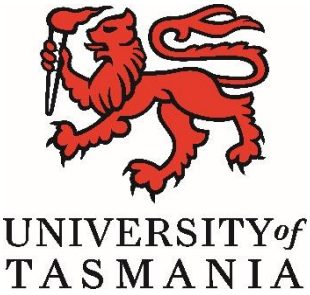

EXTENDING THE SHELF LIFE OF FRESH HORTICULTURAL PRODUCE UNDER INDUSTRIAL SETTINGS BY MODIFIED ATMOSPHERE PACKAGING SYSTEMS

by

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Statements and Declarations

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Keep moving forward.

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List of Abbreviations

CA: controlled atmosphere

GC: gas chromatography

MA: modified atmosphere

MAP: modified atmosphere packaging

MH: modified humidity

MS: mass spectrophotometry

RH: relative humidity

SPME: solid-phase microextraction

TA: titratable acidity

TSS: total soluble solids

WVTR: water vapour transmission rate

Chemical compounds:

1-MCP: 1-methylcyclopropene

BOPP: biaxially oriented polypropylene

ClO_2: chloride dioxide

CO_2: carbon dioxide

DMDS: dimethyl disulphide

DMTS: dimethyl trisulphide

H_2O_2: hydrogen peroxide

HDPE: high-density polyethylene

LDPE: low-density polyethylene

O_2: oxygen

O_3: ozone

PA: polyamide

PLA: polylactic acid

SO_2: sulphur dioxide

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Abstract

Fresh horticultural produce is subject to rapid postharvest quality deterioration and senescence due to natural plant physiological processes and microbial degradation. Extending the shelf life of fresh produce has become important to expand market reach, reduce food waste, improve consumer satisfaction and encourage repeat purchases. Postharvest technologies such as modified atmosphere packaging (MAP), sanitisation, ethylene scavenging have been suggested to improve quality retention and extend the shelf life of fruit and vegetables. However, there is a knowledge gap in defining and understanding the opportunity to combine and optimise the different technology options to deliver improved MAP-based shelf life outcomes in industrial settings. This thesis therefore investigated the potential of combination technologies for improving the shelf life of three high-value, perishable produce types: raspberries, blueberries, and broccoli. The modified atmosphere/modified humidity (MA/MH) packaging types studied in this work included microperforated bags with high water vapour transmission rates (WVTR) that were tailored for each study produce, and a mineral-clay impregnated bag with low WVTR. Complementary treatments were chosen to target the major issue limiting shelf life with each produce type such as sanitisation using SO_2 or H_2O_2 vapour-releasing technologies for raspberries and blueberries, and ethylene scavenging for broccoli. In addition, microperforated lidding film, that develops MAP in unvented punnets was also trialled for raspberries and blueberries. This approach was to help overcome the short shelf life issues with atmospheric conditions used in current vented clamshell systems.

In raspberries, MAP reduced mould growth, weight loss and anthocyanin accumulations that led to fruit darkening compared to the current vented clamshells. In the commercial trials over two harvests, a modified atmosphere of 14-15% O_2 & 7-8% CO_2, established in a microperforated bag containing 12 punnets x 125g berries, was found to extend the storability

of raspberries to 20-21 days at 2 °C. This would add 14 days extra compared to the existing industrial average shelf life of 6 days. The additions of sanitisers to MAP did not provide more benefits to the shelf life of raspberries in this study. In sealed punnets with six 70-µm perforations (creating a percentage of vented area of 5×10^{-9}) and containing 125g berries, a passive MAP formed and improved the storability of raspberries to 11 days at 2 °C, compared to the vented clamshells and packages with none to five 70-µm perforations. The aroma profiles of the raspberries stored in the MA was closer to that of the berries on day 0 with noticeably smaller peak areas for terpenes/terpenoids than the clamshell fruit.

In blueberries, MAP also reduced mould growth and weight loss during 10 weeks of the two commercial trials, as well as 8 weeks of the punnet-scale trial. An atmospheric condition of 16-18% O_2 & 2-4% CO_2, established in both mineral-clay impregnated and microperforated bags packing 12 punnets x 125 g berries, extended the shelf life of 'Legacy' blueberries to 6 weeks and 'Powder Blue' blueberries to 8 weeks at 2 °C. This is equal to 2-4 weeks extra storage compared to the current average shelf life of 4 weeks. At the dose used in this study, SO_2 effectively controlled mould growth but led to softening and bleaching at various degrees of severity. In the sealed punnets of 125g blueberries, the best quality retention was found in packages with two 70-µm perforations (providing a percentage of vented area of 1.68×10^{-9}) plus an initially reduced O_2 level of 17% or 14.5% O_2. Similar to raspberries, blueberries packed in MA had significantly smaller peak areas of terpenes/terpenoids, compared to clamshell fruit.

