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INFLUENCE OF TEMPERATURE MANAGEMENT DEFICIENCIES DURING POSTHARVEST ON THE QUALITY OF SEA EXPORTED BLUEBERRIES

A thesis presented in partial fulfilment of the requirements for the degree of

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Andres Cristobal Paniagua Gonzalez

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ABSTRACT

After harvest, fresh blueberries deteriorate rapidly due to fungal decay and softening. Postharvest softening is a major problem for the export industry, although the mechanisms for this softening are not completely understood. In order maintain product quality during the postharvest chain, blueberries need to be rapidly stored at the optimal temperature (0°C) and relative humidity (RH) (90-95%). However, rapid cooling is inhibited by the requirement of packing the product at an intermediary temperature (10 °C). Subsequently during marine export, blueberries are often shipped in reefer containers which are set up at the optimal temperature. Nevertheless, it is not known whether temperature heterogeneity within containers (4°C around the set point) constitutes an important factor affecting quality at the market place. Controlled atmosphere (CA) (8-15% CO_2 combined with O_2 > 1%) is also used in sea exported blueberries as a complementary technology to delay pathogen development, although is not clear what O_2 concentration range delivers the best quality benefits. This thesis investigated the impact of cooling delays and temperature heterogeneity during shipping on the final quality of sea exported highbush and rabbiteye blueberries, as well as the interactions of these factors with CA. In addition, the influence of moisture loss on blueberry softening was investigated as a way to improve the current understanding of this quality defect.

Cooling delays of 12 and 24 h at 10°C, simulating the packing process, were found to impact the quality of blueberries after a subsequent storage period of 6 weeks at 0°C. A delay of at least 12 h considerably increased the total moisture loss of blueberries over 6 weeks storage. However, the incidence of rotten fruit over the period of coolstorage was not affected by delays in cooling at 10°C. Considering that decay is the main factor limiting blueberry postharvest life, this result may place less relevance on accelerating the packing process despite its effect on fruit moisture loss.

A laboratory recreation of temperature heterogeneity of 4°C around the set point, as reported for reefer containers, was shown to affect the quality of blueberries by the end of a coolstorage period. Compared to optimal storage conditions, commercially packed blueberries subjected to 4°C considerably increased (up to 20 fold) the

incidence of rots after 6 weeks storage. Furthermore, this variability of 4°C led to slightly increased moisture loss from blueberries during simulated shipping. This result suggests that environments used during shipping of blueberries should minimise temperature variations below 4°C in order to improve blueberry quality.

A flow-through system was utilised to simulate 3 different storage atmospheres (2.5% O_2 + 10% CO_2, 20% O_2 + 10% CO_2 and air) and assess the effect on blueberry quality during 6 weeks of storage, confirming that CA comprising increased CO_2 concentration provides a clear effect in reducing blueberry decay incidence during storage. In addition, CA was found to slightly improve firmness retention during storage, although this benefit was not large enough to suggest a commercial impact. Moreover, increased O_2 concentrations were able to alleviate the high CO_2-induced softening during storage. The effect of CA on decreasing rot incidence was attributed to the influence of CO_2 on pathogen growth, with no benefits achieved by reducing O_2 concentration. This indicates that the export industry may benefit from improved quality outcomes and lower CA operational costs if O_2 is maintained around 20% and CO_2 increased within recommended ranges.

The gas composition of storage atmosphere influenced the impact of temperature variability within shipping containers on blueberry quality. Controlled atmosphere comprising 10% CO_2 in combination 2.5 or 20% O_2 substantially reduced by up to 50% the effect of temperature on blueberry rot incidence during storage. In contrast, atmosphere composition did not alter the effects of cooling delays on blueberry quality. Therefore, CA seems to provide a valuable protection against temperature deficiencies that occur during marine export.

Finally, an independent experiment was conducted to test the existence of a causal relationship between moisture loss and postharvest firmness for blueberries. Storage conditions were controlled so only the extent of moisture loss varied between treatments. Opposing firmness outcomes were obtained under different weight loss ranges, in addition to a high correlation between both parameters. Furthermore, different water loss patterns for firming and softening were suggested as observed in MRI analysis. This result provides evidence that fruit moisture loss plays a major role in determining firmness responses of blueberries.

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INTRODUCTION

1.1 General introduction

Blueberries are native from North America and have been cultivated since the late 1800s (Strik, 2005). The increasing demand in recent years has transformed blueberries into a major crop consumed worldwide (Brazelton and Strik, 2007). Its high nutritional value and health benefits have led marketing campaigns since 1997, resulting in high stability and good prices for fresh and processed markets (Howard and Hager, 2007; Strik, 2007).

World plantations of blueberries increased by 90% from 1995 to 2005, with 43,218 ha in 2005 producing 194,800 tons (Strik, 2007). While North America produces more than 70% of the world production of blueberries (Strik, 2005), growers from the southern hemisphere supply northern countries during the off-season (Bañados, 2006). South America and Oceania count for 9% and 1.8% of the global production, respectively (Strik, 2007), with Chile as the largest exporter of the southern hemisphere with 44,000 tonnes in 2010 (Salvo *et al.*, 2011).

Commercially grown cultivars of blueberry used for fresh market correspond to rabbiteye (*Vaccinium ashei* Reade) and highbush (*Vaccinium corymbosum* L.) species, whereas lowbush (*Vaccinium angustifolium* Ait.) blueberries are oriented for food processing (Du *et al.*, 2011). A large amount of cultivars are available for blueberry growers, with high variability of growing requirements and postharvest behaviour (Strik, 2007). Rabbiteye cultivars are generally smaller, have more seeds and thicker epidermis than highbush fruit, which have larger stem scars and tend to soften quicker during postharvest (Makus and Morris, 1993).

Blueberries deteriorate rapidly due to decay, softening and shrivelling (Forney, 2009). Acceptable quality can be maintained for up to 2 and 4 weeks in highbush and rabbiteye cultivars, respectively, by storing the fruit at 0°C and 90-95% relative

humidity (RH) (A. Kader, 2003; Perkins-Veazie, 2004). Further postharvest life extension of blueberries is achieved by using controlled atmosphere (CA) (Ehlenfeldt, 2002), which is being increasingly preferred for exports from the southern hemisphere (Bañados, 2006).

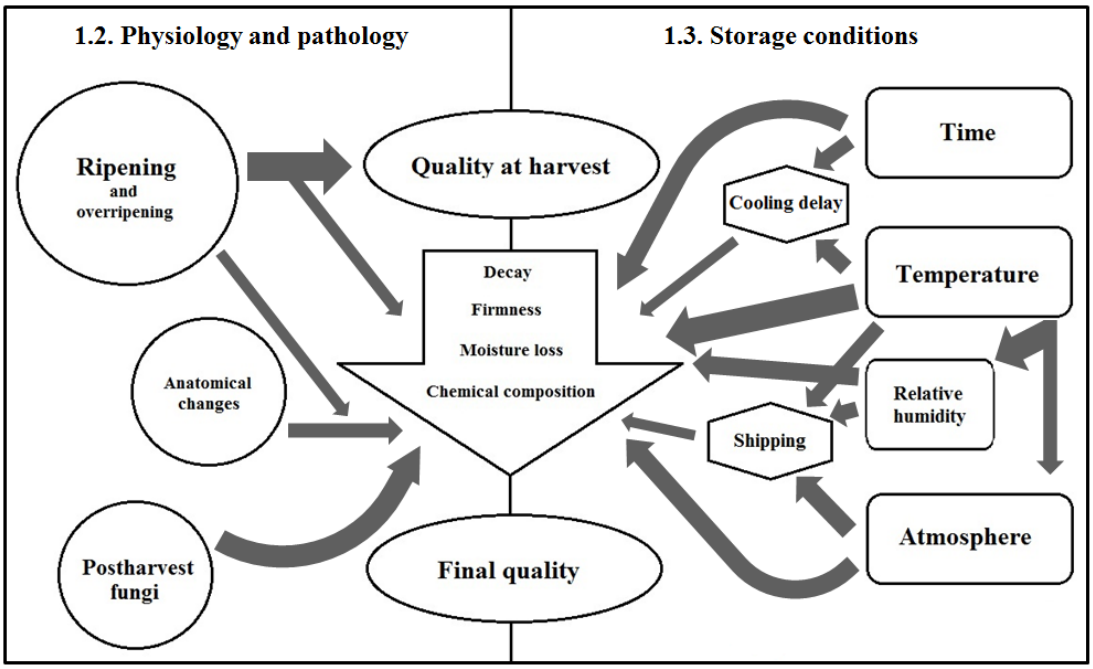

Figure 1-1. Conceptual diagram of the literature review. The physiological, physical and pathological processes defining blueberry quality are discussed in section 1.2. Section 1.3 details the effects storage factors on blueberry quality evolution, with a focus on marine export. The impact of each effect is represented by the arrow width.

Temperature management after harvest is very important to maintain the quality of fresh blueberries (Forney, 2009). It is known that extended periods between harvest and cooling (cooling delays) enhance blueberry deterioration (Thompson *et al.*, 2001). Furthermore, storage temperature higher than optimum reduce considerably the postharvest life of this commodity. Therefore, the temperature variability within reefer containers (Tanner and Amos, 2003), and its effect on RH distribution may lead to blueberry quality loss, though this question has not been addressed so far.

Beneficial CA effects on blueberry quality rely on its fungistatic properties, whereas inappropriate gas concentrations trigger physiological damage (Ehlenfeldt, 2002). CA is successfully used for marine exports of fresh blueberries to distant markets.

However, internal gas concentrations in blueberry are affected by temperature, which modifies skin resistance, gas solubility and metabolic rate (Beaudry *et al.*, 1992).

The main focus of this research is to evaluate the possible interactions between temperature management deficiencies during blueberry postharvest and the storage atmosphere, in terms of blueberry quality outputs. The influence of container temperature variability on CA efficacy and risk of phytotoxicity, as well as the effects of atmosphere on temperature deficiencies impact over blueberry quality are assessed in this work, with a focus on the marine export process. The literature review details and discusses the postharvest physiology of blueberry and the quality responses of this fruit against storage conditions, in order to identify research questions and to provide the necessary background for the further experimental analysis (Figure 1-1).

1.2 Physiology and pathology of blueberry fruit

1.2.1 Changes during ripening

After growth, fruit undergo the important biochemical and structural changes of ripening, which is considered the first step of senescence as it is comprised mostly of degradative steps (Wills *et al.*, 2007). Among major modifications occurring during ripening are skin colour development, variations in chemical composition, textural changes and production of aroma (Rhodes, 1970). The plant hormone ethylene often plays an important role in regulating ripening related changes, whereas respiration rate can accelerates the rhythm of these modifications in some species (Wills *et al.*, 2007). Fruits are classified as climacteric and non-climacteric according to the presence of a respiration rate peak (climacteric) and an autocatalytic phase of ethylene production during ripening (Rhodes, 1970). To understand the physiological behaviour of fresh produce is a primary step for further analysis of its life span management. Therefore, the major physiological changes occurring in blueberry fruit during ripening are reviewed in this section in order to inform the research.

1.2.1.1 Respiration and ethylene

Early studies describing the respiratory behaviour of blueberry fruit were inconsistent in detecting a climacteric rise of respiration rate during ripening. While in the first attempt to describe the respiration pattern of blueberry a climacteric peak was reported (Bergman, 1929), later studies failed to detect this respiration rise at ripening (Forsyth, 1969; Frenkel, 1972), generating a controversy in terms of the respiratory behaviour of these species (Windus *et al.*, 1976). Further experiments consistently provided strong evidence of the presence of a respiratory peak during the ripening of lowbush (Ismail, 1969), highbush (Windus *et al.*, 1976) and rabbiteye (Shimura *et al.*, 1986) blueberries which supports the classification of blueberry as a climacteric fruit (Mitcham *et al.*, 2011; Perkins-Veazie, 2004). The exact ripening stage at which the respiratory peak develops varies between blueberry species and cultivars, but consistently the climacteric rise has been reported to happen when fruit is at pink red stage in lowbush blueberry (Ismail, 1969), green in rabbiteye blueberry (Shimura *et al.*, 1986), and between green and pink red in highbush blueberries (Figure 1-2) (Bergman, 1929; Ismail, 1969; Shimura *et al.*, 1986; Windus *et al.*, 1976).

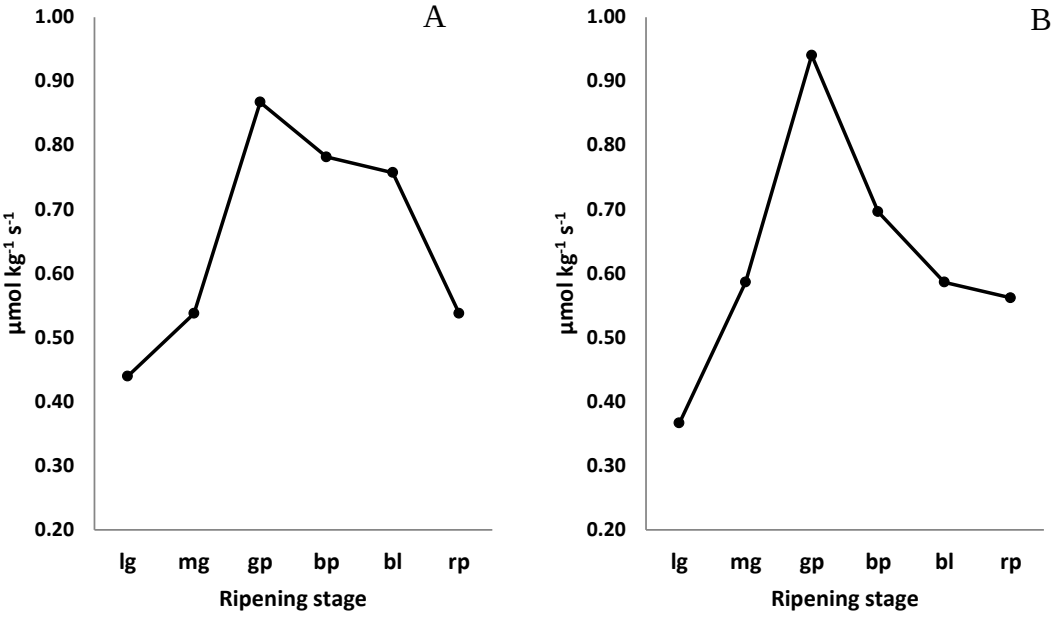

Figure 1-2. Respiration rate of highbush blueberry fruit cultivar Lateblue (A) and Bluecrop (B) at different ripening stages, measured at 23°C as rate of CO_2 production. Ripening stages are indicated by skin colouration as light blue (lg), mature green (mg), green pink (gp), blue pink (bp), blue (bl) and blue ripe (rp). Adapted from Windus *et at.* (1976)

Ethylene production also increases during blueberry ripening, presenting a sharp peak simultaneously or just before the climacteric at the transition between green and pink colouration (Figure 1-3) (El-Agamy, 1982; Lipe, 1978; Shimura *et al.*, 1986; Suzuki *et al.*, 1997a; Windus *et al.*, 1976). This pattern confirms the existence of an autocatalytic phase of ethylene production, supporting the characterisation of blueberry as a climacteric fruit. Additionally, a small secondary ethylene peak has been reported at dark blue stage in some cases (El-Agamy, 1982; Shimura *et al.*, 1986), although not consistent among cultivars (Shimura *et al.*, 1986). Ethylene production rate at 18-20°C varies from 6.11 to 24.43 ρmol kg^{-1} s^{-1} in highbush blueberries (Suzuki *et al.*, 1997a), to 122.15 ρmol kg^{-1} s^{-1} in rabbiteye blueberries (El-Agamy, 1982).

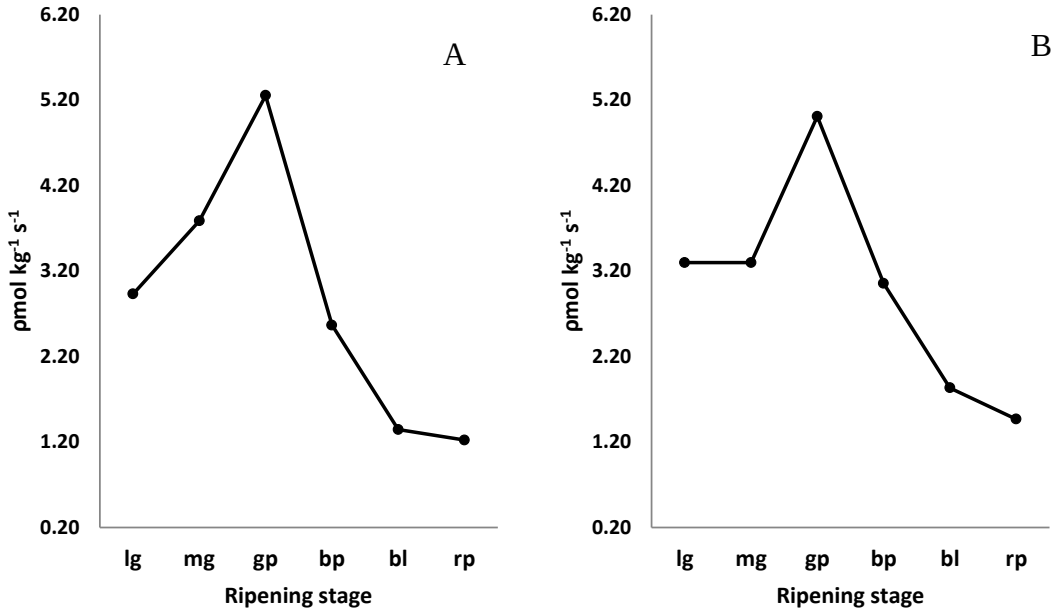

Figure 1-3. Rates of ethylene production of highbush blueberry fruit cultivar Lateblue (A) and Bluecrop (B) measured at 23°C in different ripening stages. Ripening stages are indicated by skin colouration as light blue (lg), mature green (mg), green pink (gp), blue pink (bp), blue (bl) and blue ripe (rp). Adapted from Windus *et at.* (1976)

Treatments regulating ethylene production have the potential to alter the quality attributes of blueberry depending on the ripening stage of the fruit at the moment of the treatment. Applications of 2-chloroethylphosphonic acid (i.e. ethephon) and 1-aminocyclopropane (ACC), precursors of ethylene, at preharvest on unripe blueberries accelerated ripening by stimulating ethylene synthesis, total solid concentration, anthocyanins and colour development, and decreased firmness and

acidity (Ban *et al.*, 2007; Forsyth *et al.*, 1977; Suzuki *et al.*, 1997b). However, inhibition of ethylene synthesis by applying aminoethoxyvinylglycine (AVG) or perception by using 1-methylcyclopropene (MCP) on ripe blueberries after harvest have not affected levels of total solids, titratable acidity, firmness or anthocyanin content during storage for 9 weeks (Chiabrando and Giacalone, 2011; DeLong *et al.*, 2003; Graddick, 1986). Accordingly, evidence is clear indicating that ethylene has little influence on blueberry quality attributes at postharvest since blueberries are harvested at fully ripe stage, where most biochemical changes have been induced and almost completed.

1.2.1.2 Skin colour evolution

Skin colour of blueberries evolves from green to blue during ripening (Bergman, 1929; Ismail, 1969), correlating positively with an increase in the total soluble solids to acidity ratio (Ballinger and Kushman, 1970). As such, berry colour is used as the most common harvest maturity indicator by industry, with 100% of blue colouration used (Mitcham *et al.*, 2011; Perkins-Veazie, 2004). The green colour of unripe blueberries is due to a high chlorophyll content whereas anthocyanins accumulate throughout the ripening period generating rose and purple hues (Ballinger *et al.*, 1970). Anthocyanins contributing to skin colour are located in the epidermal and hypodermal cells, being synthesized from the calyx towards the stem end of the fruit (Ballinger *et al.*, 1972). Anthocyanins continue being produced after harvest, increasing the anthocyanin content by 55% after 21 d of storage at 5°C (Mitcham, 2007), which enables blueberries not harvested at full blue stage to develop colour during storage (El-Agamy, 1982).

Colour of blueberries is influenced by total anthocyanin content (Kushman and Ballinger, 1975), distribution of individual anthocyanins (Ballinger and Kushman, 1970) and epicuticular waxes (Albrigo *et al.*, 1980; Sapers *et al.*, 1984). Once ripe, the quantity and the structure of the waxes generates different blue colour intensities, rather than other factors such as total or individual anthocyanin content or pH (Sapers *et al.*, 1984). High amounts of wax structures deposited as rodlets and upright platlets (classified mainly as β-diketones) are responsible for blue and light

blue cultivars, whereas in dark blue and black cultivars flat platelets and annealed patches of wax are the predominant structures (Albrigo *et al.*, 1980; Sapers *et al.*, 1984). Lighter blue tones are derived from the light scattering properties of upright wax structures (Sapers *et al.*, 1984). During ripening the conformation of the blueberry epicuticular waxes undergoes structural modifications, with the proportion of upright rodlets decreasing in relation to flat platlets, resulting in a darker colouration at the overripe stage (Albrigo *et al.*, 1980). Consequently, only ripe blueberries are able to express the characteristic tones of each cultivar since at this stage the epidermis is completely covered by anthocyanins and the characteristic proportion of wax structures has been developed.

1.2.1.3 Synthesis of volatiles

According to Saftner *et al.* (2008), flavour is one of the most relevant quality attributes for consumers in blueberries, often considered more important than texture and appearance. Major volatile compounds found on blueberries correspond to alcohols, ketones, aldehydes and terpenoids (Du *et al.*, 2011), although the volatile profile varies among blueberry species and cultivars. Esters such as trans-2-hexenal, trans-2-hexenol, cis-3-hexenol and terpene alcohols such as linalool, citronellol, nerol and geraniol are most common compounds found in highbush blueberry (Hirvi and Honkanen, 1983a; Parliment and Kolor, 1975), while among the volatiles detected in rabbiteye blueberry are ethylacetate, trans-2-hexenol, heptanol, cineralone, carveol and eugenol (Horvat and Senter, 1985). From more than 50 volatile compounds detected in different blueberry genotypes, only a few have been identified as contributing to aroma and flavour (Simon *et al.*, 1996). Trans-2-hexenal, trans-2-hexenol, cis-3-hexenol, linalool, and geraniol are consistently reported as the main contributors (Du *et al.*, 2011; Horvat and Senter, 1985; Parliment and Kolor, 1975). These volatile compounds are known to vary throughout ripening largely as a result of cell membrane degradation (Dudareva *et al.*, 2006). Linalool and geraniol increase their concentration during ripening up to blue colour stage and then subsequently decrease, whereas trans-2-hexenal and trans-2-hexenol are high when blueberries are unripe and reach minimal levels when fully ripened (Horvat and Senter, 1985).

1.2.1.4 Chemical composition changes

Glucose and fructose are the most abundant sugars in ripe blueberries, being found at equal proportions and in a concentration of approximately 70 mg g^{-1}, whereas sucrose is present below 10 mg g^{-1} (Darnell *et al.*, 1994; Hirvi and Honkanen, 1983b; Kader *et al.*, 1993). This is a result of an increasing activity of invertase during ripening, which degrades sucrose into glucose and fructose (Kader *et al.*, 1993). As in most fruits, total sugars measured as sugar content or total soluble solids increase during blueberry ripening (Ayaz *et al.*, 2001; Ballinger and Kushman, 1970; Kushman and Ballinger, 1968), whereas during storage ripe blueberries kept at low temperature (i.e. 0°C to 5°C) for up to 3 weeks do not vary their total soluble solid content (Chiabrando and Giacalone, 2011; Prange *et al.*, 1995; Schotsmans *et al.*, 2007; Smittle and Miller, 1988).

Citric, quinic, malic and chlorogenic acids are the main organic acids found in blueberries, with citric acid present at higher concentrations (Ayaz *et al.*, 2001; Kushman and Ballinger, 1968; Markakis *et al.*, 1963). Although the concentration of most of these acids do not vary throughout ripening, citric acid does decrease strongly up to ripe stage which produces an overall decline of total acidity during fruit ripening (Kushman and Ballinger, 1968; Markakis *et al.*, 1963). However, the titratable acidity of fully ripened blueberries shows an increasing trend during refrigerated storage (Chiabrando and Giacalone, 2011; Prange *et al.*, 1995; Schotsmans *et al.*, 2007; Smittle and Miller, 1988).

Blueberries have become popular due to their high concentration of phytochemicals which are believed to be health promoting (Giusti and Jing, 2007). Among these compounds, polyphenols are considered the most important group, with anthocyanins, procyanidins and chlorogenic acid the main phytochemical compounds. The levels of these phytochemicals vary between blueberry species, cultivars and in some cases during ripening. Total anthocyanin content is in the range of 0.2-2.7 mg g^{-1} and 0.1-5.2 mg g^{-1} in highbush and rabbiteye blueberry, respectively (Howard and Hager, 2007). More than 25 individual anthocyanins are present in different concentrations among blueberry genotypes (Kalt *et al.*, 1999b). Concentrations of procyanidins and chlorogenic acid in blueberry fruit reach the

range of 1.8-3.3 mg g^{-1} and 0.3-1.6 mg g^{-1}, respectively (Gu *et al.*, 2002; Howard and Hager, 2007). Moreover, whereas chlorogenic acid remains unchanged during ripening, anthocyanin concentrations increase as blueberry ripen and procyanidins decrease from unripe to ripe stage (S.Y. Wang, 2007).

1.2.1.5 Texture modifications at ripening

The main textural properties related to fruit quality are firmness, crispness and juiciness, among which firmness is considered a major quality attribute (Harker *et al.*, 2010). Modifications of the cell wall components cellulose, hemicellulose and pectin during ripening are associated to fruit softening in many species (Brummell, 2006; Jarvis *et al.*, 2003). For blueberries, firmness is an important quality property which limits product marketability (Forney *et al.*, 1998; Prussia *et al.*, 2006; Slaughter and Rohrbach, 1985). The cell wall changes involved in blueberry softening at ripening are discussed in the present section, while the different factors affecting the firmness of this commodity at postharvest are reviewed in section 1.2.3.

Depolymerisation, solubilisation and loss of sugar side chains in major cell wall components are associated with fruit softening (Brummell, 2006). Solubilisation of sugar arabinose from pectin and hemicellulose, and hemicellulose depolymerisation have been found to increase during blueberry ripening from green to blue stage, whereas pectin solubilisation has been detected to occur from unripe stage towards 75% blue stage (Figure 1-4) (Proctor and Peng, 1989; Vicente *et al.*, 2007). However, according to Angeletti *et al.* (2010), sugar loss and pectin solubilisation have been detected to continue in ripe blueberries during storage. Even when no pectin depolymerisation was detected in these studies the activities of enzymes pectinmethylesterase and endopolygalacturonase, both involved in pectin breakdown, were observed to peak at red and purple stage, respectively (Proctor and Miesle, 1991), suggesting the possibility for late pectin depolymerisation to occur at overripe stage. Nevertheless, blueberry firmness has been shown to decrease progressively up to blue stage concurrently with cell wall modifications during ripening, with no further decline at blue ripe stage (Vicente *et al.*, 2007). These results agree with previous studies showing firmness decreases as blueberry ripen from green to purple

stage, with subsequently little change from this point to overripe stage (Ballinger *et al.*, 1973; Proctor and Miesle, 1991). Therefore, evidence suggests that blueberry softening during ripening would be mainly associated with arabinose loss and hemicellulose depolymerisation, and possibly to pectin depolymerisation to a minor degree at overripe stage. In fact, arabinose loss and depolymerisation of hemicelluloses and pectins have been previously associated with fruit softening in peaches (Brummell *et al.*, 2004), apples (Pena and Carpita, 2004) and tomatoes (Brummell, 2006), respectively. While the influence of arabinose loss on fruit softening would be explained by weakening of the pectin network, pectin and hemicellulose depolymerisation affect the structure of the cell wall directly, decreasing mechanical resistance of cells (Brummell, 2006).

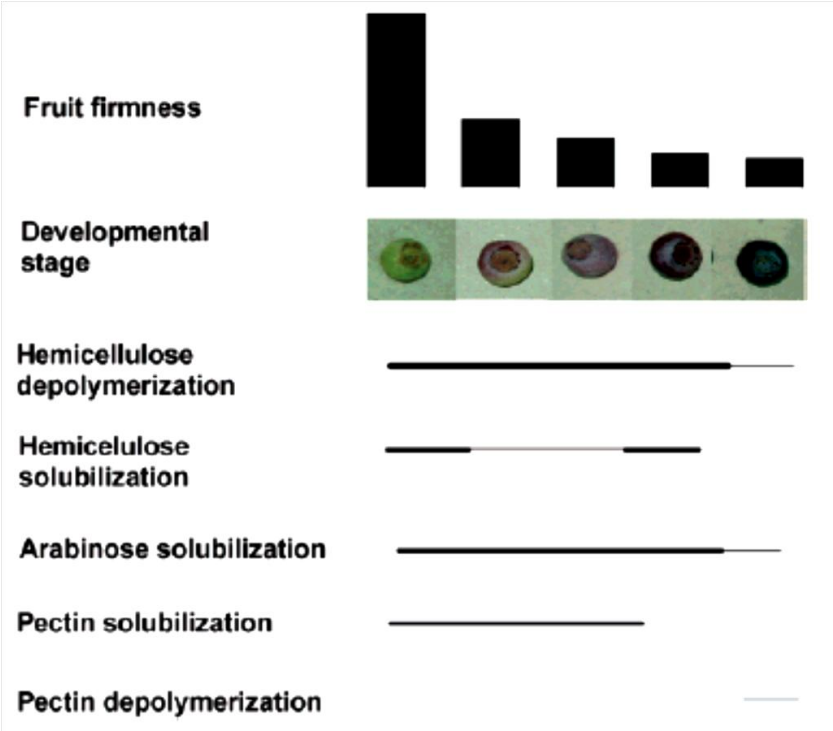

Figure 1-4. Evolution of cell wall modifications and firmness throughout different fruit skin colourations (indicating ripening stages) of blueberry. Reproduced from Vicente *et al.* (2007)

1.2.2 Fruit anatomy affecting moisture loss

Water is the main component (80 to 90%) of fresh commodities (Wills *et al.*, 2007). Moisture loss can generate product shrivelling which may affect their appearance, texture, organoleptic value and consumer acceptability (Ku *et al.*, 2000; Wills *et al.*,

2007). In experimental conditions, the loss of water is normally quantified as weight loss. For blueberries, 5% to 8% of weight loss has been indicated as the limit where fruit become unsalable due to excessive shrivelling (Forney *et al.*, 1998; Sanford *et al.*, 1991). Furthermore, moisture loss from fresh products incurs a direct financial penalty due to decrease of saleable weight (Wills *et al.*, 2007). In this section, the main anatomical features of blueberries affecting their water loss are discussed, in order to inform this research. The influence of environmental variables on fresh produce water loss is not covered here, being reviewed in section 1.3.1.

Blueberry fruit are true berries, with a round to oblate shape and varying size of up to 25 mm of equatorial diameter (Gough, 1994; Strik, 2007). Blueberries do not have lenticels, although some stomata are found concentrated in the calycinal cavity in a range of 27 to 91 stomata per mm2, depending on the cultivar (Vega *et al.*, 1991). Blueberries are covered by a single layer of epidermal cells and a cuticle which develops a waxy coat (known as bloom) during fruit growth (Figure 1-5) (Fava *et al.*, 2006; Gough, 1994). At the blossom end blueberries present a ring shaped scar as a product of corolla and style abscission (i.e. calyx scar) whereas at the stem end blueberries have a picking scar (i.e. stem scar) since they are harvested without peduncle (Figure 1-5) (Ehlenfeldt, 2002; Gough, 1994). Moreover, an important characteristic affecting the moisture loss from blueberries is the area to volume ratio, which correlates positively with increased weight loss (Makus and Morris, 1993; Vega *et al.*, 1991).

The stem scar is a primary pathway for moisture loss from blueberries during their postharvest handling and storage (Albrigo *et al.*, 1980; Ehlenfeldt, 2002; Moore, 1965). The stem scar exposure allows water evaporation (Wills *et al.*, 2007), and according to Perkins-Veazie *et al*. (1995b), its diameter correlates positively with weight loss in blueberry fruit at storage. The diameter of the stem scar varies between cultivars and species, being approximately 0.7-1.2 mm in rabbiteye and between 1.3-2.2 mm in highbush (Perkins-Veazie *et al.*, 1995b). Mainland (1995) reported that a period of delayed cooling of 8 h at 18°C prior to storage stimulates stem scar curing and decreases decay, although its effect on moisture loss during subsequent storage was not reported.

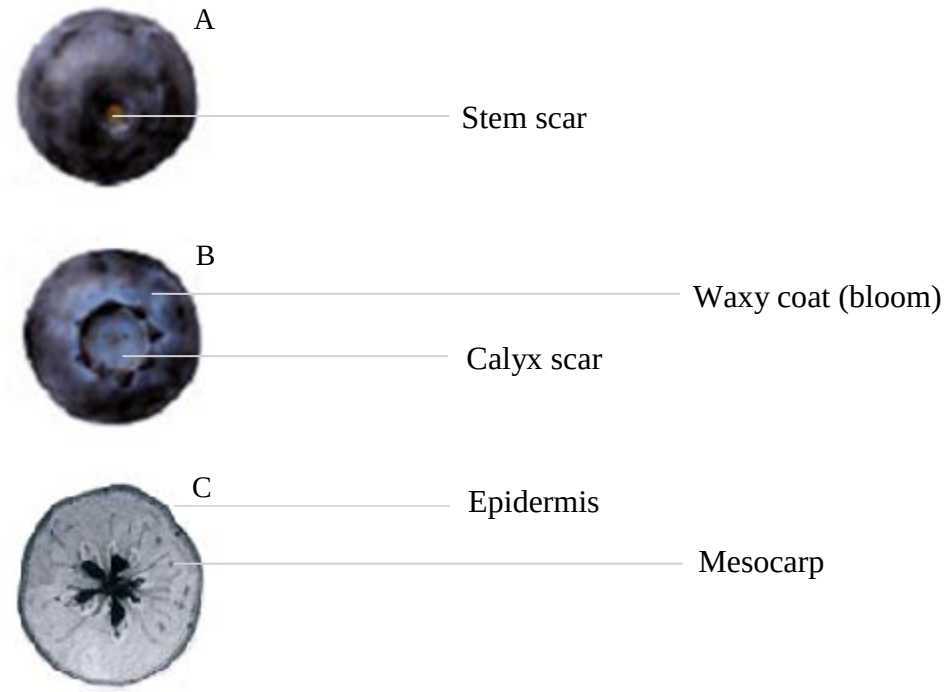

Figure 1-5. Anatomic characteristics of blueberry shown in an intact ripe fruit (A, B) and in a transverse section (C). Adapted from Gough (1983)

The cuticle is the main physical barrier regulating the transpiration of aerial plant organs used as commodities (Wills *et al.*, 2007). The cuticle forms a continuous thin film over the epidermal cells, and is composed of polymer cutine in association with intracuticular and epicuticular waxes (Riederer and Schreiber, 2001). For blueberries, the cuticle has a thickness of 5 μm and contains important epicuticular waxes contributing to bloom (Freeman *et al.*, 1979; Vega *et al.*, 1991). Freeman *et al.* (1979) reported β-diketones, triterpenoids and primary alcohols as the main wax types, with β-diketones representing up to 62%. The same study identified paraffins, fatty acids and primary alcohols as the major intracuticular waxes in blueberry cuticle. Wax conformation of blueberry fruit comprises a layer of flat patches above which are arranged vertical rodlets and upright platelets (Sapers *et al.*, 1984).

Water loss through the cuticle is considered a form of transpiration since it involves a physical resistance (Taiz and Zeiger, 2006). During this process, water first diffuses as a liquid across the cuticular matrix to be subsequently evaporated in a gaseous phase (Kerstiens, 1996). As such, epicuticular waxes play an important role in the control of moisture loss from blueberries (Albrigo *et al.*, 1980; Vega *et al.*, 1991).

Albrigo *et al.* (1980) reported that ripe blueberries with 43% more total waxes than overripe fruit, resulted in 68% less weight loss after 48 h at room temperature. In this study, weight loss decreasing was specifically associated with high concentrations of vertical structures and paraffin content. Likewise, overripe blueberries which have reduced proportion of upright structures tend to lose more water than ripe and unripe fruits (Albrigo *et al.*, 1980). On the other hand, no relationship has been found between wax structural features in ripe blueberries across several highbush cultivars and their weight loss during storage (Vega *et al.*, 1991).

