegrated upon touching, was recorded as ‘rotten’. Rots incidence was expressed as a percentage of total number of berries having rots.

3.3 Results

3.3.1 Weight loss

3.3.1.1 Batch 1

As expected, WL increased over time and was influenced by storage temperature with WL being higher at 3.5°C compared to 0.5°C. The lower RH of 70.4% at 3.5°C contributed to even further WL than that at 0.5°C (at 87.8% RH). At 7 days in storage, WL at 0.5°C in the EU cultivar was 1.0 ± 0.1% while it was 0.8 ± 0.1% in the FB cultivar (Figure 3.3). At the same time, WL in the 3.5°C treatment was nearly double than that at 0.5°C with 1.8 ± 0.1% and 2.2 ± 0.0% in the EU and FB cultivars, respectively, where WL in FB cultivar was significantly higher (P<0.05) than EU cultivar. At day 21, WL at 2.5°C (3.7 ± 0.2%) in the EU cultivar was significantly higher (P<0.05) than that at 0.5°C and 1.5°C but slightly lower than the 3.5°C (4.2 ± 0.5%) treatment. At day 21 in the FB cultivar, WL at 3.5°C (3.0 ± 0.3%) was significantly higher (P<0.05) than that of the remaining treatments. At the end of the storage period, WL in the EU cultivar was significantly higher than the FB cultivar at all storage temperatures (Appendix 1). The dynamics of WL in Batch 1 was different than Batches 2 and 3, probably due to the punnet type (jumbo vs standard type).

3.3.1.2 Batch 2

Batch 2 (and Batch 3 shown below) had berries in standard punnets and hence, the WL patterns were similar as opposed to Batch 1.

At 7 days in storage, WL at 0.5°C in the EU cultivar was 0.6 ± 0.0% while it was significantly higher (P<0.05) in the FB cultivar (1.0 ± 0.1%; Figure 3.3). WL at 1.5°C, 2.5°C and 3.5°C did not differ (P>0.05) significantly due to storage temperatures or cultivars at days 7 (averaged at 0.9%) and 14 (averaged 1.5%). At day 21, WL at 3.5°C (2.9 ± 0.3%) was significantly higher (P<0.05) than the remaining treatments in the EU cultivar. However, at day 21 in the FB cultivar, WL at 2.5°C and 3.5°C (averaged 2.7%) did not differ (P>0.05) but their average was higher than the 0.5°C and 1.5°C treatments (averaged 1.5%). At day 28 in the EU cultivar, WL at 0.5°C, 1.5°C, 2.5°C and 3.5°C was 2.7 ± 0.2%, 2.5 ± 0.2%, 3.7 ± 0.3% and 3.8 ± 0.4%, respectively. Corresponding values at day 28 in the FB cultivar were, 1.9 ± 0.1%, 1.8 ± 0.1%, 3.5 ± 0.1%, and 3.9 ± 0.1%, respectively (Figure 3.3). Moreover, after 28 days, WL in the EU cultivar was significantly higher than the FB cultivar at the 0.5°C and 1.5°C treatments but did not differ due to cultivars for the remaining treatments (Appendix 2).

3.3.1.3 Batch 3

The pattern of WL in Batch 3 was similar to that in Batch 2 – WL values increased over time and was higher at higher storage temperatures. On day 7, WL at 0.5°C and 1.5°C did not differ (P>0.05) due to storage temperature in the EU cultivar, however, WL at 2.5°C was significantly higher (P<0.05) than that at 3.5°C, with values of 1.5 ± 0.1% and 2.4 ± 0.2%, respectively (Figure 3.3). Contrastingly, in the FB cultivar on day 7, WL values did not differ between the 2.5°C and 3.5°C treatments. WL of 3.5°C treatment (4.1 ± 0.4%) on day 14 dropped to 2.9 ± 0.2% on day 21; the reason to this anomaly could not be explained. At day 28, WL at 3.5°C in the EU cultivar (6.0 ± 0.4%) was significantly higher (P<0.05) than the remaining treatments and also significantly higher than (P<0.05) that of the FB cultivar (4.9 ±

0.2% at 3.5°C; Figure 3.3). Similar to Batch 1 results, WL in the EU cultivar was significantly higher than the FB cultivar at all storage temperatures at the end of the storage period (Appendix 3).

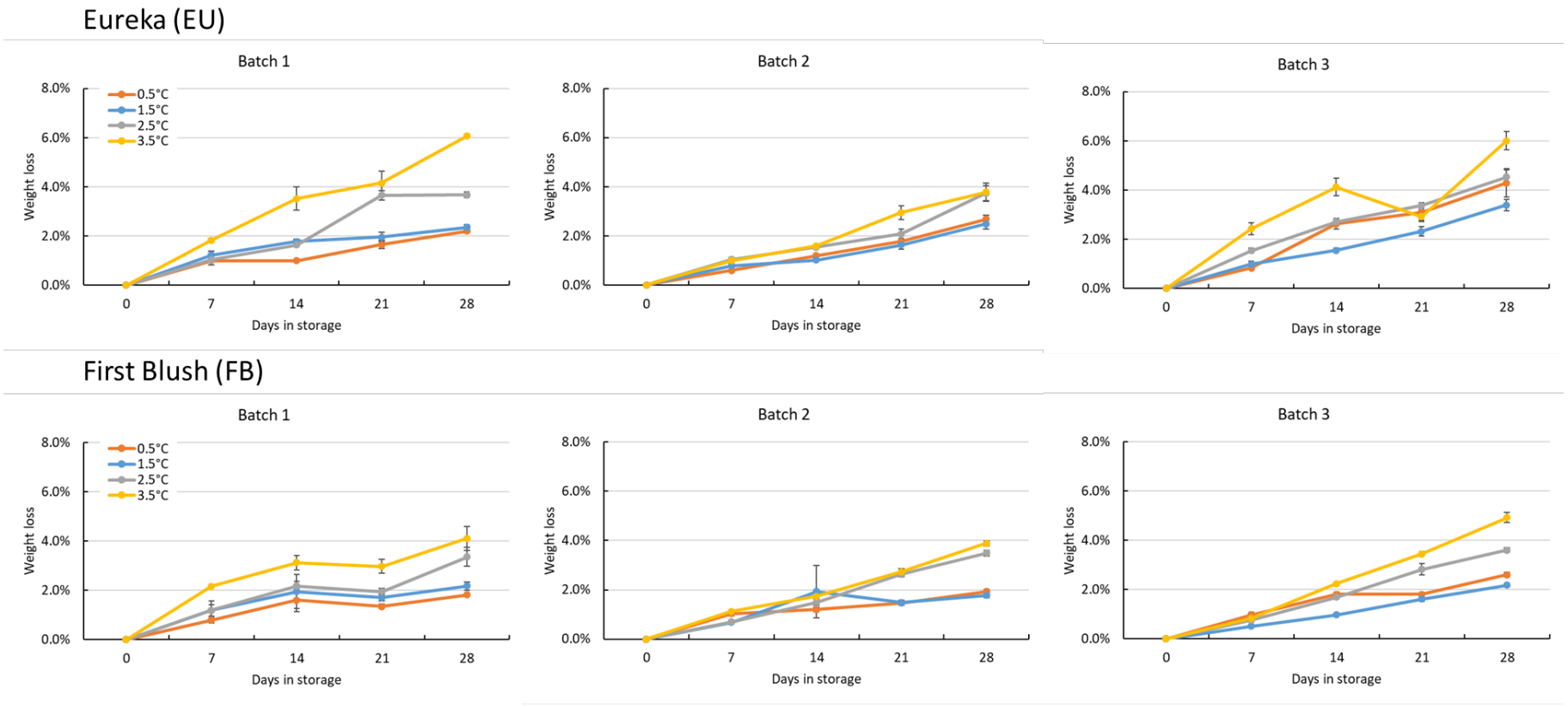

Figure 3.3. Weight loss (%) of blueberries in the trial due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 6 in Batch 1; n = 4 in Batches 2 and 3). A datapoint denotes 320-berries (Batch 1) or 450-berries (Batches 2 and 3).

3.3.2 Firmness

Previously reported results have shown that softening rate is higher as storage temperature increases (Moggia et al., 2017a; Paniagua et al., 2013). In this current trial, firmness generally did not differ statistically due to storage temperatures, however, numerically, berry firmness was higher at lower storage temperatures. Both cultivars also showed a slight 'firming' effect (i.e. increase in firmness) at all storage temperatures and this phenomenon has been reported in several studies Chiabrando et al. (2009); Duarte et al. (2009); Forney et al. (1998); Paniagua et al. (2013).The increase in firmness levels is expected to be caused due to corrugation of epidermal cell walls and thickening of the parenchyma cell walls due to lower temperatures (Allan-Wojtas et al., 2001). Chiabrando et al. (2009) observed an increase in hardness, gumminess, and a decrease in cohesiveness and springiness of highbush blueberries that occurred simultaneously with an increase in WL during storage.

3.3.2.1 Batch 1

Initial firmness on day 0 was 209.1 ± 8.5 and 217.7 ± 8.1 g/mm in the EU and FB cultivars, respectively and thereafter, a firming effect was observed in both cultivars up to day 14 in storage. However, this effect was more pronounced at 0.5°C and 1.5°C treatments (Figure 3.4). Firmness in the EU cultivar at day 7 did not differ (P>0.05) due to storage temperatures and averaged 236.5 g/mm across the four temperatures. Firmness in the FB cultivar on day 7 also did not differ (P>0.05) due to storage temperatures and averaged 234.5 g/mm. Firmness changes at day 14 were negligible compared to day 7. At day 28, firmness of the EU cultivar at 0.5°C (265.5 ± 27.3 g /mm) was significantly higher (P>0.05) than that at 3.5°C (168.2 ± 33.8 g/mm). Similarly, for the FB cultivar, firmness at 0.5°C (245.5 ± 35.0 g /mm) was significantly higher (P>0.05) than that at 3.5°C (202.3 ± 32.7 g/mm). Firmness at 1.5°C and 2.5°C throughout the storage period did not differ (P>0.05) due to storage temperatures in either of the two cultivars (Figure 3.4).

3.3.2.2 Batches 2 and 3

The rate of decrease in firmness in Batches 2 and 3 was very similar for both cultivars. For Batch 2, firmness at day 0 was 203.9 ± 8.3 and 186.7 ± 4.7 g/mm in the EU and FB cultivars, respectively (Figure 3.4). Due to firming effect, it increased to 242.7 g/mm on an average, at day 7 in the EU cultivar. Firmness did not significantly (P>0.05) differ due to temperatures on days 14, 21 and 28 in both cultivars. When averaged across storage temperatures, firmness at day 28 in EU and FB cultivars was 224.2 and 220.9 g/mm, respectively (Figure 3.4).