Broccoli is highly perishable with rapid quality loss under abuse temperatures and exposure to ethylene. MAP systems with > 1% O_2 and < 15% CO_2 maintained the chlorophyll contents and GC-MS aroma profiles of broccoli closest to day 0, while minimising weight losses to < 2% under three simulated shipping conditions. The scenarios studied included high-value, long domestic routes (7 days) with either good temperature management (2 °C) or broken

cool chain (13 ºC) and refrigerated sea-freight for exportation (42 days at 2 ºC). In contrast, top-icing adversely affected the visual quality and aroma profiles of broccoli, particularly at 13 ºC. Because MA was also developed in the packages with ethylene scavengers, it was not possible to examine the sole effects of ethylene scavenging on broccoli quality in this trial.

This research has therefore demonstrated that MAP can extend the storability of raspberries, blueberries, and broccoli beyond current average values under commercial scales and settings. The extended shelf life would allow longer domestic routes and exportation with good end quality. The specific atmospheric conditions, benefits of bulk-pack MAP, the model of retail MAP and considerations for further research have been reported to inform the berry and broccoli industries and help justify establishing new MAP-based extended shelf life offerings. In addition, the design approach used in this Thesis allowed the testings of various MAP designs from one film and one gas mixture. This methodology can benefit future trials and optimisations of retail packaging for other products.

Keywords: modified atmosphere packaging, microperforations, blueberries, raspberries, sanitisation, sulphur dioxide, hydrogen peroxide, broccoli, ethylene scavenging, top-icing, cold storage, shipping.

Chapter 1: Introduction

1.1 Research background

Consistent supply of high quality of fresh produce with prolonged seasonality has been identified as the key competitive advantage to ensure repeat purchases by consumers (Grunert, 2005; Hewett, 2006) and acceptance in export markets (George et al., 2006). The interest in and the consumption of fruits and vegetables has been increasing significantly due to consumer perceptions of their potential health benefits (Dodd & Bouwer, 2014; Kyriacou & Rouphael, 2018). Meanwhile, reducing food waste and food loss to address food insecurity has been gaining attention from both public and governmental sectors (Xue & Liu, 2019). Globally, it was reported that approximately one-third of food production went to waste (Gustavsson et al., 2011). The report also highlighted that about 29-42% of the fruit and vegetable harvest was lost during distribution, retail, and consumption. For Australia, the estimated volumes of food waste generated in these sectors were 83,000 tonnes in wholesale distribution, 179,000 tonnes in retail and 2.7 million tonnes in households (Verghese et al., 2013). In the Sustainable Development Goals, the United Nations has set a goal to reduce food waste at the retail and consumer levels by half per capita by 2030, as well as reduce food losses in production and supply chains (SDG 12.3). Quality deteriorations during transport and storage, and consumer perception of quality and safety among the main causes of food waste (Raak et al., 2017).

The quality of fresh produce cannot be improved postharvest but can rapidly deteriorate due to several internal and external factors (Gontard & Guillaume, 2010). Fruit and vegetables are perishable products of which physiological processes (respiration, transpiration, and senescence) continue after harvest (Kader, 1992). These activities lead to the breakdown of organic matter, dehydration and eventually, cell death. Senescence and related quality loss can also be triggered and accelerated by ethylene in many produce types that are sensitive to

this natural plant hormone (Saltveit, 1999). In addition, fresh fruit and vegetables are susceptible to microbial attacks and injuries that can happen at any point of the supply chain (Barkai-Golan, 2001; Kader, 1992). Therefore, while efforts have been made to improve produce quality preharvest, several postharvest technologies have been introduced to maintain it. The main strategies to extend the shelf life of fresh produce include the suppression of respiration rates, reduction of transpiration/moisture loss, control microbial growth and protection against physical damages (Mahajan et al., 2014). Controlling ethylene is also known to be helpful for ethylene-sensitive produce (Hu et al., 2019).