1.2.3 Postharvest firmness

For blueberries, the softening induced by ripening is concentrated up to fully ripe stage, as discussed in section 1.2.1. However, firmness in blueberries has also been associated with other fruit features such as moisture loss (Forney *et al.*, 1998), skin toughness and presence of stone cells (Allan-Wojtas *et al.*, 2001; Bunemann *et al.*, 1957). Since blueberries are harvested fully ripe, firmness evolution at postharvest may be influenced by a combination of factors rather than restricted just to cell wall changes, as is often inferred. Postharvest softening is one of the major problems for the blueberry industry (Slaughter and Rohrbach, 1985), and therefore to review the aspects affecting firmness during storage becomes essential to understand the postharvest behaviour of this commodity.

1.2.3.1 Relationship between water loss and firmness

Water provides physical support to plant tissues since it is attracted into cells producing turgor pressure within the plasma membrane which is contained by the cell wall (Taiz and Zeiger, 2006). Water loss affects textural attributes of many fruits and vegetables, by decreasing product firmness and crispness due to loss of turgor (Ku *et al.*, 2000; Paull, 1999; Wills *et al.*, 2007). For blueberries, turgor loss has been suggested to induce softening during storage, although this parameter has not been directly evaluated (Allan-Wojtas *et al.*, 2001; Chiabrando and Giacalone, 2011; Forney *et al.*, 1998). Nevertheless, weight loss during postharvest has shown

interesting correlations with blueberry firmness. Tetteh *et al.* (2004) found that different weight loss magnitudes from 1% to 9% correlated with higher firmness loss from 3% to 33% in ripe rabbiteye blueberries (cv. Tifblue) during 4 d of storage at 4, 21, 27 and 32°C, whereas increasing weight loss from 4% to 7% in rabbiteye blueberries (cv. Bonita) stored for four weeks at 1°C correlated with decreasing firmness from 1.28 N to 0.65 N (Ferraz *et al.*, 2001).

Very low levels of moisture loss seem to generate blueberry firming rather than softening. Miller *et al.* (1993) obtained an increase of sensory evaluated firmness in ripe highbush and rabbiteye blueberries when weight loss was lower than 1% after 3 weeks of storage at 1°C, while weight loss from 4-5% correlated with berry softening in the same study. Similarly, weight loss of 1% and 2% in highbush blueberries (cv. Burlington) correlated with 50% and 80% of fruit firming, respectively, after 3 to 9 weeks of storage at 3°C, whereas weight loss from 4-14% showed a trend to induce fruit softening (Forney *et al.*, 1998). Explanations for the observed firming response at low moisture loss were not provided in these studies. In contrast, weight loss as high as 22% was correlated with an increase of firmness of 9% during the storage for 6 weeks at 5°C of two cultivars of rabbiteye blueberries, whereas weight loss of 3-9% produced fruit softening (Basiouny, 1988). From these experiences, the relationship between moisture loss and postharvest firmness rises as a possible key factor for blueberry quality, whose understanding could provide relevant information for the postharvest management of this fruit.

1.2.3.2 The influence of skin and microstructure on firmness

The textural properties of the epidermis, such as thickness and toughness, are known to affect to overall fruit firmness (Jackman and Stanley, 1995). The skin's resistance to compression force may produce similar firmness outputs when different internal fruit textures are assessed (Jackman and Stanley, 1995). The blueberry fruit epidermis, commonly referred as skin, comprises a single layer of cells forming a tough tissue which provides physical resistance against mechanical damage (Allan-Wojtas *et al.*, 2001; Fava *et al.*, 2006). Experiments relating skin characteristics to blueberry firmness are not coincident. Bunemann (1957) reported that the skin

toughness of ripe highbush blueberries stored for 6 weeks at 5°C correlated positively with fruit firmness, although both parameters were measured subjectively. In contrast, Saftner *et al.* (2008) concluded that sensory evaluated skin toughness did not correlated with compression firmness in ripe blueberries for twelve different highbush and rabbiteye cultivars.

The presence of wall lignified cells, known as stone cells, as well as the overall cell arrangement of fruit parenchyma influences the mechanical properties of fruit and can contribute to the texture variability between species and cultivars (Smith, 1935; Yarbrough and Morrow, 1947). Stone cells, also called sclereids, are found in the pericarp of many species such as pear, apple, guava and blueberry (Smith, 1935; Weinert and Vanwyk, 1988; Yarbrough and Morrow, 1947). Stone cells are present in blueberry mesocarp tissues, concentrated mainly in the zone between the epidermis and 1.4 mm into the flesh, in densities varying from 1.5 to 19.5 per μm^2 (Figure **1**-**6**) (Gough, 1983). Sclereids are disposed as individual cells or form clusters of two units or more (Gough, 1983). Sclereids in ripe blueberry have a heavily lignified and thick cell wall of 13.5 µm compared to 1.5 µm of parenchyma cells (Gough, 1983). The cell wall thickness of stone cells, their disposition to form conglomerates and to associate to parenchyma cells, as well as their size and orientation, have all been suggested to influence firmness in blueberry (Allan-Wojtas *et al.*, 2001; Gough, 1983), although these relations have not been confirmed. However, in these studies the total number of stone cells was shown to not be related to blueberry firmness.

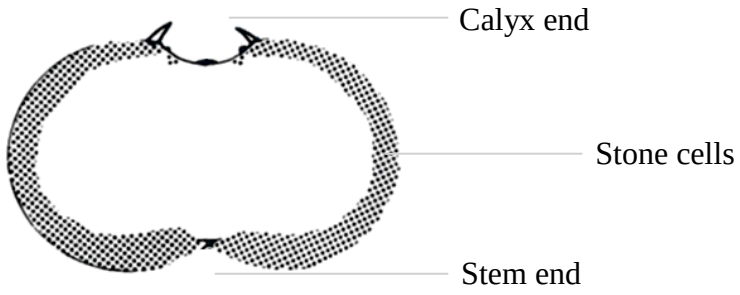

Figure 1-6. Diagrammatic distribution of stone cells in blueberry fruit represented as a longitudinal cross-section. Adapted from Gough, 1983

Modifications affecting the cell wall anatomy have been also related to blueberry firmness at postharvest. Allan-Wojtas *et al.* (2001) found that thickening of the cell

wall of parenchyma cells correlated with increasing firmness (35%) during storage of ripe highbush blueberries (cv. Burlington) for 6 weeks at 0°C, although these results were not consistent in a second year of study under similar conditions. In another work, microscopically observed corrugation of the cell walls of epidermal and hypodermal cells developed during storage for 8 weeks at 10°C generated an increase of sensory evaluated firmness in air stored ripe highbush blueberries (Bunemann *et al.*, 1957).

1.2.4 Postharvest pathology

Decay is the main factor limiting postharvest life and marketing of fresh blueberry fruit (Cappellini and Ceponis, 1977; Ehlenfeldt, 2002; Forney, 2009). The main pathogens that produce fruit quality loss and decay in blueberries are *Botrytis cinerea*, *Alternaria sp.* and *Colletotrichum sp*. (Barkai-Golan, 2001; Gough, 1994). These pathogenic fungi are widely distributed in different growing regions causing big economic losses to the blueberry industry (Gough, 1994). These fungi infect blueberries largely during the fruit growing stage, remaining dormant until blueberry ripen and decrease their chemical and mechanical resistance to pathogen development (Snowdon, 1990). However importantly, they can also be propagated during the postharvest chain, profiting from wounds and the high contact between fruit (Barkai-Golan, 2001; Snowdon, 1990). Other species of pathogenic fungi affecting blueberies in a lower incidence are *Penicillium sp.*, *Fusarium sp.* and *Cladosporium sp*. (Tournas and Katsoudas, 2005). In this section, the main characteristics of major blueberry pathogens and their importance during postharvest are reviewed, in order to inform the analysis of decay incidence data of this study.

1.2.4.1 Botrytis

Botrytis cinerea Pers. is recognised as one of the most important postharvest pathogens for a wide range of fresh horticultural products including fruits, vegetables and cut flowers (Barkai-Golan, 2001). Since *B. cinerea* lacks a specific mechanism to penetrate healthy tissues, it usually attacks weakened tissues such as senescent organs or ripe fruit (Coley-Smith *et al.*, 1980). In blueberries, this fungus is known as

grey mould and is considered the most prominent pathogen (Caruso and Ramsdell, 1995; Gough, 1994). *B. cinerea* normally infects blueberries before harvest through senescent flower remnants, although it also may inoculate and infect fruit tissues during postharvest via the stem scar, wounds or direct contact with infected fruit (Barkai-Golan, 2001; Caruso and Ramsdell, 1995). Symptoms of *B. cinerea* in blueberry postharvest comprise an abundant grey mycelium rich in conidia (i.e. asexual spores) on the fruit skin and slight berry shrivelling (Caruso and Ramsdell, 1995). Spores of *B. cinerea* need free water over the fruit surface and temperature of 20-25°C to germinate, whereas infection requires 15-20°C and 95% RH for 3 d (Caruso and Ramsdell, 1995; Gough, 1994; Lavymeir and Barkai-Golan, 1989). According to Sommer (1985), *B. cinerea* is able to grow at very low temperatures (i.e. -2°C), which allows the expression of its symptoms during cold storage.

1.2.4.2 Alternaria

The *Alternaria* genus comprises of several species of pathogenic fungi of many fruit and vegetables, such as stone and pome fruit, grapes, tomato, pepper, corn and onion (Barkai-Golan, 2001). *Alternaria alternata* (Fr.) Keissler and *Alternaria tenuissima* (Kunze: Fr.) Wiltshire species commonly attack blueberry, producing the disease known as fruit rot (Barkai-Golan, 2001). Infected blueberries become leaky and develop a compact greenish black mycelium in the calyx end (Caruso and Ramsdell, 1995). Temperatures around 28°C are optimal for spore germination of *Alternaria sp.*, whereas the infection of the fungus is enhanced by wet periods at 20°C (Caruso and Ramsdell, 1995). Hyphae of *Alternaria sp.* are able to directly penetrate the cuticle to infect fruit, remaining quiescent until the concentration of internal antifungal substances has decreased (Snowdon, 1990). *Alternaria sp.* can maintain development at temperatures as low as -3°C, and hence advance during storage and refrigerated transport (Sommer, 1985). Frequently, infected blueberries with little or no signs of contamination are harvested along with healthy fruit, generating an important propagation of the disease throughout the postharvest chain including handling, transport, packing and storage (Caruso and Ramsdell, 1995; Gough, 1994).

1.2.4.3 Colletotrichum

Fungi from the genus *Colletotrichum* are important postharvest pathogens of many fruit such as apple, grape, strawberry, avocado and citrus (Verma, 2005). *Colletotrichum sp.* are able to infect both ripe and unripe fruit due to an ability to produce an effective penetration structure called apressorium (Wills *et al.*, 2007). Two species of this genus are associated to blueberry, *Colletotrichum gloeosporioides* Penz. and *Colletotrichum acutatum* Simmonds, both having more incidence in highbush than in rabbiteye cultivars, although some highbush cultivars present resistance (Caruso and Ramsdell, 1995; Miles *et al.*, 2009; Verma, 2005). *Colletotrichum* species produce the disease known as anthracnose, where infected blueberries soften and become sunken while large masses of orange conidia are developed surrounding the calyx end of the fruit (Caruso and Ramsdell, 1995). Even though blueberries can be infected by *Colletotrichum sp.* at different developmental stages, symptoms normally appear during ripening and after fruit is harvested (Daykin and Milholland, 1984). *Colletotrichum sp.* can also be massively propagated during postharvest, when infected berries containing large amount of conidia contaminate healthy fruit (Verma, 2005). However, *Colletotrichum sp.* is unable to grow below 9°C, which limit their grow under refrigerated storage (Sommer, 1985). According to Caruso and Ramsdell (1995), once attached to the fruit surface, conidia of *Colletotrichum* genus need warm temperatures (i.e. 20-27°C) to allow germination and formation of the apressorium, whereas the infection process is favoured by high RH and warm temperatures for at least 12 h.

1.3 Storage condition effects on blueberry quality

1.3.1 Psychrometrics and humidity management in blueberry storage

Psychrometrics explains the relationship between air and water vapour based on the physical and thermodynamic properties of moist air (Thompson, 2002). Psychrometric properties allow the analysis of processes involving air humidity gradients, being important to understand moisture loss conditions and their management in horticultural products (Grierson and Wardowski, 1975). The

application of psychrometric concepts in the storage of fresh commodities is often insufficient, which limits the efficacy of produce preservation (Talbot and Baird, 1991).

Cold storage at high relative humidity (RH) reduces produce moisture loss resulting in higher turgor, less wilting and improved marketable life (Wills *et al.*, 2007). Blueberries are recommended to be stored at 90-95% RH to reduce their moisture loss (Mitcham *et al.*, 2011; Perkins-Veazie, 2004). However, high RH enhances pathogen development for this commodity, constituting an important factor of quality loss.

In this section, psychrometric concepts determining fresh produce moisture loss are reviewed, in order to provide information for the further analysis of weight loss of this study. Moreover, RH standard used in blueberry operations and the risks associated to its management are also outlined.

1.3.1.1 Psychrometrics and fresh produce moisture loss

Water loss from fresh produce is determined by the difference in moisture content between the product and the surrounding air (Wills *et al.*, 2007). Moist air is comprised of dry air and water vapour (Grierson and Wardowski, 1978). The capacity of air to retain water increases as temperature increases and ambient pressure decreases (Grierson and Wardowski, 1978). The content of moisture in the air can be described in different ways. The absolute humidity (or humidity ratio) represents the proportion between the weight of the water contained in an air volume and the weight of the dry air of the same volume (Talbot and Baird, 1991). Water vapour movement is driven by the gradient of absolute humidity between two air conditions, allowing the movement from high to low vapour concentration until the equilibrium is reached (Taiz and Zeiger, 2006). Absolute humidity is often expressed as vapour pressure in horticulture management, with both terms providing the same information (Thompson, 2002). The absolute humidity gradient between the intercellular air spaces of plant tissue and the surrounding air is the driving force for water loss, defining the rate at which fresh produce is dehydrated (Wills *et al.*, 2007).

Other psychrometric variables used in analysis of produce storage conditions are dry bulb temperature, wet bulb temperature, dew point temperature and relative humidity (RH) (Talbot and Baird, 1991). Dry bulb temperature is the temperature of the air, which combined with the wet bulb temperature, can be used to calculate the absolute humidity (Thompson, 2002). Wet bulb temperature indicates the cooling of the bulb due to water evaporation (i.e. evaporative cooling), as measured from a moist-covered bulb of a regular thermometer (Talbot and Baird, 1991). It represents the minimum temperature to which an air volume can be cooled just by increasing its moisture content (Talbot and Baird, 1991). For a given environmental pressure, the air decreases its capacity to hold water as temperature decreases, starting to condensate at a temperature referred as the dew point temperature (Grierson and Wardowski, 1975). RH indicates the percentage of the ratio between the absolute humidity and the maximum humidity which can be retained by an air mixture without varying its temperature (Wills *et al.*, 2007). RH can be directly measured by using a hygrometer (Wills *et al.*, 2007). RH is the most known and poorest applied psychrometric variable, being often miss utilised to indicate humidity gradient in postharvest environments (Talbot and Baird, 1991). RH must be always provided together with air temperature, in order to evaluate the moisture loss potential of fresh commodities in a given storage condition (Thompson, 2002).

The relationships between psychrometric variables are represented by the psychrometric chart (Figure 1-7). The horizontal axis indicates the dry bulb temperature, whereas the wet bulb temperature is shown as an axis sloping diagonally upwards from right to left (Talbot and Baird, 1991). The vertical right axis shows the moisture content of the air expressed as absolute humidity or vapour pressure, which increases as dry bulb temperature and RH increase. RH is indicated as left most upward-curved lines, with 100% RH representing the maximum moisture that can be held by an air volume at a given temperature (Thompson, 2002). The psychrometric chart is valid for a specific ambient pressure, being normally reported at atmospheric pressure. The chart allows obtaining all the properties of a given moistened air, as long as at least two variables are known. For instance, absolute humidity (humidity ratio) can be easily calculated either from the dry and wet bulb temperatures, or from the RH and dry bulb temperature. The psychrometric chart is a useful tool for packhouses and storage facilities, which facilitates the

application of psychrometric concepts on postharvest management (Talbot and Baird, 1991).

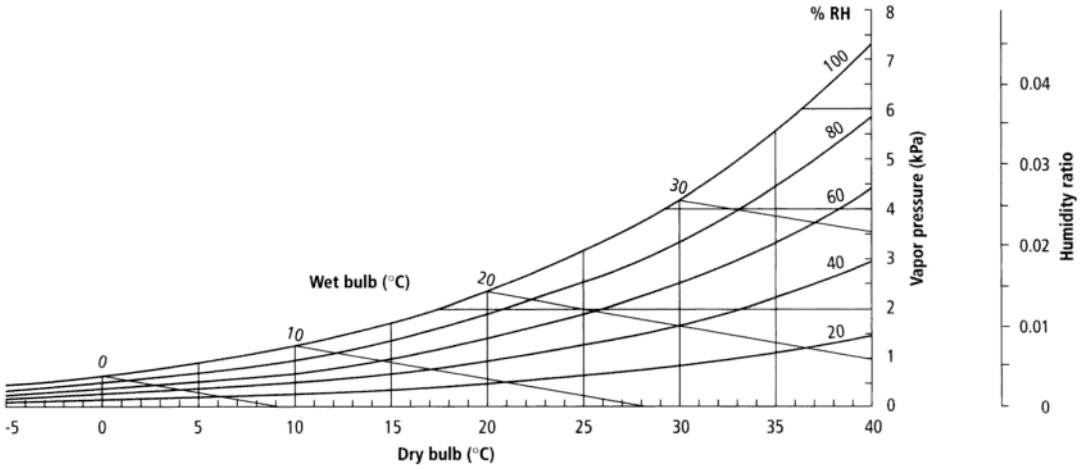

Figure 1-7. Psychrometric chart in SI (metric) units for atmospheric pressure conditions. Reproduced from Thompson (2002)

In the postharvest management of fresh commodities, the rate of moisture loss is highly related to product temperature. The temperature of fresh produce is affected by respiration heat and evaporative cooling, although it is largely determined by the ambient temperature (Grierson and Wardowski, 1975; Talbot and Baird, 1991). The temperature of the product defines its absolute humidity since RH within the tissues can be assumed to be invariably close to saturation (Grierson and Wardowski, 1978). The gradient of absolute humidity between the product and the environment increases as the product temperature increases, leading to increased moisture loss (Thompson, 2002). Consequently, a rapid product cooling is an effective management to reduce the moisture loss from fresh produce (Thompson, 2002).

1.3.1.2 Relative humidity management in blueberry

Appropriate management of humidity conditions during postharvest is a primary issue for blueberry quality. To reduce weight loss and shrivelling in blueberries, it is recommended to keep RH in the range of 90-95% during storage, together with temperatures close to 0°C (Mitcham *et al.*, 2011; Perkins-Veazie, 2004). Nevertheless, ideal air moisture contents are also optimal for the growth, infection and spore germination of main blueberry postharvest pathogens (Caruso and

Ramsdell, 1995; Snowdon, 1990). Microbial spoilage is frequently observed along blueberry supply chains, being the major reason for fruit losses (Gough, 1994). Accordingly, environmental conditions which may lead to increased RH must be avoided in order to favour the quality maintenance of this commodity.

Temperature variations and RH management in the blueberry cold chain favour condensation, resulting in free water on the produce surface which may enhance pathogen growth (Ehlenfeldt, 2002; Forney, 2009). However, literature is not consistent about the effects of condensation on blueberry decay. While Cappellini *et al.* (1983) found no influence of condensation on the decay of inoculated (*Botrytis sp.* and *Alternaria sp.*) blueberries after moving fruit from storage at 2°C and holding them for 4 d at 21°C, Cline (1997) reported greater development of *Alternaria sp*. on wet fruit after holding dry and wet inoculated blueberries for 7 d at 21°C. These different results could have resulted from different conditions of each experiment, such as pathogen specie inoculated, amount of inoculum, cultivar resistance or incubation period.

1.3.2 Temperature

Temperature is the main factor influencing the deterioration and life span of harvested horticultural products (Wills *et al.*, 2007). Temperature regulates the rate of produce respiration, ethylene production and main enzymatic reactions, affecting major quality attributes of fruits and vegetables during postharvest such as texture, colour and chemical composition (Wills *et al.*, 2007). Environmental temperature also influences product decay by regulating the growth rate of postharvest pathogens, as well as by affecting indirectly the concentration of antimicrobial compounds in fresh commodities (Barkai-Golan, 2001). Moreover, temperature determines the capacity of air to retain water, influencing the moisture content gradient between the product and the ambient and hence, the rate of produce moisture loss (Taiz and Zeiger, 2006). Consequently, refrigerated storage is considered as a primary requirement to maintain the quality of fresh fruit and vegetables and to extend their postharvest life.

As for most berry fruit, fresh blueberries are known to be extremely perishable commodities. After harvest, they must be maintained at low temperature conditions to delay physiological and microbial spoilage. Blueberry respiration rate is highly influenced by temperature, varying in CO_2 production from 0.02 μmol kg^{-1} s^{-1} at 0°C, to 0.06 and 0.21 μmol kg^{-1} s^{-1} at 10°C and 20°C, respectively (Mitcham *et al.*, 2011). Blueberries also produce considerable amounts of heat during postharvest (0.63 μmol kg^{-1} s^{-1} at 27°C), which contributes to increase the storage temperature and further accelerate their metabolism (Boyette, 1993). Furthermore, harvested blueberries are very sensitive to moisture loss and decay development, with temperature management throughout the supply chain being very important to reduce the incidence of these problems (Forney, 2009).

Refrigerated storage at 0-5°C is recommended for optimum maintenance of blueberry fruit, which keeps acceptable commercial quality up to 2 weeks for highbush cultivars and up to 4 weeks for rabbiteye blueberries (A. Kader, 2003; Mitcham *et al.*, 2011; Perkins-Veazie, 2004). However, the extent of impact of temperature at storage on each quality attribute is different (Forney, 2009), and varies between blueberry species and cultivars (Makus and Morris, 1993; NeSmith *et al.*, 2005). Hence it is necessary to review temperature effects on each blueberry properties for an adequate understanding.

1.3.2.1 Temperature influence on decay

Blueberries deteriorate quickly with temperatures higher than 10°C, developing visible signs of decay in no more than 12 h (Boyette, 1993). This phenomenon is significantly delayed by lower temperatures as first reported by Woodruff and Dewey (1959), who observed that postharvest pathogens developed much slower on blueberries kept at 2°C than at 10°C. Accordingly, the positive effect of low temperature on reducing decay incidence in blueberries has been evaluated in numerous subsequent works (Table 1-1). Cappellini *et al.* (1972) obtained a reduction of at least five fold in rot incidence after 4 d of storage of three cultivars of highbush blueberries held at 10°C instead of 27°C. Further temperature decline from 8°C to 1°C decreased decay from 10.8% to 1.5% in fruit of highbush (cv. Bluecrop) after four weeks of storage (Remberg *et al.*, 2003). When multiple temperatures

between 0°C and 20°C were compared after storage, a progressive increase of decay was obtained as the temperature increased (Table 1-1), with 20°C resulting in almost three times more decayed fruit than 0°C (Nunes *et al.*, 2004; Sanford *et al.*, 1991).

Table 1-1. Effect of storage temperatures on blueberry decay

Storage		*Evaluation after storage*			
Temp (°C)	Period (d)	Decay (%)	Cultivar (specie)	Comments	Reference
10	4	0,7	Weymouth	Relative humidity (RH) not regulated	Cappellini *et al.*, 1972*
27	4	24	(Highbush)		
10	4	0,7	Bluecrop		
27	4	11,3	(Highbush)		
10	4	1,3	Jersey		
27	4	7,4	(Highbush)		
0	22	0.6 a	Herbert	RH not regulated	Borecka and Pliszka, 1985
2	22	16.2 b	(Highbush)		
0	14	0 a	*Wild genotype* (Lowbush)	RH not regulated	Sanford *et al*., 1991
5	14	0.6 a			
10	14	3.5 b			
20	14	5.6 c			
0	21	*NS*	Burlington	RH 85-95% for all treatments	Forney *et al.*, 1998
3	21		(Highbush)		
1	28	1.5 a	Bluecrop	RH 95% for all treatments	Remberg *et al.*, 2003
8	28	10.8 b	(Highbush)		
0	12	6	Patriot (Highbush)	RH 95-100% for all treatments	Nunes *et al.*, 2004*
5	12	8,5			
10	12	10			
15	12	13			
20	12	17			

*No statistical analysis reported for this data. *NS*: non-significant

Ballinger *et al.* (1978) measured the time required for four cultivars of highbush blueberries to reach 20% of decayed fruit at 1, 10 and 22°C, obtaining an average of 36, 11 and 3 d, respectively, with all the cultivars following a similar trend. Later, Boyette (1993) demonstrate the storage temperature influence on the evolution of decay incidence in packaged blueberries during 40 d of storage. In this study, blueberries stored at 22°C and 10°C reached 80% decay after 6 and 13 d, respectively, while after the whole period of storage blueberries stored at 1°C developed less than 70% of decayed fruit.

Evidence clearly shows the effectiveness of storing blueberries close to 0°C in reducing decay, however, when temperatures within the 0-5°C range are compared, the beneficial effect of lower temperature was not as consistent. Increasing storage temperature from 0°C to 5°C increased decay from 6% to 8.5% after 12 d in highbush blueberry (cv. Patriot) (Nunes *et al.*, 2004). Similarly, fruit of highbush blueberries cultivar Herbert increased from 0.6% to 16.2% rot incidence when maintained 22 d at 0°C and 2°C, respectively (Borecka and Pliszka, 1985). In contrast, temperature differences of 0°C and 5°C had no effect on decayed fruit in wild lowbush blueberry held for 14 d (Sanford *et al.*, 1991) and highbush blueberries (cv. Burlington) developed equal levels of decay after 21 d of storage regardless if they were stored at 0°C or 3°C (Forney *et al.*, 1998). Different factors such as interaction with other quality attribute (e.g. moisture loss) or experimental differences might explain these variable responses. In addition, it is known that blueberry genotypes have considerable variability in terms of fruit physical and chemical resistance to pathogen infection (Ehlenfeldt, 2002; Magee, 1999; Makus and Morris, 1993).

1.3.2.2 Effects of temperature on moisture loss

Higher storage temperatures increase the moisture loss rate between the produce and the environment (Talbot and Baird, 1991). At constant temperature and RH, produce moisture loss normally follows a linear increase during storage since it is mainly a physically-driven phenomenon (Nunes *et al.*, 2004). Special attention must be paid to RH conditions (i.e. regulation, control) when comparing different studies in terms of temperature influence on moisture loss.

From own experience, blueberries normally lose around 2-3% of their weight due to dehydration in the commercial chain when airfreighted at 0°C from Chile to United States, with weight loss increasing up to 5-7% after blueberries are shipped by sea between these countries at the same temperature. Under experimental conditions, the magnitude of weight loss reported at diverse temperatures is highly variable between studies, which would be explained by storage settings and cultivar variability. Sanford *et al.* (1991) obtained a progressive increase of weight loss from 5.3% to 17.1% between blueberries stored for 14 d at various temperatures from 0°C to 20°C,

where RH varied from 80% to 50% at 0°C and 20°C, respectively (Table 1-2). Weight loss also increased with higher temperatures, although within a narrower range, when blueberries were held at similar temperatures with RH regulated between 95% and 100% (Nunes *et al.*, 2004). Likewise, Tetteh *et al.* (2004) obtained 4% and 9% of weight loss in rabbiteye blueberries stored for 3 d at 4°C and 21°C, respectively, with RH fixed at 95%. NeSmith *et al.* (2005) also reported a progressive weight loss increase after 7 d of storage as temperature increased from 1°C to 32°C, although they found important differences between weight loss values of various rabbiteye cultivars used in the experiment (Table 1-2).

Table 1-2. Effect of storage conditions on blueberry weight loss

Storage		*Evaluation after storage*			
Temp (°C)	Period (d)	Weight loss (%)	Cultivar (specie)	Comments	Reference
0	22	0.3 a	Herbert	Relative humidity (RH) not reported	Borecka and Pliszka, 1985
2	22	1.4 b	(Highbush)	Fruit covered with plastic bag	
0	14	5.3 a			
5	14	7.6 a	*Wild genotype*	RH not regulated, RH varied	Sanford *et al.*, 1991
10	14	12.8 b	(Lowbush)	from 80% at 0°C to 50% at 20°C	
20	14	17.1 c			
0	21	*NS*	Burlington	RH 85-95% by placing fruit	Forney *et al.*, 1998
3	21		(Highbush)	inside clamshell and plastic bag	
4	3	4,0	Tifblue	RH controlled at 95% by computer	Tetteh *et al.*, 2004*
21	3	9,0	(Rabbiteye)		
0	12	1.8 a			
5	12	2.4 b	Patriot	RH 95-100% by placing fruit	
10	12	2.5 b	(Highbush)	inside clamshell and plastic bag	Nunes *et al.*, 2004
15	12	3.4 c			
20	12	3.6 c			
1	7	1,5			
12	7	3,4	Climax		
22	7	5,9	(Rabbiteye)		
32	7	15,1			
1	7	0,3			
12	7	0,8	Tifblue		
22	7	4,7	(Rabbiteye)	RH > 90% by placing fruit	
32	7	8,4		inside clamshell and plastic bag	NeSmith *et al.*, 2005*
1	7	0,2			
12	7	1,2	Brightwell		
22	7	3,0	(Rabbiteye)		
32	7	10,1			
1	7	1,3			
12	7	2,9	Powderblue		
22	7	4,7	(Rabbiteye)		
32	7	20,2			

*No statistical analysis reported for this data. *NS*: non-significant

Variations of temperatures in the range of 0°C to 3°C does not seem to result in big water loss differences. Borecka and Pliszka (1985) did report weight loss of 0.3% and 1.4% when blueberries (cv. Herbert) were stored at 0°C and 2°C, respectively for 22 d. However, Forney *et al.* (1998) did not obtain different weight loss levels after storing (cv. Burlington) blueberries for 21 d at 0°C or 3°C.

1.3.2.3 Firmness responses to temperature variations

Blueberry firmness during postharvest is determined by a combination of different factors such as water loss, cell wall degradation and fruit anatomy features, among which water loss seems to be a key factor influencing firmness evolution. This relationship between moisture loss and postharvest firmness behaviour is influenced by different storage temperature conditions. When lowbush and rabbiteye blueberry have been stored at temperatures within the range of 0-32°C, firmness has decreased progressively as temperature increases, accompanied by a simultaneous weight loss increase (NeSmith *et al.*, 2005; Sanford *et al.*, 1991) (Table 1-3). Similar to the weight loss responses, NeSmith *et al.* (2005) found important differences in firmness magnitudes between cultivars. In addition, Tetteh *et al.* (2004) reported a firmness loss of 20% and 33% after keeping blueberries at 4°C and 21°C, respectively, for 3 d which correlated with an increase of weight loss. However, Forney *et al.* (1998) found no differences either in firmness nor weight loss when highbush blueberries (cv. Burlington) were maintained for 21 d at 0°C or 3°C at regulated RH. Furthermore, small variations on firmness were obtained after storing blueberries (cv. Bluecrop) at 0, 10, 20 and 30°C under constant RH conditions (Forney, 2009) (Table 1-3).

Table 1-3. Effect of storage temperatures on blueberry firmness

Storage		*Evaluation after storage*			
Temp (°C)	Period (d)	Firmness	Cultivar (specie)	Comments	Reference
		Compression (N)			
0	14	28.9 a	*Wild genotype*		
5	14	15.5 b	(Lowbush)	Weight loss varied from 5.3%	Sanford *et al.*, 1991
10	14	12 c		at 0°C to 17.1% at 20°C	
20	14	11 c			
		Compression (g mm^{-1})	Burlington		
0	21	*NS*	(Highbush)	Weight loss was also *NS*	Forney *et al.*, 1998
3	21			between temperatures	
		Firmness loss (%)	Tifblue		
4	3	20	(Rabbiteye)	Weight loss was 4% at 4°C	Tetteh *et al.*, 2004*
21	3	33		and 9% at 21°C	
		Firmness loss (%)	Tifblue		
1	8	4.7 a	(Rabbiteye)		
22	8	35 b		Weight loss not evaluated	Nunez-Barrios *et al.*, 2005
1	8	1.5 a	Brightwell		
22	8	32 b	(Rabbiteye)		
		Firmness loss (%)			
1	7	5,0	Climax		
12	7	8,4	(Rabbiteye)		
22	7	11,8			
32	7	18,5			
1	7	6,7			
12	7	10,1	Tifblue		
22	7	11,8	(Rabbiteye)	Weight loss varied from 0.2%	
32	7	28,6		at 1°C to 20.2% at 32°C	NeSmith *et al.*, 2005*
1	7	6,7			
12	7	8,4	Brightwell		
22	7	8,4	(Rabbiteye)		
32	7	10,1			
1	7	3,4			
12	7	5,0	Powderblue		
22	7	18,5	(Rabbiteye)		
32	7	63,8			
		Compression (g mm^{-1})			
0	8	1,1	Bluecrop	Relative humidity constant	
10	8	1,3	(Highbush)	for all temperatures.	Forney, 2009*
20	8	1,2		Weight loss not evaluated	
30	8	1,1			

*No statistical analysis reported for this data. *NS*: non-significant

1.3.2.4 Temperature influence on additional quality attributes

The influence of storage temperature on total solids, titratable acidity anthocyanin content and flavour is variable among experiments, although it seems to be associated mainly to the effect of high temperature enhancing either ripening related processes or fermentation. Sanford *et al.* (1991) obtained a small but progressive increase of total soluble solids from 10.6% to 11% when lowbush blueberries were held at several temperatures from 0°C to 20°C after 14 d, while total acidity also showed a progressive increase of up to 100% and fruit presented a darker colouration (i.e. lower *L* values), as temperature increased. On the other hand, Remberg *et al.*

(2003) found no significant changes on total soluble solids nor titratable acidity after storing highbush blueberries (cv. Bluecrop) for 28 d at 1°C or 8°C. For these studies, fruit picked at slightly different ripening stages could explain these variable responses on total soluble solids at diverse temperatures, while acidity normally increases when blueberries are maintained under storage conditions. Moreover, anthocyanins are known to increase during storage, although the extent of this increase is influenced by temperature. Total anthocyanin content increased 18% and 15% throughout 8 d of storage of Bluecrop blueberries at 20°C and 30°C, respectively, although this parameter did not vary its levels during storage when fruit was kept at 0°C or 10°C for the same period (Kalt *et al.*, 1999a). Additionally, Nunes *et al.* (2004) reported off-flavours, associated to fermentation products, after 8 d of storage at 15°C and 20°C, whereas this was not observed when blueberries were held at 0°C, 5°C or 10°C for up to 12 d in the same experiment.

1.3.3 Cooling delays

Fresh produce quality deteriorates rapidly under high temperature environments. As such, moving product quickly from the field to the packhouse and to cooling as soon as possible extends postharvest life (Wills *et al.*, 2007). The period of time elapsed from harvest until the produce reaches the cool storage is referred to as a cooling delay, which usually includes harvest handling, transport and packing (Ferraz *et al.*, 2001; Tetteh *et al.*, 2004). Longer cooling delays and higher temperatures during this period can decrease the product storage life and affect the final quality of many fresh commodities (Thompson *et al.*, 2001). Alternatively, rapid cooling of fresh commodities such as forced air, hydro or vacuum cooling are utilised for many perishable fruit and vegetables to quickly remove the field heat and hence to contribute to improve their shelf life (Wills *et al.*, 2007).