In the case of Batch 3, day 0 firmness was 181.8 and 200.7 g/mm in the EU and FB cultivars, respectively. In the EU cultivar, there was a tendency of the firmness levels at 2.5°C to coincide with the levels at 3.5°C while the firmness at 0.5°C coincided with 1.5°C; although, it was not statistically significant at each time point in storage (Figure 3.4). In the FB cultivar, firmness did not differ (P>0.05) due to storage temperatures, at days 7, 14, 21 and 28. At day 28, when averaged across the storage temperatures, firmness in the EU cultivar (207.7 g/mm) was significantly lower (P>0.05) than the FB cultivar (229.7 g/mm) (Figure 3.4).

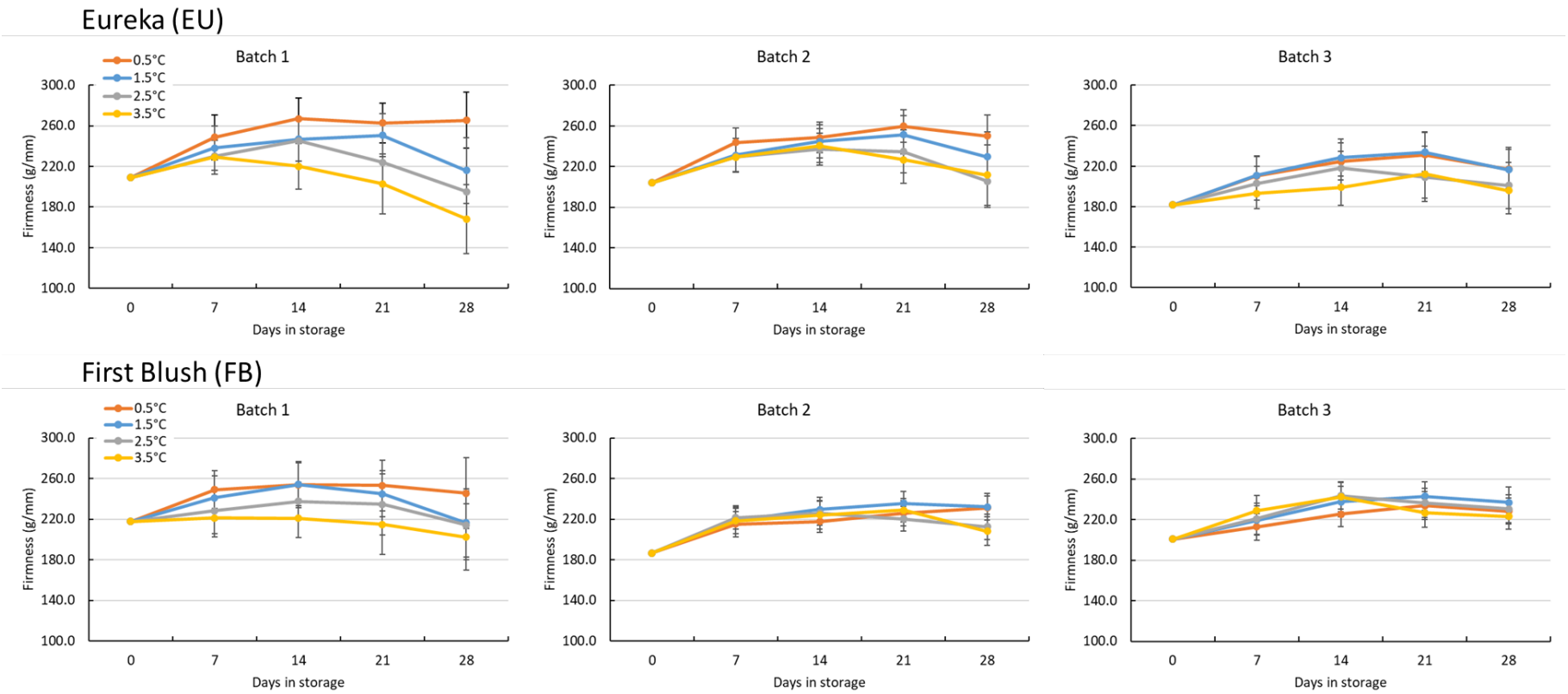

Figure 3.4. Firmness of blueberries in the trial due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE of all berries within 6 jumbo punnets (Batch 1) or 4 standard punnets (Batches 2 and 3). A datapoint denotes 320-berries (Batch 1) or 450-berries (Batches 2 and 3).

3.3.3 Incidence of rots

When expressed as a percentage of berries having rots, Batch 1 had 1% and 7% rots in the 2.5°C and 3.5°C treatments, respectively, at the end of the storage period in the EU cultivar (Table 3.2; Figure 3.5), however, the FB cultivar had 1, 4, 6 and 12% rots at the 0.5°C, 1.5°C, 2.5°C, and 3.5°C temperatures, respectively (Table 3.3).

Batches 2 and 3 had a negligible rot incidence in the FB cultivar at all temperatures (Table 3.3). The EU cultivar recorded 3 and 6% in the 2.5°C, and 3.5°C temperatures, respectively in Batch 2, and it was 6 and 12% in Batch 3, at the same temperatures (Table 3.2). No statistical analysis was performed for this data.

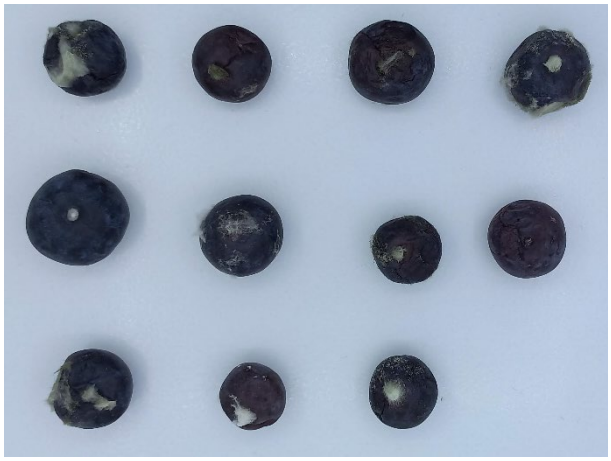

Figure 3.5. Rotten Eureka berries at 28 days in the 3.5°C treatment of the trial.

Days in storage					
Treatments	0	7	14	21	28
Batch 1					
0.5°C	0	0	0	0	0
1.5°C	0	0	0	0	0
2.5°C	0	0	0	0	1
3.5°C	0	0	0	0	7
Batch 2					
0.5°C	0	0	0	0	0
1.5°C	0	0	0	0	4
2.5°C	0	0	0	3	6
3.5°C	0	0	0	0	12
Batch 3					
0.5°C	0	0	0	0	0
1.5°C	0	0	0	1	0
2.5°C	0	0	0	0	3
3.5°C	0	0	0	0	6

Table 3.2. Incidence of rots (%) in Eureka berries after 28 days in storage at four different temperatures.

Days in storage					
Treatments	0	7	14	21	28
Batch 1					
0.5°C	0	0	0	0	1
1.5°C	0	1	0	0	4
2.5°C	0	0	0	0	6
3.5°C	0	0	0	0	12
Batch 2					
0.5°C	0	0	0	0	0
1.5°C	0	0	0	0	1
2.5°C	0	0	0	0	0
3.5°C	0	0	0	0	0
Batch 3					
0.5°C	0	0	0	0	0
1.5°C	0	0	0	0	0
2.5°C	0	0	0	0	0
3.5°C	0	0	0	0	1

Table 3.3. Incidence of rots (%) in First Blush berries after 28 days in storage at four different temperatures.

3.4 Discussion

WL is largely not a physiological process, rather a physical movement of water vapour from within the berry via the stem scar and epidermis, to the surrounding environment. As a result, a bigger sized berry (higher volume to surface ratio) has a proportionally bigger stem scar. Both berry diameter and berry weight are the distinguishing factors (shown in Table 3.1) in this current study when compared to data reported by Rivera et al. (2021) and Jorquera-Fontena et al. (2017) (values reported in Section 3.1 above).

WL is dependent on storage temperature and relative humidity which coincides with firmness loss (Moggia et al., 2017a; Paniagua et al., 2013). Moreover, WL caused due to fruit respiration is significantly less when compared to the physical WL process. Respiration rates of EU and FB varieties were not significantly different in this current study (data not shown). A recent review by Moggia and Lobos (2023) indicates the importance of fruit dehydration and how it is a valuable alternative to estimate the travel capacity of blueberries to distant destinations.

Rivera et al. (2021) investigated the effect of storage humidity on 'Nui' and 'Rahi' blueberry cultivars over a 28-day storage period. They stored the berries at 4°C and altered the humidity to 54, 75, 89, 92 and 96%. After 28 days in storage, they found the maximum WL ranged from 13.4-16.7% at 54% RH, while lowest WL of 0.57-1.32% occurred at 96% RH. At 75% RH, WL ranged from 9.5-12%. The closest comparison of Rivera et al. (2021) to this current trial, in terms of storage temperature, is the 3.5°C treatment in which RH was 70.4% during the 28-storage period. The QPods in our trial had a relatively static system of refrigeration causing negligible airflow changes over time. Consequently, WL values in our trial at day 28 ranged from 4.1-6.1%, 3.8-3.9%, and 4.9-6.0% across the two cultivars in Batches 1, 2 and 3, respectively. These values are almost half than that of the values found in Rivera et al. (2021), evidently because of a dynamic airflow system in their study and different cultivar characteristics. However, if the QPods could, for instance, were able to consistently maintain the same, 90-95% RH across all storage temperatures, theoretically, water loss would be significantly reduced but with higher number of rots. The cultivars used by Rivera et al. (2021) are conventional berries that are significantly smaller (hence lower volume to surface ratio) than the ones used in this current trial affecting WL characteristics. Nunes et al. (2004) stored blueberries at five different storage temperatures and reported WL of ~2% at 0°C when over a 14-day storage period. The WL values in our study at 0.5°C at day 14 are similar or slightly less than that of Nunes et al. (2004). The Chilean blueberry industry does not allow WL of more than 5–7% in a commercial 3-week period stored at 0°C (Paniagua et al., 2014). However, the New Zealand blueberry industry operates at 2°C and hence, rates of WL are expected to be higher.

The blueberry cultivar played an important role in WL during postharvest storage. Moisture loss in blueberries mainly occur via the stem scar and the cuticle while the calyx, and stomata are less drastic pathways (Paniagua et al., 2013). Moggia et al. (2017a) suggested that the cuticle is the major route of WL at higher temperatures, however, the stem scar is the main

WL pathway at lower temperatures such as in cool storage, also implying that stem scar was the main cause of WL in this current study. NeSmith et al. (2005) investigated the WL and firmness patterns of six rabbiteye cultivars in storage and found that berries with higher at-harvest firmness had a longer storage period. Moreover, cultivar differences can play a key role in the softening dynamics due to increase in storage temperatures. In this current study, firmness or WL differences were not industrially significant across the two cultivars, however, the FB cultivar had a lower tendency of WL and berry firmness reacting to storage temperature differences. Moreover, closer observation and handling of the berries during the trial indicated that the FB cultivar had a much stronger 'bloom' (i.e., cuticular wax layer) than the EU cultivar, although the bloom thickness was not measured in this trial. Studies such as Martin and Rose (2014) and Lara et al. (2014), have shown that the thickness of the bloom layer can play a significant role in postharvest handling and storage.