Food packaging has been continually evolving since the 1800s to meet modern requirements as lifestyles change (Risch, 2009). Conventionally, packaging serves the primary functions of (1) protection from external effects and damage, (2) containment, and (3) means of communication between producers and consumers (Coles et al., 2003). Novel packaging technologies allow traceability and increase convenience and safety (Marsh & Bugusu, 2007; Robertson, 2012), as well as preserve product quality and prolong shelf life (Gontard & Guillaume, 2010). While the uses of packaging remain controversial (Fernqvist et al., 2015; White & Lockyer, 2020), it has been shown that the energy input for packaging is about 10% of the supply chain. However, it could save the remaining 90% from being wasted (Verghese et al., 2013).

Modified atmosphere packaging (MAP) has been extensively developed and shown to be an effective tool to maintain quality and extend storability of many fresh produce. Conventionally, MAP refers to the sealing of food packages with a reduced O_2 and elevated CO_2 atmosphere that can be created passively by produce respiration, or actively by gas flushing (Mangaraj et al., 2009). More recently, studies on MAP also explored noble gases and non-conventional uses of O_2/CO_2 such as super atmospheric O_2 (> 70% O_2) and CO_2 "shock" (> 20% CO_2) (Moradinezhad et al., 2018; M.D. Wilson et al., 2019a). These

atmospheric conditions are capable of suppressing the produce respiration, ethylene production and enzymatic activities, as well as controlling microbial growth (Beaudry, 2000; M.D. Wilson et al., 2019a). Furthermore, MAP creates an in-package environment with high relative humidity that reduces water vapour deficits and moisture loss (Bovi et al., 2016). The major challenge of MAP is to achieve and maintain the desirable atmospheres to guarantee the shelf life extension (Mangaraj et al., 2009; Vermeulen et al., 2018).

Perforation technologies and modified humidity (MH) packaging have been developed to alleviate the limitations of non-perforated MAP (Jalali et al., 2017; Rodriguez-Aguilera & Oliveira, 2009). Perforations refer to the macroscopic (> 200 μm) and microscopic (50-200 μm) holes in the packaging material (Rennie & Tavoularis, 2009). The perforations facilitate the gas exchange and improve the packaging permeability to water vapour to reduce moisture condensation. An efficient perforated MAP should be tailor-made to meet the produce respiration and transpiration rates at a certain temperature (Hussein et al., 2015). However, perforated MAP might have to be designed with higher O_2/CO_2 exchange rates than the optimal levels to prevent the risks of exceeding produce tolerances to O_2 and CO_2, which would result in physiological stress and cellular degradation (Kader et al., 1989).

Combinations of multiple technologies have been suggested to better overcome the limitations of individual treatments and achieve an enhanced overall shelf life for fresh produce (Pinela & Ferreira, 2017). Additional strategies to extend produce shelf life and potentially compensate for suboptimal MA include controlling microbial growth and ethylene for ethylene-sensitive produce. Furthermore, novel preservation technologies are now available in convenient formats such as sachets/pouches or sheets that could be used as complementary treatments to MAP (Álvarez-Hernández et al., 2019; Erickson, 2017; Saito & Xiao, 2017). Technologies are also available which blend the active ingredients into packaging materials during manufacture (Yildirim et al., 2018). These products offer safe and

easy applications without high capital investments, compared to fumigation and irradiation (Guzel-Seydim et al., 2004; Mahajan et al., 2014).

1.2 Industry-focus of the project

Tasmania has geographical advantages to produce cool-climate, high-quality fruit and vegetables. However, its isolated island location also requires longer times for postharvest transportation to reach other regions of Australia and for exportation. For example, the high-value domestic routes to reach North Queensland or Western Australia by sea and road take up to 6-7 days. The long transit times can reduce the produce quality and shelf life at retail and households, particularly for highly perishable products. Additionally, Tasmania has relatively limited higher cost productive agricultural land and high input costs, compared to many other production areas for the same products (C.R. Wilson, 2014). Therefore, producing and maintaining superior quality is important for Tasmanian produce to remain competitive in the markets.