Cooling delays affect the quality and postharvest life of fresh blueberries in subsequent storage (Thompson *et al.*, 2001), hence considerable effort has been made in the blueberry industry to improve the cooling management (Boyette, 1993). The optimisation of postharvest logistics by lowering prepacking temperatures and by reducing supply chain times is considered a primary challenge for blueberry

growers and marketers (Ferraz *et al.*, 2001; Forney, 2009). The effect of cooling delays on blueberry quality is dependent on the holding temperature and total time of the delay period (Ferraz *et al.*, 2001). In addition, each of the quality attributes varies in its response to cooling delay (Tables 1-4 and 1-5). The studies evaluating the residual effect of cooling delays on fresh blueberries are reviewed in this section, in pursuance of providing the necessary information to analyse the storage delay results of this study.

Experimental results on the residual effect of cooling delays on decay and chemical parameters are consistent. When highbush and lowbush blueberries were held from 2 to 48 h at temperatures close to 20°C, a progressive increase of pathogen development after the subsequent storage was observed as delay periods were longer (Table 1-4) (Ceponis and Cappellini, 1979; Jackson *et al.*, 1999). Likewise, Ceponis and Cappellini (1982) reported a positive correlation between longer delays and higher incidence of decay after storage when highbush blueberries were subjected to holding periods at 30°C (Table 1-4). In all studies, the levels of total soluble solids and titratable acidity are not affected after storage by cooling delays (Table 1-4).

Table 1-4. Residual effect of different cooling delays on decay and chemical parameters of blueberries after storage

Delay		*Storage*		*Evaluation after storage*		Cultivar		
Period (h)	Temp (°C)	Period (d)	Temp (°C)	Decay	Chemicals	(specie)	Comments	Reference
				% Incidence		*Not*	Evaluated after	
2	20	4	1,5	7.4 a	not	*reported*	3 extra days	Ceponis & Cappellini, 1979
48	20	4	1,5	28.5 b	measured	(Highbush)	at 21°C	
				% Incidence				
2	30	14	2	2	not	Bluecrop	Evaluated after	Ceponis & Cappellini, 1982*
48	30	14	2	6,9	measured	(Highbush)	1 extra day	
72	30	14	2	10,8			at 21°C	
				Count (log_{10} g^{-1})	*TSS, TA*	*Not*		
3	19	21	0	5.1 a		*reported*	Packing	
9	19	21	0	5.3 b	*NS*	(Lowbush)	temperature	Jackson *et al.*, 1999
21	19	21	0	5.4 c			not reported	
48	19	21	0	5.5 d				
					TSS, TA			
0	30	28	2					
2	30	28	2	not developed	*NS*	Bonita	Evaluated after	Ferraz *et al.*, 2001
4	30	28	2			(Rabbiteye)	1 extra day	
6	30	28	2				at 20°C	
8	30	28	2					

*No statistical analysis reported for this data. *NS*: non-significant

Table 1-5. Effects of cooling delays on weight loss and firmness of blueberries at storage

Delay		*Storage*		*Evaluation after storage*		Cultivar		
Period (h)	Temp (°C)	Period (d)	Temp (°C)	Weight loss (%)	Firmness	(specie)	Comments	Reference
					Compression (N)			
0	30	28	2	5.2*				
2	30	28	2	5,8		Bonita	Evaluated after	Ferraz *et al.*, 2001
4	30	28	2	6,3	*NS*	(Rabbiteye)	1 extra day	
6	30	28	2	6,8			at 20°C	
8	30	28	2	7,2				
8	32	3	4	6,1	not	Tifblue		Tetteh *et al.*, 2004*
24	32	3	4	8,5	measured	(Rabbiteye)		
					Firmness loss (%)			
0	20	10	4,5	not	10 a	Brightwell	Compression	NeSmith *et al.*, 2002
24	20	10	4,5	measured	16 b	(Rabbiteye)	Firmness	
					Compression (N)			
4	10	14	0	7.87*	0.9 a			
8	10	14	0	7,55	1.0 a	Maru	Delay at 10°C	Paniagua *et al.*, 2012
20	10	14	0	9,52	0.7 b	(Rabbiteye)	applied after	
4	10	21	0	8.83*	0.8 a		6 h at 20°C	
8	10	21	0	9,46	0.8 a			
20	10	21	0	11,51	0.6 a			

*No statistical analysis reported for this data. *NS*: non-significant

Longer holding periods and higher temperatures before cooling impact both moisture loss and firmness of fresh blueberries during subsequent storage. Increasing delay at 20°C from 0 to 24 h lowered firmness in rabbiteye blueberries (cv. Brightwell) after a subsequent 10 d storage at 4.5°C (NeSmith *et al.*, 2002), while delays of 8 and 24 h at 32°C generated weight loss of 6.1% and 8.5%, respectively, after 3 d of subsequent storage at 4°C in rabbiteye (cv. Tifblue) (Tetteh *et al.*, 2004). Nevertheless, when the duration of the cooling delay has been shorter than 9 h, its effect on weight loss and firmness after storage has not been consistent. Ferraz *et al.* (2001) obtained progressive increase of weight loss after storage as the delay period increased, but no differences in terms of firmness (Table 1-5). In contrast, Paniagua (2012) reported no firmness differences after storage between 'Maru' blueberries previously held at 10°C for 4 or 8 h (Table 1-5). Accordingly, evidence shows that cooling delay effect on firmness of blueberries is clearer when the period of delay lasts minimum for 24 h, for holding temperatures of at least 10°C. Moreover, the influence of delay increasing water loss during storage is more consistent, especially at higher holding temperatures.

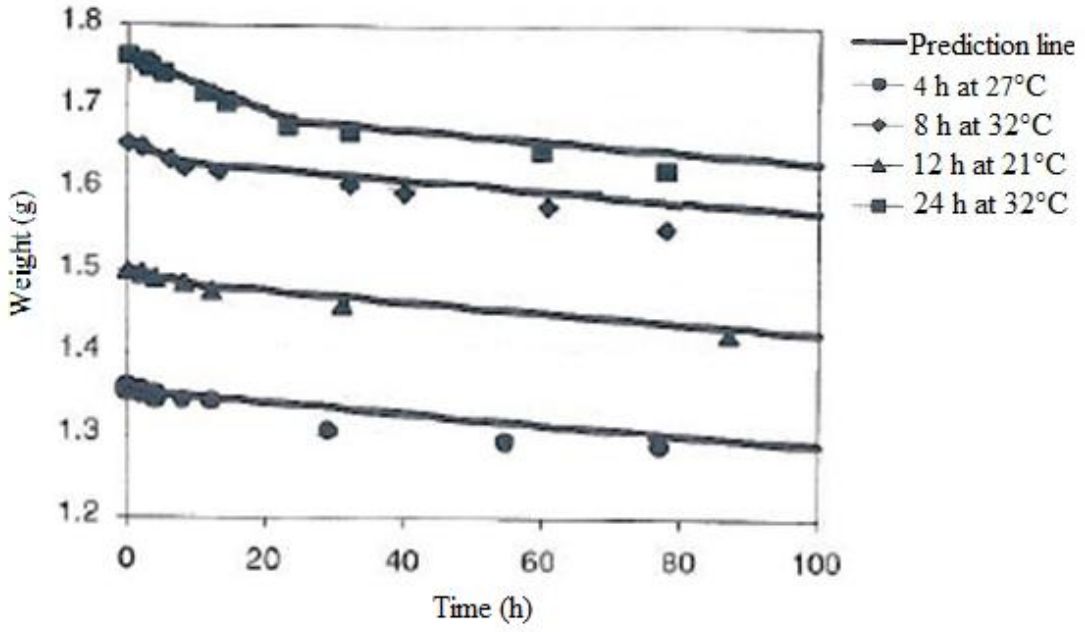

Figure 1-8. Weight evolution of rabbiteye blueberries cv. Tifblue during different cooling delay treatments and throughout subsequent storage at 4°C and 95% RH. Adapted from Tetteh *et al.* (2004)

The effect of the delay duration on water loss at storage seems to vary directly with to the period of exposure to the moisture content gradient between the blueberry fruit and environment during the holding period, which increases at higher temperatures. This would lead to a residual effect on product moisture loss at storage in terms of magnitude, without affecting the rate of water loss during this period, as shown by Tetteh *et al.* (2004) after comparing the residual effect of cooling delay periods at 21°C, 27°C and 32°C on blueberry weight during subsequent storage at 4°C for 4 d (Figure 1-6). Furthermore, this effect on moisture loss seems to be influencing to some extent the residual effect of delays on blueberry softening at storage, although some degree of inconsistency indicates the participation of other factors as well. Moreover, cooling delay effect on blueberry decay during storage seems to be exclusively explained by a direct influence of holding period and temperature on the growth rate of pathogens.

1.3.4 Controlled atmosphere

Controlled atmosphere (CA) is the widely used technology of manipulating the gaseous composition of postharvest environments to improve the storability of perishable fruit and vegetables. In complement with proper refrigerated conditions, CA is able to extend the postharvest life of many fresh products, and hence utilised in storage facilities and sea freight exports around the world (Wills *et al.*, 2007). CA systems are based on strict monitoring and regulation of gas levels during the storage period, unlike modified atmosphere techniques which relies on semipermeable films and produce properties to achieve the desired atmospheres (Ben-Yehoshua *et al.*, 2005). Gas composition during CA is commonly manipulated by reducing oxygen (O_2) and increasing carbon dioxide (CO_2) concentrations, creating atmospheric conditions able to decelerate produce and pathogens respiration, as well as to inhibit ethylene production (Yahia, 2009). Therefore, ripening and senescence related processes are delayed under CA storage, whereas decay incidence is significantly reduced (Ben-Yehoshua *et al.*, 2005).

CA is commercially used for fresh blueberries in extended transoceanic shipments throughout the season and in a minor extent in land-based storage facilities, with clear positive results in improving the postharvest life (Alsmairat *et al.*, 2011). When combined with optimal storage temperatures, CA can double or triple blueberry life span (Forney, 2009). Most beneficial effects of CA on blueberries storability are due to the direct effect of high CO_2 inhibiting decay growth, whereas CA influence on ripening related attributes has less impact on blueberry quality since these fruit are harvested fully ripe, and hence unresponsive to ethylene (Alsmairat *et al.*, 2011; Chiabrando and Giacalone, 2011; Ehlenfeldt, 2002). Accordingly, for an effective reduction of blueberry decay at storage it is recommended to manage CA levels from 8% to 15% CO_2 whereas the O_2 concentration must be maintained above 1% to avoid fermentation damage (Ceponis and Cappellini, 1979, 1983, 1985). Additionally, concentrations of O_2 between 1% and 10% have been shown to contribute to further decay reduction in some studies (Fan *et al.*, 1993; Kim *et al.*, 1995; Prange *et al.*, 1995). Moreover, increased CO_2 concentrations above recommended ranges may also lead to anaerobiosis and tissue toxicity, affecting the overall quality of blueberries and making the product unsalable (Forney, 2009; Kubo *et al.*, 1990). The

susceptibility of blueberry fruit to physiological damage is a function of several factors such as temperature, duration of exposure and genotype (Pesis, 2005). The influence of CA on postharvest decay and overall blueberry quality is reviewed in this section, in addition to discuss CA interaction with storage temperature and its implications for blueberry quality. The information detailed in this section provides valuable information for the analysis of CA effects of this study.

1.3.4.1 CA effects on decay

Altered gas composition generates inhibitory effects on the metabolism of many postharvest pathogens, decreasing their growth during storage and consequently the incidence of produce decay. Oxygen concentrations lower than 1% decrease the respiration rate of many pathogens, while high CO_2 levels inhibit the activity of some respiratory enzymes and generate an imbalance among carbon compounds, leading to the suppression of respiration (Barkai-Golan, 2001; Kubo *et al.*, 1990; Sommer, 1985). Additionally, the effect of CA delaying ripening and senescence can result in increased levels of antimicrobial substances in some products, which also contributes to reduce decay development (Ben-Yehoshua *et al.*, 2005).

The main postharvest pathogens associated with blueberry fruit are affected by CA. Under *in vitro* conditions, *Botrytis cinerea, Alternaria alternata* and *Cladosporium sp.* have decreased their spore germination by 50% at oxygen concentrations lower than 1%, whereas enriched CO_2 atmospheres have inhibited the growth of *Botrytis cinerea* and suppressed by 90% the germination of its spores (Agar, 1990; Wells and Uota, 1970). However, *Alternaria alternata* showed its ability to germinate normally under conditions of up to 32% of CO_2. Despite the high responsiveness of these pathogens against O_2 and CO_2 modifications, commercial CA application to decrease postharvest decay in blueberry fruit relies mainly on the fungistatic action of high CO_2, due to the high susceptibility of fresh blueberries to low O_2.

Table 1-6. Examples of CA effects on the decay incidence of blueberries after storage

Gas levels (%)		*Storage*	*Evaluation after storage*			
O_2	CO_2	Period (weeks)	Decay (%)	Comments	Cultivar (specie)	Reference
20,9	0	2	19.2 a	Evaluated after 3 extra days at 21°C	*Mixture of cv.* (Highbush)	Ceponis & Capellini, 1985
20,9	10	2	13.5 ab			
20,9	15	2	11.7 bc			
20,9	20	2	6 c			
2	0	2	19.6 a			
2	10	2	11.8 bc			
2	15	2	11.1 bc			
2	20	2	5.7 c			
5	10	6	10,0	Evaluated after 3 extra days at 15°C	Climax (Rabbiteye)	Smittle & Miller, 1988*
5	15	6	6,0			
5	20	6	2,0			
5	10	6	25,0		Woodard (Rabbiteye)	
5	15	6	6,0			
5	20	6	6,0			
1	0	5	37.9 a	Not held in poststorage period	Bluecrop (Highbush)	Fan *et al.*, 1993
1	5	5	20.2 b			
1	10	5	*no data*			
1	15	5	11 c			
2	0	5	42.3 a			
2	5	5	17.5 b			
2	10	5	11.6 c			
2	15	5	11.1 d			
16,8	0	5	36.5 a			
16.8	5	5	23.7 b			
16.8	10	5	8.9 c			
16.8	15	5	15.9 d			
20,9	0	3	12.3 a	3 extra days at 20°C	Ivanhoe (Highbush)	Beaudry *et al.*, 1998
2	8	3	1.3 b			
20,9	0	7	8.5 a	Not held in poststorage period	Duke (Highbush)	Harb & Streif, 2004
18	6	7	6.6 a			
18	12	7	2.5 b			
18	18	7	4 b			
18	24	7	4.3 b			
2	12	7	3.3 b			
2	18	7	4 b			
20,9	0	7	35 a	Not held in poststorage period	Bluecrop (Highbush)	Harb & Streif, 2006
18	6	7	33 a			
18	12	7	15 b			
18	18	7	1 c			
18	24	7	0.5 c			
2	12	7	11 b			
2	18	7	0.5 c			

*No statistical analysis reported for this data

Numerous studies evaluating CA benefits on fresh blueberry decay have shown a clear reduction of rot incidence when CO_2 is managed within ranges from 10% to 24%, while CO_2 levels below 10% have not been consistently effective. For example, when highbush blueberries (cv. Duke and Bluecrop) were kept under several CO_2

concentrations within the range of 6-24% CO_2 combined with 2% or 18% O_2, the proportion of fruit developing decay after the period was progressively lower as CO_2 increased from 12% to 24%, whereas 6% CO_2 did not generate significant differences with the control (air stored) (Harb and Streif, 2004, 2006) (Table 1-6). However, Fan *et al.* (1993) found that 5% CO_2 was effective in reducing decay in 'Bluecrop' after 5 weeks of storage, although further increases of CO_2 concentration to 10% and 15% resulted in greater decay control (Table 1-6). Similarly, CA with 8% CO_2 generated significantly less rot incidence compared to air storage when highbush blueberries cv. Ivanhoe were stored for 3 weeks (Beaudry *et al.*, 1998). For rabbiteye blueberries, Smittle and Miller (1988) also reported a reduction of decay development as CO_2 increased from 10% to 20% in 'Climax' and 'Woodard' cultivars held for 6 weeks under constant 5% O_2 (Table 1-6).

Oxygen concentrations above the limit of physiological damage have not been consistent for reducing decay development in blueberries. Regardless if combined with 2% O_2 or air, Ceponis and Cappellini (1985) obtained a progressive reduction of decay incidence as CO_2 levels increased, in a mixture of highbush cultivars (Table 1-6). Likewise, Harb and Streif (2004, 2006) found that decay incidence in 'Duke' and 'Bluecrop' blueberries subjected to increased CO_2 atmospheres was not affected by O_2 levels (2% or 18%) (Table 1-6). In contrast, 'Bluecrop' blueberries stored for 5 weeks at O_2 atmospheres of 1% and 2% had less decay incidence than when kept at 16.8% O_2, independent of CO_2 concentration (Fan *et al.*, 1993) (Table 1-6). In another experiment, decay was lower in 'Coville' blueberries stored for 10 d at 3% and 9% O_2 than at 15% O_2, at a given CO_2 concentration (Kim *et al.*, 1995). Similarly, Prange *et al.* (1995) obtained a rot incidence reduction of 60% and 50% with atmospheres of 5% and 2% O_2 (in comparison to air), respectively, after keeping fruit of two lowbush blueberry cultivars for a period of 4 weeks. Therefore, the question still remains whether low O_2 concentrations (1-10%) above the fermentation limit could improve the effect of high CO_2 on the control of decay in fresh blueberries.

1.3.4.2 Quality attribute responses to CA

Compared to air storage, benefits of CA on firmness have been rarely reported for fresh blueberries. In contrast, blueberry firmness decreases during storage as CO_2 concentration increases above 10-15%, in a phenomenon which seems to be related to fruit tissue toxicity. The limit on which this effect begins to occur varies among blueberry genotypes and possibly with O_2 level. In several studies, CO_2 levels from 10% to 25% after 4 to 7 weeks of storage have consistently resulted in lower blueberry firmness as CO_2 concentration increases (Fan *et al.*, 1993; Forney *et al.*, 2003; Harb and Streif, 2004, 2006; Schotsmans *et al.*, 2007) (Table 1-7). However, softening was triggered at CO_2 levels lower than 10% when 'Bluecrop' blueberries were kept at 1% and 2% O_2 for 5 weeks, but not when held at 16.8% (Fan *et al.*, 1993), suggesting increased tolerance to CO_2 damage at higher O_2 concentrations. Similarly, fruit from 'Duke' and 'Bluecrop' showed firmness reduction after being stored for 7 weeks at 12% or higher CO_2 levels, with 2% or 18% O_2, although cv. Bluecrop softened much less at 18% than at 2% O_2 (Harb and Streif, 2004, 2006). Furthermore, Forney *et al.* (1997) reported variable responses on firmness after subjecting five cultivars of highbush blueberries to 12.5% CO_2 for 9 weeks, obtaining no differences between 'Bluegold', 'Brigitta' and 'Burlington' and their initial firmness, whereas 'Coville' and 'Reka' softened 23% and 41% after the period, respectively. Also Alsmairat *et al.* (2011) obtained progressive lower firmness as CO_2 increased, compared with air stored blueberries, after keeping highbush cultivars 'Brigitta', 'Jersey', 'Legacy' and 'Liberty' at various CO_2 concentration from 6% to 19% for 8 weeks, while fruit from 'Duke', 'Ozarkblue' and 'Toro' cultivars had similar firmness in all CA treatments and air.

It has been observed in several studies that blueberry softening at high CO_2 is accompanied by increased tissue discolouration, development of off flavours and the increase of fermentation products (i.e. ethanol and ethyl acetate) (Forney *et al.*, 2003; Harb and Streif, 2004, 2006; Krupa and Tomala, 2007). On the other hand, cell wall thickening and corrugation, possibly associated to blueberry firming during storage, has been inhibited by 15% CO_2 which could be indicating changes at microstructural level (Allan-Wojtas *et al.*, 2001; Bunemann *et al.*, 1957).

Table 1-7. Effect of CA composition on blueberry firmness after storage

Gas levels (%)		*Storage*	*Evaluation after storage*			
O_2	CO_2	Period (weeks)	Firmness	Comments	Cultivar (specie)	Reference
			*MTG index***			
1	0	5	408 a			
1	5	5	365 b			
1	10	5	*no data*			
1	15	5	349 c			
2	0	5	498 a			
2	5	5	369 b	Not held	Bluecrop	
2	10	5	344 c	in poststorage	(Highbush)	Fan *et al.,* 1993
2	15	5	351 d	period		
16,8	0	5	421 a			
16.8	5	5	455 b			
16.8	10	5	423 c			
16.8	15	5	306 d			
			Compression (N mm^{-1})			
15	0	6	1.3 a	Not held		
15	10	6	0.85 b	in poststorage	Burlington	Forney *et al.*, 2003
15	15	6	0.73 c	period	(Highbush)	
15	20	6	0.5 d			
15	25	6	0.5 d			
			Compression (g mm^{-1})			
20,9	0	7	160 ab			
18	6	7	183 a			
18	12	7	141 b	Not held	Duke	
18	18	7	110 c	in poststorage	(Highbush)	Harb & Streif, 2004
18	24	7	95 c	period		
2	12	7	142 b			
2	18	7	105 c			
			Compression (g mm^{-1})			
20,9	0	7	180 a			
18	6	7	175 a			
18	12	7	155 b	Not held	Bluecrop	
18	18	7	110 d	in poststorage	(Highbush)	Harb & Streif, 2006
18	24	7	85 d	period		
2	12	7	120 c			
2	18	7	105 d			
			Compression (N)	Not held	Maru, Centurion	
20,9	0	4	3.5 a	in poststorage	(Rabbiteye)	Schotsmans *et al.*, 2007
2,5	15	4	2.8 b	period		

**Momentum Transfer Generation (firmness indicator by impact assesment)

As long as gas concentrations leading to anaerobiosis are avoided, CA does not have a clear influence on other quality attributes. Different CA compositions have not modified weight loss responses in comparison to air storage after storage periods of up to 7 weeks (Duarte *et al.*, 2009; Schotsmans *et al.*, 2007; Smittle and Miller, 1988). Likewise, total soluble solids and titratable acidity have not been affected by CA in terms of their evolution during storage, for highbush blueberries (Beaudry *et*

al., 1998; Forney *et al.*, 2003; Harb and Streif, 2006; Krupa and Tomala, 2007; Zheng *et al.*, 2003) and rabbiteye cultivars (Schotsmans *et al.*, 2007; Smittle and Miller, 1988). Moreover, whereas Remberg *et al.* (2003) and Schotsmans *et al.* (2007) obtained slight reductions on antioxidant capacity after storing blueberries under CA compared to air, others studies have not reported differences between CA and air storage for the same indicators and for total anthocyanin content (Duarte *et al.*, 2009; Krupa and Tomala, 2007; Song *et al.*, 2003). Therefore, evidence suggests that CA effect on ripening related quality attributes is minimum, which agrees with the condition of fully ripened fruit of blueberry at the moment of harvest and the minor role of ethylene during postharvest for this product.

1.3.4.3 Interaction of CA with temperature

Temperature has an important influence on CA effects in fresh produce, by modifying gas solubility, their internal concentration in the product, and the resistances to gas diffusion across the pathway. In plant tissues, the solubility of O_2 and CO_2 decreases as temperature increases, affecting their concentration and their diffusion within the cell sap and between cells and intercellular spaces (A. Kader *et al.*, 1989). For instance, CO_2 solubility in water and at atmospheric pressure is 18% lower at 5°C than at 0°C (Carroll *et al.*, 1991), and O_2 is 14% less soluble at 10°C than at 5°C in a 0.25 M sucrose solution (Reynafarje *et al.*, 1985). Furthermore, higher temperatures accelerate the respiration rate in plant cells leading to increased O_2 uptake and CO_2 production, which potentially modifies the internal concentrations of these gases and their diffusion gradients if the resistances across the pathway do not modify at the same extent (Beaudry *et al.*, 1992). For many fruit, the epidermis is the main resistance to O_2 and CO_2 diffusion into tissues (Burg and Burg, 1965). Skin resistance can be increased with shrivelling, as it has been reported in apples and oranges (A. Kader *et al.*, 1989), however it can be reduced by direct effect of higher temperatures (Lange *et al.*, 1991).

Studies using modified atmosphere packaging (MAP) in blueberries have provided evidence about the increased sensitivity of this commodity to low O_2 as temperature increases. Fermentation can be detected by a rise in the respiratory quotient (RQ), which is the ratio between the CO_2 production and O_2 uptake rates (Cameron *et al.*,

1994). Beaudry *et al.* (1992) showed that as temperature increased, higher O_2 concentrations were needed in the package to avoid the RQ rise (breakpoint). This response would be explained by an increased O_2 consumption and reduced solubility of the gas (Beaudry *et al.*, 1992; Cameron *et al.*, 1994). On the other hand, blueberry skin has shown to increase its permeability to O_2 at higher temperatures, although this response would be slower than variations in respiration and solubility (Cameron *et al.*, 1994; Lange *et al.*, 1991).

At increased storage temperatures, blueberry fruit have been shown to consistently improve its tolerance to elevated concentrations of CO_2, which has been related to lower internal concentrations of the gas due to reduced CO_2 solubility. Terry *et al.* (2009) found that highbush blueberries (cv. Burlington) stored for 9 weeks at 3°C had less discolouration damage at increased CO_2 than fruit held at 0°C (Figure 1-9). Also Saltveit and Ballinger (1983) reported carbonic acid-like flavour after holding rabbiteye blueberries under 100% CO_2 at 0°C for one hour, whereas a similar response was not detected until 3 h when blueberries were kept at 20°C and 30°C.

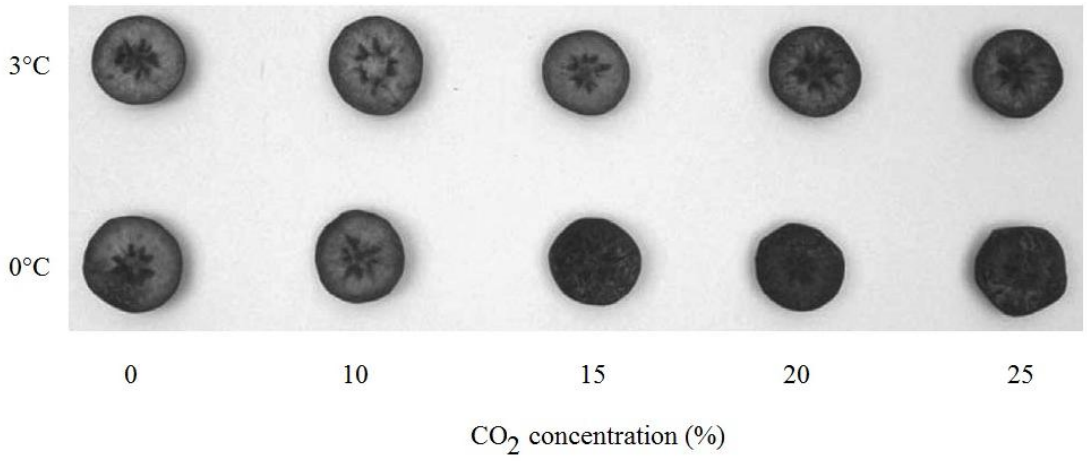

Figure 1-9. Flesh discolouration in blueberries after 9 weeks of storage under various CO_2 concentrations at 0°C or 3°C, plus an additional week in air at 7°C. Reproduced from Terry *et al.* (2009).

The softening effect of high concentrations of CO_2 on blueberries has been also reduced by storing the fruit at higher temperatures. When Forney *et al.* (1998) held 'Burlington' blueberries under 15 to 25% CO_2 for up to 9 weeks, they found that

fruit softened significantly less at 3°C than at 0°C, with the resulting differences in firmness being as high as 40% (at 15% CO_2) (Figure 1-10). In this experiment, CO_2-induced softening was also enhanced by storage duration. In a similar study, Forney *et al.* (1999) looked for the minimum CO_2 level required to trigger softening in highbush blueberries (cv. Burlington, Coville and Jersey) as a function of temperature. At 0°C, softening was induced at 12% CO_2 in 'Burlington' and at 10% for both 'Coville' and 'Jersey', whereas at 3°C softening was induced at 17% CO_2 for 'Burlington' and at 12% for Coville and Jersey cultivars.

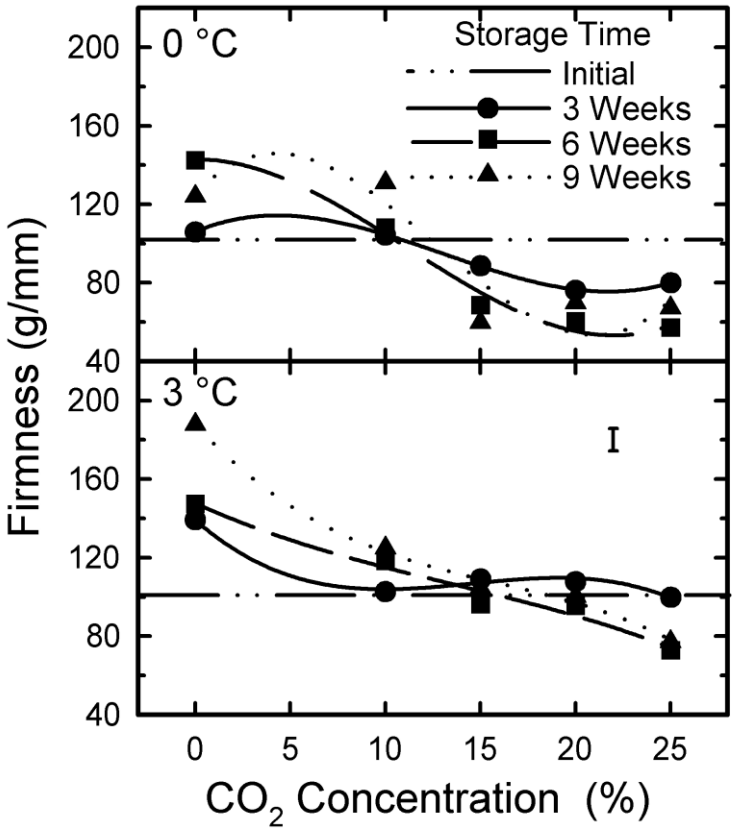

Figure 1-10. Firmness of 'Burlington' blueberries during 9 weeks of storage in various CO_2 concentrations combined with 15% O_2 at 0 or 3°C. Standard error is shown. Reproduced from Forney *et al.* (1998).

Increased temperatures in combination with CA seem to favour the firming effect observed under some conditions during storage. Bunemann (1957) found that blueberries stored for 2 weeks under various CA ranging from 5% to 25% CO_2 had higher cell wall corrugation and firming when kept at 10°C than at 0°C. The

corrugation response of cell walls, which correlated with increased sensorial firmness, was inhibited by high CO_2 atmospheres, and the associated physiological damage due to fermentation. However, Forney *et al.* (1998) found that firmness increased more in blueberries held at 3°C than at 0°C, with both kept at 0% CO_2 plus 15% O_2 which suggests that another mechanism, not related to CO_2, could be also involved (Figure 1-10).

1.3.5 Temperature variability in shipping containers

Although air freight has been historically used for blueberry exports, sea freight in reefer containers combined with controlled atmosphere is being increasingly preferred by exporters from the southern hemisphere to send their fruit to North America and Europe, since its cost is about three times lower than air freight (Bañados, 2006; Beaudry *et al.*, 1998). However, the use of marine shipping in the blueberry marketing chain has extended the export period to 2 or 3 weeks depending on the destination, making the temperature control during this period one of the most important issues in the postharvest of blueberries (Beaudry *et al.*, 1998).

Modern reefer containers accurately maintain low average temperatures during the shipping period, by generating refrigerated air in the cooling unit (located at the container end) and distributing it along the floor through T-shaped aluminium channels (Figure 1-11) (Lawton *et al.*, 2011). The air flows vertically through the cargo to subsequently return to the refrigeration unit via a grill located close the container ceiling (Figure 1-11) (Brecht *et al.*, 2009). An adequate circulation of air inside the container is required to effectively remove the product heat. As a result, the temperature distribution within the container is dependent on product primary packaging, carton design, pallet type and cargo stowage (Lawton *et al.*, 2011).

Refrigerated containers are widely used for marine shipping of perishable commodities, with the 40’ container length most commonly utilised for sea exports of fresh produce worldwide (Lawton *et al.*, 2011). Most fresh export products are marketed in trays or flats stacked over 1.2 x 1.0 m pallets (ISO Standard), which are subsequently loaded into containers or charter vessels (Lawton *et al.*, 2011). ISO

Standard pallets are disposed in groups of twenty units per each 40’ container, normally using a stowage system known as the 9-11 pattern (Lawton *et al.*, 2011). This standard container packing arrangement is also applied in the blueberry industry (Boyette, 1993).

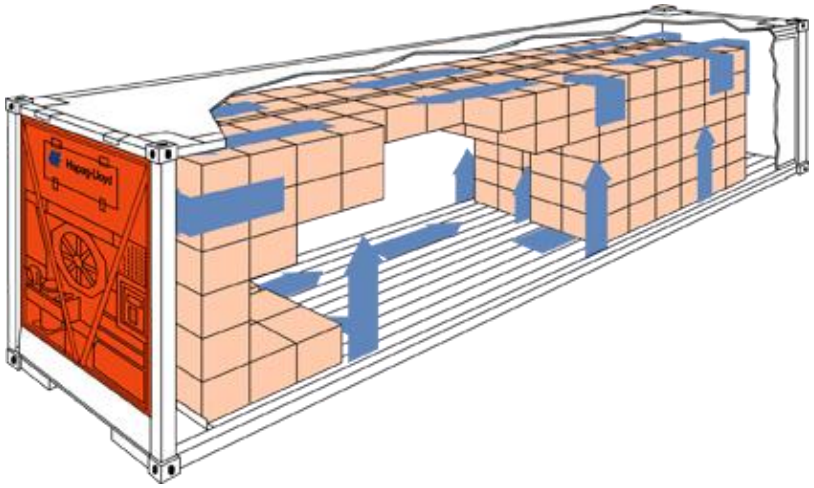

Figure 1-11. Air circulation in a reefer container. Reproduced from Hapag-Lloyd (2011)

Several studies have quantified the temperature heterogeneity within reefer containers during the shipping period, although most of them have been done on 20’ containers (Tanner and Smale, 2005). Amos and Sharp (1998) reported temperature variability of 2°C after monitoring 20’ containers loaded with oranges during 16 d, whereas Billing *et al.* (1998) found a similar temperature range within 20’ kiwifruit containers after repeated years of evaluation. For 40’ containers, Tanner and Amos (2003) evaluated the temperature variability inside a container loaded with fresh kiwifruit (setup at -0.5°C) during shipping from New Zealand to Belgium in a 26 d journey. They installed thermocouples in different points of each pallet and across the container width, recording their readings with data loggers to monitor the temperature distribution during the trip. Results showed that the area close to the door end had the maximum temperatures throughout the period, while the period travelling over the equator generated the highest differences in temperature distribution within the container (Figure 1-12). At that point, higher temperatures were always recorded on the container side where the ventilation unit was located (i.e. right side seen from door end), since the air flow was limited by the high

humidified air coming from the external ambient, causing frosting on the evaporator coil, resulting in the majority of cooling occurring on the opposite side. Important temporal variability was also generated by defrost cycles, as the cooling unit suspends the air flow to melt the ice deposited over the evaporator. As a result, even if the average temperature was maintained near to the set point during the trip, spatial and temporal variability led to at least 4°C of temperature heterogeneity across the shipping period.