Several studies have indicated that during the ripening of berries, firmness loss occurs due to immense changes to the structure and composition of the cell wall (solubilisation and degradation due to high pectin methyl esterase and β-galactosidase), stone cells, turgor, cell membranes damage and dehydration (Liu et al., 2019; Montecchiarini et al., 2018; Olmedo et al., 2021; Rivera et al., 2021). For instance, Moggia et al. (2022a) found that higher amounts of ursolic acid and lupeol in ripe fruit caused higher WL and firmness loss in Duke berries compared to that of Brigitta berries. Softening rates are also dependent on the cultivar differences (flowering window, cuticular wax thickness), cultivar's sensitivity to the environment (season, location, orchard orientation), and agronomic management (type and frequency of picking, nutrient regimes) (Moggia and Lobos, 2023).

Firmness loss is one of the major factors governing consumer acceptance and the marketing of fresh blueberries (Chiabrando et al., 2009; Vicente et al., 2007). Lobos et al. (2014) indicated that cultivar differences and maturity stages during harvesting can also affect postharvest firmness. Although our study did not record industrially significant firmness differences due to cultivars (EU and FB) (because of high variability), the tendency was that EU had higher firmness until day 21 until it reached a point beyond which a rapid firmness decline occurred. Storage temperatures did not affect the firmness of FB as significantly as that of EU. The main reason for high variability in our study and other blueberry studies is that our manual harvest procedure is dependent on visual judgement of the picker, which makes it difficult to recognise differences in physiological maturity at harvest [i.e., semi-ripe vs ripe vs overripe berry; (Lobos et al., 2018)]. Moreover, the variability is further increased due to an extended flowering period and asynchrony of fruit ripening.

3.5 Conclusion

The New Zealand blueberry industry operates at a storage temperature of 2°C which is higher than other countries (e.g., Chile uses 0°C), hence, rates of WL are expected to be higher. As a result, the punnets in New Zealand are packed with approximately 1-2% extra weight at-packing to account for the WL. If efforts are diverted to decrease the storage temperature to

~0°C and focus on a more consistent coolchain, then we can achieve a reduced WL, improvise quality and increase our financial returns by packing more accurately. Hence, in order to understand the storage potential of these newer cultivars used in the current study, future studies should focus on attributes such as TA and TSS contents, bloom thickness, respiration rates and skin permeance characteristics. And if these characteristics are significantly different, then each cultivar may have a different optimum storage temperature, which should be investigated.

Chapter 4 Effect of packaging technologies on moisture loss and firmness during storage

4.1 Introduction

Chapter 3 showed the importance of temperature and humidity management during storage of blueberries and how it can affect WL (due to dehydration), firmness retention, and rots. Among these modes of failure, decays/rots are the most important cause of postharvest losses and have a much greater significance due to lower customer tolerance, leading to export rejections (Paniagua et al., 2013; Vicente et al., 2007). Fungal infections including botrytis, anthracnose and *Pestalotiopsis* spp. are more pronounced during ripening which can accelerates senescence Hosking (2023). Previous studies (Cappellini and Ceponis, 1977; Cappellini et al., 1972) reported that pathogens such as *Alternaria* spp. and *Colletotrichum* spp. are more prevalent when storage temperatures exceed 2°C.

During the pre-harvest phase, there are limited options that can extend storage potential, including breeding for more resilient cultivars. However, there are promising post-harvest techniques that have been proven to significantly prolong storage period without compromising quality. These include technologies such as low-temperature storage (coolstorage), controlled atmosphere (CA) (Schotsmans et al., 2007), calcium dips (Hanson et al., 1993), plant-originated antimicrobial agents (Wang et al., 2010), and edible coatings (Duan et al., 2011).

In addition, packaging technologies such as modified atmosphere packaging (MAP), in combination with an adequate (<4°C) temperature control (Hancock et al., 2008; Schotsmans et al., 2007) has also proven to be an effective storage extension technique.

The CA approach involves modification of gas concentrations of O_2 and CO_2 during cold storage, consequently leading to lower the fruit's respiratory metabolism rate thereby extending its storability (Falagán et al., 2020; Yahia, 2009). Recommended CO_2 concentrations range from 10-12% with higher levels causing negative effects on firmness, fruit splitting, flavour profile, flesh discolouration and TA (Forney et al., 2022; Harb and Streif, 2004),

The MAP techniques is more advantageous over CA because it can be used during the whole supply chain, maintaining high RH (>95%) until fruit reaches the consumer. However, MAP cannot maintain CO_2 concentrations higher than 6-8%, and consequently, other technologies for controlling rots may be required to use in combination with MAP such as sodium metabisulfite (sulphur dioxide) pads.

Xtend® packaging solution (StePac L.A., Tefen, Israel) is one of the manufacturers of MAP bags. The company claims that their MAP bags are breathable, multi-layered, polyamide-based packaging designed to not only regulate headspace gas composition, but also relative humidity. They use co-extruded polyamide-based films with relatively high water vapour

transmission rates and embedded antifog for bulk packaging of blueberries that minimises WL and helps retain bloom. They regulate the O_2 and CO_2 of the film using laser perforations to achieve the desired modified atmosphere, which slows down respiration and aging processes and helps reduce the risk of microbial decay.

Berryguard™ (OKSU, Chile) is one of the manufacturers of SO_2 pads. The company claims that these pads have sodium metabisulfite laminated polymers which allow the controlled emission of SO_2 gas upon reaction with moisture. They also claim that their pads are capable of releasing very low dosage of SO_2 to the fruit causing inhibition of the *Botrytis cinerea* rot pathogen.

The reaction of sulphite salts of sodium or potassium with moisture releases SO_2. Based on the fruit type, cultivar, size of the packaging enclosure, the sodium metabisulfite chemical is carefully embedded within the pads which ultimately reacts with the moisture released during cold storage (Li et al., 2023). Chapter 2 discusses the detailed mode of action of these sulphite salts with moisture.

Loss of pigmentation due to SO_2 fumes, also known as 'bleaching', is a commonly reported problem that has previously been reported in several studies on blueberries, the reason to it is attributed to cultivar, bloom levels, and fluctuations in storage temperatures and humidity levels (Cantín et al., 2012; Rodriguez and Zoffoli, 2016; Saito et al., 2020; Saito and Xiao, 2017).

The effect of SO_2 fumigation (Rivera et al., 2013a) and SO_2 pads (Saito et al., 2020; Saito and Xiao, 2017) in blueberries have been tested in previous studies, and there are several types of MAP and SO_2 products commercially available, however, the use of MAP bags in combination with SO_2 pads has not been tested in the modern cultivars such as the varieties used in this study. Moreover, the negative effects such as softening and bleaching, if any, due to the use of both MAP and SO_2 technologies on these cultivars is not known.

The following experiment was designed to investigate the effect of both MAP and SO_2-generating pads on postharvest quality attributes such as firmness, WL and incidence of rots in Eureka (EU) and First Blush (FB) blueberry cultivars.

4.2 Materials and methods

4.2.1 Sample collection, transport and experimental design

Similar to Chapter 3, this trial was repeated in two experimental sets, i.e., Batches 1 and 2. For each Batch, 18.0 kg berries of each cultivar were acquired from Yieldia packhouse (Te Puke, New Zealand) in the form of commercially packed 125 g 'standard' punnets. Each of these punnets contained approximately 70-80 berries.

12 punnets × 125 g × 6 storage timings × 2 treatments, equated to 18 kg berries of the EU cultivar and likewise for the FB cultivar. Batches 1 and 2 were acquired on 1 and 9 December 2021, respectively. Dimensions of the punnets used were 115 mm × 100 mm × 35 mm (L × B

× H), had 22 ventilation holes (6.95 mm diameter), providing 2.46% ventilation area as a proportion of the total surface area of the punnet.

Prior to transferring the punnets from the packhouse to the storage site, all punnets were weighed for a 'time zero' measurement in the coolstore (maintained at ~2°C) of the packhouse, using a digital balance (SDAB323, Wedderburn Instruments, Mount Maunganui, New Zealand) with a precision of 0.001 g.

As described in Chapter 3, upon arrival at Start Afresh premises, all acquired punnets were transferred into the QPods maintained at 2.0 ± 0.5°C and 76.6% RH.

The trial was setup as a randomised block design with two treatments (MAP+SO_2 vs control) and two cultivars as the factors. At each storage time, 12 punnets (i.e., replicates) of each treatment and each cultivar at each storage time were tested for WL, firmness and defects (12 punnets × 2 treatments × 2 cultivars, equated to 48 punnets tested at each storage time). These measurements were performed at weekly intervals using the same methods and instruments described in Chapter 3.

An analysis of variance (ANOVA) was performed for WL and firmness data and treatment differences were tested using Tukey's test (95% at confidence interval) using the statistical software Minitab version 19.0 (Pennsylvania, US). All data presented here are mean ± SE (n = 12).

4.2.2 Treatments

A standard commercial tray consists of 12 punnets (125 g each), with six on the bottom and another six as the top layer of the tray (Figure 4.1-A). This tray was the 'untreated control' for the trial.

The SO_2 pads were sourced from Berryguard™ (OSKU, Chile). The Berryguard SO_2 pad (EPA Registration No. 56799-3; measuring 21 cm × 31 cm) designed for 1.5 kg of fresh produce containing 3 g of sodium metabisulfite, was used for this trial.

The 1.5kg blueberry MAP bags (Cat. No. 885-BL46/L/a) are a proprietary formulation sourced from Xtend® packaging solution (StePac L.A., Tefen, Israel). This product is a co-extruded polyamide-based transparent flexible packaging designed to provide optimum modified atmosphere for the produce item within, thereby prolonging storage and transportation life. The film is characterised by high clarity, excellent antifog properties and good printability and complies with food contact regulations (EEC/FDA) and standards 5113, 202/72/EC, 204/19/EC, 205/79/EC. It is laser-perforated with a thickness ranging from 25-35 µm and designed to deliver a modified atmosphere of approximately 15% O_2 and 5% CO_2 at 1°C storage temperature.

For the MAP+SO_2 treatment, one SO_2 pad was placed between the top six and the bottom six punnet layers followed by putting all punnets along with the SO_2 pad in a MAP bag. Prior to sealing the bag using a zip tie (Figure 4.1-B), a handheld vacuum pump was used to extract the ambient air out of the bag. After sealing, the punnets were placed in a similar commercial

configuration within the tray (Figure 4.1-C). The methodology used is very similar to that of Saito et al. (2020).