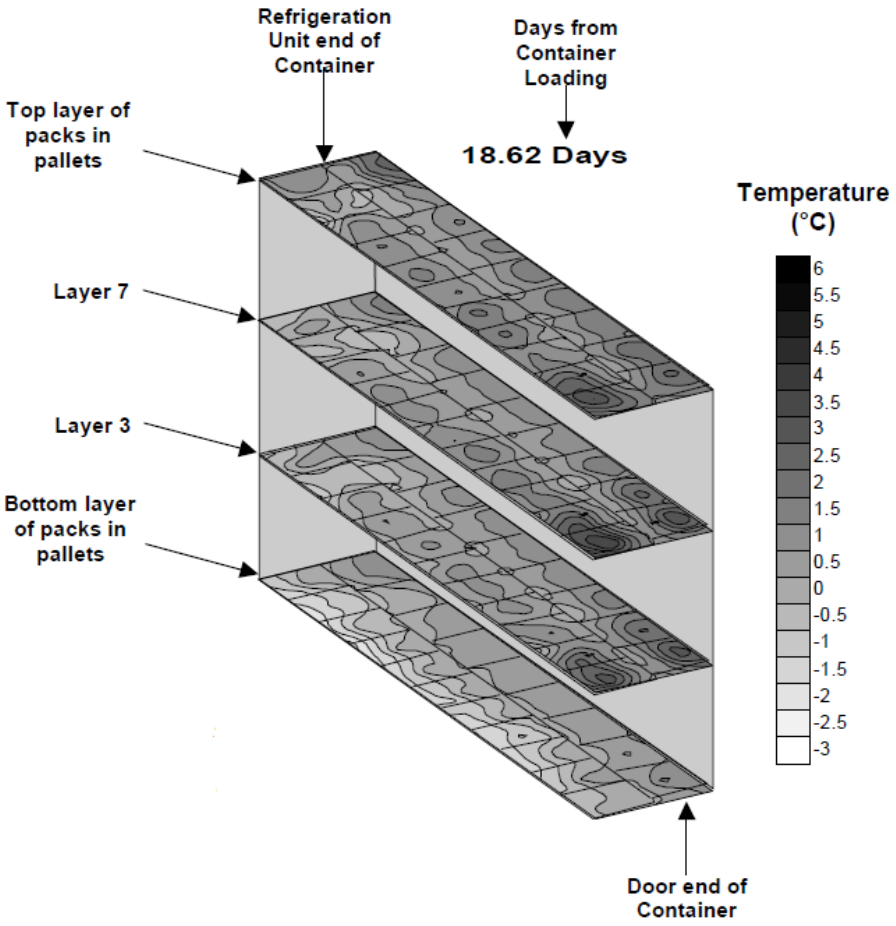

Figure 1-12. Container temperature distribution at the Equator. Reproduced from Tanner & Amos (2003)

Temperature has an important effect on blueberry quality, influencing decay incidence, moisture loss, firmness and chemical composition. The temperature behaviour of containers during marine shipments represents a potential source of quality variability for sea freighted blueberries which has not been evaluated so far. Higher temperature spots within blueberry containers may enhance microbial growth

which potentially could spoil the whole cargo. Furthermore, to maintain a high quality consistency among exported fruit shipments is as important as achieving buyer's quality requirements. Fruit trading relationships are built on fruit quality as well as on the accomplishment of commercial programs, which rely on agreed volumes (Wills *et al.*, 2007). Therefore, the impact of temperature heterogeneity within containers on blueberry quality rises as a key issue affecting the acceptability of this commodity in the market place.

1.4 Research questions and objectives

After harvest, fresh blueberries deteriorate rapidly due to decay, softening and shrivelling. The evolution of their quality from harvest to market is determined by diverse physiological, physical and pathological processes. Although blueberries are classified as climacteric fruit, ethylene related ripening changes have a minor influence on their postharvest deterioration since they are fully ripe at the moment of harvest. On the other hand, pathogenic fungi associated to blueberry are able to grow across the entire postharvest chain, constituting the main factor of blueberry quality loss. Furthermore, moisture loss has an important impact on the postharvest of this commodity by causing fruit shrivel and also reducing the total saleable weight.

Postharvest softening in blueberries is a major problem for the export industry. The mechanisms for this observed softening are not completely understood. With most of the cell wall modifications occurring before harvest during the ripening process, fruit moisture loss seems to have an important role in defining the firmness changes of blueberries at postharvest. Previous experiments have reported interesting correlations between these two parameters, though no studies have been conducted to investigate this relationship. Improving the current understanding of moisture loss influences on blueberry firmness could provide useful information for the postharvest management of this product.

Temperature is the most important environmental factor affecting blueberry quality. Fresh blueberries must be stored at temperatures close to 0°C and 90-95% RH for maximum postharvest life (2 and 4 weeks for highbush and rabbiteye cultivars,

respectively). Higher temperatures accelerate blueberry and pathogen metabolism and increase the moisture loss gradient between the fruit and the ambient, leading to higher decay, softening and moisture loss at storage. However, temperatures in the range of 0-5°C have not resulted in consistent differences on blueberry quality attributes. Moreover, the range of temperature heterogeneity within refrigerated containers has been reported to be 4°C during fresh fruit shipping. As containerised sea freight is being increasingly used for blueberry exports, the question remains, if temperature heterogeneity during shipping could constitute an important variability factor affecting blueberry final quality at the market place.

Storage delays at temperatures higher than the optimum range have shown to decrease the postharvest life of blueberries during subsequent storage. The residual effect of cooling delay at storage has shown to be function of the holding period and the temperature, though its effects considering blueberry packing conditions (i.e. 10°C) have not been fully tested on decay and weight loss, nor for more than 3 weeks storage. As blueberries are pre stored at packing temperature until they reach the process line, to evaluate the incidence of this period on blueberry final quality should provide relevant information for the packing logistics.

Controlled atmosphere with 8-15% CO_2 and O_2 concentration above 1% is widely used in marine exports to extend the postharvest life of fresh blueberries. The success of CA in increasing blueberry storability relies mainly on the effect of CO_2 delaying pathogen development, although some studies have reported a further decay reduction when O_2 is maintained below 10%. On the other hand, either increased CO_2 concentrations or lower O_2 can trigger physiological damage in blueberries which leads to softening, off-flavours and decolouration, constituting an important risk for the export industry. However, elevated O_2 concentrations (i.e. 17-18%) have been suggested to reduce softening damage induced by high CO_2. Therefore, further evaluations of O_2 influence on blueberry quality are still required to improve the use of CA in blueberries.

Temperature management deficiencies during the postharvest chain (i.e. cooling delays, container temperature variability) could also interact with the shipping atmosphere, altering quality responses of blueberries. It has been reported that

storage temperature affects gas concentrations within blueberry tissues in modified atmosphere systems. Increased temperatures decrease CO_2 and O_2 solubility in aqueous solutions, as well as accelerate the CO_2 production and O_2 uptake by increasing the respiration rate. Consequently, whether temperature heterogeneity during the shipping period could alter CA effects on blueberry quality attributes remains an open question. Furthermore, that CA might modify the effects of temperature variations or cooling delays on quality responses is also a possibility which needs to be explored.

The following objectives have been defined for this study in order to address the exposed research questions:

- To investigate the relationship between moisture loss and postharvest firmness for blueberries

- To evaluate the impact of potential temperature variability within shipping containers on blueberry quality outputs

- To assess the impact of potential cooling delays at blueberry packing temperature on the quality attributes of this fruit

- To compare the effects of different O_2 concentrations on main blueberry quality indicators

- To investigate possible interactions between temperature management deficiencies during blueberry postharvest and the storage atmosphere, in terms of blueberry final quality

These questions were investigated over a single season by using one cultivar of both highbush and rabbiteye blueberries. A single large experiment considering a full matrix of delay times, temperatures and storage atmospheres was setup, in order to address the questions related to storage condition effects on blueberry quality and their possible interactions during the postharvest chain. A second small experiment

was conducted specifically to investigate the influence of weight loss on blueberry firmness at postharvest and the possible mechanisms involved in this relationship. It is expected that the outputs of this research will contribute with useful and applicable information to improve the quality of fresh blueberries at the market place and to the further development of the blueberry export industry of the southern hemisphere.

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

This chapter details the materials and methods for the experiments conducted in the study. Some common issues such as fruit origin, quality measurements and data analysis are detailed here once as a way to avoid repetition and to highlight the most important aspects of each experiment.

2.2 Fruit source

The fruit used in this study comprised two cultivars of two different blueberry species. ‘Brigitta’, a modern global cultivar of northern highbush blueberry (*Vaccinium corymbosum L.*) developed in Australia (Bañados, 2006; Hancock, 2006) and ‘Maru’, a local cultivar of the rabbiteye specie (*Vaccinium ashei* Reade) which was developed in New Zealand during the 1990’s from crossing of ‘Tifblue’ and ‘Homebell’ cultivars.

Fruit were obtained from a commercial orchard located near Hastings, Hawke’s Bay, New Zealand, and collected from a single block with homogeneous plant condition and common technical management (i.e. nutrition, irrigation, pruning and pest control). Berries were hand harvested on the same day by farm pickers according to commercial practices and normal harvest index (i.e. 100% blue colouration). After picking, fruit were immediately moved to the farm packhouse and kept at 10°C. Subsequently, fruit was placed into chilly bins at 18°C and transported by car for 2 h to the Postharvest Laboratory of the Institute of Food Nutrition and Human Health (IFNHH), Massey University, Palmerston North.

2.3 Data analysis

All statistical analysis was conducted using MINITAB version 16.1.0 (Minitab Inc., Pennsylvania, USA). Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) command after checking assumptions of normal distribution and homogeneity of variance. Tables presented in this work were created using Microsoft Excel 2010 whereas figures were designed by using Microsoft Excel 2010 and MINITAB version 16.1.0 (Minitab Inc., Pennsylvania, USA).

2.4 Effects of temperature management deficiencies on blueberry quality under simulated sea freight conditions (Experiment 1)

2.4.1 Introduction

The main objective of this experiment was to evaluate the effects of temperature management deficiencies during postharvest on blueberry quality under different storage atmospheres in simulated sea freight conditions. The experiment was conducted in parallel on two cultivars from different species. Temperature deficiencies were created by subjecting the fruit to cooling delays and subsequently storing at two different temperatures with equivalent absolute humidity to simulate variability within export containers. Delays represented the duration of prestorage managements (i.e. prepacking, packing) on blueberry quality (Ferraz *et al.*, 2001), whereas storage conditions simulated the temperature range reported for 40’ reefer containers (Tanner and Amos, 2003) combined with or without controlled atmosphere. Direct influences of cooling delay, temperature and atmosphere on quality parameters throughout storage were also investigated. All the storage conditions and quality attribute measurements were conducted at the Postharvest Laboratory of the IFNHH, Massey University, Palmerston North.

2.4.2 Fruit material

The fruit material used in this experiment corresponded to 32 kg per cultivar of 'Brigitta' and 'Maru' cultivars. Picking and transport was conducted following the guidelines stated previously, with 'Brigitta' picked on 02/02/2011 and 'Maru' on 11/02/2011.

2.4.3 Sample configuration

Once at 20°C in the laboratory, fruit were mixed to homogenise and hand graded to eliminate major visual defects (e.g. peduncle, scars and cracks). For each cultivar, 182 samples of 140 g of fruit were setup in vented polyethylene clamshells. Each sample comprised a sub-sample of 22 fruit contained in a previously weighed cotton mesh bag of 10 cm x 10 cm. Each sub-sample was identified, weighed and placed inside a clamshell which was subsequently filled with additional fruit to reach 140 g net. Samples were randomised and finally identified and labelled on the clamshell lid. Clamshells used in this experiment corresponded to the model utilised by the blueberry industry for marketing of 125 g (T2907, Flight Plastics, Auckland, New Zealand).

2.4.4 Experimental design

For each cultivar, three equal groups of samples were subjected as open clamshells to one of three storage delay options (0, 12 or 24 h delay) at 10°C in a controlled temperature room, simulating normal prestorage periods for the blueberry industry (NeSmith *et al.*, 2005). Immediately after completing the delay period, clamshells were closed and placed in the storage environment. The storage condition corresponded to either air or one of two controlled atmospheres (10 kPa CO_2 + 2.5 kPa O_2 or 10 kPa CO_2 + 20 kPa O_2) within the range recommended for blueberries (Ceponis and Cappellini, 1979, 1985; Fan *et al.*, 1993; Harb and Streif, 2006), combined with a temperature of either 0°C or 4°C, for a maximum period of 6 weeks. Temperatures were chosen since 0°C is recommended as the optimum storage

temperature for blueberries (Forney, 2009; Perkins-Veazie, 2004), while a variability of 4°C was reported by Tanner & Amos (2003) to occur within 40' reefer containers during extended marine shipments. In order to represent the effect of potential container temperature variability on relative humidity (RH) distribution within containers, a specific RH was associated to each storage temperature, being 90% and 67% RH used for 0°C and 4°C, respectively. Whereas 0°C and 90% RH are the optimum conditions recommended for blueberry storage (Perkins-Veazie, 2004), 67% RH corresponds to the same absolute humidity of the air than at 0°C and 90% RH, but expressed as RH at 4°C (Talbot and Baird, 1991). This value of RH was calculated by using Eq 1. RH conditions were constantly monitored and maintained by using humidifier and dehumidifier equipment. Thus, a multifactorial design was created by the full combination of these three factors (i.e. delay, atmosphere and temperature), defining a total of 18 treatments which were conducted in duplicate. Each treatment replication was kept inside an air tight closed polyvinylchloride (PVC) container of 13.5 L and comprised five destructive samples which were maintained under storage conditions until quality evaluation.

Eq 1

$$\text{Partial pressure of vapour } (Pa) = 611 \exp\left\{17.27 \left(\frac{\text{Temperature } (°C)}{\text{Temperature } (°C) + 237.3}\right)\right\} \times \frac{RH}{100}$$

2.4.5 Storage system

Controlled atmosphere conditions were created by using a flow-through system which supplied the atmospheres from a mixer to the PVC containers. Gas cylinders of food grade oxygen (O_2) and carbon dioxide (CO_2) were connected to the mixer and used as continuous supply for the system. Nitrogen (N_2) and dry air were supplied to the mixer from an air compressor equipped with a N_2 generator. The atmospheres were established in the mixer by regulating each gas flow rate with a needle valve using a portable ADM 2000 gas flowmeter (Agilent Technologies, Delaware, USA) and combining the gases at the desired proportion (Figure 2-1). The

three different storage atmospheres were supplied from the mixer to a manifold which was used to split each gas mixture into 12 outputs (Figure 2-1), one for each PVC container (Figure 2-2). Atmospheres required 12 h to be established within the PVC containers, initially and after the subsequent samplings. Thirty six PVC containers were used in total, with five clamshells of each cultivar per container. Gas levels were checked three times per week during the experiment by taking samples from the manifold and the containers using 100 µL gastight syringes and obtaining the O_2 and CO_2 concentrations from an O_2/CO_2 analyser equipped with an O_2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK), with O_2-free N_2 as carrier gas. The gas mixtures were humidified by bubbling them through a glycerol solution before each container (Figure 2-2), with the volume maintained by replacing water evaporated weekly. Two glycerol solutions of different concentration were used to create relative humidity conditions (34.20% and 66.24% glycerol for 90% and 67% RH, respectively). Each atmosphere was supplied continuously to the containers using a flow rate of 50 mL min^{-1} which was setup individually for each container at the manifold regulating a needle valve with the portable flow meter.

Temperature conditions during storage were maintained by having the experimental equipment located in temperature controlled rooms at 0°C and 4°C respectively. The relative humidity was adjusted and monitored during the experiment to match RH setup inside the containers.

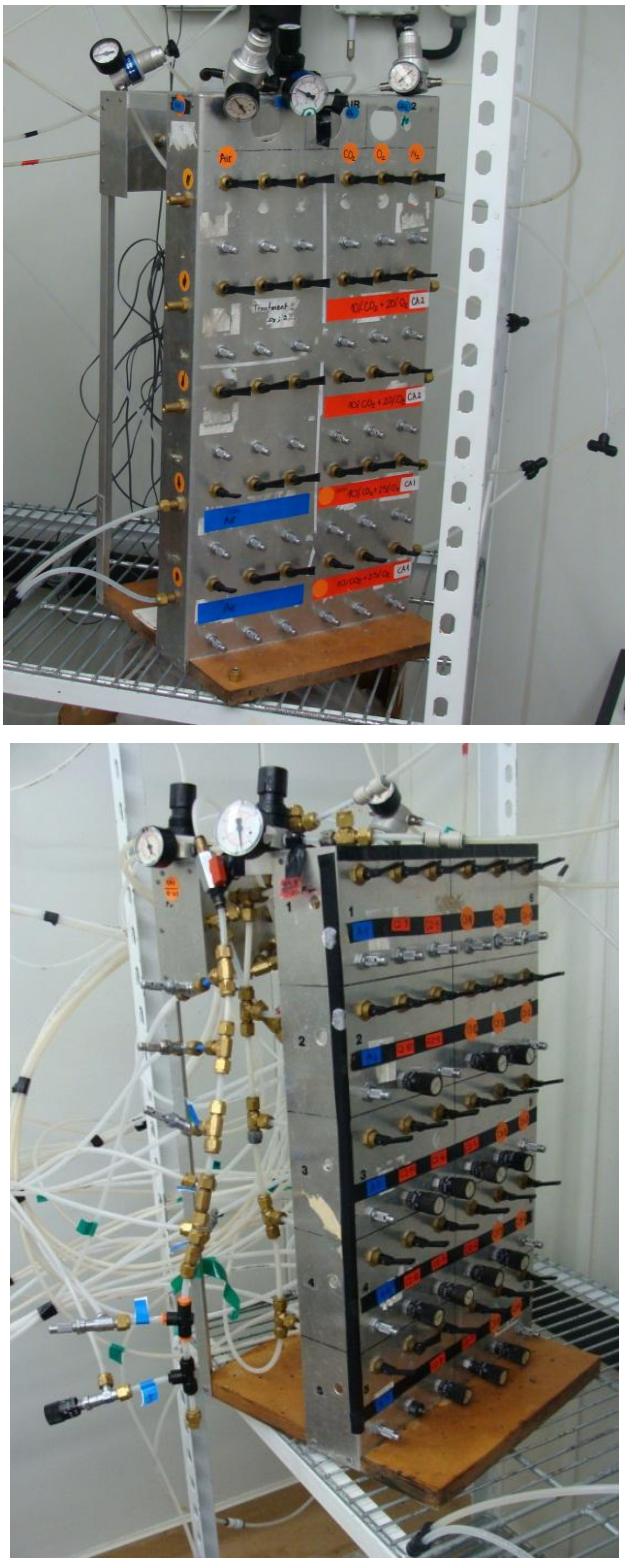

Figure 2-1. Mixer used to combine gases and create the atmospheres (A). Manifold with needle valves used to split the atmospheres received from the mixer and to supply them to the PVC containers (B).

Figure 2-2. PVC containers and jars filled with glycerol solution utilised in Experiment 1.

2.4.6 Evaluation

The following description details sampling methodologies and evaluation times for each cultivar during the experiment.

Two samples of 140 g of fruit each were evaluated before the storage delay period at the beginning of the experiment, corresponding to the initial quality (week 0) for all the treatments. Further evaluations of the treatments during storage were conducted weekly after an initial 2 weeks of storage, up to a total of 6 weeks by removing one sample from each container and conditioning it for 1 h at room temperature (20°C)

before measuring. Samples were removed from the storage environment and evaluated in random order to avoid bias. The containers were immediately closed after each sampling to allow recovery of the storage atmosphere. All samples were eliminated after the evaluation process. Quality attributes evaluated in this experiment were weight loss, firmness and rot incidence which were assessed in this sequence.

2.4.7 Statistical methods

The data set was analysed by testing the effect of each factor and their interactions on each quality attributes using ANOVA on untransformed data. Differences between means were assessed by using Tukey HSD (Honest significant difference) test. Factors considered in the model were delay, temperature, atmosphere, and evaluation time (i.e. week). Both cultivars were analysed independently as two different trials although they shared methodologies and storage systems. Significant differences were considered at 5%. Regression analysis was performed on weight loss data of each replication to obtain slopes, which were analysed with the same statistical methods.

2.5 Quality measurements

2.5.1 Weight loss

Weight loss was obtained for each sample as the difference between the initial and the final weight of the mesh bag containing fruit, after the subtraction of the mesh bag weight. The result was expressed as percentage according to Eq 2. A digital balance (PG503-S Mettler Toledo, Switzerland) of 0.001 g of precision was used for this purpose. The weight loss rate was calculated for each sample as the value of the slope obtained from a linear regression analysis.

Eq 2

$$\left[\frac{(\text{initial weight - bag weight}) - (\text{final weight - bag weight})}{(\text{initial weight - bag weight})}\right](100)$$

2.5.2 Firmness

Twenty blueberries were randomly taken from each sub-sample and subsequently evaluated for firmness. Fruit firmness was measured using a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., UK) equipped with a 5 kg load cell (Chiabrando *et al.*, 2009), a heavy duty platform and a 25 mm cylindrical aluminium probe (Figure 2-33) (ASAE, 2003). Firmness was individually assessed for each blueberry using a non-destructive compression test, simulating a very gentle squeeze with the fingers in terms to simulate consumer perception (Slaughter and Rohrbach, 1985). Collapsed fruit or fruit presenting fungal mycelium were not selected for firmness evaluation. Each blueberry was compressed 1 mm (Ferraz, 2001; Saftner *et al.*, 2008), equatorially (Donahue and Work, 1998), using a test speed of 0.8 mm/s (Chiabrando *et al.*, 2009), a pre-test speed of 1.6 mm/s, and a trigger force of 0.5 g. The peak force (N) necessary to achieve the target distance was recorded (Chiabrando *et al.*, 2009; Saftner *et al.*, 2008; Schotsmans *et al.*, 2007). A flat metal ring of 10 mm internal diameter, 35 mm external diameter and 1 mm height was fixed above the centre of the platform to support blueberries before measurement (Figure 2-33).

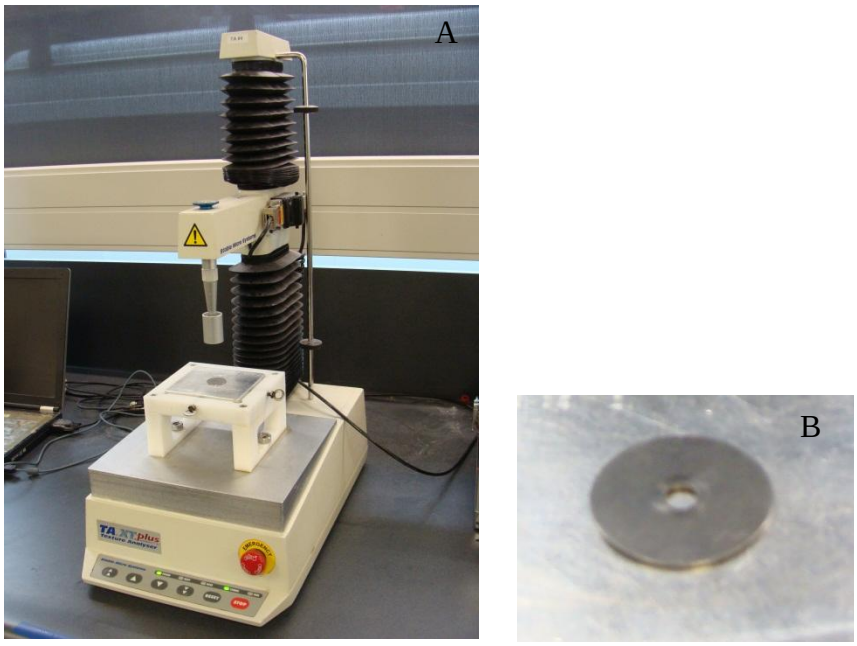

Figure 2-3. TA.XT Plus Texture Analyser equipped with heavy duty platform utilised to measure firmness of blueberries (A). Flat metal ring used to support samples (B).

2.5.3 Rot incidence

For rot incidence determination, fruit presenting fungal mycelium development on their surface, juice leaking or collapsed consistency due to decay were separated and weighed from each sample. The total weight of rotten fruit was related to the total weight of the fruit contained in the clamshell and expressed as percentage of rot incidence as detailed in Eq 3.

Eq 3

$$\left(\frac{(\text{rotten fruit weight})}{(\text{total fruit weight})}\right)(100)$$

CHAPTER 3

WEIGHT LOSS

3.1 Introduction

Moisture loss compromises the quality of fresh blueberries and constitutes a direct loss of saleable weight. For blueberries, excessive dehydration measured as weight loss (i.e. ≥ 5-8%) generates fruit shrivelling and has been related to postharvest softening (Forney *et al.*, 1998). Managing the critical postharvest steps which enhance blueberry moisture loss is a primary issue in maintaining acceptable quality.

Temperature management during postharvest has an important impact on blueberry weight loss. Higher storage temperature increases the rate of weight loss in fresh produce (Wills *et al.*, 2007), including blueberries (Nunes *et al.*, 2004; Sanford *et al.*, 1991), which results in higher total weight loss after storage. Fresh blueberries must be stored at 0-3°C and high relative humidity (RH) to reduce their weight loss. However, the temperature range in shipping reefer containers has been reported to be as much as 4°C around the set point (Tanner and Amos, 2003), which could generate a commercially relevant increase of blueberry weight loss during marine exports. Furthermore, cooling delays at temperatures higher than the optimum range increases the total weight lost from blueberries during subsequent storage (Ferraz *et al.*, 2001). Nevertheless, whether holding periods at 10°C simulating the prepacking storage and packing process could vary the weight loss response during blueberry storage is a question which still needs to be addressed.

The use of controlled atmosphere (CA) improves the postharvest life of blueberries by reducing pathogen development (Ceponis and Cappellini, 1985). CA has been reported to not affect weight loss for this fruit (Schotsmans *et al.*, 2007; Smittle and Miller, 1988). Gas concentrations within blueberry tissues are modified by temperature variations (Beaudry *et al.*, 1992; Cameron *et al.*, 1994). Therefore, whether temperature heterogeneity along the supply chain could modify CA effect on blueberry weight loss remains an open question. On the other hand, CA might

modify the weight loss responses generated from cooling delays and variable transport temperature.

In order to address these questions, the objectives of this chapter were:

- To evaluate the effect of potential temperature variability within refrigerated containers on blueberry weight loss

- To assess the influence of delay times at a common packing temperature used in the blueberry industry on blueberry weight loss during subsequent storage

- To evaluate possible interactions between shipping atmosphere and temperature management deficiencies on the weight loss of blueberry

The results of this chapter will permit the evaluation of the commercial relevance of temperature management deficiencies on the weight loss of sea shipped blueberries.

3.2 Experimental methods

The research questions were investigated by setting up an experiment which considered a full matrix between delay times, storage temperatures and controlled atmospheres. The experiment was conducted on a single cultivar of both highbush and rabbiteye blueberries, in pursuance of including in the investigation the high genotypic variability between blueberry species. Full details about the materials and methodologies of the experiment are found in Chapter 2.

A particular configuration of the RH conditions was utilised in the experiment, in order to represent the effect of container temperature variability on the RH distribution within the shipping environment. The temperatures included in the experiment matrix were 0°C and 4°C, simulating a container setup with the ideal conditions for blueberry storage (i.e. 0°C and 90% RH) and a spatial temperature variability of 4°C. A constant absolute humidity was deliberately maintained between the two temperatures of the model, resulting in different RH settings for

each temperature (Talbot and Baird, 1991). From psychrometric calculations, 67% RH was considered for 4°C, whereas 90% RH was used for 0°C. These RH settings should represent accurately the impact of temperature variability within containers on the absolute humidity gradient between the fruit and the environment, allowing assessment of its influence on blueberry weight loss. However, it must be clarified that the conditions applied to the fruit were finally altered by the use of packaging (clamshells), which should have limited the water vapour flux from the produce to the environment, in a similar way as they do in the industry context.

3.3 Results and discussion

Weight loss obtained during the storage period was lower than own experiences from blueberry export industry. After 3 weeks of storage, the total weight loss was 0.9% for both 'Brigitta' and 'Maru'. By the end of the storage period (6 weeks) the total weight loss for both varieties reached just 1.3%. From previous industry experience, the blueberry industry considers 5-7% of fresh weight as the maximum weight to be lost during 2-3 weeks of containerised marine export (at 0°C and 90-95% RH), with this difference being calculated between after packing and post container opening measurements. The differences in total weight loss between these experimental data and commonly industry values could be explained by logistical differences between both processes. Industrial practice contains some less controlled additional factors between weight measurements which could increase blueberry weight loss during the period, such as 2 h of forced air cooling (to rapidly cool to 0°C), 1-3 d of preshipping storage (also at 0°C and 90-95% RH), the container loading process and the time required for container condition establishment. These additional factors, not included in this experiment, may increase blueberry weight loss during industrial shipping inducing higher total weight loss beyond that observed in this study.

Previously reported weight loss for blueberries is highly variable, probably due to different experimental conditions. For instance, Borecka and Pliszka (1985) obtained just 0.3% of weight loss after storing highbush 'Herbert' blueberries at 0°C and high RH (i.e. inside sealed plastic bags) for 22 d, whereas Forney (1998) found that highbush blueberries (cv. Burlington) decreased their weight by 1% after 21 d at 3°C

and 85-95% RH. On the other hand, when lowbush blueberries were stored for 14 d at 0°C or 5°C with no RH regulation, the total weight loss was 5.3% and 7.6%, respectively (Sanford *et al.*, 1991). As such, the weight loss magnitudes obtained in this research agree with the results from other experiments which maintained high RH conditions together with low temperature.

Data of this experiment was analysed by performing ANOVA in order to assess the effect of each factor (delays in cooling, atmosphere and temperature) and their interactions on weight loss. The analysis of weight loss was conducted on means and on slopes, representing total weight loss and weight loss rate, respectively. Full details about the statistical methods used are provided in the section 2.4.7. The statistical analysis revealed that there were no major interactions between experimental factors; hence the results for each factor are discussed independently in this chapter. Storage time did not have a significant interaction with the experiment factors for weight loss.

3.3.1 Temperature effect

Higher temperature had a significant influence on weight loss during storage, consistently increasing the rate of weight loss and hence the total weight loss after storage. The rate of weight loss was approximately double at 4°C than at 0°C, for both cultivars (Table 3-1). Consequently, fruit stored at 4°C lost significantly more weight than at 0°C over the 6 weeks of storage, with differences between the temperatures of 0.3% and 0.4% for ‘Brigitta’ and ‘Maru’, respectively (Table 3-1).

The differences in weight loss rate and magnitude between the temperature conditions are expected results due to the increased absolute humidity gradient established in the 4°C treatment. The ambient temperature is the main factor determining the temperature of the stored product, which defines moisture content changes within plant tissues (Thompson, 2002). As intercellular air spaces can be assumed to be always saturated with vapour (i.e. 100% RH), higher temperatures of the product result directly in a rise of its absolute humidity (Talbot and Baird, 1991). In this experiment the absolute humidity of the storage environment was deliberately

designed to be constant irrespective of temperature, and therefore the treatments of 0°C at 90% RH and 4°C at 67% RH resulted in the same air absolute humidity (0.0034 kg kg^{-1}). Hence, the higher absolute humidity within fruit stored at 4°C should have resulted in an increased absolute humidity gradient between the fruit and the storage environment and consequently in a higher rate of moisture loss.

Table 3-1. Total weight loss and weight loss rate (per week) of blueberries over 6 weeks of storage, as influenced by storage temperature, delay duration at 10°C, and atmosphere for cultivars Brigitta (A) and Maru (B). Honest significant difference (HSD) values are used to report differences within factors. For each cultivar values followed by a letter in common are not different at 5% level. Number of independent measurements (n) is indicated.

A. 'Brigitta'

factor	*value*	*total weight loss (%)*	*weight loss rate (%/week)*
Temperature	0°C	0.93 a	0.10 a
n=90 (total weight loss)	4°C	1.20 b	0.17 b
n=18 (weight loss rate)	*HSD*	*0,08*	*0,05*
Delay	0 h	0.83 a	0.11 a
n=60 (total weight loss)	12 h	1.19 b	0.15 a
n=12 (weight loss rate)	24 h	1.18 b	0.15 a
	HSD	*0,11*	*0,08*
Atmosphere	Air	1.04 a	0.16 a
n=60 (total weight loss)	20%O_2 + 10%CO_2	1.12 a	0.12 a
n=12 (weight loss rate)	2.5%O_2 + 10%CO_2	1.04 a	0.13 a
	HSD	*0,11*	*0,08*

B. 'Maru'

factor	*value*	*total weight loss (%)*	*weight loss rate (%/week)*
Temperature	0°C	0.79 a	0.09 a
n=90 (total weight loss)	4°C	1.23 b	0.21 b
n=18 (weight loss rate)	*HSD*	*0,06*	*0,043*
Delay	0 h	0.61 a	0.14 a
n=60 (total weight loss)	12 h	1.17 b	0.17 a
n=12 (weight loss rate)	24 h	1.24 b	0.14 a
	HSD	*0,09*	*0,06*
Atmosphere	Air	0.97 a	0.13 a
n=60 (total weight loss)	20%O_2 + 10%CO_2	0.96 a	0.14 a
n=12 (weight loss rate)	2.5%O_2 + 10%CO_2	1.10 b	0.18 a
	HSD	*0,09*	*0,06*

From the psychrometric knowledge is possible to calculate that fruit absolute humidity should have risen from 0.0036 kg kg^{-1} to 0.005 kg kg^{-1} between 0°C and 4°C, respectively, whereas the ambient value was designed to remain unchanged at 0.0034 kg kg^{-1} for both conditions, resulting in an increase of the moisture content gradient of 8 fold (0.0002 kg kg^{-1} to 0.0016 kg kg^{-1}) between conditions. This difference in absolute humidity gradient should have been reflected in the rate of weight loss, which, although showed an increased value for 4°C, presented differences of just 2 fold between both temperatures (Table 3-1). As mentioned previously, the use of clamshells results in the environmental conditions to which the fruit are subjected to within the clamshell being potentially different to that supplied outside the clamshell. Enclosed fruit packages help to maintain the moisture of fresh products by acting as extra physical barriers against vapour diffusion, in addition to limiting air flow within the package. Water vapour from produce transpiration tends to accumulate within the package, increasing the humidity and hence reducing subsequent product weight loss (Wills *et al.*, 2007). The package used in this study corresponds to vented polyethylene clamshells used as standard by the industry, which has been evaluated to decrease effectively the weight loss of different species of berry fruit (Singh, 1992). Consequently, it is likely that during this experiment the final RH within the clamshell was an intermediate situation between the environmental conditions applied to the clamshell and the equilibrium condition due to the design of the clamshell.

The statistically significant differences on blueberry weight loss generated by 0°C and 4°C in this experiment are small and probably not relevant in the industry context. Considering that the total weight loss experienced by the industry during the export period (2-3 weeks) is around 5% and that this magnitude would be highly influenced by additional prestorage managements, differences on weight loss rate of 0.1% per week between both temperatures do not seem to constitute an important impact on the quality of blueberries nor the total saleable weight. The ‘buffer effect’ of clamshells attenuating the temperature incidence on the absolute humidity gradient appears as a key issue decreasing the impact of temperature heterogeneity on blueberry weight loss. Accordingly, this experiment suggests that temperature variability within shipping containers would not represent an important impact on the weight loss of blueberries, at least for ‘Brigitta’ and ‘Maru’ cultivars, and for a

storage period of up to 6 weeks. Additional factors during the postharvest chain such as the forced air cooling after packing and the period of container condition establishment could constitute more relevant factors affecting the weight loss of this commodity which might require further investigation.

3.3.2 Cooling delay effect

Cooling delays at 10°C simulating packing temperature increased the total weight loss of blueberries over the subsequent storage period. Regardless of the delay duration (i.e. 12 or 24 h), cooling delay led to 0.4% and 0.6% more total weight loss at storage than no delay in 'Brigitta' and 'Maru', respectively (Table 3-1). On the other hand, the rate of weight loss during the subsequent storage was not affected by the cooling delay times, in either cultivar (Table 3-1).

The observed residual effect of delayed cooling on the total weight loss at storage is an expected consequence of the increased moisture loss during the delay period. As moisture loss from fresh produce depends on the absolute humidity gradient between the product and the environment (Talbot and Baird, 1991), higher temperatures and longer times during the delay increase the produce weight loss during this period. Total weight loss after subsequent storage is therefore a direct consequence of the weight loss during the delay, which is simply amplified by the storage conditions. This has been previously confirmed for blueberries, where different temperature/time delay combinations have modified the total weight loss after subsequent storage, but not the weight loss rate during storage (Tetteh *et al.*, 2004). As such, the results obtained in this study are in agreement in that total weight loss of blueberries after storage increased as delay duration increased from 0 to 12 h, without altering the rate of weight loss. However, it was also expected that 24 h delay would lead to higher weight loss than 12 h delay, although this was not observed for either cultivar. A possible explanation for this could be a progressive drying and suberisation of the stem scar as delay period was extended beyond 12 h. The stem scar is known to be one of the primary pathways for weight loss in blueberries (Perkins-Veazie *et al.*, 1995a), and periods of 8 h at 18°C have been reported to stimulate the drying of the stem scar in blueberries (Mainland, 1995). Consequently, the rate of weight loss

during the delay period would have been reduced, resulting in non-significant differences between 12 and 24 h delay at the subsequent storage.

The results of the experiment agree with previous research evaluating the influence of cooling delays on blueberry quality, which have consistently reported increased total weight loss after subsequent storage. For example, increasing delay periods within 0-24 h range at high temperatures (30-32°C) have resulted in progressive increases of total weight loss of up to 2% after subsequent cold storage (Ferraz *et al.*, 2001; Tetteh *et al.*, 2004). Likewise, when delays have included intermediate temperatures, such as packing conditions (10°C), they have also led to increased total weight loss after storage (Jackson *et al.*, 1999; Paniagua *et al.*, 2012). However, the residual effect of delay at these temperatures has not been clear for periods shorter than 20 h. Paniagua *et al.* (2012) suggested higher total weight loss after storage of 'Maru' blueberries due to increased cooling delay times at 10°C from 4 to 20 h, although this was not confirmed. Accordingly, the weight loss data in this study confirms the residual effect of cooling delays shorter than 20 h at packing temperatures on total weight loss of blueberries after subsequent storage.

When applying these results to the blueberry industry context, the effect of cooling delay at 10°C on weight loss obtained in this experiment could imply a relevant impact of extended packing periods on blueberry quality. The difference of average weight loss between no delay and 12-24 h delay was 0.4% and 0.6% for 'Brigitta' and 'Maru', respectively. This approximately 0.5% increase in weight loss could constitute a serious risk of reaching shrivelling threshold levels (5-8%) during the export period (2-3 weeks), assuming that the additional factors along the commercial chain of blueberries which lead to increased weight loss remain. Similarly, since total weight loss higher than 2% have been correlated to blueberry softening, this potential increase of weight loss due to cooling delays could also lead to further quality reduction expressed as softening. Furthermore, a weight loss increase of 0.4-0.6% during the supply chain should mean a relevant loss of saleable weight for blueberry exporters. On the other hand, it should be considered that the particular conditions of this experiment for the delay simulation led to similar total storage weight loss between 12 h and 24 h delay, although a further increase of this parameter should be expected under commercial conditions as delay duration increases. In fact, different

studies conducted on blueberries have reported a progressive increase of total weight loss after storage when delay times have been increased (Ferraz *et al.*, 2001; Tetteh *et al.*, 2004). This could mean that the potential impact of delays at packing conditions on blueberry weight loss outputs could be greater than the values obtained in this experiment, especially when delay times exceed 12 h. Nevertheless, the effect of this factor could be partially minimised at the end of the packing process, where all the clamshells are normally checked and adjusted for weight. Consequently, improving packhouse logistics in order to reduce delay times at packing temperature below 12 h should effectively reduce the weight loss of blueberries, decreasing the risk of fruit shrivelling and the loss of saleable weight during the postharvest chain.

3.3.3 Atmosphere effect

Controlled atmosphere (CA) did not consistently influence blueberry weight loss during storage. There were no differences in total weight loss across the atmospheres tested for 'Brigitta', whereas for 'Maru' fruit kept at low O_2 (2.5%) atmosphere generated slightly higher total weight loss after storage than the other atmospheres (Table 3-1). Previous studies have reported no effects of storage atmosphere on weight loss of blueberries (Duarte *et al.*, 2009; Schotsmans *et al.*, 2007; Smittle and Miller, 1988), agreeing with the results for cv. Brigitta. The slightly higher total weight loss observed for 'Maru' blueberries kept at low oxygen atmosphere could be associated to accelerated tissue metabolism due to anaerobiosis. Although weight loss has not been evaluated in blueberries when fermentation damage has occurred, textural changes and tissue decolouration are known to happen in this fruit under anaerobic conditions (Fan *et al.*, 1993; Harb and Streif, 2004). As shown in other fruit such as tomatoes, anaerobic conditions can also lead to increased weight loss due to increased sugar loss and accelerated senescence (Park *et al.*, 1994). Fermentation has been reported to occur consistently at O_2 concentrations below 1% in blueberries (Ceponis and Cappellini, 1985), though symptoms associated to anaerobiosis have been detected at 2% O_2 in some cases (Fan *et al.*, 1993). Furthermore, gas threshold levels associated to fermentation in blueberries vary among species and cultivars (Pesis, 2005). Internal O_2 concentrations within blueberry tissues are known to decrease with increased temperatures (Beaudry *et al.*, 1992; Cameron *et al.*, 1994), and hence temperature variations at storage could have

contributed to a further decrease of O_2 concentrations at some stage during the experiment. However, this effect could not have been consistent since there was no interaction between storage atmosphere and temperature in the data of this study.

Results from the experiment confirm practically no influence of CA on the weight loss of blueberries during storage, showing no additional benefits for the industry in terms of avoiding fruit shrivelling and reducing saleable weight loss. Slight differences observed for 'Maru' blueberries between atmospheres (0.1% over 6 week storage) would not represent a relevant weight loss variation for the export industry. Nevertheless, the potential effect of low O_2 tissue toxicity on blueberry weight loss might be taken on account and possibly addressed in future experiments.

3.3.4 Atmosphere and temperature deficiencies interaction

Storage atmosphere did not influence weight loss responses produced by temperature management deficiencies during blueberry postharvest chain. There were no interactions for weight loss between atmosphere and temperature during the period of storage of 'Brigitta' and 'Maru', indicating that weight loss obtained was a result of the storage temperature (i.e. 0°C or 4°C), regardless if the atmosphere utilised was 2.5% O_2+10% CO_2, 20% O_2+10% CO_2 or air. Similarly, no interactions were found between cooling delays and atmosphere for 'Brigitta' and 'Maru', showing that delay times influenced blueberry weight loss after storage independently of the atmosphere chosen to maintain the fruit. Both results are in agreement with the null influence of storage atmosphere on weight loss of fresh blueberries reported by previous studies (Duarte *et al.*, 2009; Schotsmans *et al.*, 2007; Smittle and Miller, 1988). In terms of the export industry context, the use of CA during marine shipments would represent no benefits in reducing the impact of cooling delays at 10°C and temperature containers variations on the weight loss of fresh blueberries.

3.4 Conclusion

Variations of temperature in the range reported for 40'' containers over transoceanic fruit sea freight (i.e. temperature increases of 4°C from the set point) seems to slightly affect the total weight loss of commercially packed blueberries during the shipping period. Compared to optimum conditions (0°C and 90% RH), subjecting fresh blueberries at 4°C would increase the weight loss of highbush 'Brigitta' and rabbiteye 'Maru' blueberries during 6 weeks of storage; however, this difference would generate a non-relevant impact on the fruit quality and the total saleable weight.

Delay times at the common packing temperature (i.e. 10°C) would generate a residual effect on the total weight loss of fresh blueberries after subsequent cold storage. Prestorage holding periods of 12 h or more prior to cooling seems to increase the total weight loss of blueberries cv. Brigitta (highbush) and cv. Maru (rabbiteye) after 6 weeks of storage, without affecting the weight loss rate during this period. Reducing delay times below 12 h during the packing process would be a significant way to avoid blueberry fruit shrivelling and saleable weight loss over the shipping period.

As stated by previous research, atmosphere composition seems to not affect the weight loss of fresh blueberries during cold storage. The use of CA compared to air storage would not alter the weight loss responses of 'Brigitta' and 'Maru' blueberries over a shipping or storage period of up to 6 weeks. Furthermore, CA seems to not provide additional benefits than air in reducing the effects of temperature management deficiencies (i.e. cooling delays and temperature container variability) on blueberry weight loss. Consequently, in terms of weight loss outputs, the use of controlled atmosphere during the blueberry export process would not be justified.

CHAPTER 4

FIRMNESS

4.1 Introduction

Firmness is a major factor influencing the consumer appeal for fresh blueberries and is often indicated as the most critical quality attribute after decay incidence (NeSmith *et al.*, 2002). Harvested blueberries soften during the postharvest chain, which limits marketable life and leads to important economical loses to the fruit industry (Ehlenfeldt, 2002; Prussia *et al.*, 2006). The mechanisms defining postharvest softening in blueberries are not completely understood, although fruit moisture loss (Forney *et al.*, 1998) and cell wall modifications (Allan-Wojtas *et al.*, 2001; Angeletti *et al.*, 2010) have been related to this phenomenon. New research focused on a better understanding of blueberry firmness responses to postharvest variables is still required in order to improve the quality outcomes of this crop.

Storage temperature influences firmness of fresh blueberries. According to recommendations, harvested blueberries must be stored at 0°C and 90-95% RH to delay deterioration (Ehlenfeldt, 2002; Perkins-Veazie, 2004). Increased temperature has shown to reduce the firmness of blueberries during storage (Nunez-Barrios *et al.*, 2005; Sanford *et al.*, 1991). However, whether a temperature variability of 4°C around the set point in the storage environment, as reported for reefer containers, would generate a commercially relevant impact on firmness during the storage of blueberries needs to be confirmed.

Cooling delays can potentially generate a residual effect on blueberry firmness during storage. Delayed cooling for at least 20 h at temperatures higher than optimal has consistently resulted in decreased firmness of blueberries during subsequent storage (NeSmith *et al.*, 2002; Paniagua *et al.*, 2012). When evaluating cooling delay times at 10°C, which is a common temperature used for blueberry packing, 20 h delay resulted in softer blueberries than 4 h delay after 2 weeks of storage, with no differences after 3 weeks (Paniagua *et al.*, 2012). The questions remaining are if

shorter delay times at 10°C would produce a significant firmness reduction during subsequent storage and whether the residual effect of cooling delays at 10°C would last for more than 2 weeks during extended blueberry storage up to 6 weeks.

Controlled atmosphere (CA) is widely used for blueberry marine export due to its inhibitory effect on rot development and hence extension of postharvest life (Alsmairat *et al.*, 2011). CA concentrations higher than 15% CO_2 and lower than 1% O_2 have consistently generated fermentation damage in stored blueberries, although anaerobiosis threshold levels vary between cultivars (Ehlenfeldt, 2002; Forney, 2009). Compared to air storage, CA has rarely been reported to provide benefits reducing softening of harvested blueberries. In contrast, CO_2-induced fermentation is known to produce softening in blueberries during storage (Fan *et al.*, 1993; Forney *et al.*, 2003; Schotsmans *et al.*, 2007). However, a few experiments have reported that O_2 concentrations above 16% have alleviated CO_2-related softening, even when CO_2 concentrations have been maintained within the recommended range (8-15%) (Fan *et al.*, 1993; Harb and Streif, 2004, 2006). More evidence is still required to confirm the beneficial effect of high O_2 CA (> 16%) in reducing anaerobic softening risks for commercially used CO_2 concentrations.

It is known that higher temperatures reduce the solubility of CO_2 and O_2 in blueberry fruit tissue (Beaudry *et al.*, 1992; Cameron *et al.*, 1994). Accordingly, blueberries have shown increased sensitivity to low O_2 and better tolerance to elevated CO_2 concentrations at increased temperatures (Saltveit and Ballinger, 1983; Terry *et al.*, 2009). In fact, CO_2-induced softening in blueberries has been reduced by increased storage temperatures (Forney *et al.*, 1999; Forney *et al.*, 1998). Therefore, whether temperature variations in the storage environment of blueberries, as reported within shipping containers, could modify the internal CO_2 and O_2 concentrations and hence influence the CA induced softening responses is a question which needs to be addressed.

In order to address these questions, the objectives for this chapter were:

- To assess the effects of potential temperature heterogeneity within shipping containers on blueberry firmness

- To investigate the impact of different cooling delay times, at a temperature commonly used for blueberry packing, on blueberry firmness during extended storage (up to 6 weeks)

- To confirm the efficacy of atmospheres with more than 16% O_2 in reducing the fermentation related softening generated by commercially used CO_2 concentrations

- To evaluate potential interactions between storage atmosphere and temperature variability during postharvest affecting the firmness outcomes of blueberries

The outcomes of this experiment should contribute to understand how the different variables of the postharvest chain of blueberries impact on the firmness of this crop.

4.2 Experimental methods

To investigate these questions, an experiment considering a full matrix of 3 cooling delay times at 10°C, 2 storage temperatures and 3 controlled atmospheres was conducted. A single cultivar of both highbush and rabbiteye blueberries was included in the experiment to represent the high genotypic variability of this crop. Fruit was stored for 6 weeks, with evaluation weekly from 2 weeks storage. Firmness was individually assessed for each fruit using a non-destructive compression test, in order to simulate consumer perception by a gentle squeeze with the fingers (Slaughter and Rohrbach, 1985). The peak force required to achieve 1 mm compression was used as the firmness output of the measurement (Ferraz, 2001; Saftner *et al.*, 2008). Full details of the materials and methods used in the experiment are provided in Chapter 2.

4.3 Results and discussion

Fruit firmness increased in all the atmospheres and both temperatures during the storage of both cultivars compared to the initial condition of the fruit ($p < 0.05$), reaching maximum values at week 5 or 6 depending on storage factors (Figure 4-1). 'Brigitta' blueberries were 63% firmer at week 6 than at the beginning of the storage, whereas 'Maru' blueberry firmness increased 44% from week 0 to the end of storage. Compression firmness values stayed above 1 N during the storage of both blueberry cultivars (Figure 4-1).

Firming behaviour has previously been reported to occur in stored blueberries under experimental conditions. Blueberry moisture loss has been suggested to induce postharvest softening due to reduced turgor (Allan-Wojtas *et al.*, 2001; Forney *et al.*, 1998), although this particular firming response has previously correlated with low total weight loss (≤ 1-2%) during long term storage of blueberries (Chiabrando and Giacalone, 2011; Duarte *et al.*, 2009). The possible relationship between the firming obtained here and low weight loss agrees with the low levels of total weight loss (up to 1.3%) obtained in this experiment for both cultivars (section 3.3). Fruit 'drying and hardening' were suggested to be involved in blueberry firming due to reduced weight loss (Chiabrando *et al.*, 2009), although this has not been further investigated. While being previously reported in laboratory trials, the increased firmness obtained in this experiment does not represent the normal evolution of firmness in industry, and therefore this fact should be taken into account when analysing the effects of other postharvest factors on firmness.

The potential connection between moisture loss and firmness responses appears as a key relationship to investigate in order to improve blueberry quality. Miller *et al.* (1993) obtained increasing sensory evaluated firmness during storage when weight loss of blueberries was lower than 1%, whereas weight loss higher than 1% correlated with softening in the same experiment. Similarly, Forney *et al.* (1998) reported firming of 'Burlington' blueberries together with 1-2% weight loss but fruit softening when weight loss was 4-14%. Possible explanations for this behaviour

were not provided in these experiments. This relationship is further evaluated and analysed in an independent experiment in Chapter 5 of this work.

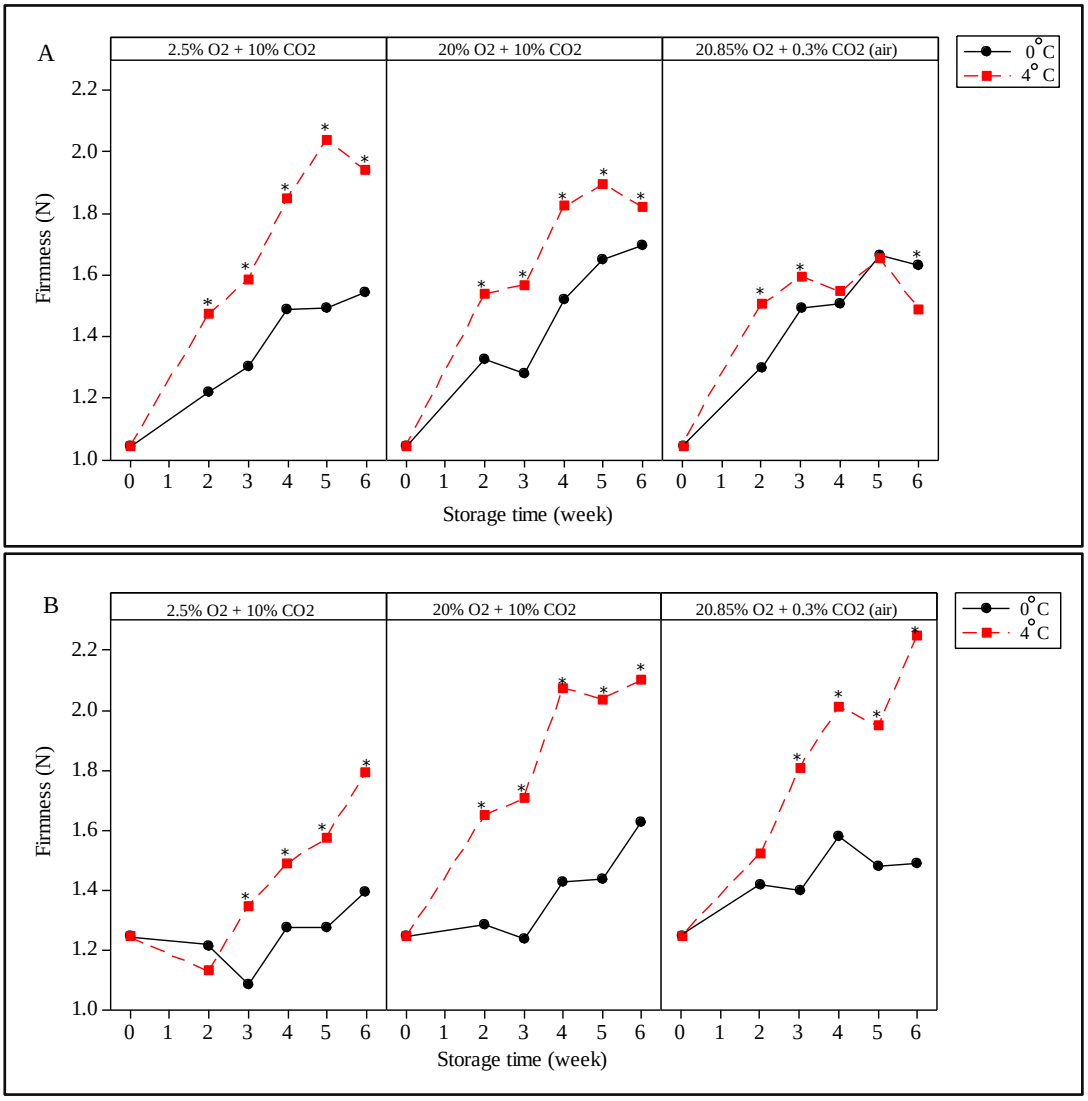

Figure 4-1. Firmness of 'Brigitta' (A) and 'Maru' (B) blueberries during storage, as affected by temperature and storage atmosphere. *represents significant differences between storage temperatures for each week at 0.05 level as determined by Tukey's test. Each data point represents 120 independent data measurements (n=120).

Blueberries maintained commercially acceptable firmness during the storage period of the experiment. Subjective evaluation by using 'touch firmness' is largely the preferred method used in the industry to assess the firmness of blueberries (Forney *et al.*, 1998). Despite the use in research work of several instruments able to measure blueberry firmness accurately, these equipments are not used extensively by blueberry traders due to their high price and low portability (Chiabrando *et al.*, 2009; Li *et al.*, 2011). Consequently, there is not an objective value used as a firmness

standard for this crop. However, previous experiments have delivered useful comparisons between compression firmness and sensorial evaluations. According to Ehlenfeldt and Martin (2002), firmness values in the 1.3-1.4 N range would represent an ‘average firmness’ for blueberries compressed up to 1 mm, while 1.5-1.6 N can be considered as ‘superior firmness’. In another study, Beaudry *et al.* (1998) classified blueberries presenting an average firmness of 0.87-0.89 N (1 mm compression) mainly as ‘moderately firm’. Consequently, it can be assumed that firmness of blueberries in the present experiment was maintained within commercial ranges from harvest up to the end of subsequent storage.

Data of this experiment was analysed with ANOVA, assessing the effects of cooling delays, temperature and atmosphere individually and the interactions between these factors. The analysis was conducted on untransformed firmness data and the measurement of each individual berry was counted as an independent subject for this purpose. The statistical methodologies used to carry this analysis are fully detailed in the section 2.4.7. Statistical analysis revealed that storage time did not have a major interaction with experiment factors, whereas the major factors affecting firmness responses in both cultivars were individual factors as well as the interaction between storage temperature and atmosphere. Therefore, the result discussion of the chapter follows this logic.

4.3.1 Temperature effect

Increased temperature resulted in firmer blueberries during the storage period. Regardless of atmosphere, fruit was firmer when stored at 4°C than at 0°C, with differences between both temperatures of 13% for ‘Brigitta’ and 28% for ‘Maru’ (Table 4-1). This result does not agree with most of the experimental evidence available for blueberries. It has largely been reported that increased temperatures lead to decreased firmness in fresh blueberries stored for periods ranging from 3 to 21 d (NeSmith *et al.*, 2005; Nunez-Barrios *et al.*, 2005; Sanford *et al.*, 1991; Tetteh *et al.*, 2004). This has been related to accelerated cell wall modifications and increased fruit moisture loss. As such, blueberries are recommended to be stored at 0°C to maintain acceptable quality.

However, a few previous experiments have reported increased blueberry firmness at higher temperatures during storage. Bunemann (1957) obtained higher sensory firmness and skin toughness in blueberries stored at 10°C than at 4.5°C which correlated to increased corrugation of the cell walls of epidermal and hypodermal cells. Forney *et al.* (1998) found that firmness of 'Burlington' blueberries increased during storage together with weight loss levels below 2%, whereas a storage temperature of 3°C resulted in a further firming compared to 0°C. No explanations were provided for these responses. According to these experiences, it appears that the particular effect of higher storage temperature generating an increase of blueberry firmness in this experiment would be related to the firming response, possibly associated to moisture loss, indicated previously in this chapter. Whether the higher weight loss (below 1-2%) obtained by increasing the temperature from 0°C to 4°C (section 3.3.1) would have led to increased dehydration of epidermal and hypodermal cell layers and therefore to a higher blueberry skin toughening perceived as higher firmness is a possibility. The weight loss and firmness relationship is further investigated in Chapter 5 of this work.

In terms of the industry scenario, the temperature effect on firmness observed in this experiment does not represent the normal behaviour of fresh blueberries subjected to temperature variations during the supply chain, which tend to soften at increased storage temperatures. Considering that in commercial conditions the total weight loss of blueberries is approximately 5-7% by the end of 2-3 weeks of export process (personal experience), the possible relationship between blueberry firming and low total weight loss indicated here appears to be determining the firmness response to temperature obtained in the present experiment. Nevertheless, the potential to obtain firmer berries during storage by increasing temperature under low weight loss conditions raises an issue of great interest for blueberry traders due to the relevance of this quality attribute.

4.3.2 Cooling delay effect

Cooling delays at common packing temperature did not have a residual effect on blueberry firmness during subsequent storage. Delay times of 12-24 h at 10°C before cooling did not generate significant differences on fruit firmness compared to 0 h delay after 2-6 week storage for both varieties (Table 4-1). This result conflicts with most previous work evaluating the influence of cooling delays on blueberry firmness during storage. Cooling delays at 20°C or more for at least 24 h have led to reduced firmness of blueberries during subsequent storage (NeSmith *et al.*, 2002). Furthermore, when delayed cooling at 10°C was evaluated in 'Maru' blueberries, a residual effect on firmness caused by 20 h delay was found after 2 weeks storage (Paniagua *et al.*, 2012), although in that trial firmness declined significantly in storage. Consequently, for this experiment it could have been expected that at least 24 h delay at 10°C would affect firmness, especially considering that the same cultivar was used. It seems possibly that the particular firming response obtained in this experiment could be masking the cooling delay effect on blueberry firmness reduction during storage. Future work focussed on evaluating the effect of delayed cooling on the firmness of this crop should ensure that storage conditions recreate the normal softening behaviour of blueberries during the postharvest chain.

In terms of the possible implications of these results for the fruit industry, it appears that blueberry traders could benefit from the low impact of cooling delays on subsequent firmness if the storage conditions which generated the firming observed in this experiment could be replicated in a commercial scale. In this case, reducing the cooling delay effect on blueberry firmness during storage would allow the industry to concentrate efforts on improvements in other steps of the supply chain, rather than increasing the time efficiency of packing process. A more holistic analysis considering other quality attributes relevant for consumer appeal is presented in Chapter 7 of this work. Assessment of this response in other blueberry cultivars is also necessary in order to evaluate this hypothetical scenario.

4.3.3 Atmosphere effect

Atmosphere influenced the firmness of blueberries during storage, although its effect varied depending on cultivar. Both controlled atmospheres (CA) generated slightly higher firmness (5% increase) compared to air during the storage of 'Brigitta' blueberries. However, 'Maru' blueberries were 19% firmer when stored at air or 20% O_2 + 10% CO_2 than at low oxygen CA (2.5% O_2 + 10% CO_2) (Table 4-1). These different firmness responses between cultivars agree with the high variability among blueberry genotypes for CA outcomes reported in previous work. When Forney *et al.* (1997) stored five highbush blueberry cultivars in 12.5% CO_2 during 9 weeks at a given O_2 concentration, 'Coville' and 'Reka' blueberries softened 23% and 41% after the period, respectively, whereas 'Bluegold', 'Brigitta' and 'Burlington' blueberries did not vary from their initial firmness. In other work, Alsmairat *et al.* (2011) compared the effect of atmospheres combining 6-19% CO_2 with 2-15% O_2 for 8 weeks on blueberry firmness obtaining a progressive firmness reduction as CO_2 increased and O_2 decreased for 'Brigitta', 'Jersey', 'Legacy' and 'Liberty' blueberries, while the firmness of 'Duke', 'Ozarkblue' and 'Toro' blueberries was not clearly affected by gas concentration. The skin of blueberries has been identified as a major resistance to gas inflow in blueberries (Cameron *et al.*, 1994), whereas the picking scar exposure enhances the entry of gas into the fruit (Paul *et al.*, 2012). Anatomical differences between blueberry cultivars such as epidermal thickness (Makus and Morris, 1993), epicuticular wax configuration (Sapers *et al.*, 1984) and stem scar diameter (Magee, 1999) could influence CO_2 and O_2 diffusion into blueberry tissue, affecting internal gas concentrations and hence contributing to genotypic variability in response to atmospheric change.

Fermentation induced by high CO_2 is known to produce softening in blueberries during storage (Fan *et al.*, 1993; Forney *et al.*, 2003; Schotsmans *et al.*, 2007), although anaerobiosis threshold levels vary between cultivars (Ehlenfeldt, 2002; Forney, 2009). Firmness behaviour observed for 'Brigitta' blueberries exposed to commercially used CO_2 concentrations has not been consistent, which could be in part related to different instrumental techniques used to measure firmness. 'Brigitta' blueberries were softer, as measured by using a durometer, when stored in 6-15% CO_2 (combined with 6-15% O_2) than in air after 8 weeks (Alsmairat *et al.*, 2011),

while CA comprising 10% CO_2 + 5% O_2 decreased blueberry firmness (measured as puncture test) compared to air storage after 7 week storage of 'Brigitta' blueberries (Duarte *et al.*, 2009). In contrast, the compression firmness of 'Brigitta' blueberries was not affected by 12.5% CO_2 atmosphere during a storage period of 9 weeks (Forney *et al.*, 1997). Compression firmness is considered the most accurate method to measure blueberry firmness (Angeletti *et al.*, 2010; Buran *et al.*, 2012; Cantín *et al.*, 2012; Duan *et al.*, 2011; Li *et al.*, 2011; Lopez *et al.*, 2010; Yang, 2009). Furthermore, while Chiabrando *et al.* (2009) reported a low correlation (Pearson's coefficient of 0.34) between durometer readings and compression firmness in blueberries, the puncture test is preferred to assess skin toughness rather than firmness in this fruit (Silva *et al.*, 2005). Consequently, compression firmness obtained in this experiment to 10% CO_2 regardless of O_2 concentration could indicate that 'Brigitta' blueberries did not undergo fermentation related softening during storage. This would agree with the results reported by Forney *et al.* (1997) for this cultivar, suggesting that fermentation threshold for this cultivar would be at a higher CO_2 concentration.

CA resulted in higher firmness than air for 'Brigitta' blueberries during storage in the present experiment, disagreeing with most of the previous work evaluating CA influence on blueberry firmness irrespective of cultivar. These experiments have reported either null influence of CA on firmness, or fermentation induced softening (Cantín *et al.*, 2012; Fan *et al.*, 1993; Forney *et al.*, 2003; Harb and Streif, 2004, 2006; Schotsmans *et al.*, 2007). In contrast, Prange *et al.* (1995) reported that lowbush blueberries were firmer when kept at 0°C in 5-15% CO_2 combined with 1-5% O_2 CA than in air during 2-6 week storage. Likewise, CA improves firmness retention in strawberries compared to air storage (Pelayo *et al.*, 2003; Van der Steen *et al.*, 2002; Wszelaki and Mitcham, 2000). High CO_2 concentrations during storage increase apoplastic pH in strawberry tissue (Nunes *et al.*, 1995), which leads to greater cell to cell adhesion and hence better firmness retention (Harker *et al.*, 2000). Additionally, a decrease in the expression of the pectin breakdown enzyme polygalacturonase (PG) has been detected in strawberry exposed to 20% CO_2 (Ponce-Valadez *et al.*, 2004). For blueberries, most of the cell wall disassembly occurs before harvest maturity (full ripening) (Vicente *et al.*, 2007), although PG enzyme has shown to peak at late stages of ripening (Proctor and Miesle, 1991) and

pectin depolymerisation has been reported to occur in the overripe stage (Vicente *et al.*, 2007). Coincidently, even if major firmness decrease occurs up to the fully ripe stage in attached blueberries (Proctor and Miesle, 1991), enzyme mediated softening is also occurring at a minor extent in overripe fruit (Ballinger *et al.*, 1973). Therefore, it might be possible that CA increased 'Brigitta' firmness compared to air storage in this work by altering the cell wall modifications which enhance softening in overripe fruit. CA comprising 10% CO_2 would have enhanced intercellular adhesion or reduced PG synthesis in this blueberry cultivar, as reported for strawberries.

'Maru' blueberries seem have been affected by CA in a different way than 'Brigitta' cultivar in this experiment. The firmness decrease observed for 'Maru' blueberries maintained in the low oxygen CA (2.5% O_2 + 10% CO_2) compared to air appears to be associated with CO_2-induced softening, although increased O_2 would have alleviated this effect in 20% O_2 + 10% CO_2 atmosphere. As reported in a number of previous studies, CA comprising CO_2 within the commercial range has decreased blueberry firmness. At a given O_2 concentration, 10% CO_2 have previously led to softer blueberries for 'Bluecrop' (Fan *et al.*, 1993) and 'Burlington' cultivars (Forney *et al.*, 2003) in comparison to 0% CO_2 atmospheres. This is in agreement with the only study testing the effects of CA on 'Maru' blueberries, which found that 15% CO_2 CA in combination with 2.5% O_2 resulted in softer fruit than air storage during 6 weeks (Schotsmans *et al.*, 2007). Consequently, it seems that the CO_2 threshold concentration which triggers softening in 'Maru' blueberries would be somewhere around 10-15% CO_2.

Table 4-1. Average firmness of blueberries over 6 weeks of storage, as influenced by storage temperature, delay duration at 10°C, atmosphere and temperature by atmosphere interaction for cultivars Brigitta (A) and Maru (B). Honest significant difference (HSD) values and different letters are used to report differences within factors. Number of independent measurements (n) is indicated.

a. 'Brigitta'

factor	***value***	***Firmness (N)***
Temperature	0°C	1.58 a
n=1800	4°C	1.69 b
	HSD	*0.03*
Delay	0 h	1.57 a
n=1200	12 h	1.59 a
	24 h	1.59 a
	HSD	*0.04*
	Air	1.54 a
Atmosphere	20%O_2 + 10%CO_2	1.62 b
n=1200	2.5%O_2 + 10%CO_2	1.60 b
	HSD	*0.04*
	0°C*Air	1.52 a
	4°C*Air	1.56 a
*Temperature*Atmosphere*	*HSD*	*0.04*
n=600	0°C*20%O_2 + 10%CO_2	1.50 a
	4°C*20%O_2 + 10%CO_2	1.73 b
	HSD	*0.05*
	0°C*2.5%O_2 + 10%CO_2	1.41 a
	4°C*2.5%O_2 + 10%CO_2	1.78 b
	HSD	*0.05*

b. 'Maru'

factor	***value***	***Firmness (N)***
Temperature	0°C	1.38 a
n=1800	4°C	1.76 b
	HSD	*0.03*
Delay	0 h	1.55 a
n=1200	12 h	1.59 a
	24 h	1.58 a
	HSD	*0.05*
	Air	1.69 a
Atmosphere	20%O_2 + 10%CO_2	1.66 a
n=1200	2.5%O_2 + 10%CO_2	1.36 b
	HSD	*0.05*
	0°C*Air	1.47 a
	4°C*Air	1.91 b
*Temperature*Atmosphere*	*HSD*	*0.06*
n=600	0°C*20%O_2 + 10%CO_2	1.40 a
	4°C*20%O_2 + 10%CO_2	1.91 b
	HSD	*0.06*
	0°C*2.5%O_2 + 10%CO_2	1.25 a
	4°C*2.5%O_2 + 10%CO_2	1.47 b
	HSD	*0.07*

Increased O_2 concentration would have alleviated the softening effect of 10% CO_2 for ‘Maru’ blueberries in this experiment. Contrary to the softening obtained for this cultivar in 2.5% O_2 + 10% CO_2 atmosphere, ‘Maru’ blueberries stored in 20% O_2 + 10% CO_2 had the same firmness as fruit maintained in air. This result agrees with Fan (1993), who found that CO_2-induced softening of ‘Bluecrop’ blueberries stored for 5 weeks was triggered by 5-15% CO_2 concentration when it was combined with 1 and 2% O_2, but not when O_2 was at 16.8%. Similarly, ‘Bluecrop’ blueberries showed notoriously less softening (14% versus 33%) compared to air storage in 12% CO_2 CA when combined with 18% O_2 instead of 2% O_2 (Harb and Streif, 2006).

The mechanism explaining the positive effect of elevated O_2 reducing CO_2-related softening would be possibly associated to a shift in the CO_2 fermentation trigger as O_2 varies. Ceponis and Cappellini (1985) reported the detection of off-flavours attributed to fermentation in highbush blueberries kept in 10-15% CO_2 and 2% O_2, while same CO_2 concentrations did not generate off-flavours when combined with 20% O_2. Likewise, O_2 concentrations of 2% or less enhanced the off-flavours produced by CO_2 in ‘Bluecrop’ and ‘Duke’ blueberries (Harb and Streif, 2006). An interaction between CO_2 and O_2 concentrations for fermentation was demonstrated by Beaudry *et al.* (1993) for ‘Bluecrop’ blueberries kept in modified atmosphere packaging, which proved that the tolerance to low O_2 declines as CO_2 concentration increases. In that experiment, it was suggested that O_2 levels would alter the tolerance to CO_2, although no data has been presented to confirm this so far. Elevated CO_2 suppresses respiration by inhibiting the activity of enzymes which use O_2 as substrate such as cytochrome oxidase (Kubo *et al.*, 1990). Therefore, it might be possible that increased O_2 could alter the CO_2 concentration trigger to activate fermentation by enhancing the activity of specific respiratory enzymes.

The slightly enhanced firmness observed for CA stored ‘Brigitta’ blueberries in the present experiment would probably not constitute a relevant issue in the fruit industry context. Small differences between compression firmness values as obtained in this case (5%) are not likely to be detected by sensorial firmness evaluation (Beaudry *et al.*, 1998; Ehlenfeldt and Martin, 2002), and hence influence consumer appeal. Additionally, the possible cell wall mechanism altered by CA and leading to this response would need to persist under the conditions to which blueberries are

subjected in industry, which seems unlikely due to the consistent softening which is known to occur during postharvest.