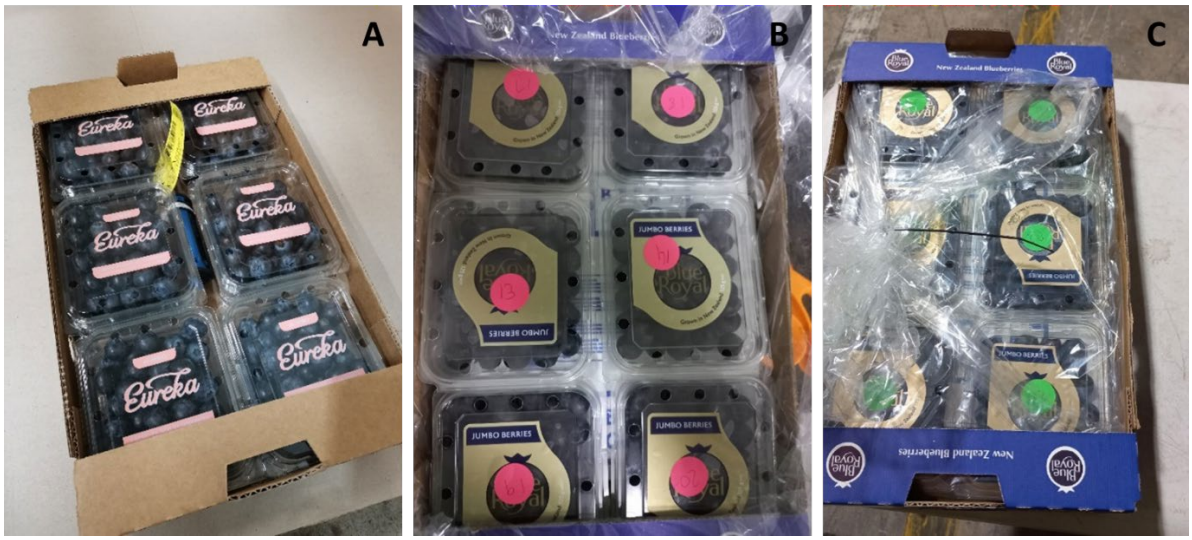

Figure 4.1. A standard commercial tray containing six punnets on the top layer and six on the bottom layer depicting the 'control' treatment of the trial (A); the 12 punnets in an MAP bag prior to sealing where the SO_2 pad was placed between the top and bottom layers (B); the same tray shown in panel-B after sealing using a zip tie (C).

Treatments were termed 'MAP' (that included the SO_2 pad in a MAP bag) and an untreated 'control' where the 12 punnets were placed in a tray without any intervention (i.e., no SO_2 pad and no MAP bag). All trays (MAP+SO_2 and the controls) were stored in the same QPods used in Chapter 3 that had an average temperature of 2.0 ± 0.5°C and an average RH of 76.6 ± 0.6%. The RH within the MAP bags could not be measured.

All berries within a punnet per replicate were tested at 14, 21, 28, 35 and 42 days in storage. At each storage time, prior to opening the MAP bags for measurements, O_2 and CO_2 concentrations within the bags were measured using Dansensor® CheckPoint 3 (Ametek Mocon, Minnesota, USA). The gas concentrations did not change drastically throughout the 42-day storage period and stayed at an average of 18.0% O_2, 3.7% CO_2 (Figure 4.2). After gas measurement, the bag was opened, and each individual punnet was weighed to determine the WL in the coolstore. Followed by measuring the punnet weight in the coolstore for WL, the respective punnets were conditioned at room temperature (20ºC) for 1 hour prior to being processed on the FirmTech for firmness.

Immediately prior to firmness testing, the number of berries with rots and SO_2 bleaching from each punnet was recorded and expressed on a percentage basis. In the case of rots, the type of rot was not quantified, however, a berry with superficial fungal growth that disintegrated upon touching, was recorded as 'rotten'. A softened spot with loss of pigmentation area greater than 5 mm was recorded as 'bleached'.

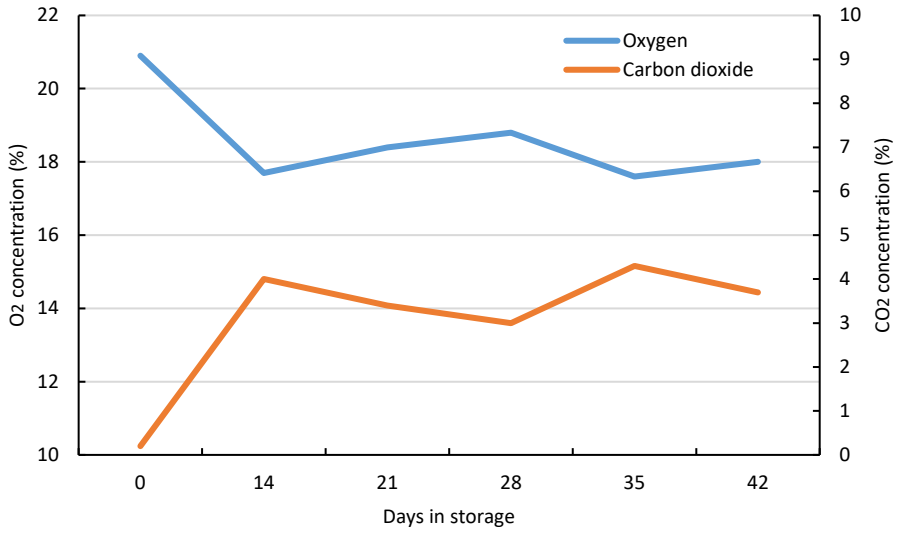

Figure 4.2. Changes in O_2 and CO_2 concentrations within a MAP bag containing blueberries with SO_2 pad over a 42-day storage period stored at 2.0 ± 0.5°C.

4.3 Results

4.3.1 Weight loss

When compared to the control, significantly less (P<0.05) WL occurred in the MAP treatment in both Batches 1 and 2 (Figure 4.3). Moreover, for both Batches, WL did not differ across the cultivars (P>0.05) in MAP. While WL did not differ significantly (P>0.05) due to the cultivars in the control treatment on most storage timings.

In Batch 1, WL at day 14 in the MAP treatment averaged across both cultivars was 0.6-0.7% while it was 1.5 ± 0.09% and 1.5 ± 0.04% in the controls of the EU and FB cultivars, respectively, which did not differ significantly (P>0.05). At 28 days, 1.0 ± 0.03% WL occurred in the MAP treatment (identical in both cultivars), while it was 3.7 ± 0.31% and 3.5 ± 0.13% in the controls of the EU and FB cultivars, respectively which did not differ significantly (P>0.05). At 42-day, 1.5% WL occurred for both cultivars in the MAP treatment on an average. In the controls, WL was 7.8 ± 0.44% and 6.9 ± 0.30% in the controls of the EU and FB cultivars, respectively with significantly higher (P<0.05) WL in the EU cultivar (Figure 4.3).

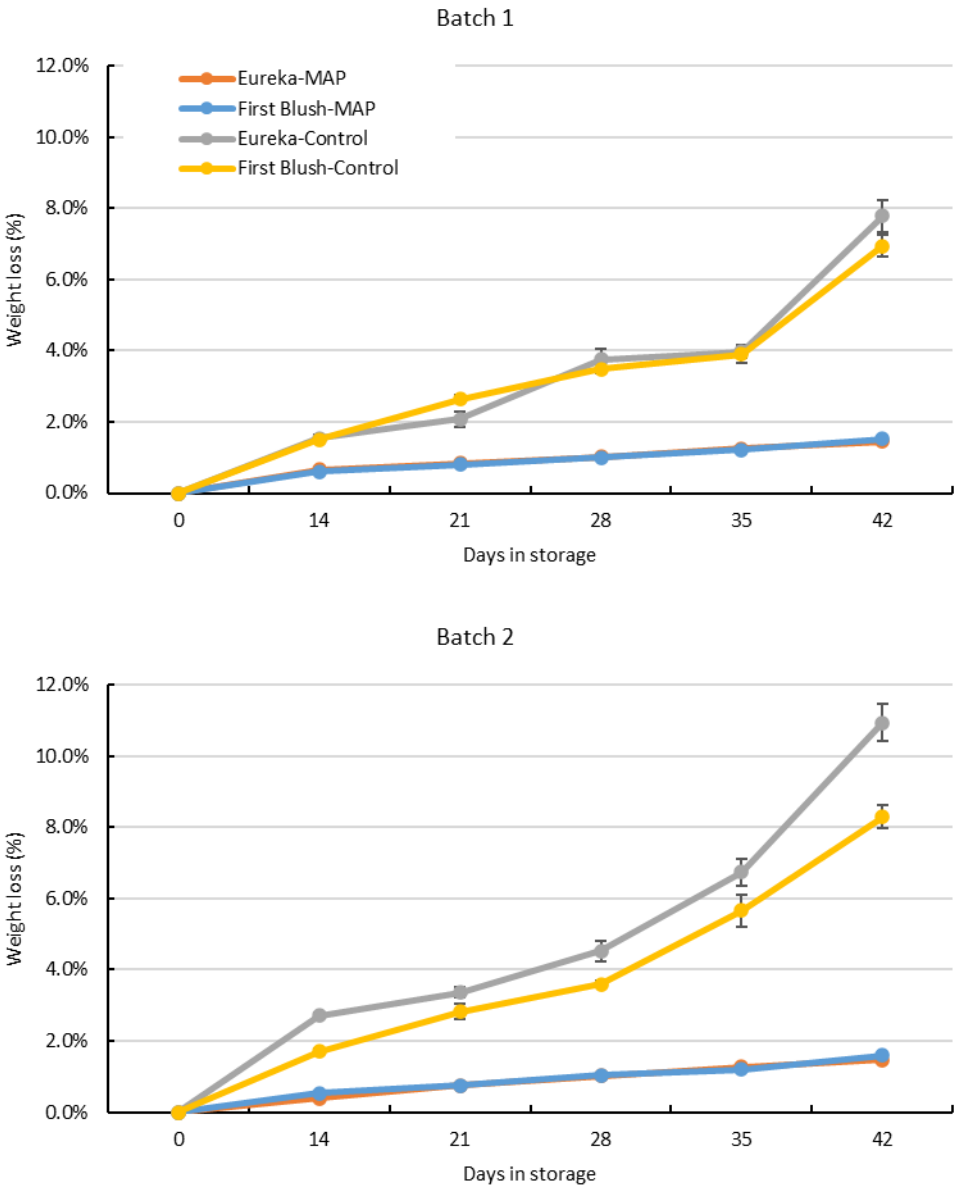

Figure 4.3. Weight loss (%) of blueberries in Batch 1 (top panel) and Batch 2 (bottom panel) of the trial due to packaging intervention over a 42-day storage period. Each datapoint is an average ± SE (n = 12). A datapoint denotes average of ~900-berries.

In Batch 2, the dynamics of WL were almost identical in the MAP treatment with maximum WL of 1.6% occurring at day 42 for both cultivars which did not differ significantly (P>0.05; Figure 4.3). However, WL in the control treatment was significantly higher (P<0.05) than the MAP treatment on all days from day 14 to day 42. Moreover, WL in the control treatment of the EU cultivar was significantly higher (P<0.05) than that in the FB cultivar at days 28, 35 and 42. At day 42, WL in the controls of the EU cultivar was 10.9 ± 0.5% which was significantly higher (P<0.05) than the WL in the FB cultivar (8.3 ± 0.3%).

4.3.2 Firmness

As expected, firmness decreased over the 42-day storage period in both treatments and cultivars. A slight increase in firmness in the first 21 days after storage was observed. This 'firming effect' has been reported in several studies (Forney et al., 2022; Paniagua et al., 2013; Rivera et al., 2022b). Firming was also observed in Chapter 3 and the effects and probable reasons have been discussed in that chapter.

At each storage timing, a large statistical variation (higher SE values) was observed for each firmness datapoint, resulting in no statistically significant differences. Whenever significant differences occurred, the differences were of a smaller magnitude which may probably not be relevant to affect consumer perception.

For Batch 1, firmness at day 0 in the EU cultivar was 203.9 ± 8.3 g/mm, which was significantly higher than that of the FB cultivar (186.7 ± 4.7 g/mm) (Figure 4.4). In all treatments, a firmness increase was observed at day 14, which decreased thereafter.

Overall, the effect of MAP over the control in the EU cultivar was slightly more pronounced than that of the FB cultivar. By the end of the storage period, at day 42, firmness in the EU-MAP and EU-control treatments was 182.1 ± 17.9 and 179.0 ± 22.9 g/mm, respectively, which did not differ significantly ($P>0.05$). Firmness in the FB-MAP treatment was 178.3 ± 16.1 g/mm which was significantly lower ($P<0.05$) than that in the FB-control (207.1 ± 10.3 g/mm) (Figure 4.4).

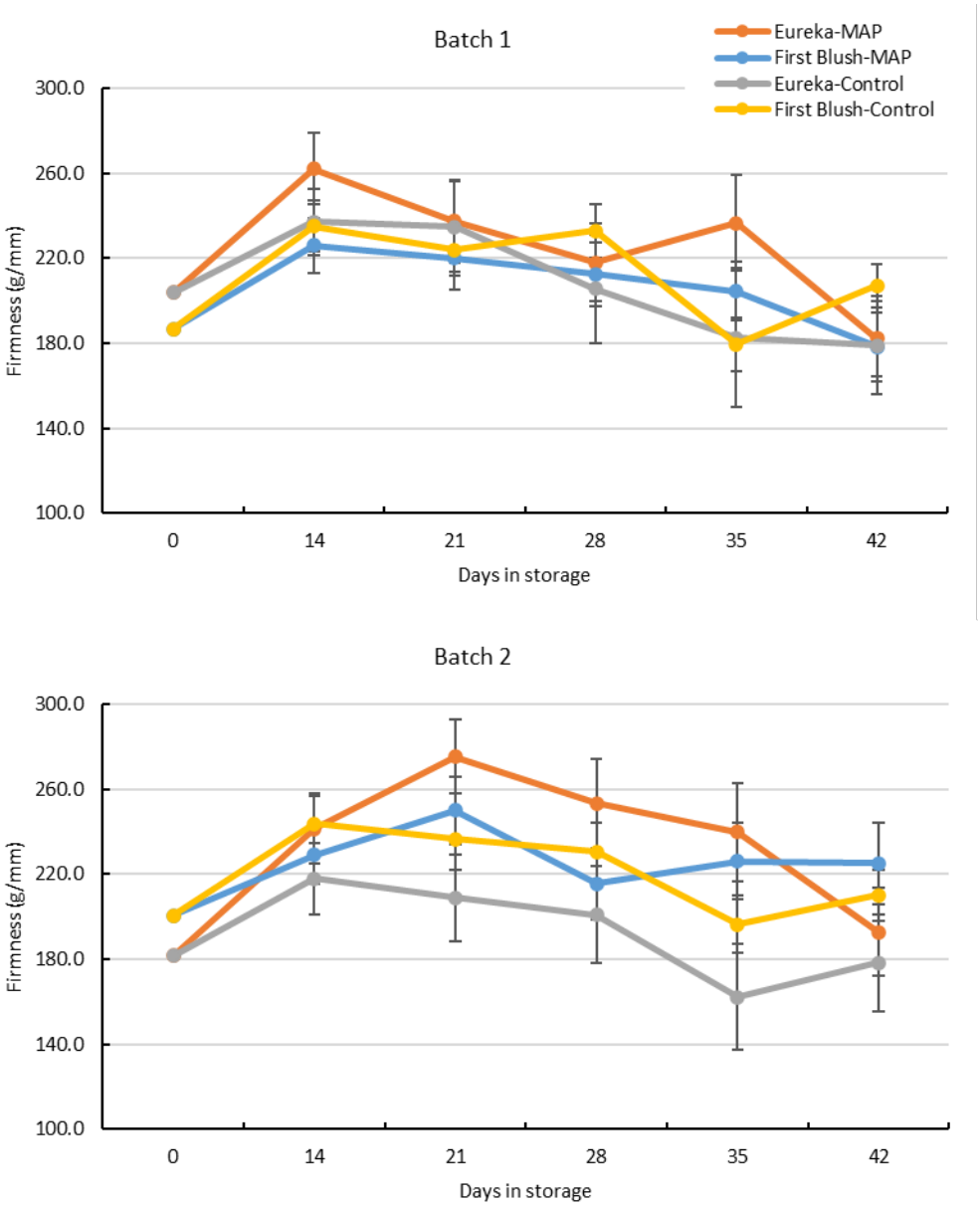

Figure 4.4. Changes in the firmness of blueberries in Batch 1 (top panel) and Batch 2 (bottom panel) of the trial due to packaging intervention over a 42-day storage period. Each datapoint is an average ± SE of all berries within 12 standard punnets. A datapoint denotes an average of ~900-berries.

In Batch 2, day 0 firmness in the FB cultivar (200.7 ± 3.4 g/mm) was significantly higher (P<0.05) than the EU cultivar (181.8 ± 8.7 g/mm). Firmness in the EU-control and FB-control increased on day 14 and then decreased over the remaining storage period. Firmness also increased in the EU-MAP and FB-MAP treatments on day 14 and continued to increase on day 21 but decreased thereafter until day 42 (Figure 4.4, bottom panel). Firmness at day 21 in the EU-MAP was 275.3 ± 17.6 g/mm which was significantly higher (P<0.05) than that of EU-control (209.0 ± 20.4 g/mm). Corresponding values in the FB-MAP and FB-control was 250.0 ± 15.8 and 236.6 ± 14.5 g/mm, respectively that did not differ significantly (P>0.05). At day 42, firmness of EU-control (178.4 ± 22.8 g/mm) did not significantly

(P>0.05) differ from EU-MAP (192.8 ± 20.7 g/mm) and likewise in the case of FB-control (210.2 ± 12.0 g/mm) vs FB-MAP treatments (225.1 ± 19.4 g/mm) (Figure 4.4, bottom panel).

4.3.3 Incidence of rots

Blueberries stored in MAP treatments showed significantly lower (P<0.05) incidence of rots for both cultivars (Table 4.1). However, the number of rots in the control treatment of the EU cultivar was generally higher than the FB cultivar. In Batch 1, at 42 days, EU-control had 24% rots while EU-MAP had 3%. In Batch 2, these values corresponded to 25 and 8%, respectively. For the FB cultivar, control had 4% rots and 0% rots in the MAP treatment, for both Batches 1 and 2.

	Days in storage					
Treatments	0	14	21	28	35	42
Batch 1						
Eureka-MAP+SO_2	0	1	0	1	3	3
Eureka-Control	0	0	3	6	16	24
First Blush-MAP+SO_2	0	0	0	0	0	0
First Blush-Control	0	0	0	0	1	4
Batch 2						
Eureka-MAP+SO_2	0	1	1	2	4	8
Eureka-Control	0	0	0	3	5	25
First Blush-MAP+SO_2	0	0	0	0	1	0
First Blush-Control	0	0	0	0	0	4

Table 4.1. Incidence of rots (%) in berries for 42 days in storage treated with MAP and SO_2 pads vs a control.

4.3.4 Bleaching due to SO_2

Visual observation at each storage time showed that SO_2 bleaching occurred at the stem-end of all berries on all storage times for both cultivars which used the SO_2 pads (Figure 4.5). This was a visual observation only and was not quantified or statistically analysed.

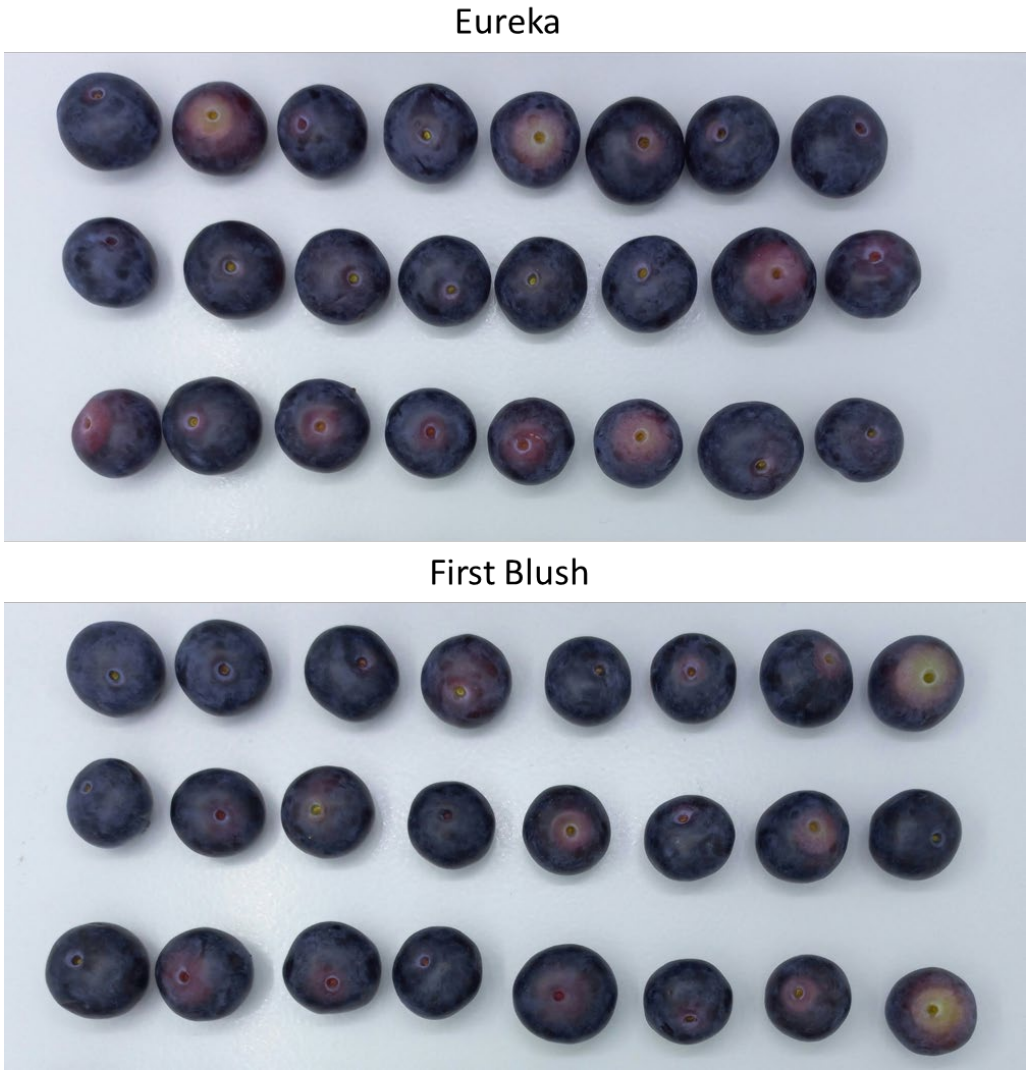

Figure 4.5. Bleaching damage caused due to SO_2 fumes observed at day 42 in storage.