The alleviation of CO_2-induced softening obtained for 'Maru' blueberries by increasing O_2 concentrations may contribute to improve the safety of CA use in the blueberry export chain. In agreement with the results reported by a few studies in which 16.8-20% O_2 CA storage has decreased CO_2 injury in blueberries (Ceponis and Cappellini, 1985; Fan *et al.*, 1993; Harb and Streif, 2004, 2006), the response observed in this experiment would confirm a way to reduce the CA risks able to compromise blueberry quality. By using CA with increased O_2 concentration, blueberry exporters could benefit from elevated CO_2 concentrations to optimise decay control and simultaneously decrease the chances of high CO_2-related disorders. However, it is necessary to evaluate this interaction in main commercially grown cultivars due to the high genotypic variability for O_2 and CO_2 fermentation thresholds and CO_2 efficacy in suppressing decay. Future research should investigate the minimum O_2 concentrations required to achieve this potential benefit for each cultivar, as well as the maximum CO_2 concentrations which can be reached without leading to toxicity, for each O_2 concentration. Furthermore, it might also worth to evaluate if the use of CA with increased O_2 could reduce CA operational costs since gas levels could potentially be obtained just by modifying the CO_2 concentration, rather than both O_2 and CO_2 as it is currently done.

4.3.4 Atmosphere and temperature interactions

CA modified the effect of temperature on blueberry firmness during storage depending on cultivar. For 'Brigitta' blueberries stored in air, temperature did influence firmness (Table 4-1), although 4°C produced significantly firmer blueberries than 0°C after 2 and 3 weeks (Figure 4-1). However, during storage in 20% O_2 + 10% CO_2 and 2.5% O_2 + 10% CO_2 atmospheres blueberries were consistently firmer when kept at 4°C than 0°C (Figure 4-1), with firmness differences between temperatures of 15% and 26%, respectively (Table 4-1). On the other hand, temperature effect on firmness was consistent between storage atmospheres for 'Maru' blueberries. Regardless of the atmosphere, 4°C generated firmer fruit than

0°C during storage (Table 4-1), producing significant differences from the beginning of the storage in 20% O_2 + 10% CO_2 CA and from week 3 onwards in low oxygen atmosphere and air (Figure 4-1). Temperature effect on firmness was greater in 'Maru' blueberries maintained in 20% O_2 + 10% CO_2 and in air than in 2.5% O_2 + 10% CO_2, reaching firmness differences between temperatures of 36% and 30%, respectively, compared to 18% (Table 4-1).

The firming effect produced by higher temperature seems to have been reduced in 'Brigitta' blueberries stored in air compared to fruit kept in CA. As discussed in section 4.3.3, CA led to a slight increase of blueberry firmness in comparison to air possibly by modifying the normal cell wall changes associated to overripe softening. Therefore, it might be possible that these softening associated cell wall changes (such as pectin depolymerisation) could have been enhanced by increased temperature in air storage, resulting in smaller firmness differences between 0°C and 4°C for this atmospheric condition. In contrast, the temperature influence leading to higher firmness is clearly expressed in CA storage resulting in larger differences between both storage temperatures, which together with the CA effect would explain the maximum firmness values obtained for blueberries maintained in CA at 4°C (Table 4-1). Consequently, it appears that CA comprising 2.5-20% O_2 in combination with 10% CO_2 enhances the firming effect produced by higher temperature under low moisture loss conditions in 'Brigitta' blueberries.

Although CA did not largely modify the effect of storage temperature on firmness of 'Maru' blueberries, the CO_2-induced softening apparently occurred in 2.5% O_2 + 10% CO_2 decreasing the firmness of fruit stored at both temperatures and reducing the firming effect of 4°C in comparison to 0°C. Cell wall corrugation of outer fruit cell layers, which has been associated with blueberry firming, has previously been inhibited by 15% CO_2 in parenchyma and sclerenchyma cells (Allan-Wojtas *et al.*, 2001) and in epidermal and hypodermal cells (Bunemann *et al.*, 1957). However, since 20% O_2 CA seems to have alleviated the anaerobic injury triggered by CO_2 in the low O_2 atmosphere, the temperature effect on blueberry firmness would have been expressed in a more similar extent in this atmosphere than in air.

Applying these interactions between storage atmosphere and temperature to the industry, it seems that depending on cultivar differences and as long as harmful gas levels are avoided, CA would either not alter or improve the effect of temperature in increasing blueberry firmness under particular low dehydration conditions. Nevertheless, postharvest conditions able to replicate the particular firming response obtained in the present experiment should be achieved in a commercial scale in order to get benefits from these interactions.

4.3 Conclusion

Temperature heterogeneity of 4°C around the set point, as reported for reefer containers, would result in enhanced blueberry firmness at increased temperature under the particular conditions which led to fruit firming during storage in this experiment. The mechanism involved in the firming response, possibly associated with fruit moisture loss within 0-2% is further investigated in Chapter 5. The enhancement of blueberry firmness by higher temperatures during storage could potentially become an interesting response to attempt to replicate in the commercial postharvest chain.

Cooling delays at 10°C, a common packing temperature used in blueberry, appears to not have a residual effect on firmness during subsequent storage when the storage conditions which produce blueberry firming are present. Under this scenario, delay times of 12-24 h at 10°C before cooling did not modify blueberry firmness outcomes compared to prompt cooling after 2-6 week of subsequent storage. This could constitute a valuable issue for fruit exporters as long as postharvest conditions which generate blueberry firming are replicated in industry.

Controlled atmosphere (CA) within recommended gas ranges was found to be able to slightly improve firmness retention during blueberry storage. CA potentially alters cell wall changes which have been associated to overripe softening in attached blueberries. However, the positive influence of CA in reducing blueberry softening appears to be too small to generate a commercial impact, especially when the conditions which normally lead to postharvest softening are present.

This study confirms that CA with increased O_2 concentrations can potentially alleviate the softening generated by high CO_2 in stored blueberries. Atmospheres comprising 20% O_2 seem to shift the fermentation CO_2 trigger for 'Maru' blueberries compared to low oxygen CA, enhancing the tolerance of fruit to high CO_2, even when CO_2 concentration is maintained within the recommended range. Future research should be focused on to find the O_2 range able to produce this beneficial effect for main blueberry cultivars, as well as on to test the maximum CO_2 concentrations which can be reached without compromising quality. The export industry could benefit from CA systems which ensure a better safety in terms of maintaining blueberry firmness and from low operational costs during shipping due to possibly cheaper CA gas settings.

It appears that as long as harmful gas levels are avoided, CA would either not alter or improve the effect of temperature variability within shipping containers (4°C around the set point) on firmness under storage conditions which produce blueberry firming. Postharvest conditions leading to increased firmness of blueberries during storage should be replicated in the commercial chain in order to benefit from a potential firmness increasing produced by CA at higher temperatures.

CHAPTER 5

MOISTURE LOSS AND FIRMNESS RELATIONSHIP (Experiment 2)

5.1 Introduction

Firmness is one of the most critical quality attributes influencing consumer appeal and marketing of fresh blueberries (NeSmith *et al.*, 2002). Blueberries normally soften during the postharvest chain which compromises final quality (Ehlenfeldt, 2002). Important volumes of blueberries are rejected at the marketplace due to firmness levels below retail standards (Prussia *et al.*, 2006). New research oriented to improve firmness retention in blueberries during postharvest has potentially great value for the fruit industry.

Blueberry is considered a climacteric fruit (Shimura *et al.*, 1986; Suzuki *et al.*, 1997b), which softens as ripening progresses in a phenomenon mainly associated with cell wall breakdown (Proctor and Miesle, 1991). Hemicellulose depolymerisation and arabinose loss have been identified as the main cell wall modifications during blueberry ripening (Vicente *et al.*, 2007). However, blueberries are harvested fully ripe from the bush (Perkins-Veazie, 2004), with most of the cell wall disassembly completed (Vicente *et al.*, 2007). Some cell wall changes would occur up to the overripe stage (Angeletti *et al.*, 2010), although attached blueberries are known to soften very little after full ripening (Ballinger *et al.*, 1973; Proctor and Miesle, 1991).

Postharvest softening of blueberries is not well understood. Reduced turgor due to fruit moisture loss has been related to softening of harvested blueberries (Forney *et al.*, 1998), although no experimental data has been reported to support this hypothesis. Turgor loss has been shown to reduce firmness in apples and potato tissue (Lin and Pitt, 1986). Nevertheless, blueberry moisture loss (measured as

weight loss) has correlated to decreased firmness in numerous experiments (Angeletti *et al.*, 2010; Cantín *et al.*, 2012; Miller *et al.*, 1984; Tetteh *et al.*, 2004). Moisture loss appears to influence blueberry firmness differently depending on the extent of fruit dehydration. Total weight loss below 1-2% has been reported together with a particular firming behaviour in blueberries (Chiabrando and Giacalone, 2011; Duarte *et al.*, 2009; Forney *et al.*, 1998; Miller *et al.*, 1993). This blueberry firming during storage has been associated with microscopically observed thickening of the cell wall of parenchyma cells (Allan-Wojtas *et al.*, 2001) and corrugation of the cell walls of epidermal cells (Bunemann *et al.*, 1957). Whether the variable firmness responses obtained under different conditions of blueberry moisture loss imply a relationship of causality is a question which still needs to be addressed. The present experiment is an attempt to induce different firmness outcomes in blueberries by creating variable moisture loss conditions during storage.

The objectives defined for this experiment were:

- To manipulate moisture loss of blueberries to induce softening and firming responses
- To confirm the potential relationship of causality between moisture loss and firmness for blueberries during storage

The results of this study will contribute to a better understanding of postharvest softening for blueberry and could be potentially used to modify postharvest management in order to improve blueberry firmness.

5.2 Experimental methods

To address these questions, diverse weight loss conditions were artificially created and monitored throughout the experiment. Firmness was taken to characterise the condition of the fruit.

5.2.1 Fruit material

One kg of ‘Centurion’ rabbiteye blueberries were harvested and transported on 06/05/2011, according to information indicated in section 2.2 of this work. ‘Centurion’ is a traditional cultivar released in 1978 by the North Carolina Agricultural Experiment Station and the Department of Agriculture of the United States (Schotsmans *et al.*, 2007).

5.2.2 Sample configuration

Fruit was standardised on size and quality by hand grading at room temperature (20°C). Thirty nine samples in total were randomly established, each one comprising 20 blueberries contained in a previously weighed cotton mesh bag of 10 cm x 10 cm. Glass jars of 0.578 L were used to contain three weighed and labelled samples each. Additional non-destructive samples were established for periodic evaluation by using Magnetic Resonance Imaging (MRI). Each non-destructive sample consisted of a 51 mm x 71 mm x 1 mm acrylic flat platform, with 4 blueberries glued on its surface (2 equatorially oriented and 2 supported on the stem scar) (Figure 5-1).

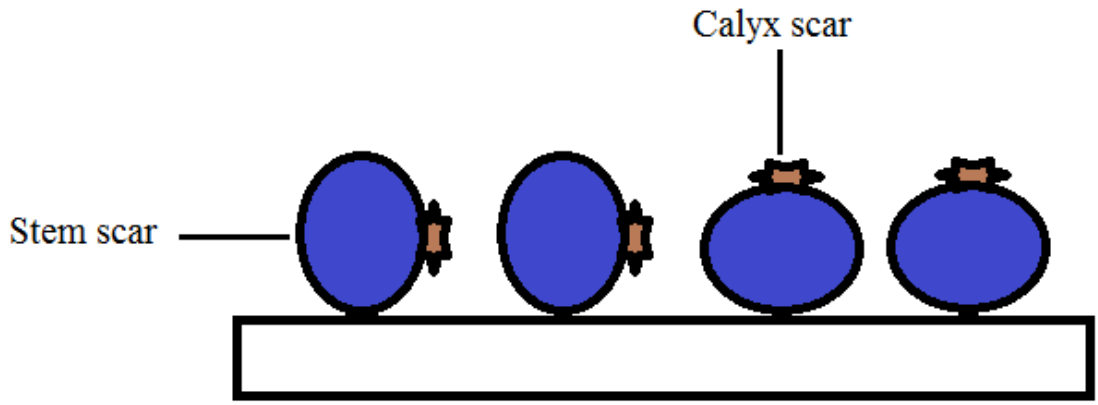

Figure 5-1. Diagram (lateral view) showing the positioning of blueberries on a platform as a non-destructive sample utilised for MRI measurements.

5.2.3 Experimental design

Controlled weight loss conditions were created by supplying dry air to the jars containing the samples at different flow rates. Four treatments were configured based on four air flow rates: 0 (control), 15, 30 and 60 mL min^{-1}. The objective of the treatments was to influence the rate of weight loss only, with other factors remaining invariable. All treatments were conducted in triplicate and kept at 4°C for a total period of 3 weeks. A MRI non-destructive sample was included in one jar of each treatment.

Each glass jar containing fruit was closed using an air tight lid modified by drilling two holes in it and covering each hole with a rubber septum. The air flow rates were regulated by using needle valves located in a manifold and supplied to the jars by tubing through one of the septa whereas the other septa was utilised as the air outlet by opening it it to ensure air renewal. For the control, both air inlet and outlet were left open to ensure air renewal and avoid atmosphere modification. Each replication of each treatment was setup in a different jar and regulated independently by a needle valve. Flow rates were checked and adjusted every two days using a portable ADM 2000 gas flowmeter (Agilent Technologies, Delaware, USA). All jars were maintained at controlled 4°C throughout the storage period.

5.2.4 Evaluation

Treatments were evaluated weekly for a total period of 3 weeks by removing randomly one sample from each jar for evaluation and subsequent elimination (i.e. destructive measurement). Samples were evaluated after conditioning them for 1 h at room temperature (20°C). The jars were immediately closed after samples were removed. A common initial evaluation at experiment initiation was conducted. Non-destructive samples for MRI were removed weekly for weight and MRI evaluation, simultaneously with normal samples, from week 1 to 3 and placed back into the same jar. Parameters evaluated in each evaluation were weight loss, firmness, shrivel count and MRI.

5.2.4.1 Weight loss

Weight loss was obtained for each sample as detailed previously (section 2.5.1). In the case of the non-destructive samples, weight loss was calculated as the difference between the initial and the final weight of the platform containing fruit, after the subtraction of the platform.

5.2.4.2 Firmness

Firmness was measured on individual blueberries as detailed previously (section 2.5.2). All the 20 fruit of each sample removed from the jar were evaluated.

5.2.4.3 Shrivel count

For each sample, shrivelled blueberries were separated, counted and related to the total number of fruit of the sample. The result obtained was expressed as percentage by using Eq 4. Fruit were considered as shrivelled if they presented skin corrugation detectable by naked eye(Figure 5-2).

$$\left(\frac{(\text{shrivelled fruit count})}{(\text{total fruit count})} \right) (100) \qquad \text{Eq 4}$$

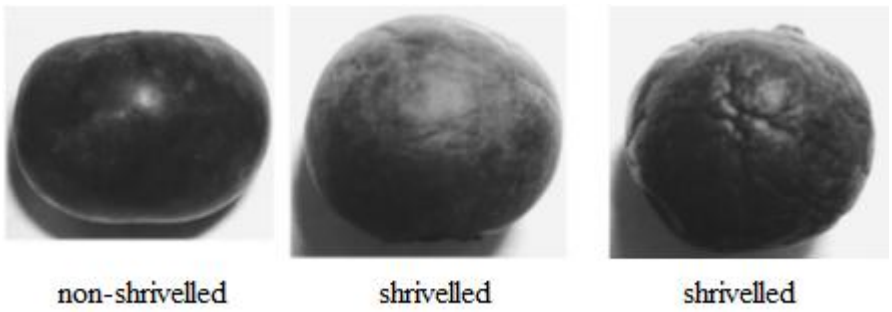

Figure 5-2 Examples of non-shrivelled and shrivelled blueberries used as references in shrivel count. Adapted from Schotsmans *et al.* (2007).

5.2.4.4 Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) was used to visualise water distribution within blueberries of non-destructive samples. Variable water content was visualised as different colour intensities within a monochromatic range image. The MRI equipment used to obtain the images consisted of an Oxford instruments 4.7 T superconducting magnet (200 MHz for 1H resonance frequency) equipped with a Bruker AMX 200 console. An axial image of the transverse relaxation from the centre of the fruit was taken simultaneously for the 4 blueberries of each non-destructive sample in each evaluation. Final images were obtained from multiple scans according to parameters used by Gamble (1994) for blueberries.

The transversal diameter of individual fruit was obtained for each non-destructive sample by analysing the final MRI pictures with the Bruker AMX 200 console software. Percentage of shrinking was calculated for each fruit over the storage period as the difference between initial and final diameter as related to the initial diameter (Eq 5).

Eq 5

$$\left(\frac{(\text{initial diameter}) - (\text{final diameter})}{(\text{initial diameter})} \right) (100)$$

5.2.5 Statistical methods

Treatment effects were determined by using ANOVA on untransformed data whereas correlations were analysed using Pearson's correlation test. Differences between means were checked by using Tukey HSD (Honest significant difference) test. Significant differences were considered at 5%.

5.3 Results and discussion

5.3.1 Weight loss

Weight loss progressively increased during storage for all treatments (Figure 5-3), which is a predicted response as a result of the fruit moisture loss due to transpiration. Blueberry weight loss was higher at increased air flow rates, reaching maximum cumulated values of 1.13, 6.89, 9.46 and 15.06% after 3 weeks for 0, 15, 30 and 60 mL min^{-1}, respectively (Figure 5-3). This higher weight loss obtained at increased air flow rates is an expected consequence of the air renewal effect in increasing the gradient of water vapour content (absolute humidity) between fruit and the air contained within the glass jars. The absolute humidity gradient between the intercellular spaces of plant tissue and the surrounding air is the driving force for water loss, defining the rate at which fresh produce is dehydrated (Wills *et al.*, 2007). Higher rates of air flow should have generated a quicker outflow of air from the jars to the external environment, therefore leading to a faster removal of the water vapour contained in the jar as a product of fruit transpiration. Consequently, the gradient between fruit absolute humidity and the water vapour content of the jar should have been increased at higher air flow rates resulting in greater moisture loss of blueberries.

Weight loss values observed in the present experiment are similar to magnitudes commonly observed by industry during blueberry export or found by previous research work. According to personal experience, the fruit industry considers a weight loss of 5-7% to be usual during 2-3 weeks of containerised marine export of blueberries (at 0°C and 90-95% RH). Weight loss observed in our own previous work is highly variable, which is probably due to differences in experimental conditions. While blueberries stored inside controlled atmosphere containers lost less than 1.5% weight loss after 42 d at 0°C (Table 3-1), weight loss was around 10% in blueberries subjected to cooling delay times at 20°C and 10°C plus a subsequent 21 d period at 0°C (Paniagua *et al.*, 2012). Similarly, Borecka and Pliszka (1985) obtained just 0.3% of weight loss after storing blueberries at 0°C in sealed polyethylene bags for 22 d, whereas Sanford *et al.* (1991) reported 5.3% and 7.6% weight loss, respectively, in blueberries stored for 14 d at 0°C or 5°C with no RH regulation.

Accordingly, the weight loss values considered by industry and previously reported in research work are included within the weight loss range obtained in this experiment, which validates further comparison to be done with industry scenario and experimental experience.

5.5.2 Firmness

The evolution of firmness during storage was different between treatments. For 0 mL min^{-1} treatment, fruit firmness significantly increased (approximately 14%) after 1 week storage compared to initial firmness (1.74 N) and reached a maximum value of 2.13 N, approximately 23% firmer 3 weeks after harvest (Figure 5-3). Firmness also showed an increasing trend after 1 week storage for 15 and 30 mL min^{-1} treatments, although these differences were not statistically significant from the initial firmness (Figure 5-3). Later, blueberries were 14% and 34% softer in comparison to the initial firmness value after 3 weeks for 15 and 30 mL min^{-1} treatments, respectively (Figure 5-3). For 60 mL min^{-1} treatment, firmness decreased by 14% after 1 week of storage compared to the firmness at the beginning of the experiment (Figure 5-3). The minimum firmness observed was 0.73 N for fruit stored for 3 weeks in 60 mL min^{-1}treatment, 58% softer than those evaluated at the beginning of the experiment.

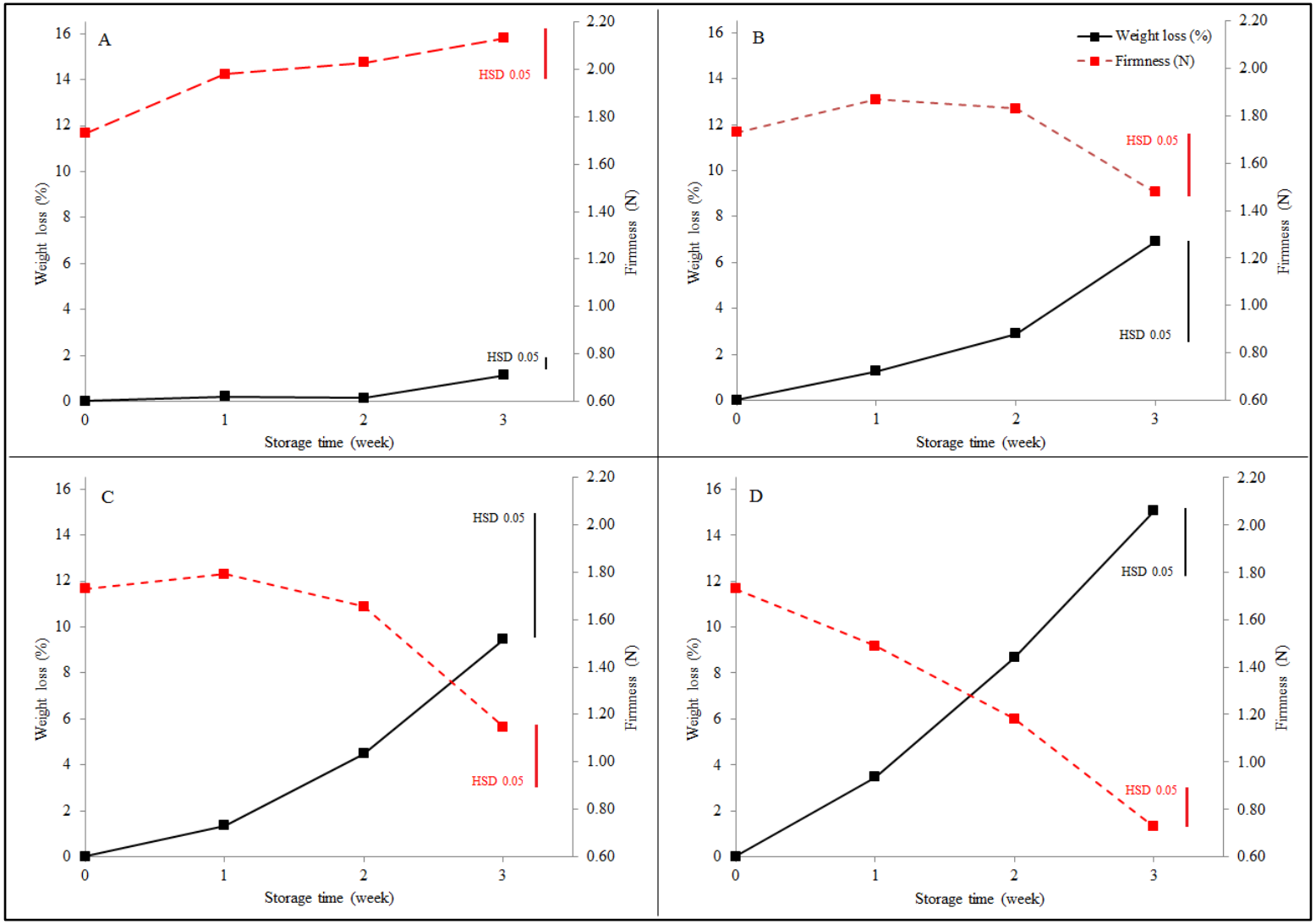

Figure 5-3. Weight loss (n= 3) and firmness (n= 60) evolution of blueberries during 3 weeks storage, as influenced by 0 (A), 15 (B), 30 (C) and 60 (D) mL min^{-1} air flow. Bars represent Honest significant difference (HSD) at 0.05 level for each variable across the storage period as determined by Tukey's test.

According to the work of Ehlenfeldt and Martin (2002), blueberries used in this experiment would have presented a firmness condition which can be considered as 'superior firmness' (1.5-1.6 N) at the beginning of the storage period (Figure 5-3), with this status being further improved in the case of treatments which resulted in an increased fruit firmness. In contrast, blueberries reached firmness levels below those defined as 'moderately firm' (0.87-0.89 N) by Beaudry *et al.* (1998) after 3 weeks storage at an air flow of 60 mL min^{-1} (Figure 5-3) which could indicate that the firmness of that fruit may have fallen to levels considered as commercially unacceptable. While no formal sensory analysis was conducted in the present experiment, the perception of the firmness status over the evaluation period agrees with these previous classifications. Hence the range of experimental conditions used resulted in providing a range of blueberry fruit that represent substantially different quality.

Firmness of blueberries has been reported to either decrease (Angeletti *et al.*, 2010; Cantín *et al.*, 2012; Miller *et al.*, 1984) or increase (Chiabrando and Giacalone, 2011; Duarte *et al.*, 2009; Forney *et al.*, 1998) during storage under experimental conditions. This variable firmness behaviour observed in blueberries in previous work agrees with the responses obtained in this experiment which led to either blueberry softening or firming over the period of storage. This suggests that both firmness responses were successfully induced in the present study by manipulating the rate of air flow during storage.

In the industry context, blueberry firmness is known to decrease considerably during the postharvest chain which leads to important fruit losses (Ehlenfeldt, 2002; Prussia *et al.*, 2006). This is in agreement with what was found in the present experiment for 15, 30 and 60 mL min^{-1} treatments over the storage period. Factors causing postharvest softening of blueberries are not fully understood. With most of the cell wall disassembly occurring during ripening and very little once blueberries are commercially mature (fully ripened) (Vicente *et al.*, 2007), modifications of the cell wall components would have a minor role in firmness evolution of harvested blueberries.

Firming of blueberries has been found to occur in a number of previous studies (Chiabrando and Giacalone, 2011; Duarte *et al.*, 2009; Forney *et al.*, 1998), agreeing with the results observed in the present experiment for 0 mL min^{-1} treatment. Nevertheless, the possible causes leading to this increased firmness have not been addressed. Bunemann (1957) reported a correlation between higher sensory firmness and increased corrugation of the cell walls of epidermal and hypodermal cells for 'Rubel' blueberries stored at 4.5 and 10°C for 8 weeks. Furthermore, Allan-Wojtas *et al.* (2001) associated firming of blueberries with microscopically observed thickening of the cell wall of parenchyma cells of 'Burlington' blueberries during 6 week storage at 0°C, although these results were not consistent in a second year of evaluation. Overall it appears that microstructural changes in the cell wall of outer cell layers of blueberry fruit might be involved in the expression of this firming behaviour in blueberries, although the reasons triggering these responses remain unclear.

Firmness evolution of blueberries during storage could have been influenced by weight loss in this experiment since opposing firmness outcomes were obtained in different weight loss ranges. Regardless of the air flow rate, blueberry firming occurred consistently at the same time as low levels of weight loss (0.22-1.34%) whereas softening was simultaneously observed with higher weight loss (3.47-15.06%) (Figure 5-3). This is in agreement with the work of Miller *et al.* (1993) who obtained increasing sensory evaluated firmness during storage when weight loss of blueberries was lower than 1%, whereas weight loss higher than 1% correlated with softening in the same experiment. Similarly, Forney *et al.* (1998) reported firming of 'Burlington' blueberries simultaneously with 1-2% weight loss but fruit softening when weight loss was 4-14%. Possible explanations for this behaviour were not provided in these studies. Furthermore, firmness was found to correlate to fruit weight loss during storage in the present experiment, with a high Pearson's coefficient obtained for weight loss and firmness correlation (Figure 5-4). This high correlation agrees with our own previous research (Paniagua *et al.*, 2012) and supports the hypothesis that blueberry firmness outcomes are influenced by weight loss.

Moisture loss has been previously suggested to induce postharvest softening in blueberries due to reduced turgor (Allan-Wojtas *et al.*, 2001; Forney *et al.*, 1998), while no evidence beyond this statement has been provided. Nevertheless, a decrease in turgor loss has been shown to contribute to firmness reduction in apple and potato (Lin and Pitt, 1986). Many previous studies have reported blueberry softening to occur together with weight loss levels ≥ 2% (Almenar *et al.*, 2010; Angeletti *et al.*, 2010; Cantín *et al.*, 2012; Ferraz *et al.*, 2001; Miller *et al.*, 1984; Tetteh *et al.*, 2004). Similarly, blueberries soften during the postharvest chain under commercial conditions, where 5-7% weight loss is known to be normally lost after 2-3 weeks of export process (Prussia *et al.*, 2006). Consequently, the result observed in the present experiment supports the possibility of turgor loss enhancing blueberry softening and agrees with what has been reported in previous work and how weight loss and firmness behave in the industry context.

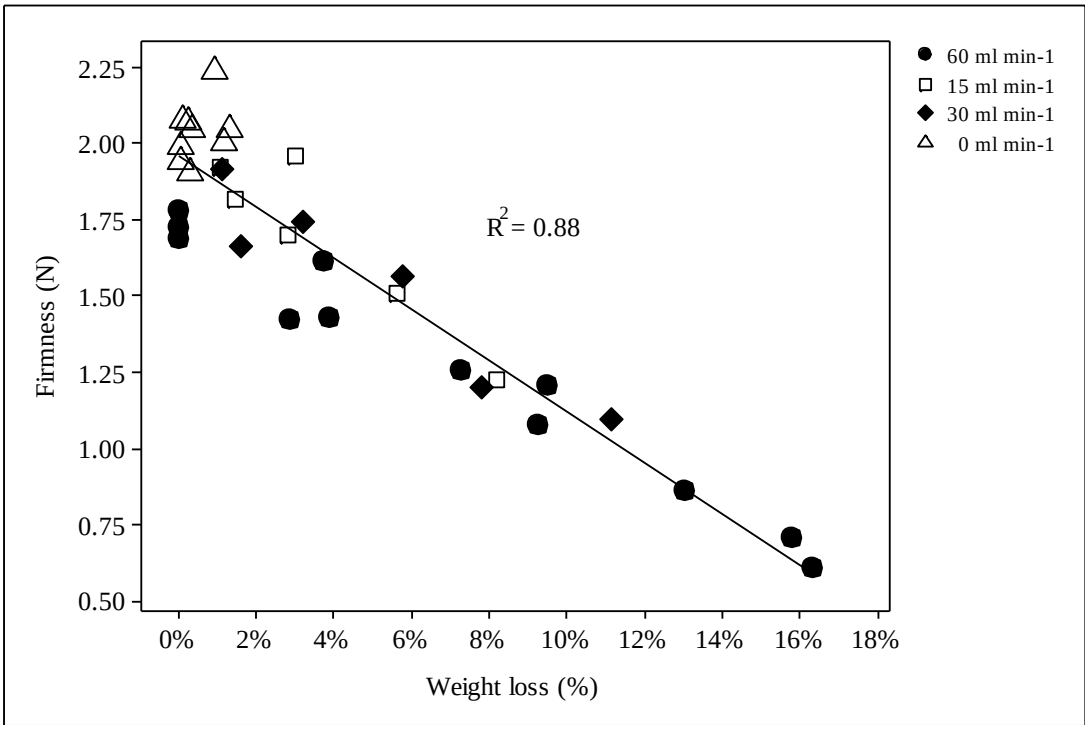

Figure 5-4. Correlation between firmness and water loss means, grouped by air flow treatment (0, 15, 30 and 60 mL min^{-1}).

Fruit 'drying and hardening' due to reduced fruit moisture loss have been suggested to produce blueberry firming (Chiabrando *et al.*, 2009), although this has not been investigated. Firming response has previously correlated with low total weight loss (≤ 1-2%) during the storage of fresh blueberries in a number of previous works

(Chiabrando and Giacalone, 2011; Duarte *et al.*, 2009; Schotsmans *et al.*, 2007). Cranberries have also been found to increase their firmness up to 9% during storage together with weight loss below 1% (Gunes *et al.*, 2002). While observed corrugation and thickening of the cell walls of epidermal (Bunemann *et al.*, 1957) and parenchyma cells (Allan-Wojtas *et al.*, 2001) have correlated to a higher firmness in blueberries, increased 'skin toughness' has also been noticed to increase as part of this firming response (Bunemann *et al.*, 1957). The mechanical resistance of the epidermis is known to affect fruit overall firmness (Jackman and Stanley, 1995). Skin drying results in increased firmness of lychee fruit during cool storage, as reported by Mahajan and Goswami (2004). Accordingly, it seems possible that low levels of weight loss (≤ 1.34%) in the present experiment could have led to increased dehydration of the outer cell layers of blueberry and therefore resulted in a higher skin toughening perceived as increased firmness.

Applied to the industry scenario, the potential causal relationship between blueberry moisture loss and firmness responses could have an important impact on the postharvest management of this crop and its final firmness outcomes. According to this data, maintaining weight loss levels below 8-15% during the postharvest chain would minimise excessive softening of blueberries (Figure 5-3). Therefore, firmness retention might be improved along the commercial chain by considering technologies and materials which limit weight loss such as palletised modified atmosphere bags or less vented clamshell designs. Postharvest managements could also be oriented to minimise weight loss, making efforts to minimise the steps of the supply chain which enhance fruit moisture loss such as delays in cooling and cold chain breakages. Furthermore, firmness evolution during postharvest could be more easily controlled by monitoring the losses in weight, potentially avoiding firmness levels below commercial standards by taking opportune correction measures or destining fruit batches to less exigent or less distant markets.

The evidence provided by this experiment indicates a causal relationship between moisture loss and firmness for blueberries. Direct evaluations of turgor, epidermis resistance and changes in cell morphology should be included in future research to confirm the potential mechanism of this relationship. Additionally, more cultivars

should be evaluated in order determine the consistency of the response across the genetic variability.

5.5.3 Shrivel count

Fruit shrivel was observed for 30 and 60 mL min^{-1} treatments after 3 and 2 week storage, respectively, although the only statistically significant shrivel count was 13.67% obtained after 3 weeks for the 60 mL min^{-1} air flow rate (Figure 5-5). This result is an expected consequence of increased fruit moisture loss. According to Wills *et al.* (2007), just 5% moisture loss can affect the quality of fresh produce, by generating shrivel, wilting or affecting organoleptic attributes. In the present experiment, fruit shrivel occurred together with weight loss ≥ 8.66% although was not observed simultaneously with weight loss ≤ 6.89% (Figures 5-3 and 5-5) which could indicate that the limit of weight loss in which shrivelling starts would be in between these two values, at least for 'Centurion' blueberries.

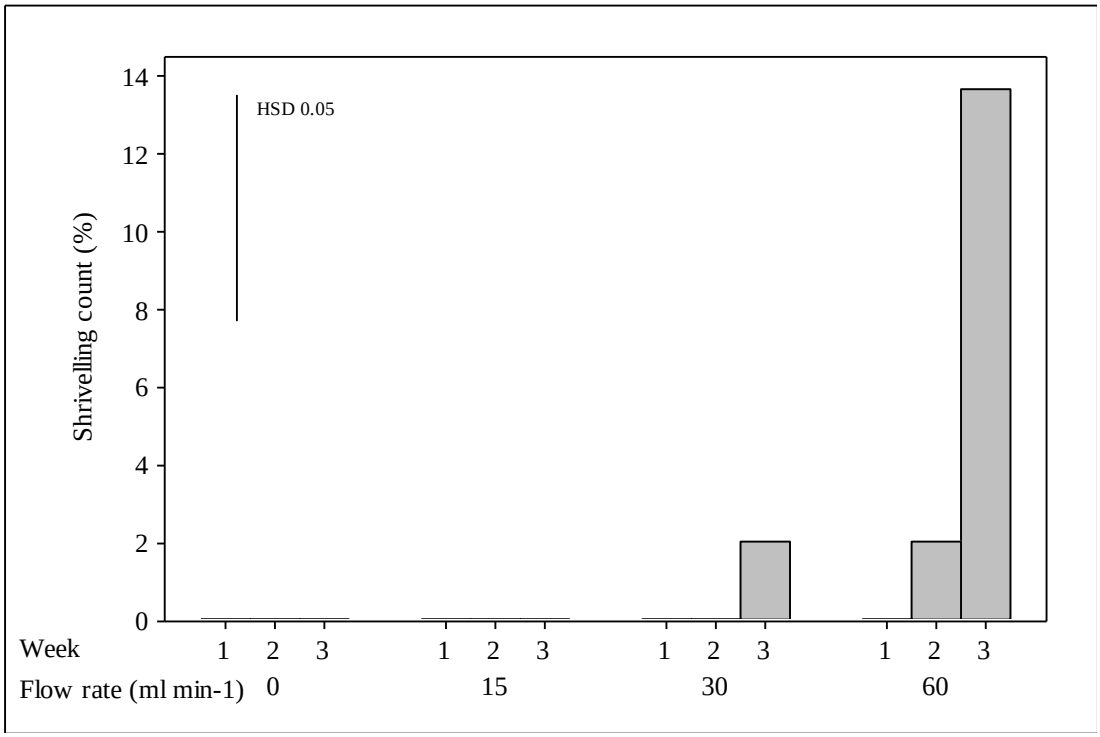

Figure 5-5. Average shrivel count (n= 3) evolution of blueberries during 3 week storage, as influenced by different air flow rates. Bar represents Honest significant difference (HSD) at 0.05 level as determined by Tukey's test.