4.4 Discussion

Berries stored in MAP bags with SO_2 pad, showed reduced WL of ~1.5% when compared to berries in standard packaging that lost 6.9-10.9% moisture (8.5% average) after 42 days in storage at 2.0 ± 0.5°C. This is a significant WL benefit of 7% due to the use of MAP bags. For this trial, 36 kg of berries were sourced for each Batch (Section 4.2.1). As an estimate, the WL benefit of 7% equates to approximately 2.52 kg of berries that could be saved by using this technology (7% of 36 kg by calculating the difference between 8.5% WL in control vs 1.5% in MAP).

An important consideration, however, is that the 'net effect' of using 'MAP only' in this trial was not investigated. The MAP technology is costly due to being relatively new (in comparison to using standard polyliner) and hence, future studies should focus on comparing the efficacy of using MAP vs a standard polyliner. This will allow to make a decision if using a cheaper option (i.e., the polyliner only) could be equally beneficial in reducing WL.

Studies (Falagán et al., 2020; Forney et al., 2022; Schotsmans et al., 2007) show that 2-5% O_2 and 10-12% CO_2 in CA is recommended in controlling postharvest rots. A minimum of 6% CO_2 concentrations has been found as the minimum for fungistatic to occur that would inhibit natural infections among blueberry fruit during cold storage (Cantín et al., 2012; Duarte et al., 2009; Harb and Streif, 2004; Schotsmans et al., 2007). Koort et al. (2018) investigated the effect of storing two blueberry cultivars (a lowbush and a half-highbush cultivar 'Northblue') using regular atmosphere, low-density polyethylene (LDPE) packaging and Xtend MAP stored at 3 ± 1°C. They found that Xtend MAP could extend the storage life by 9-15 days for both cultivars and concluded that genetic differences played the most significant role with the half-highbush cultivar more firmer and less shrivelled. Moreover, at the end of their 37-day storage period, O_2 and CO_2 concentrations were 13.0% and 9.3%, respectively. The authors compared their achieved gas concentrations with other literature and suggested that their concentrations were not sufficient in controlling rots. The CO_2 concentrations in the MAP treatment of this current study were 3.7% on an average (with 18.0% O_2) further reflecting Xtend MAP's differences in maintaining atmospheres at difference storage temperatures and/or controlling the rots.

Saito et al. (2020) in their MAP+SO_2 treatment could reduce the WL to 0.1-0.4% during storage at 1°C for three weeks. The WL values in their study also differed across different cultivars. The WL in the control treatments of Saito et al. (2020) was 4.3-4.7%. The WL did not differ due to cultivars in this current study which is contrasting to some previous studies using MAP (Cantín et al., 2012; Saito et al., 2020). When compared to the storage period of 21 days, WL was 0.7% in the MAP treatment in this current study, compared to that of 2.8-3.8% in the control treatments of Saito et al. (2020). The differences in WL in this current study and Saito et al. (2020) may be due to the differences in storage temperatures (1°C vs 2.0 ± 0.5°C). Although humidity within the packaging was not monitored in this current trial, the significantly lower WL observed in the MAP treatments compared to the controls, indicate that humidity was higher in the MAP treatments resulting in decreased WL and aided in firmness retention (Rodriguez and Zoffoli, 2016). The use of MAP treatment has a significant storage benefit that could be important to facilitate longer shipping transits.

In the current study, firmness in the MAP treatment was slightly higher (but did not differ significantly) than the control treatment for both cultivars. This is accordance with Saito et al. (2020) who used 'Emerald', 'Jewel' and 'Misty' blueberry cultivars with MAP, SO_2, and MAP+SO_2 treatments, and did not achieve a significantly higher firmness in either of the treatments. Moreover, firmness values at the end of the three- and six-week storage periods in their trial were almost similar to that found in the current study ranging between 149-218 g/mm across the cultivars used. Schotsmans et al. (2007) conducted an experiment to test the effect of CA (2.5% O_2, 15% CO_2) vs regular atmosphere, on two rabbiteye cultivars, 'Maru' and 'Centurion' stored at 1.5°C. Despite these differences in softening rate, the authors did not find significant differences between firmness of the two cultivars.

Rodriguez and Zoffoli (2016) investigated the effect of different levels of CA (CO_2%/O_2%, 5/1, 7/15, 15/5) on 'Brigitta' highbush cultivar stored for 30-45 days at 0°C. CO_2

concentrations >8% and O_2 <2% caused fruit softening. The CA ratio of 7 % CO_2/ 15% O_2 and the regular atmosphere could maintain significantly higher firmness than the remaining CA ratios. Moreover, the CA ratio of 15 % CO_2/ 5% O_2 had significantly lower rots than the remaining CA ratios and control. The authors also investigated the effect of using perforated polyethylene bags vs MAP on the storability of 'Legacy' cultivar. They found that decay was significantly and similarly controlled in both, indicating packaging in a ventilated bag was the best and economically viable option because it avoided the risk of MAP to develop CO_2 phytotoxicity during long term storage.

Cantín et al. (2012) investigated the combined effect of SO_2 fumigation and different CA ratios (3% O_2 + 3, 6, 12, or 24% CO_2) on firmness and decay of eight fresh blueberry cultivars and found that 3% O_2 and 6 or 12% CO_2 were the best combination in reducing decays and extend storability.

In a MAP system, there is a dynamic interaction between O_2 consumption (of the berries and the resulting CO_2 generation) and the transfer of gases through the MAP (O_2 input and CO_2 output). Studies show that an MAP-equilibrium is dependent on the type of product to be packed, the temperature at which the package is stored, the weight and the rates of respiration and permeance of the berries and the permeation rate of the packaging system that is used.

The aim of SO_2 as fumigation or pads during cold storage is well known to prevent storage rots in blueberries (Cantín et al., 2012; Rivera et al., 2013a; Rodriguez and Zoffoli, 2016; Saito et al., 2020; Saito and Xiao, 2017). The current study did not investigate the type of rots observed, but studies (Hosking, 2023; Wang et al., 2010) show that the main type of rots are caused due to *Botrytis* sp. and *Colletotrichum* sp. Using SO_2 pads significantly reduced the number of rots in this current study. Cultivar differences with respect to the number of rots were also observed in this study where the EU cultivar was more prone to rots than that of the FB cultivar. Cultivar differences were also observed in previous studies (Cantín et al., 2012; Saito et al., 2020) and were attributed to the varying levels of SSC and TA contents of the berries.

The reduced number of rots due to SO_2 pads was indeed a significant marketing benefit but also had a significant disadvantage in the form of bleaching. Bleaching was observed on all storage timings throughout the duration of the study indicating that the SO_2 loading from the pads was in excess for the two cultivars in question. Saito and Xiao (2017) observed that the SO_2 pads in their study released 166 µL/L of SO_2 within the first 24 h in cold storage causing significant bleaching. Saito et al. (2020) observed significantly reduced bleaching injury when the concentration was maintained at 10 µL/L of SO_2 in southern highbush blueberries and this concentration could still significantly reduce rot incidence.

Although SO_2 concentrations were not measured in this current study; it is assumed that either these two modern cultivars are more sensitive to the SO_2 levels used, or may be the dosage from the pads used was significantly higher than that used in previous studies. It is also possible that SO_2 from the pads was released relatively faster than expected at the storage temperature used. Hence, future studies using EU and FB cultivars should focus on

sourcing SO_2 pads of lower dosage and/or investigate the SO_2 release patterns at storage temperatures of <2°C. In its current dosage, the use of SO_2 pads was a fatal flaw for both cultivars – causing bleaching to the berries.

4.5 Conclusion and recommendations

MAP can significantly reduce WL during storage, and while in combination with SO_2 pads, was successful in reducing rot incidence. However, the SO_2 pads used in this study caused bleaching damage, probably due to the sensitivity of the current cultivars to SO_2 and/or the levels of SO_2 generated over the storage period. Temperature plays a key role in the release of SO_2 gas from the pads and the storage temperature of 2°C may have accelerated its release in the current study. The effect of these SO_2 pads should be further investigated at lower temperatures (~0°C) and controlled humidity (90-95%). Future studies should focus on creating SO_2 pads with a slower release rate and/or lower amount of active ingredient, customised for these new cultivars.

MAP did not provide a significant firmness benefit and its potential benefit in reducing rots could not be individually assessed. Refinements to the MAP bags should be investigated to match the respiration of the product with the permeation rates of the packages. Using a polyliner in controlling WL could be cheaper and efficient option and hence should be assessed either individually or in combination with SO_2 pads. Future studies should also focus on investigating the effect of SO_2 pads and/or other rot inhibitors (Chiabrando and Giacalone, 2011).

Chapter 5 Conclusion and recommendations

Blueberries can be stored up to 2-4 weeks with an acceptable quality by storing the fruit at 0°C and 90-95% RH (Kader, 2003; Perkins-Veazie, 2016). Disruptions in the temperatures during postharvest handling and storage can cause excessive moisture loss, firmness loss, shrivelling, and increase in rots. Despite the importance of lower temperatures during storage, New Zealand's blueberries are coolstored at 2°C post-packing (Alan Mclean, per. comm.). Moreover, the blueberry punnets are packed with approximately 1-2% extra weight at-packing to account for the WL during storage and handling. It is therefore important to understand if further refinements to the current coolchain can be employed.

Novel blueberry cultivars such as Eureka (EU) and First Blush (FB) have been imported to New Zealand and are picked and packed together and commercially sold in the same punnet. Novel cultivars can have different storage potential due to differing attributes such as respiration rate, skin thickness, wax content, stem scar size, among other factors. Whether these cultivars should be picked and packed separately based on these attributes, require further investigation.

Two experiments were conducted with a primary focus on modes of failure including moisture loss (dehydration), softening and rots during storage.

- Assess the effect of four storage temperatures on moisture loss and firmness during storage (Chapter 3).
- Assessing the effect of packaging technologies (MAP and SO_2 pads) alongside standard packaging in standard storage conditions on moisture loss and firmness during storage (Chapter 4).

This current study, for the first time, reports the effect of different storage temperatures on the EU and FB cultivars. When stored at four storage temperatures, WL increased over time with highest WL (3.8-6.1%) at 3.5°C and lowest (1.8-4.3%) at 0.5°C. Cultivar differences were observed with relatively higher WL in the EU cultivar. Firmness at 0.5-1.5°C after 28 days was higher than the at-harvest firmness for both cultivars. Rot incidence was numerically higher at 3.5°C but statistically could not be verified.

Previous studies (Forney et al., 2022; Paniagua et al., 2013; Rivera et al., 2022b) have reported an increase in firmness (firming effect) that was also observed in both cultivars and at all storage temperatures in this current study. These studies also alluded that firming effect is more pronounced at lower (0-2°C) storage temperatures and shorter storage periods (0-21 days). In this current study, interestingly, firming had a strong influence throughout the storage period in both cultivars at 0.5°C and 1.5°C. This is an important finding and unique in the case on these novel cultivars because firmness after 28 days in storage was still higher than the at-harvest firmness. Provided that firmness is interlinked to WL, this finding has a greater significance at the relatively higher levels of WL observed. These results demonstrate that both cultivars should be packed separately due to the propensity of the EU cultivar to lose more moisture affecting the quantity of extra weight that is packed conventionally.

Further studies should focus on the reasons of higher WL while maintaining firmness. Quantifying the stem scar, respiration rates and skin permeance can be useful in determining the physiology of WL and firmness changes. The 28-day storage period in this current study simulated the transit period that New Zealand blueberries would experience when being shipped to distant markets. However, extending the storage period at each storage temperature may have helped in elucidating treatment differences (both in terms of rots and firmness) that otherwise were not statistically different during the storage period of 28 days. If this experiment could be repeated, the net effect of differing storage temperature at a constant RH would be beneficial in correlating the findings with vapour pressure deficit and aid in separating cultivar differences.

Using MAP+SO_2 in Experiment 2 could reduce the WL by 7 times compared to the control (6.9-10.9%). Firmness generally did not differ due to cultivars or treatments. The MAP+SO_2 treatment had significantly less rots than the control. Cultivar differences were also observed with higher number of rots in the EU cultivar; however, this was not tested statistically. A more pronounced effect of rot incidence and firmness changes due to treatments could have been elucidated if the storage period was longer than 42 days. The MAP+SO_2 treatment caused SO_2 bleaching to both cultivars observed at all storage timings.

Quantifying the rates of stem scar healing, the thickness and composition of the wax content and host defence mechanisms of these cultivars can provide more insights into their rot incidence. An important consideration, however, is that the 'net effect' of using 'MAP only', 'polyliner only' and 'polyliner + SO_2' in this trial was not investigated and should be considered for future trials. Future studies should focus on comparing the efficacy of using MAP vs a standard polyliner and understand the role of different cultivars and their potentially different respiration rates and its consequent effect on RH within the MAP. This will allow the industry to make a decision if using a cheaper option (i.e., the polyliner only with perforations) could be equally beneficial in reducing WL.

The current configuration of the SO_2 pads is not recommended for these cultivars. Future studies should focus on creating SO_2 pads with a slower release rate and/or lower amount of active ingredient customised for these new cultivars. Future studies should also focus on investigating the effect of SO_2 pads in combination with other rot inhibitors.

Future research should investigate the optimum storage temperatures of other imported cultivars, if those results are similar to the current study, then a combined picking and packing should be performed. Overall efforts should focus on storage temperature of ≤1°C and focus on a consistent coolchain, then the industry can achieve a reduced WL, improvised quality and increase our financial returns by packing more accurately.

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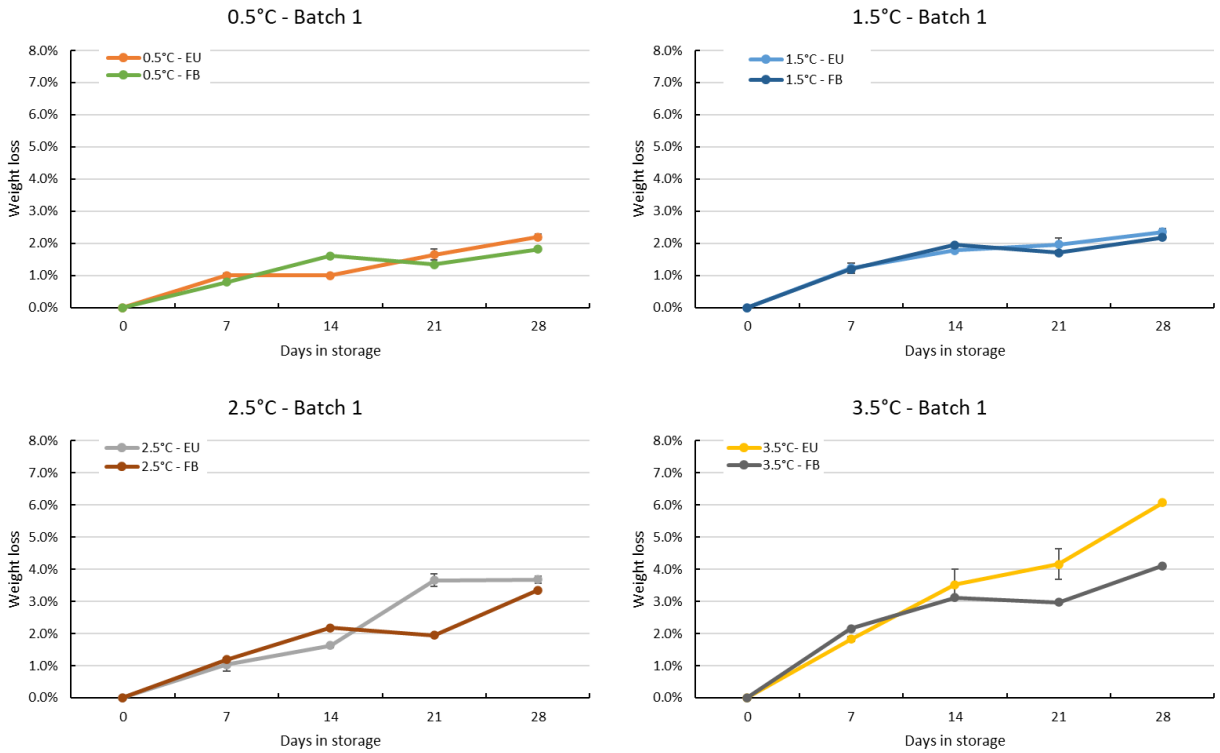
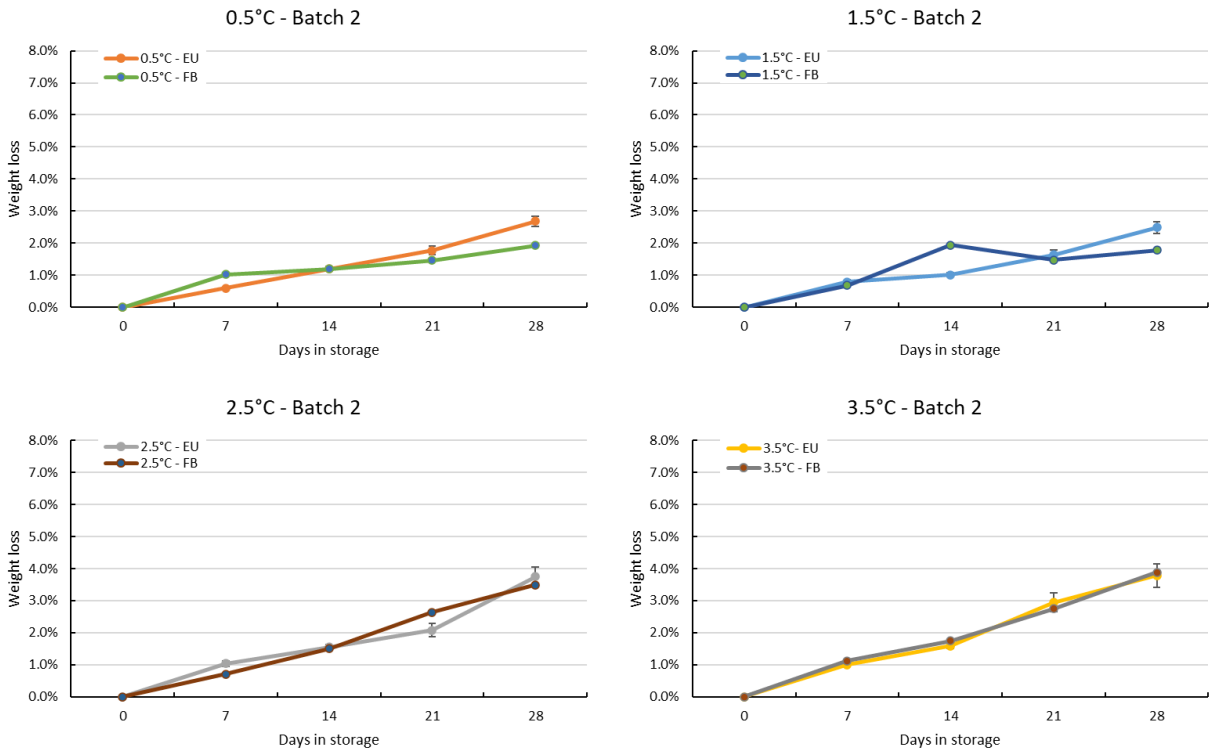
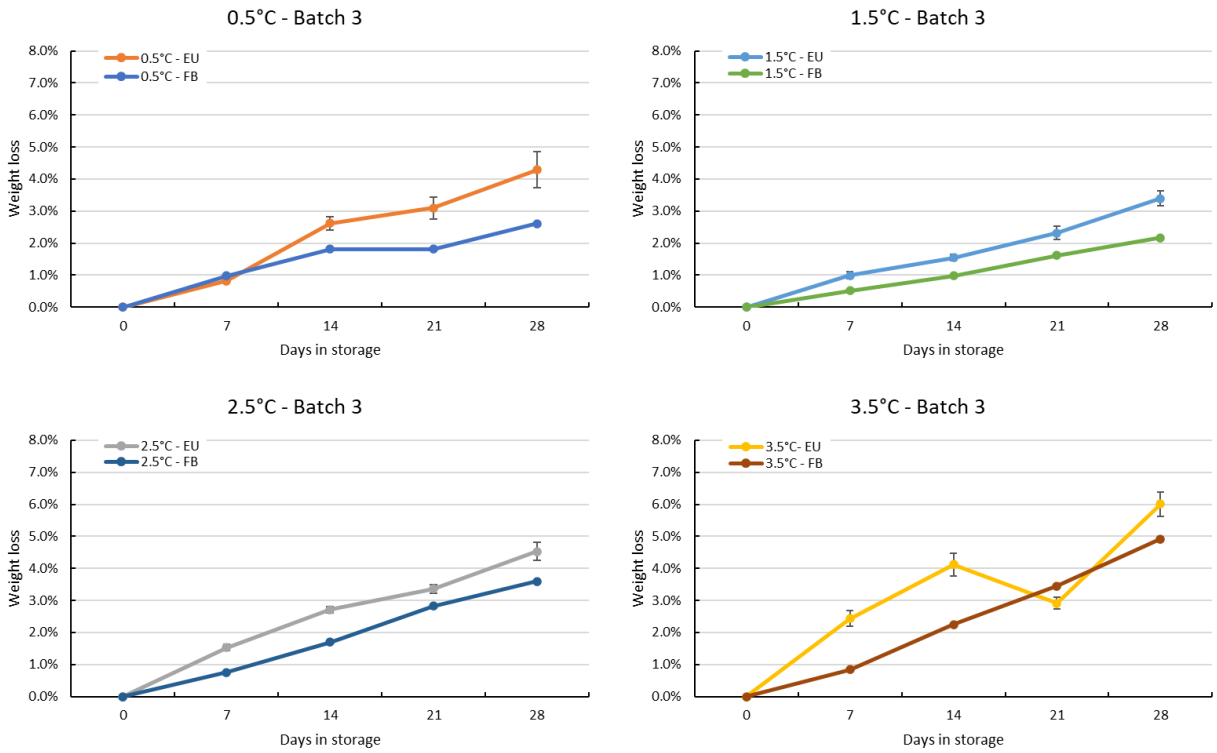
Appendices