The results obtained in this experiment for fruit shrivel are not dissimilar to what has been reported previously. Schotsmans *et al.* (2007) reported that shrivelling of 'Centurion' and 'Maru' blueberries occurred at weight loss levels of approximately 8% or higher. Likewise, Sanford *et al.* (1991) observed that wild lowbush blueberries shrivelled during storage with weight loss levels ≥ 8% and Forney *et al.* (1998) found that weight loss of 5% or higher correlated to shrivel in 'Burlington' blueberries. Overall it would seem that this experiment confirms that the limit of weight loss in which shrivel becomes evident is approximately 5-8% depending on cultivar.

5.5.4 MRI outcomes

Some areas near to the epidermis were observed to decrease in water content (showed as dark gaps in the MRI pictures) in 0 mL min^{-1} air flow rate treatment after 2 and 3 week storage, in comparison to what was observed after 1 week of storage for this treatment (Figure 5-6). The appearance of these areas with reduced moisture content after 2 and 3 weeks were simultaneous to weight loss levels of 0.3 and 0.8%, respectively (Figure 5-6). Furthermore, fruit of this treatment were not found to shrink significantly during the period of storage (Figure 5-7). In contrast, samples of 15, 30 and 60 mL min^{-1} air flow rate treatments did not present clear dehydration near to the fruit epidermis, rather showing repetitively reduced water content close to the stem scar (Figure 5-6). This was evident after 3 weeks storage for blueberries subjected to 15 and 30 mL min^{-1} air flow rate and after 2 and 3 weeks for 60 mL min^{-1} treatment, being observed together with 8.6-19.6% weight loss (Figure 5-6). Unlike what was found for 0 mL min^{-1} air flow rate treatment, blueberries of 15, 30 and 60 mL min^{-1} treatments significantly shrank over the storage period, reaching shrinking values of 10.79, 4.49 and 5.08%, respectively, after 3 weeks storage (Figure 5-7).

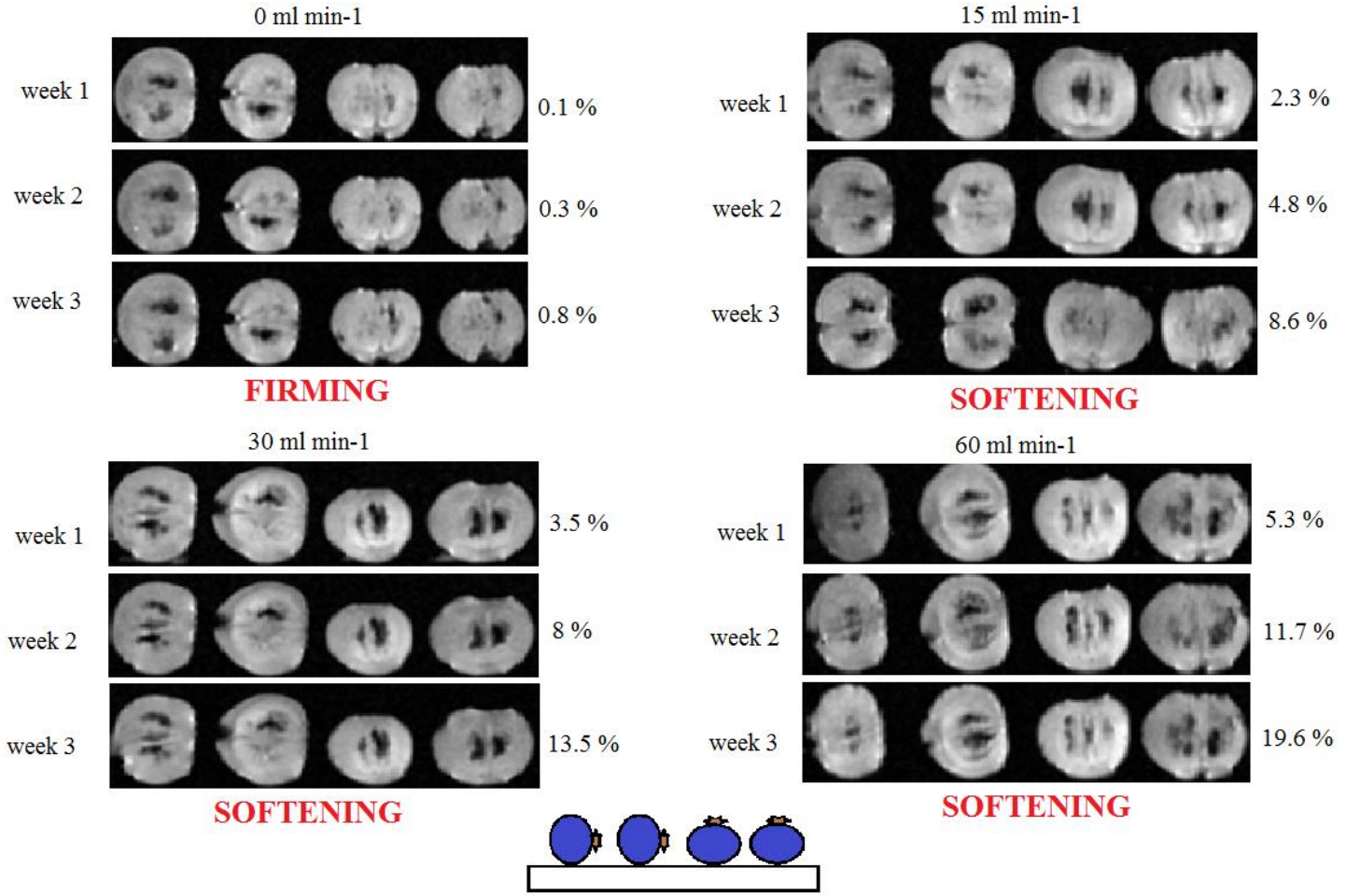

Figure 5-6. MRI pictures showing water content of non-destructive samples during storage, as affected by air flow rate. Higher colour intensity indicates higher water content. Weight loss values of samples at each week are shown beside images. A diagram of the sample orientation is included as a reference.

The reduced water content of the epidermal area and the negligible shrinking observed for 0 mL min^{-1} air flow rate treatment could be related to the increased firmness obtained for this treatment during the storage period (Figure 5-3A). This moisture loss reduction in the outer cell layers of the fruit may be associated with the previously reported corrugation and thickening of the cell walls of epidermal (Bunemann *et al.*, 1957) and parenchyma cells (Allan-Wojtas *et al.*, 2001) which have correlated to blueberry firming. Microstructural changes could occur in the outer cell layers of the fruit as a result of dehydration in low moisture loss conditions, in this case 0.3 and 0.8% weight loss (Figure 5-6). These morphological modifications may be perceived as the firming response observed in this experiment and in a number of previous studies. The insignificant fruit shrinkage obtained for this treatment (Figure 5-7) and the apparently invariable water content of inner cell layers of the fruit (Figure 5-6) would indicate that the water content of the centre of the fruit was not significantly affected. Therefore, the observations made for the 0 mL min^{-1} treatment with MRI supports the hypothesis of weight loss $\leq$ 1.34% generating dehydration of the outer cell layers of blueberry and hence leading to overall fruit firming.

The dehydration near to the stem scar area observed for 15, 30 and 60 mL min^{-1} air flow rate treatments agrees with what has been reported in previous studies indicating that the picking scar is a primary pathway for moisture loss in blueberries (Albrigo *et al.*, 1980; Ehlenfeldt, 2002; Moore, 1965). Furthermore, the fruit shrinkage observed for these treatments that correlates to weight loss levels $\geq$ 8.6-19.6% is an indication of the dehydration of the whole fruit. Excessive shrinking was obtained for 15 mL min^{-1} treatment after 3 weeks, which could be due to changes in fruit orientation (Figure 5-6) as a result of handling affecting MRI assessment. Disregarding this outlying data point for 15 mL min^{-1} at 3 weeks storage, the trend of increased fruit shrinkage with higher air flow rate is consistent especially with comparison to the insignificant shrinkage observed for 0 mL min^{-1} treatment. Accordingly, the MRI data obtained for 15, 30 and 60 mL min^{-1} air flow rate treatments suggests that moisture was lost from the epidermal area as well as from parenchymatic cells of the fruit which agrees with the possibility of overall fruit turgor loss acting as the main cause of blueberry softening.

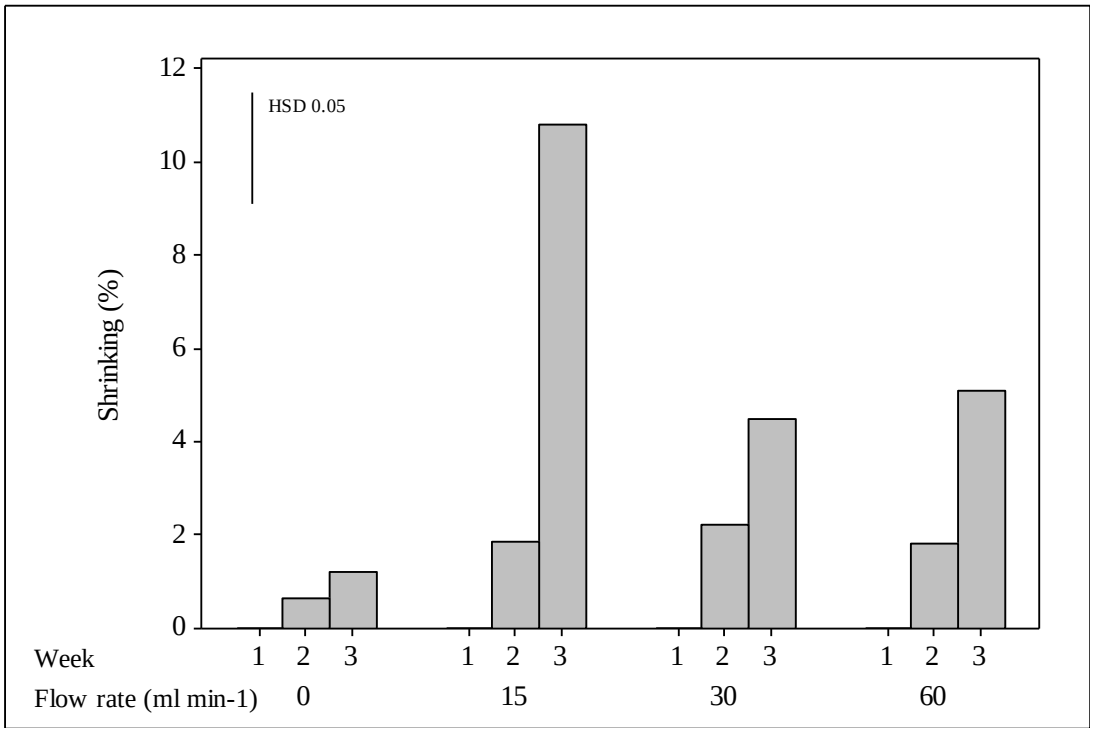

Figure 5-7. Percentage of shrinking of individual (n= 4) blueberries contained in the non-destructive MRI samples during 3 weeks storage, as influenced by different air flow rates. Bar represents Honest significant difference (HSD) at 0.05 level as determined by Tukey's test.

MRI analysis supports the causal relationship between moisture loss and firmness responses of blueberries during postharvest. The observations and data obtained from MRI are in agreement with the occurrence of different fruit moisture loss patterns for softening and firming of blueberries, which could constitute evidence to state a potential mechanism of causality between these two quality variables. Nevertheless, further research measuring parameters mentioned in the discussion of this experiment (turgor, skin toughness, and cell wall modifications) is still required to confirm this hypothesis.

5.6 Conclusion

The results of this experiment support the hypothesis of a causal relationship between moisture loss and postharvest firmness for blueberries. The opposite firmness outcomes obtained under different weight loss regimes, the high correlation found between these two quality parameters as well as the indications of different

water loss patterns for firming and softening as observed in MRI analysis suggest that fruit moisture loss plays a major role in determining firmness responses of blueberries during postharvest. Nevertheless, more research including the direct assessment of critical variables (such as turgor, skin toughness, and cell wall modifications) may elucidate the mechanism for this relationship between moisture loss and firmness. There is potential for industry to benefit from this relationship by minimising blueberry moisture loss during the postharvest chain as a way to improve firmness retention and hence increasing the final firmness status at the marketplace.

Softening and firming responses can be successfully induced in blueberries by manipulating weight loss during storage. Variable weight loss was created in this work by modifying air flow rate. This technique can be utilised as a way to either increase or decrease firmness of stored blueberries at a given storage temperature, relative humidity or atmosphere composition. These experimental settings could represent a simple, low cost but effective option to induce variable firming behaviour in this crop which might be used in future studies focussed on the moisture loss and firmness relationship in blueberries.

CHAPTER 6

ROT INCIDENCE

6.1 Introduction

Decay is the main cause of postharvest losses in the commercial chain of fresh blueberries, resulting in reduced saleable volumes (Forney, 2009). Blueberry importers have zero tolerance for the presence of rots in the marketplace (Ehlenfeldt, 2002). *Botrytis cinerea*, *Alternaria sp.* and *Colletotrichum sp.* are the most common pathogens of blueberry fruit. All can easily propagate after harvest due to their ability to proliferate under storage conditions (Gough, 1994). Therefore, activities oriented to decrease the incidence of rots during postharvest are of great interest to the blueberry export industry.

Temperature determines the growth rate of postharvest pathogens, and influences the concentration of antimicrobial compounds in blueberries (Barkai-Golan, 2001). It is recommended to maintain fresh blueberries at 0°C and 90-95% RH in order to minimise decay incidence without compromising quality (Mitcham *et al.*, 2011; Perkins-Veazie, 2004). Higher temperatures accelerate the development of rots and hence favour fruit spoilage (Boyette, 1993). Nevertheless, previous research work indicates that temperatures within the range of 0-5°C have not consistently resulted in different decay levels (Forney *et al.*, 1998; Sanford *et al.*, 1991). The question remains, if a temperature variability of 4°C around the set point exists, as reported for marine containers (Tanner and Amos, 2003), would this temperature variability significantly impact the rot incidence of blueberries during the export period.

Cooling delays at temperatures above 18°C have also been shown to increase the decay incidence of blueberries during subsequent storage (Ceponis and Cappellini, 1979; Jackson *et al.*, 1999). However, the residual effect of delayed cooling at packing temperature (10°C) on blueberry rot development has not previously been evaluated.

Controlled atmosphere (CA) is successfully used in fresh blueberry exports to reduce decay and therefore improve the postharvest life (Alsmairat *et al.*, 2011). Recommended CA gas concentrations for blueberries correspond to 8-15% CO_2 and above 1% of O_2 (Ceponis and Cappellini, 1983, 1985; Perkins-Veazie, 2004). Increased CO_2 and reduced O_2 beyond these levels generates physiological damage due to fermentation (Ehlenfeldt, 2002). The CA effect on blueberry decay is mainly due to the fungistatic action of high CO_2 which decreases microbial respiration, although O_2 concentrations between 1-10% have been suggested to lead to further reduction of decay (Kim *et al.*, 1995; Prange *et al.*, 1995). Commercial use of CA in blueberry marine shipping normally considers O_2 concentration of 2-5% (A. Kader, 2003). Whether the use of low O_2 ($\leq$ 10%) is technically justified in terms of a better control of postharvest rots in blueberry is a question which still needs to be addressed.

The solubility of CO_2 and O_2 in fruit tissue decreases as temperature increases (Beaudry *et al.*, 1992; Cameron *et al.*, 1994). Due to the risk of physiological damage, CO_2 is used at the lowest possible concentration which ensures an efficient control of decay, whereas O_2 is normally maintained above 2% to avoid anaerobiosis (Ehlenfeldt, 2002). Accordingly, temperature heterogeneity along the postharvest chain of blueberries might potentially vary the dissolved gas concentration during CA, compromising its efficacy and increasing the risk of fermentation injury. On the other hand, CA could reduce the effects of cooling delays and storage temperature on blueberry rot incidence, by directly inhibiting the fungal growth regardless of the other postharvest conditions. As such, CA would be acting as a safety buffer for temperature management deficiencies during postharvest.

In pursuance to answer these questions, the objectives chosen for this chapter were:

- To evaluate the effect of potential temperature variability within marine reefer containers on the rot incidence of blueberries

- To assess the impact of cooling delays at a common temperature used by the industry for blueberry packing on the rot incidence of blueberries

- To compare the effects of different O_2 concentrations on CA efficacy controlling decay in blueberries
- To investigate potential interactions between temperature management deficiencies during postharvest and the storage atmosphere in terms of rot incidence of blueberries

The outputs of this study should provide the relevance of the factors within the supply chain that influence decay incidence of blueberries. As such, this study will provide insight into the most important factors to control by the export industry to reduce blueberry rot incidence.

6.2 Experimental methods

An experiment consisting of a full matrix of 3 cooling delay times at 10°C, 2 storage temperatures and 3 controlled atmospheres was established in order to address these research questions. Genotypic variability among blueberry species was included in the work by conducting the experiment on a single cultivar of both highbush and rabbiteye blueberries. The storage time considered for the experiment was 6 weeks, with evaluations at weeks 2, 3, 4, 5 and 6 which simulated commercial storage periods. Rot incidence was assessed immediately after storage by separating the fruit presenting decay symptoms (mycelium growth, leaking or collapsed consistency) of each sample and relating their total weight to the total weight of the sample. The final value was expressed as percentage of rot incidence. Full details of the materials and methods used in the experiment are provided in Chapter 2.

6.3 Results and discussion

Blueberries did not develop visible decay during 4 weeks of storage irrespective of cooling delay period, atmosphere or storage temperature. After 5 and 6 weeks, both ‘Brigitta’ and ‘Maru’ presented a clear increase of rot incidence. Under optimum storage temperature, the time elapsed until the first rots appear depends on different factors such as fruit resistance, inoculum amount, storage atmosphere and free water

availability. The blueberry industry considers 2 and 4 weeks as the usual life span of refrigerated highbush and rabbiteye blueberries, respectively, which can be doubled by combining cold storage with CA (Forney, 2009). As previously discussed (section 1.3.4.1), experiments evaluating decay incidence in cold stored blueberries have reported the presence of rotten fruit from 2-5 weeks onwards. Therefore, the time elapsed in this experiment until the appearance of the first rot symptoms agrees with the common knowledge of the industry and previous research experience, suggesting that the overall conditions of the experiment represented what occurs in the blueberry commercial chain.

Maximum decay incidence obtained in the experiment reached 3.5% and 7% for 'Brigitta' and 'Maru' cultivars, respectively, after 6 weeks of storage. It is known that factors such as amount of inoculum and cultivar sensitivity can influence the expression of pathogenic fungi on cold stored blueberries (Barkai-Golan, 2001). As such, the magnitude of rot incidence obtained in previous studies evaluating similar cold storage periods without considering initial pathogen inoculation has been highly variable. While Harb and Streif (2004) obtained 0.7% of decayed berries after cold storing 'Duke' blueberries for 5 weeks in air, the same authors reported rot incidence as high as 31% for 'Bluecrop' blueberries kept under the same conditions for just 4 weeks (Harb and Streif, 2006). Consequently, the percentages of rot incidence obtained in this experiment are in agreement with the decay range reported by similar earlier studies.

Most of the decayed berries presented one of the following two sets of symptoms, either a loose grey mycelium concentrated at the stem scar of the fruit, or a dense grey-green mycelium at the calyx scar, together with an overall leaky appearance and sunken areas on the fruit epidermis (Figure 6-1). These symptoms seem to correspond to *Botrytis cinerea* and *Alternaria sp.*, respectively, which are two of the main postharvest fungi associated to blueberries (Anco and Ellis, 2011; Caruso and Ramsdell, 1995). These rot species originally inoculate in the field and proliferate in the postharvest chain due to the high contact between fruit and their ability to grow under cold storage conditions (Sommer, 1985).

The data analysis comprised the evaluation of each factor effect (cooling delays, temperature and atmosphere) individually on rot incidence, as well as their possible interactions affecting decay response. For this purpose, an ANOVA was conducted on rot incidence expressed as percentages, with this analysis being conducted on data sets corresponding to each individual week due to the high heterogeneity of variance between weeks as a result of the natural increase in incidence with time. Full details of the statistical analysis conducted can be found in the section 2.4.7. In this chapter, the results corresponding to each experimental factor are primarily presented and discussed independently as the statistical analysis revealed that there were no major interactions between factors.

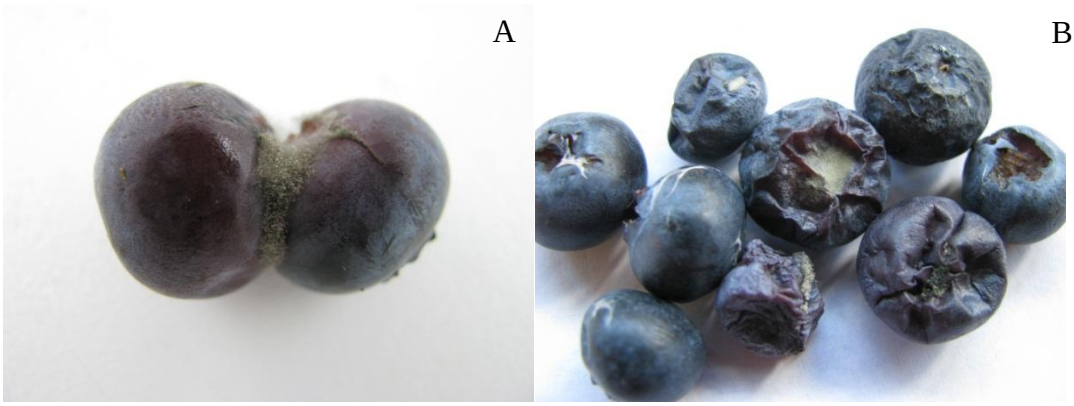

Figure 6-1. Example of the two different sets of decay symptoms observed in blueberries during the experiment. According to Anco and Ellis (2011), these could correspond to *Botrytis cinerea* (A) and *Alternaria sp.* (B).

6.3.1 Temperature effect

Higher temperature generated more rot incidence during the storage of both blueberry cultivars. Storage temperature of 4°C significantly increased rot incidence in comparison to 0°C from 5 weeks onwards for ‘Brigitta’ and after 4 weeks for ‘Maru’, reaching differences of almost 3 and 20 fold, respectively, after 6 weeks (Table 6-1). The increase of rot incidence at higher storage temperatures is an expected consequence of increased temperature accelerating pathogen development in blueberries. Higher temperature leads to increased pathogen growth rate, in addition to decreasing the concentration of antimicrobial compounds such as phenols and saponins in fresh fruit by accelerating senescence (Barkai-Golan, 2001).

Previous research studies evaluating the effect of storage temperatures within the 0-5°C range on blueberry decay have not been coincident. For instance, 'Herbert' blueberries increased from 0.6% to 16.2% rot incidence when maintained for 3 weeks at 0°C and 2°C, respectively (Borecka and Pliszka, 1985), whereas 'Burlington' blueberries developed equal decay levels after 6 weeks when stored at 0°C and 3°C (Forney *et al.*, 1998). These different responses seem to be closely related to genotypic variability among blueberry cultivars in terms of keeping phenolic compound concentrations high enough to provide chemical resistance to pathogens (Ehlenfeldt *et al.*, 2010; Polashock *et al.*, 2005). For 'Brigitta' and 'Maru' blueberries, the results of this experiment indicate that increasing the storage temperature from 0°C to 4°C results in higher rot incidence during the storage period.

The particular relative humidity (RH) settings of this experiment should not have had a big effect on the rot incidence differences between both storage temperatures. A lower RH was deliberately used for 4°C than for 0°C (67% and 90% RH, respectively) in order to represent the effect the temperature variability would have on RH distribution within reefer containers (section 3.2). On the other hand, it is known that the development of most postharvest pathogenic fungi is favoured by high RH conditions, which are required for the processes of spore germination and fruit infection (Barkai-Golan, 2001). As such, the low RH used in this experiment for 4°C may have had the potential to moderate the effect of the temperature accelerating fungal proliferation, reducing the differences between 0°C and 4°C for decay incidence. However, the main blueberry pathogens which are able to grow under storage conditions, *Botrytis cinerea* and *Alternaria sp.*, need temperatures higher than 15°C to germinate new spores and infect healthy berries, even if they are able to continue growing below 0°C (Caruso and Ramsdell, 1995; Sommer, 1985). This means that the decayed blueberries obtained during the experiment were consequence of the spore germination and the infection occurring before the storage period, with the visible rot symptoms developing as a result of the mycelium growth during storage. Since the two fungal growth processes dependent on RH are not likely to occur under the storage conditions used for blueberries, RH settings of this experiment should have had a minor influence on the rot incidence observed.

Table 6-1. Rot incidence of blueberries after 4, 5 and 6 weeks of storage, as influenced by temperature, cooling delay period and storage atmosphere. Results are presented individually for cultivars 'Brigitta' (A) and 'Maru' (B). Honest significant difference (HSD) values and different letters are used to indicate differences within factors. Number of independent measurements (n) is indicated.

A. 'Brigitta'

		rot incidence (%)		
factor	***value***	***week 4***	***week 5***	***week 6***
Temperature	0°C	0.08 a	0.66 a	0.98 a
n=18	4°C	0.25 a	2.74 b	2.59 b
	HSD	*0.29*	*1.38*	*1.32*
	0 h	0.17 a	1.57 a	1.97 a
Delay	12 h	0.32 a	1.91 a	0.94 a
n=12	24 h	0.00 a	1.62 a	2.46 a
	HSD	*0.43*	*2.41*	*2.43*
	Air	0.29 a	2.83 a	3.53 a
Atmosphere	20%O_2 + 10%CO_2	0.07 a	1.46 a	1.39 b
n=12	2.5%O_2 + 10%CO_2	0.14 a	0.81 a	0.44 b
	HSD	*0.42*	*2.03*	*1.95*

B. 'Maru'

		rot incidence (%)		
factor	***value***	***week 4***	***week 5***	***week 6***
Temperature	0°C	0.00 a	0.00 a	0.35 a
n=18	4°C	1.18 b	2.33 b	7.08 b
	HSD	*0.869*	*1.499*	*2.199*
	0 h	0.95 a	0.28 a	5.09 a
Delay	12 h	0.19 a	1.66 a	2.10 a
n=12	24 h	0.63 a	1.56 a	3.97 a
	HSD	*1.40*	*2.62*	*5.04*
	Air	1.04 a	2.34 a	6.04 a
Atmosphere	20%O_2 + 10%CO_2	0.53 a	0.56 a	3.30 ab
n=12	2.5%O_2 + 10%CO_2	0.19 a	0.60 a	1.81 b
	HSD	*1.284*	*2.214*	*3.25*

Placed in the context of the industry, the differences of rot incidence obtained between 0°C and 4°C storage indicate the potential negative impact of temperature variability within marine refrigerated containers (4°C from the set point) on the rot incidence of exported blueberries. Even if 0°C and 4°C started to generate different decay levels beyond the usual shipping period of blueberries (2-3 weeks) in this experiment, the risk for a potential expression of this difference earlier in the export

process or during the subsequent shelf life period seems to be high. The tolerance of blueberry importers for decay is zero in the marketplace, and therefore any factor during the supply chain which enhances the development of rots has potential economic consequences. As such, the blueberry export industry could benefit from improved refrigeration systems able to reduce temperature variability within marine containers, in addition to evaluate alternative shipping systems with better temperature stability.

6.3.2 Cooling delay effect

Rot incidence of blueberries during storage was not influenced by previous cooling delays at 10°C. There were no significant differences between 0, 12 and 24 h of cooling delay on the decay development of 'Brigitta' and 'Maru' blueberries across the complete storage period (Table 6-1). These results are not in concordance with previous experiments evaluating the effects of cooling delays at high temperatures on blueberry decay. The residual effect of delayed cooling on blueberry rot incidence during storage has been consistently confirmed for delay temperatures within the 20-30°C range (Ceponis and Cappellini, 1979, 1982; Jackson *et al.*, 1999). Nevertheless, the lower delay temperature considered in this experiment (10°C) to simulate the packing environment did not resulted in increased rot incidence during the subsequent storage period. This is probably related to the optimal temperatures that main blueberry postharvest fungi need to complete their growth cycle. While *Botrytis cinerea* needs temperatures of at least 20°C and 15°C for spore germination and infection processes, respectively, *Alternaria sp.* requires 20°C as a minimum to germinate and infect fruit (Caruso and Ramsdell, 1995; Sommer, 1985). Therefore, although the blueberries infected before the cooling delay period may have endured faster mycelial growth as the delay duration increased, the temperature limitation for the germination of new spores and the infection of new berries limited the delay at 10°C to generate significant differences in rot incidence after periods of storage.

These results deliver very practical information for the postharvest logistics in the blueberry industry. After the fruit is transported from the field to the packhouse, blueberries are maintained in cool stores usually at the same temperature as the packing environment (10°C) to reduce their deterioration and avoid subsequent

condensation when moved to the packing room. The duration of this pre packing storage can increase considerably during the peak of the harvesting season, which can lead to delays prior to packing of up to 48 h. Information on whether this delay impacts the final conditions of the fruit is extremely valuable. The results obtained in this experiment indicate that pre packing delays (up to 24 h) do not lead to increased decay during subsequent storage, which suggests that there is no need to improve delays at 10°C and that investment efforts should be focussed on other steps of the postharvest chain.

The results obtained in this experiment are for only 2 of many commercial blueberry cultivars. If the results obtained in the experiment are not applicable to all blueberry cultivars, to know which cultivars are negatively affected by the pre packing delay period would provide very useful information. More resistant cultivars could be held under these conditions for longer whereas susceptible ones could be prioritised. Accordingly, decay of 'Brigitta' and 'Maru' blueberries could be considered as not largely influenced by pre packing conditions, which would signify an advantage for these cultivars in terms of postharvest life. However, although rot incidence is considered as the major quality attribute in this commodity, the evaluation of the cooling delay impact on the quality of blueberries must be done in terms of the overall quality rather than analysing just its influence on decay. Further analysis of the effect of cooling delay on blueberry quality in a holistic sense is addressed in the conclusions and recommendations (Chapter 7).

6.3.3 Atmosphere effect

Controlled atmosphere (CA) significantly decreased rot incidence after storage for both blueberry cultivars. There was a clear tendency of fruit stored in air to develop the highest decay values from the appearance of the first rots at 4 weeks onwards (Table 6-1). While the low oxygen atmosphere (2.5% O_2 + 10% CO_2) significantly reduced rot incidence after 6 weeks storage in 'Maru' blueberries, both CA were able to decrease decay indistinctly in 'Brigitta' after 6 weeks of storage (Table 6-1). There were no statistical differences between 2.5% O_2 + 10% CO_2 and 20% O_2 + 10% CO_2

atmospheres on the effect on decay development during the experiment for both cultivars (Table 6-1).

The effect of CA in reducing the rot incidence of blueberries observed in this experiment corresponds to an expected response of high CO_2 concentrations inhibiting fungal growth. As discussed previously (section 1.3.4.1), high CO_2 suppresses the activity of various enzymes involved in the respiration of microorganisms, leading to decreased pathogen metabolism. Numerous studies have shown the efficacy of CO_2 in the range of 10-24% in reducing decay incidence of fresh blueberries (Ceponis and Cappellini, 1985; Fan *et al.*, 1993; Harb and Streif, 2004, 2006). Blueberries stored in 10% CO_2 have reduced their rot incidence up to 50-75% in comparison to fruit stored in 0% CO_2 (Ceponis and Cappellini, 1985; Fan *et al.*, 1993). Consequently, the decay reduction obtained in this experiment using 10% CO_2 atmospheres agrees with the existing knowledge about the effect of CO_2 on blueberry rot incidence.

The statistically null difference between the effect of 2.5% O_2 + 10% CO_2 and 20% O_2 + 10% CO_2 atmospheres on blueberry decay supports the opinion that O_2 concentration has no major relevance on CA efficacy for this commodity. To significantly reduce the respiration rate of microorganisms, O_2 concentration must be managed below 1% which is not achievable for fresh blueberries since that atmospheric condition consistently triggers fermentation (Ceponis and Cappellini, 1983, 1985). Since O_2 is managed above the 1% concentration limit for blueberries, the influence of O_2 on the rot development of this fruit is known to be small (Ehlenfeldt, 2002). Ceponis and Capellini (1985), Harb and Streif (2004) and Harb and Streif (2006) all found no differences in rot incidence when storing several highbush cultivars under 2% O_2 or 18-21% O_2. However, other works have obtained a further decay reduction in blueberries maintained at O_2 concentrations between 1 and 10% regardless of the CO_2 level. Kim *et al.* (1995) obtained lower decay in 'Coville' blueberries stored in 3% and 9% O_2 than in 15% O_2, at a given CO_2 concentration, whereas Prange *et al.* (1995) reported a rot incidence reduction of 60% and 50% with atmospheres of 5% and 2% O_2 (in comparison to air), respectively, when storing lowbush blueberries for 4 weeks. Cultivar differences in terms of skin resistance to gas inflow could explain these variable responses in terms

of O_2 efficacy reducing blueberry rot incidence. The skin is the main resistance to O_2 diffusion into fruit tissues (Burg and Burg, 1965). Skin thickness has been reported to vary up to 2 fold between blueberry cultivars (Makus and Morris, 1993). It might be possible that O_2 internal concentration reaches levels low enough to reduce pathogen metabolism when low O_2 atmospheres are utilised in blueberry cultivars with thicker skin.

These results confirm the importance of using CA for blueberry marine exports (2-3 weeks) and long term storage up to 6 weeks in order to reduce the decay incidence compared to air refrigerated storage. Even if CA led to significantly lower rot levels compared to air just after 6 weeks of storage, there was a similar trend for the appearance of the first rots (week 4) onwards (Table 6-1). The storage time elapsed until the first decay symptoms appear depends on factors such as fruit resistance, inoculum amount and free water availability, which may explain why in this case the first rotten fruit were found beyond a usual shipping period. In fact, the use of CA in blueberry sea freight is largely attributed to its benefits in reducing decay at the marketplace, which agrees with this research study. Moreover, the results obtained also suggest that the use of low O_2 CA within the 1-10% range in blueberries would not provide a clear advantage in terms of rot incidence reduction, at least for 'Brigitta' and 'Maru' blueberries. In agreement with what previous studies have reported for other popularly grown cultivars such as 'Bluecrop' and 'Duke' (Harb and Streif, 2004, 2006), it would seem that the efforts of shipping companies to maintain low O_2 concentrations in the CA systems would not be technically justified in the case of this commodity. Therefore, it might be important for blueberry exporters to focus their demands for CA improvements on more stable CO_2 delivering systems during the shipping or storage period, in order to enhance a better performance of their fruit during this period. Additionally, as the use of CA considerably increases the costs of blueberry shipping, it may be worth evaluating if CA operational costs could decrease by controlling solely the CO_2 concentration instead of also modifying the O_2 concentration as is currently done. In any case, low O_2 atmospheres must be evaluated considering its effects on overall blueberry quality, which is further addressed in the thesis conclusions and recommendations (Chapter 7). Additionally, other commonly grown cultivars must be evaluated in order to properly analyse the impact of low O_2 atmospheres on blueberry postharvest.