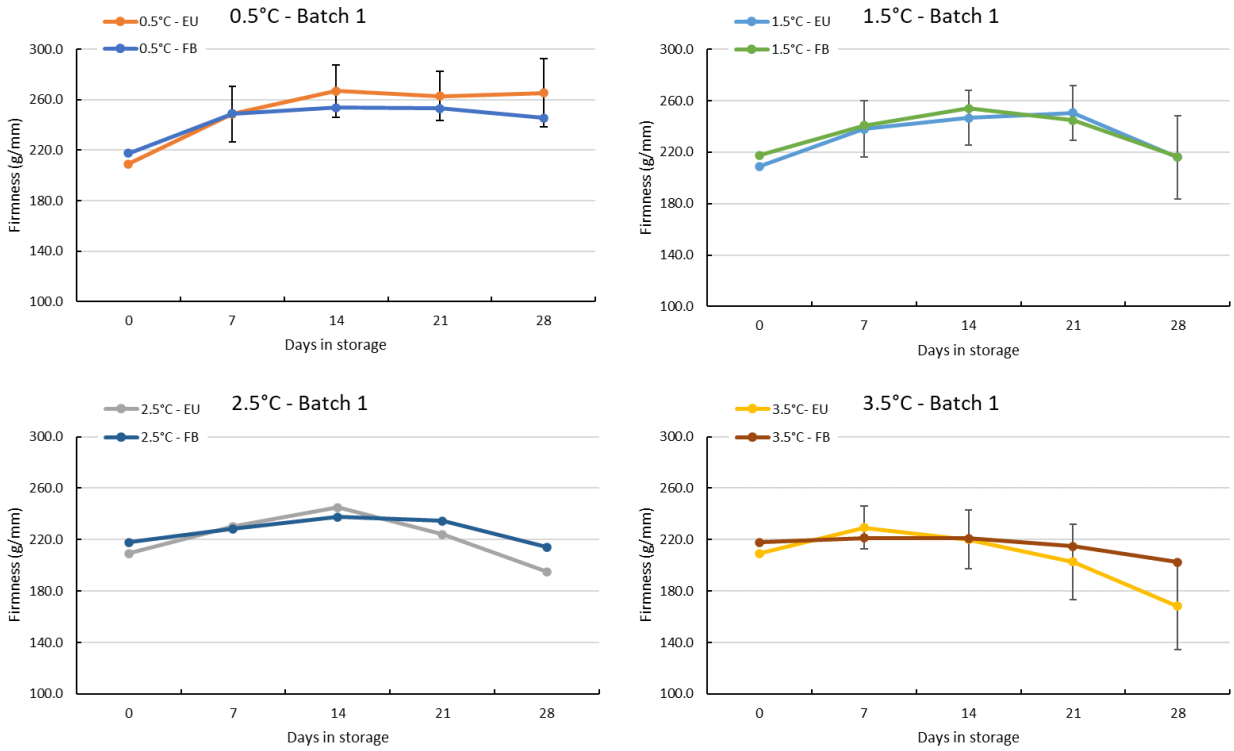
Appendix 1. Weight loss (%) of blueberries in Batch 1 of Chapter 3 due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 6). A datapoint denotes 320-berries.



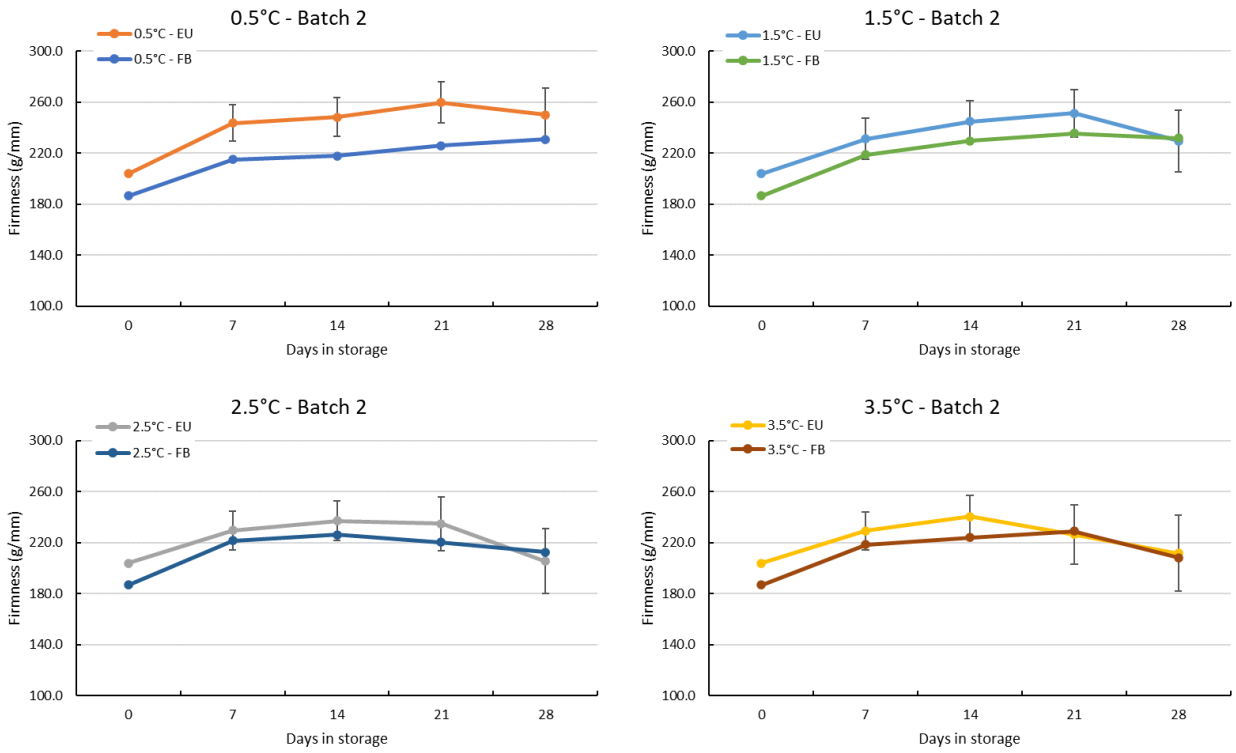
Appendix 2. Weight loss (%) of blueberries in Batch 2 of Chapter 3 due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 4). A datapoint denotes 450-berries.



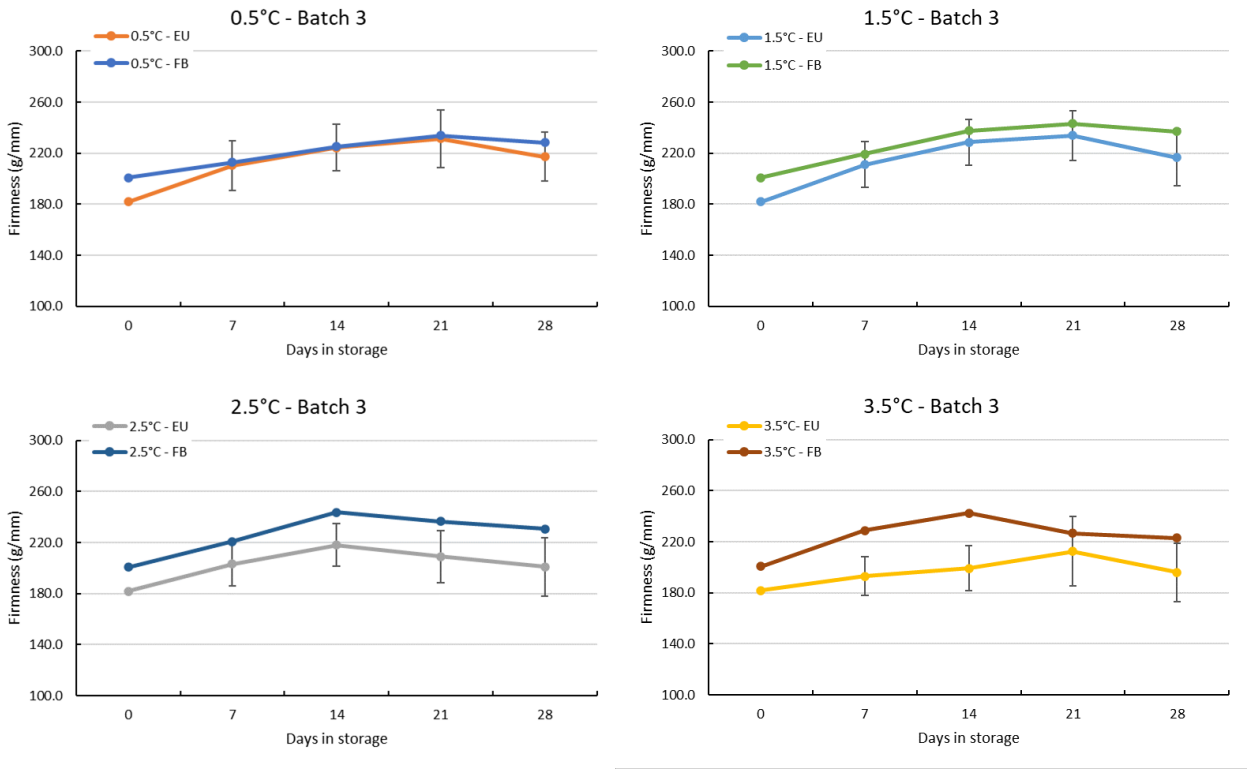
Appendix 3. Weight loss (%) of blueberries in Batch 3 of Chapter 3 due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 4). A datapoint denotes 450-berries.



Appendix 4. Firmness of blueberries in Batch 1 of Chapter 3 due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 6). A datapoint denotes 320-berries.



Appendix 5. Firmness of blueberries in Batch 2 of Chapter 3 due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 4). A datapoint denotes 450-berries.



Appendix 6. Firmness of blueberries in Batch 3 of Chapter 3 due to various storage temperatures over a 28-day storage period. Each datapoint is an average ± SE (n = 4). A datapoint denotes 450-berries.