6.3.4 Atmosphere and temperature interactions

Controlled atmospheres (CA) reduced the influence of temperature on rot incidence during the storage of blueberries. From 5 weeks onwards, 2.5% O_2 + 10% CO_2 and 20% O_2 + 10% CO_2 atmospheres significantly decreased the temperature effect on rot incidence compared to air storage for 'Maru' blueberries, leading to equal decay levels between fruit stored at 0°C and 4°C after 5 weeks of storage (Figure 6-2). For 'Maru', low oxygen atmosphere (2.5% O_2 + 10% CO_2) reduced the incidence of decay at 4°C by more than 50% after 6 weeks of storage in comparison to air storage (Figure 6-2). 'Brigitta' blueberries were also observed to have lower incidence of rots at 4°C in CA than in air storage from 5 weeks onwards, with the lowest decay incidence at 4°C in the low oxygen CA, although these differences were not statistically significant (Figure 6-2).

The influence of CA reducing the effect of temperature on blueberry decay is an expected consequence of high CO_2 inhibiting fungal growth indistinctly for both storage temperatures. As discussed previously, CO_2 concentrations in the 1-10% range have been consistently effective in decreasing decay development in cold stored blueberries. However, it is known that the solubility of CO_2 and O_2 in blueberry tissue decreases as temperature increases (Beaudry *et al.*, 1992; Cameron *et al.*, 1994), and that CO_2 reduces its solubility by 18% in an aqueous solution maintained at 5°C instead of at 0°C (Carroll *et al.*, 1991). A reduction of CO_2 sensitivity expressed as reduced fermentation damage of blueberry has previously been reported as temperature increases (Forney *et al.*, 1999; Terry *et al.*, 2009). Therefore, storing fruit at 4°C instead of 0°C could have resulted in lower internal CO_2 concentrations and hence in decreased CA efficacy to inhibit rot growth at this temperature. In contrast, the experiment reveals that CA was highly effective in reducing decay incidence at 4°C during the storage of 'Brigitta' and 'Maru' blueberries, indicating that the reduction of the CO_2 solubility generated by 4°C was not strong enough to compromise CA efficacy.

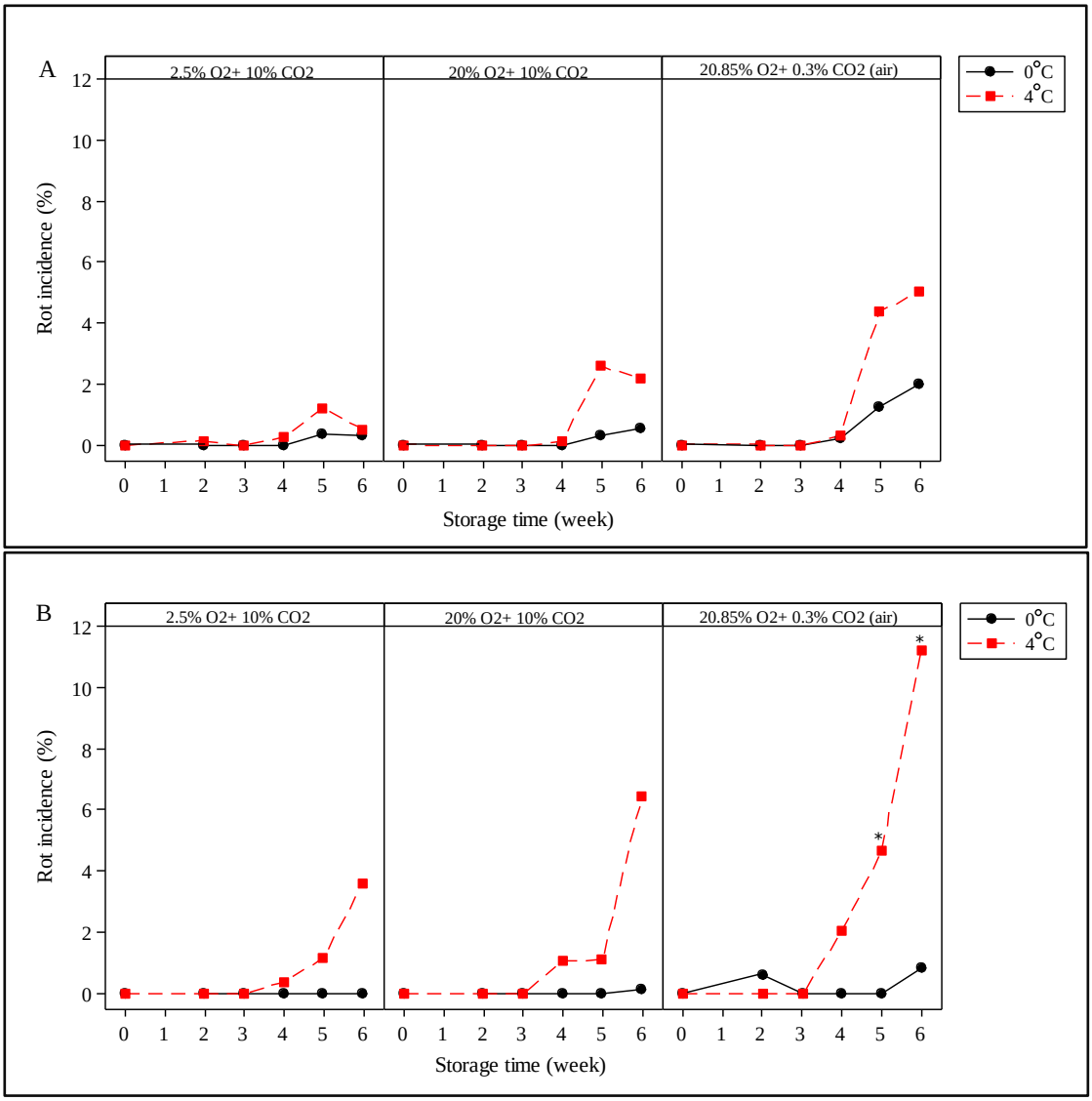

Figure 6-2. Rot incidence of 'Brigitta' (A) and 'Maru' (B) blueberries during storage, as affected by temperature and storage atmosphere. *represents significant differences between storage temperatures for each week at 0.05 level as determined by Tukey's test. Each data point represents 6 independent data measurements (n=6).

For the blueberry industry context, these results validate the importance of CA for blueberry shipping and storage as a safety buffer against the effect of temperature deficiencies enhancing decay in this fruit. With CA reducing the effect of temperature heterogeneity within reefer containers on final decay incidence represents a very interesting advantage to be considered by fruit exporters. This result adds value to CA usage in blueberries beyond its classical perspective of extending postharvest life, as an extra protection against the temperature variability that may occur in the export industry. More cultivars should be included in future evaluations considering the high genotypic variability of this fruit.

6.4. Conclusion

This work provides evidence that the temperature heterogeneity of 4°C around the set point reported within refrigerated marine containers may considerably increase the rot incidence of blueberries during the shipping period. Increasing the storage temperature from 0°C to 4°C would accelerate the growth rate of blueberry pathogenic fungi, resulting in important rot incidence differences after 6 weeks of storage. It appears that maintaining fresh highbush 'Brigitta' and rabbiteye 'Maru' blueberries at 0°C; avoiding temperature fluctuations as high as 4°C during export or long storage periods (3-6 weeks) would reduce the incidence of rot observed in the marketplace, hence decreasing importer rejections due to decayed fruit.

Cooling delays at the temperature conditions commonly used when packing fresh blueberries appear to not influence the decay incidence of blueberries during subsequent cold storage. Delayed cooling at 10°C for up to 24 h did not generate a residual effect on the decay of fresh blueberries during storage. This could constitute relevant information for the blueberry industry, which might focus its efforts to improve blueberry postharvest logistics in other steps of the supply chain. An alternative idea is to prioritise cultivars according to their sensitivity to this delay period.

Controlled atmosphere (CA) provided a clear effect of reducing decay incidence in blueberries during storage. The effect of CA on decreasing rot incidence of 'Brigitta' and 'Maru' blueberries can be largely attributed to the influence of CO_2 on pathogen growth, with no further benefit achieved by reducing the O_2 concentration from 20% to 2% during a 6 week period of storage. Consequently, the use of O_2 concentrations in the range of 1-10% does not appear to be technically justified for this fruit. The blueberry export industry may benefit from cheaper CA systems that do not require O_2 control if the limited advantage of low O_2 is confirmed for other commonly exported blueberry cultivars.

The use of CA was observed to reduce the influence of temperature variability on rot incidence during storage of fresh blueberries. Storage atmospheres with 10% CO_2

supressed the decay incidence differences generated between storing blueberries at 0°C or 4°C, for a period of 6 weeks. As such, CA provides valuable protection against temperature deficiencies occurring during the export period within reefer containers or in other steps of the postharvest chain of blueberries.

An analysis beyond rot incidence responses integrating the effects of storage temperature, cooling delays, CA and the interaction between these factors on the overall quality of blueberries is required for a more complete understanding of the results implications of this work. It is entirely possible that conditions that limit decay may needed to be compromised with conditions that favour maintenance of product quality attributes. The conclusions and possible applications to the industry of the outputs of this work are addressed in the conclusion and recommendations chapter.

CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

7.1 Impact of temperature management deficiencies on blueberry quality as affected by storage atmosphere

7.1.1 Effects of storage temperature variability

This work provides evidence that temperature heterogeneity of 4°C around the set point, as reported for reefer containers, may potentially affect the quality of fresh highbush and rabbiteye blueberries by the end of the export process. Compared to optimum storage conditions (0°C and 90% RH), the outcomes of this experiment show that commercially packed blueberries subjected to 4°C can considerably increase the incidence of rots at the marketplace, with differences as large as 20 fold between temperatures (Table 6-1). Furthermore, this variability of 4°C can also lead to slightly increased moisture loss from blueberries during simulated shipping (Table 3-1). In contrast, heterogeneity of 4°C around the set point in transport temperature enhanced blueberry firmness under the particular conditions which led to fruit firming in this experiment (Table 4-1).

Decay is the main quality defect of fresh blueberries influencing importer acceptability (NeSmith *et al.*, 2002). Storage temperature has a large influence on the growth rate of postharvest pathogens (Barkai-Golan, 2001). The results of this study show that blueberries developed more rot incidence when maintained at 4°C instead of 0°C, after simulating a sea freight period of 6 weeks with temperature heterogeneity of 4°C around the set point, as occurring within shipping containers (Table 6-1). Therefore, maintaining blueberry storage environments stable at 0°C

minimising temperature variability would reduce decay incidence during postharvest and hence decrease rejections at the marketplace.

Blueberry moisture loss during postharvest may result in important quality losses and decreased total saleable weight (Sanford *et al.*, 1991; Wills *et al.*, 2007). Higher temperatures have been found to increase blueberry water loss during storage (Nunes *et al.*, 2004), which is in agreement with the results of this experiment (Table 3-1). However, the differences in weight loss found in the present study between fruit stored at 0°C and 4°C are not large enough to affirm that a temperature variability of 4°C around the set point would generate a commercial impact on weight loss.

Technologies oriented to minimise the temperature variability within storage environments or able to provide a better fruit protection to temperature changes in postharvest should impact positively in reducing the effects of temperature heterogeneity on blueberry quality. The use of techniques which consider the addition of extra physical barriers between fruit and environment, such as wrapping films may decrease temperature heterogeneity and therefore the rot incidence and moisture loss from blueberries, hence improving postharvest life of this crop. Similarly, refrigeration systems able to achieve better spatial distribution and temporal stability of temperature within reefer containers, for instance by an improved air delivery design or by reducing defrost cycle times, respectively (Tanner and Amos, 2003), should lead to a considerable increase of the final quality of blueberries at the marketplace.

The enhancement of blueberry firmness with higher temperatures during storage observed in this study (Table 4-1) may become an interesting response to attempt to replicate in the commercial postharvest chain. Blueberries increased in firmness during the storage period of this experiment (Figure 4-1) as part of a particular phenomenon possibly related to low levels of weight loss (Chiabrando and Giacalone, 2011). This response is dissimilar to the behaviour observed for firmness in industry, where blueberries soften over the postharvest period often reaching firmness values below commercial standards and hence leading to fruit losses (NeSmith *et al.*, 2002). In the hypothetical case of this blueberry firming being replicated in industry conditions, the effect of temperature heterogeneity of 4°C

around the set point leading to a further increase of firmness would have great value for blueberry traders. Nevertheless, even though the potential benefits of a better firmness condition could be important, it appears that the gain in quality would not be large enough to negate the costs of increased decay incidence at the end of the export chain.

As the increase in firmness observed in the present study does not represent the normal evolution of this attribute in industry, it appears that avoiding temperature fluctuations as high as 4°C during marine export or long storage periods (3-6 weeks) would ensure a better final quality of blueberries. Despite the small influence of temperature variability on weight loss observed in this work, its considerable effect on decay incidence suggests that environments used during shipping or storage of fresh blueberries should minimise temperature variations below 4°C in order to improve the overall quality of blueberries at the marketplace.

7.1.2 Effects of delayed cooling

Delays in cooling at packing temperature may alter the quality of fresh blueberries after a subsequent shipping or storage period. Prestorage holding periods at 10°C for at least 12 h considerably increased the total moisture loss of highbush and rabbiteye blueberries over 6 weeks storage in this experiment, with the rate of weight loss remaining invariable over this period (Table 3-1). Moreover, firmness during storage was not influenced by previous cooling delays at 10°C (Table 4-1), although this response, which is dissimilar to previous research data, appears to be conditioned by the particular firming behaviour observed in this work (Figure 4-1). The incidence of rotten fruit over a subsequent simulated marine export process was not affected by delays in cooling at 10°C (Table 6-1).

A delayed cooling could lead to important losses in blueberry quality on a commercial scale. According to the results of this experiment, the residual effect of cooling delays is a potential weight loss increase of up to 0.6% during storage which may lead to blueberry shrivelling and reduced saleable weight. Furthermore, as increased weight loss has been associated to blueberry softening under commercial

storage conditions (Forney *et al.*, 1998), the influence of cooling delays might also result in decreased firmness during storage. Therefore, limiting delays to cooling at packing temperatures to less than 12 h would be a significant way to avoid losses in blueberry quality and saleable weight after the shipping period.

In agreement with our previous studies (Paniagua *et al.*, 2012), this work confirms that blueberries are more sensitive to delayed cooling than previously reported by other authors. In the present experiment, a cooling delay time of just 12 h at packing temperature (10°C) was found to impact the quality of blueberries after storage (Table 3-1), which is considerably shorter than the limit of 21 h at similar temperatures (12°C) stated by Jackson *et al.* (1999). Likewise, delay periods of 24 h at 20°C (NeSmith *et al.*, 2002) and at 32°C (Tetteh *et al.*, 2004) were previously found to reduce blueberry quality after subsequent storage. Accordingly, our experimental data indicates that blueberries undergo quality deterioration when subjected to delays times as short as 12 h at intermediate temperatures, such as when maintained at 10°C before packing, with these changes influencing quality outcomes after storage.

The change in weight loss induced by cooling delays at 10°C occurs during the delay time rather than modifying the rate of weight loss in subsequent storage (Table 3-1). This is consistent with our own previous work (Paniagua *et al.*, 2012) which found that both fruit weight and firmness were modified in storage as a consequence of changes happening in the delay period without affecting the subsequent rates of quality change. Similarly, Tetteh *et al.* (2004) also reported that different cooling delays regimes affected the weight loss and firmness of blueberries after subsequent storage but not the rate at which these changes occurred in this period. Overall it would appear that the residual effects of delayed cooling on the final quality of blueberries are likely to be consequence just of physical processes, such as moisture loss, with probably no biological mechanisms involved.

The null influence of cooling delays at 10°C on the decay incidence of blueberries during subsequent cold storage observed in this study (Table 6-1) could constitute interesting information for the export industry. Considering that fruit decay is the main factor limiting the acceptability of blueberries at the market place (NeSmith *et*

al., 2002), this result places less relevance on the importance of accelerating the packing process despite its effect on other quality attributes such as fruit shrivel. By adopting additional packing or shipping technologies oriented to minimise the risk of moisture loss-related problems, the industry may be well advised to pay less attention to decrease packing times and to focus its efforts to improve other steps of the supply chain such as shipping conditions in order to minimise decay incidence.

7.1.3 Interaction between temperature management deficiencies and storage atmosphere

The gas composition of storage atmosphere would influence the impact of temperature variability on blueberry quality. According to the results of this work, controlled atmosphere (CA) comprising 10% CO_2 in combination 2.5 or 20% O_2 does not modify how the weight loss responses are affected by storage temperature (Table 3-1), but it does substantially reduce, by up to 50%, the effect of temperature on blueberry rot incidence during storage (Table 6-1). Controlled atmosphere was also found to enhance the positive effect of higher temperature on blueberry firmness when the firming phenomenon occurs during storage, leading to up to 26% of firmness differences between 0°C and 4°C (Table 4-1). In contrast, atmosphere composition did not alter the effects of cooling delays on blueberry quality during a subsequent cold storage period (Tables 3-1, 4-1 and 6-1).

In the industry context, the use of CA would considerably minimise the effect of temperature variability within shipping containers (4°C above the set point) on decay incidence during the shipping of fresh blueberries. Storage atmospheres with 10% CO_2 were shown in this experiment to supress the differences in decay incidence generated between storing blueberries at 0°C or 4°C, for a period of up to 6 weeks (Table 6-1). Additionally, it appears that as long as gas levels which compromise blueberry quality (O_2 < 1-2% and CO_2 > 8-15%) are avoided, CA either does not alter or even favours the expression of the temperature effect on firmness under storage conditions which produce blueberry firming (Table 4-1). While postharvest conditions leading to increased firmness of blueberries during storage should be commercially replicated in order to benefit from a potential CA and temperature

interaction for firmness, CA seems to provide a valuable protection against temperature deficiencies that occur during the export period within reefer containers though limiting decay incidence.

7.1.4 Future work

The impact of temperature heterogeneity within refrigerated containers on blueberry quality should include the evaluation of other quality attributes such as flavour, aroma and anthocyanin content in order to determine other possible implications for the fruit caused by the environmental variability in which exported blueberries may be subjected to during shipping. It is known that storage temperature can influence the evolution of anthocyanin content (B.C. Wang *et al.*, 2010), chemical attributes such as total solids and acidity (Sanford *et al.*, 1991), and volatiles (Dudareva *et al.*, 2006) of blueberry. However, whether the temperature variability of 4°C above the set point, as reported within reefer containers, could lead to important changes related to the organoleptic and health promoting properties of blueberries is a question which should be addressed in future research.

Although a storage temperature variability of 4°C above the set point led to increased firmness in this experiment (Table 4-1), this response is likely to be related to the fruit firming observed simultaneously with low weight loss in this work (Figure 4-1). It would be interesting to evaluate the effect of the temperature heterogeneity within reefer containers on the normal softening behaviour that blueberries undergo under commercial conditions in postharvest. If blueberry softening is associated to fruit moisture loss as it has been suggested (Forney *et al.*, 1998), it should be expected that enhancement of this softening process occurs at increased storage temperatures. However, the effect of the temperature variability reported within shipping containers could be not large enough to produce a considerable impact on firmness.

A temperature variability as reported within reefer containers was found to clearly increase the incidence of decay during blueberry shipping period in the present study (Table 6-1). Considerable differences in rot incidence were observed between ‘Brigitta’ and ‘Maru’ blueberries stored at 0°C and fruit maintained at 4°C after 6 weeks storage, although there were important differences in terms of the magnitude

of this response between cultivars. By the end of the storage period, 'Maru' blueberries had 20 times more decay incidence at 4°C than at 0°C, whereas for 'Brigitta' blueberries this difference was just of 3 fold (Table 6-1). According to these results, it appears that genotypic variability can have a large influence on the responses in decay incidence to temperature heterogeneity. As it is frequent that blueberry export shipments comprise a mixture of several cultivars, it would be useful to know the susceptibility of main cultivars to this variability. This should allow managing fruit batches in order to minimise crossed contamination risks. Consequently, future research should aim the test the impact of temperature variability within reefer containers on a wider pool of popular blueberry cultivars used for export.

Further research evaluating the effects of processing logistics on blueberry quality should try to confirm the sensitivity of firmness responses to cooling delays at intermediate temperatures (10-12°C), as used during packing of blueberries. Our previous work found that a delay period of 20 h at 10°C, compared to 4-8 h delays at the same temperature, was able to considerably reduce firmness after subsequent storage (Paniagua *et al.*, 2012). Similarly, studies in lowbush blueberries have shown that delays in cooling of 48 h at 12°C reduced blueberry firmness after subsequent storage (Jackson *et al.*, 1999). Nevertheless, although in the present experiment firmness during storage was not influenced by previous cooling delays of 12-24 h at 10°C (Table 4-1) this result may have been conditioned by the firming response observed in this study (Figure 4-1). Therefore, due to the constant efforts made in the industry to improve the dynamics of the packing process, it would be interesting to evaluate the effect of cooling delays shorter than 20 h at packing temperature on blueberry firmness under the storage conditions which normally lead to postharvest softening of this crop.

The expression of pathogenic fungi during storage is affected by factors such as amount of initial inoculum, fruit resistance and postharvest conditions (Barkai-Golan, 2001). Even if the decay incidence in storage was not affected by previous cooling delay times at 10°C in this experiment (Table 6-1), it would be interesting to know if rot incidence changes would be still unresponsive to delayed cooling in growing seasons with higher pressure of fungal inoculum. Since temperatures during

cooling delays at 10°C and subsequent storage would not allow neither fungal spore germination nor the infection of new blueberries (Caruso and Ramsdell, 1995; Sommer, 1985), the potential effect of delayed cooling on rot incidence during storage would be limited to the direct result of an increased mycelial growth at increased cooling delay durations. Whether a higher level of initial fungal inoculum might be able to develop considerably during the cooling delay period and to generate a significant impact during a subsequent storage is a question which could be useful to address in future research. Artificial inoculation could be considered in future experiments as a way to mimic higher fungal pressure scenarios.

Due to the apparent large influence of the firming behaviour obtained in this study on the blueberry firmness responses to temperature and cooling delay managements, it seems important that future research aiming to simulate industry outcomes ensures that fruit softening is properly induced by the experimental conditions. According to the experiment conducted as part of this research to evaluate the weight loss and firmness relationship, it is possible to induce fruit softening at increased weight loss (above 8-15%) by manipulating air flow rates (Figure 5-33). Even when the present experiment the relative humidity (RH) of the storage environments was manipulated to mimic the shipping conditions (Section 3.1), the total weight loss after 6 weeks storage was around 1.3% (Table 3-1) which is considerably lower than weight loss magnitudes commonly observed in industry (5-7% after 2-3 weeks of shipping) (personal experience). Industry practice contains less controlled additional factors which would increase weight loss during export process such as force air cooling after packing (to rapidly cool to 0°C), 1-3 d of preshipping storage, and the container loading process (Beaudry *et al.*, 1998). Therefore, in order to simulate firmness responses according to industry context, future experiments should adopt additional techniques able to induce blueberry softening such as manipulating air flow rates in the storage environments to obtain 8-15% weight loss.

It would have been interesting to repeat this experiment across several growing seasons to evaluate the consistency of the results as influenced by environmental conditions and agronomic practices. It is known that environmental conditions to which the fruit is subjected in the field during the growing period (such as temperatures, light intensity and rain) can affect the quality of blueberries by

modifying fruit features such as cuticular waxes (Gough, 1994; Makus and Morris, 1993). Likewise, cultural practices during the fruit growing season such as irrigation, nutrition and pruning affect final size, flesh firmness and chemical composition of blueberries in postharvest (Angeletti *et al.*, 2010; Gough, 1994). Further research evaluating the evolution of blueberry quality parameters during postharvest should try to evaluate of 2-3 growing seasons in order to include this source variability in the experiment design.

As the information for temperature variability within shipping containers used in this experiment was taken from evaluations made for kiwifruit export (Tanner and Amos, 2003), the range of temperature heterogeneity should be confirmed for blueberry export conditions. The temperature distribution within a reefer container is dependent on product primary packaging, carton design, pallet type and cargo stowage (Lawton *et al.*, 2011). Even if container length, pallet dimensions and stowage system are similar between kiwifruit and blueberry industries (Boyette, 1993; Lawton *et al.*, 2011), differences in primary packaging such as tray design and the use of clamshells may have a large influence on the temperature variability within containers.

7.2 Controlled atmosphere

7.1.1 Influence of increased oxygen on controlled atmosphere effects

This experiment provides evidence that CA has the potential to improve firmness retention during blueberry storage (Table 4-1). In addition to the well documented effect of CA in reducing blueberry decay incidence in storage (Ceponis and Cappellini, 1983, 1985; Harb and Streif, 2006), the use of CA during shipping may potentially alter cell wall changes which have been associated to overripe softening in blueberries (Vicente *et al.*, 2007), assuming that conditions that induce anaerobiosis are avoided. Nevertheless, this potential benefit of CA in reducing softening does not seem to be large enough to generate a magnitude of commercial significance for blueberry quality.

The results obtained in this work confirm that CA with increased O_2 concentrations is able to alleviate the high CO_2-induced softening during the storage of blueberries. Similarly to what was observed in two previous studies (Fan *et al.*, 1993; Harb and Streif, 2006), atmospheres comprising elevated O_2 in this study seemed to modify the softening CO_2 trigger for blueberries compared to low oxygen CA, hence enhancing the tolerance of blueberries to excessively high CO_2. 'Maru' blueberries were 19% firmer when stored at 20% O_2 + 10% CO_2 than when maintained at 2.5% O_2 + 10% CO_2 (Table 6-1). By considering the use of high O_2 CA, the export industry could benefit from CA systems which ensure a better safety in terms of blueberry firmness retention.

In agreement with previous research and the common knowledge of the industry, the outcomes of this experiment show that CA comprising increased CO_2 concentration provides a clear effect in reducing blueberry decay incidence during storage. Compared to air storage, CA reduced decay incidence in up to 90% during the storage period (Table 6-1). According to our results, the effect of CA on decreasing rot incidence of blueberries can be largely attributed to the influence of CO_2 on pathogen growth, with no benefits achieved by reducing O_2 concentration. This is not dissimilar to what has been found in previous studies (Ceponis and Cappellini, 1983, 1985), although it does not agree with results found by Kim *et al.* (1995) and Prange *et al.* (1995) which suggested a further reduction of rot incidence due to O_2 concentrations below 10%. Consequently, the use of O_2 concentrations in the range of 1-10% does not appear to be technically justified in terms of reducing decay incidence for this crop.

The blueberry export industry may benefit from improved quality outcomes and lower CA operational costs during shipping if O_2 is maintained around 20% and CO_2 increased within recommended ranges. The use of CA comprising 8-15% CO_2 in combination to high O_2 concentrations would provide protection against CO_2-induced softening and simultaneously ensuring decay control efficacy. Simpler CA gas settings could be required to maintain storage atmospheres with elevated O_2 (similar to air) in comparison to current systems which keep 2-5% O_2 in addition to CO_2 regulation, resulting potentially in lower operational costs for marine export.

7.1.4 Future work

Future research should investigate in more detail the interaction between CO_2 and O_2 in CA systems, in terms of the alleviation of CO_2-induced softening by elevated O_2 for blueberries, in addition to checking other possible implications of this interaction on blueberry quality. Future experiments may aim to optimise the O_2 range able to produce this beneficial effect across the main blueberry cultivars, in addition to testing the maximum CO_2 concentrations which can be utilised without compromising quality. Furthermore, it would be important to know whether other quality disorders which are triggered by excessive CO_2 (flesh decolouration, off-flavours, and off-odours) can also be alleviated by increasing O_2 concentration.

There is further potential to investigate if the unresponsiveness of rot incidence to O_2 concentration in the 1-10% range is consistent among the main blueberry cultivars. It is known that fruit anatomical features such as skin properties (Cameron *et al.*, 1994) and picking scar exposure (Paul *et al.*, 2012) influence gas inflow to the fruit. Since blueberry cultivars have a high genotypic variability for epidermis thickness (Makus and Morris, 1993), stem scar diameter (Magee, 1999), and epicuticular wax configuration (Sapers *et al.*, 1984), these properties might vary the internal O_2 concentration to which pathogens are subjected, hence leading to different responses in terms of decay incidence in storage.

7.2 Moisture loss and firmness relationship

7.2.1 Impact of moisture loss on postharvest firmness

The results of this experiment indicate the existence of a causal relationship between moisture loss and postharvest firmness for blueberries. The different firmness responses obtained under different weight loss regimes (Figure 5-3), the confirmation of a high correlation between these two parameters (Figure 5-4) as well as the indications of different water loss patterns for firming and softening as observed in MRI analysis (Figure 5-33) provide evidence that fruit moisture loss plays a major role in determining firmness responses of blueberries. Since softening is one of the

most important problems for the blueberry export industry, this relationship of causality between moisture loss and firmness open new possibilities in terms of postharvest managements oriented to improve blueberry quality at the marketplace.

There is a clear potential for industry to benefit from this relationship by minimising moisture loss during the postharvest chain as a way to improve firmness retention of blueberries. Maintaining weight loss levels below 8-15% would minimise excessive softening of blueberries. Firmness might be improved along the commercial chain by considering technologies and materials which limit weight loss such as palletised modified atmosphere bags, less vented clamshell designs, or edible fruit coatings. Furthermore, relative humidity (RH) within the storage or shipping environments should be maintained as high as possible over the whole postharvest chain, in order to reduce the rate moisture loss. Nevertheless, any postharvest management leading to an increase of the RH of the environment in direct contact with the fruit surface should be complemented with fungicidal treatments to avoid the enhancement of decay development. For instance, the use of palletised modified atmosphere bags alone or the combination with extra fungicidal postharvest treatments (such as sulphur dioxide, chlorine dioxide, ozone and ultra violet radiation) could become an interesting option for blueberry exporters to replace conventional containerised CA systems. Finally, postharvest managements could also be oriented to minimise the steps of the supply chain which enhance blueberry moisture loss such as delays in cooling and cold chain breakages.

The relationship between moisture loss and postharvest firmness could be also used as a practical way to monitor the firmness status of blueberry during the postharvest chain. Firmness evolution during postharvest could be easily estimated by monitoring the weight losses through the process, potentially taking opportune correction measures or redirecting fruit batches to less exigent or less distant markets to avoid firmness levels below commercial standards from occurring. In addition, the firmness condition at arrival or in retail conditions could be approximated from knowledge of the common weight loss that blueberries undergo during the export period to a particular market. Consequently, quality control systems could improve their accuracy and efficacy in order to ensure good blueberry firmness standards at the marketplace and consumer level.

The technique utilised in the present experiment of manipulating weight loss by modifying air flow rate and therefore to successfully induce softening and firming responses in blueberries could be adopted in future experiments. This technique can be utilised as a way to either increase or decrease firmness of stored blueberries at a given storage temperature, relative humidity or atmosphere composition. These experimental settings could represent a simple, low cost but effective option to induce variable firming behaviour in this crop which might be used in future studies focussed on the moisture loss and firmness relationship in blueberries.

7.2.2 Future work

Even if this experiment confirms the existence of a causal relationship between moisture loss and postharvest firmness for blueberries, the mechanism explaining this relationship still needs to be clarified. More research including the direct assessment of critical variables (such as turgor, skin toughness, and cell wall modifications) may elucidate the processes determining the relationship between moisture loss and firmness. The utilisation of other methodologies for analysis of fruit microstructure and water content such as scanning electron microscopy and near infrared radiation, respectively, could also deliver some useful information about the mechanisms involved.

Although weight loss levels below 8-15% would minimise blueberry softening during postharvest, the high genetic variability among species and cultivars makes it necessary to validate this limit for the main blueberry cultivars. Fruit characteristics such as amount of waxes covering the skin and the picking scar diameter might potentially modify the patterns for moisture loss, altering the weight loss ranges at which blueberry firming and softening are expressed. Additionally, to elucidate if specific microstructural differences between blueberry cultivars, such as the number or distribution of stone cells, may influence how moisture loss and firmness responses are related is question which should be addressed in future studies.

Due to the important implications of the causal relationship between moisture loss and postharvest firmness for blueberry quality, future breeding programs for new cultivars should consider anatomical improvements able to impact positively on firmness retention by limiting the moisture loss of harvested blueberries. Blueberry cultivars with smaller stem scars and a higher amount and endurance of epicuticular waxes may result in much firmer blueberries at the end of the export process. Also the facilitation of the peduncle detachment during harvesting could be oriented to favour a small stem scar exposure that quickly dries to minimise water loss from blueberries.

Efforts should be also made in order to standardise firmness assessments in both experimental and industry conditions. Although compression firmness is largely preferred in research as the methodology to measure this quality attribute in blueberries (Angeletti *et al.*, 2010; Buran *et al.*, 2012; Cantín *et al.*, 2012; Duan *et al.*, 2011; Li *et al.*, 2011; Lopez *et al.*, 2010; Yang, 2009), there are other studies utilising other techniques such as puncture tests (Duarte *et al.*, 2009) or durometer readings (Alsmairat *et al.*, 2011) which deliver firmness outcomes which are difficult to compare to other experiment results. Even for the case of compression tests, there is not complete agreement in terms of the outputs to use from this test. While peak force at a compression distance of 1-2 mm is the main preference, the slope for a similar deformation is also used in some cases (Marshall *et al.*, 2008). Furthermore, more research is required to correlate instrumental firmness measurements to the sensorial firmness perceived by customers and traders in industry. Very limited information is available in this matter (Beaudry *et al.*, 1998; Ehlenfeldt and Martin, 2002), which is probably one of the reasons why the industry prefer sensorial evaluation (‘touch firmness’) as the method of assessment in quality control (Forney *et al.*, 1998). Additionally, validation of techniques to evaluate blueberry firmness with a higher portability and at lower costs is still needed in order to establish an objective standard for firmness in the industry context.

Although it is known that transpiration through the fruit epicuticular waxes (Albrigo *et al.*, 1980) and the stem scar (Cappellini and Ceponis, 1977; Moore, 1965) are the main pathways for moisture loss from harvested blueberries, it is not known in what proportion these factors determine water loss from this crop. As the stem scar of

blueberries has been shown to dry during the fruit exposure to warm temperatures (8h at 18°C) (Ehlenfeldt, 2002), it would be useful to evaluate how much moisture can be lost through each pathway during the postharvest chain. This information could lead to practical recommendations to apply by blueberry growers and traders, such as considering an initial period of stem scar curing or even promoting the harvesting to be made with pedicel.

7.3 Final considerations

By conducting this research study it has been possible to gain considerable understanding about how the produce physiology and storage factors influence the evolution of quality during the postharvest of fresh horticultural products. Taken blueberry as a model, the author has been able to approach to the physiological fundaments underlying the changes that main attributes undergo during ripening process and which are subsequently determining final quality. Similarly, by reviewing the effects of selected environmental factors (temperature, relative humidity, cooling promptness, and storage atmosphere) on blueberry storage life the author has incorporated valuable knowledge to understand postharvest managements oriented to maintain produce quality. The comparison of multiple sources of information, sometimes not coincident, has been crucial to develop a personal opinion of the main postharvest issues affecting the storage life of this crop.

The design and conduction of the experiments included in this work allowed the author to test part of the personal understanding gained through the review of the literature, in addition to learn instrumental techniques able to quantify the main quality attributes of blueberries and to simulate consumer perception. The evaluation made for the influence of postharvest management deficiencies during shipping environments on blueberry quality delivered useful information to apply in industry in terms of storage temperature, cooling delays and CA, whereas the confirmation of the causal relationship between blueberry weight loss and firmness provided a new fundamental information which may have considerable impact on the postharvest management of this crop.

It would have been interesting to include in this study the evaluation of other postharvest techniques and non-conventional berry fruit species in order to gain a wider understanding about how postharvest managements can impact fruit quality. Prestorage and storage treatments such as prestorage fruit fumigation with ozone or CA comprising super atmospheric O_2 concentrations have been successful controlling decay and improving quality attributes. While fumigations with ozone have had great efficacy controlling decay in table grapes (Gabler *et al.*, 2010), O_2 concentrations of 40-100% during storage have reduced rot incidence and increased anthocyanin content in blueberries (Zheng *et al.*, 2003). These two techniques could constitute valuable tools to extend postharvest life of berry fruit. Additionally, non-conventional berry fruit species such as 'murtilla' (*Ugni molinae*), a Chilean berry which is being started to be commercially grown, could have been included in the present study in order to provide valuable information for the quality management of recently grown berry fruit species .

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