Despite the continuous development of packaging and preservation techniques, current fresh produce industry mainly relies on a cold supply chain to maintain the product quality. MAP technology has been available for more than 50 years but there are relatively few applications for fresh fruit and vegetables (Madrid, 2019). In interviews conducted by Verghese et al. (2013), growers and wholesalers expressed that there needed to be more collaborative research and evidence for the shelf life improvements to lead to commercial uptake of new technologies. Due to the extra costs associated with establishing new post-harvest packaging technologies, initial studies would better be conducted on higher-value, perishable fresh produce to provide evidence of benefits. Communications with industrial partners and packaging experts in the early stages of this project identified raspberries, blueberries and broccoli as important Tasmania fruit and vegetables where shelf life and packaging should be improved.

Berries account for a large segment of fruit grown in Tasmania, with strawberries, blueberries, raspberries and blackberries dominating the market. Compared to strawberries, raspberries and blueberries have smaller production volumes but higher market prices, and increasing demand worldwide (Graham & Brennan, 2018; Retamales & Hancock, 2018). For the financial year ending June 2019, the production of Tasmanian raspberries and blackberries (as *Rubus* berries) was 2721 tonnes (28% of Australia production) (Hort Innovation, 2019a). This value for blueberries was approximately 1236 tonnes (7% of Australian production). Fresh Tasmanian raspberries are often available between November and May, with peak production between December and March (Hort Innovation, 2019a). The expansion of domestic and international markets for raspberries is limited by their extremely perishable nature and short shelf life of 2-5 days (Perkins-Veazie, 2016b). Blueberries have relatively longer storability of about 2-4 weeks (Jackson et al., 1999; Perkins-Veazie et al., 1994), but a very short season, usually between January and March (Hort Innovation, 2019a). Currently there is a three-month gap (April – June) between the end of the Tasmanian supply and the start of next blueberry season in other regions. Prolonging the blueberries supply for one or two months after the season peaks, therefore, would improve returns (Bounous et al., 1996; Hancock et al., 2008).

Broccoli is one of the most important vegetable crops grown in Tasmania, with Tasmanian broccoli attracting premium prices in major Australian markets (Hort Innovation, 2019b). About 4295 tonnes of broccoli (including baby broccoli) were produced in Tasmania in 2018/2019, accounting for 4% of total Australian production (Hort Innovation, 2019c). The quality of broccoli can be lost quickly under abuse temperatures and with exposure to ethylene. This is characterised by wilting, yellowing and formation of off-odours (Toivonen & Forney, 2016). Therefore, commercial shipments of broccoli often use top-icing in polystyrene boxes or in liners with plastic crates to offset the temperature fluctuations during

transportation (Gillies & Toivonen, 1995; Jacobsson et al., 2004a). However, the disadvantages of this method could outweigh its benefits. There needs to be clean water source for ice-making and extra costs for energy and handling (Ekman, 2017). The meltwater can create favourable conditions for the microbial growth and spreading contamination (Felt et al., 1983) and pose safe handling hazards if accumulating on the floor (Brecht et al., 2019). Furthermore, the use of non-recyclable polystyrene boxes is undesirable for environmental concerns and fire safety (Overholt et al., 2011).

1.3 Thesis aims and hypothesis

This project is a part of the Australian Research Council Training Centre in Innovative Horticulture Products which was funded in collaboration with Woolworths, a major supermarket chain in Australia. The industry-defined study overall aimed to determine and assess innovative packaging techniques for maintaining quality and extending shelf life of fresh produce from grower to consumer. The prolonged storability could therefore potentially improve consumer satisfaction for repeat purchase and reduce food waste.

The research focused on raspberries, blueberries, and broccoli as examples of Tasmanian high-value, perishable fresh produce. It was hypothesised that the shelf life of blueberries, raspberries and broccoli could be improved under industrial conditions. The research specifically aimed to:

- Extend the industrially achievable storage periods of raspberries and blueberries to expand reach for national and international markets, and
- Find alternative methods to top-icing shipment of broccoli to prevent associated quality loss, the use of non-recyclable polystyrene packaging, and lower costs.

The results found in this research could potentially be applicable to other fresh produce sharing similar characteristics and issues. In addition, while the research was placed in a Tasmania-mainland context, there are similar regional production circumstances over the

world which could benefit from the findings of this work. These could include transporting raspberries from Mexico to the United States, blueberries from Chile/Peru to North America and Europe, or broccoli from the United States to Canada and Japan/Taiwan (Boriss & Brunke, 2005; Nonnecke et al., 2013; Retamales & Hancock, 2018).

1.4 Research questions

The following science research questions were developed to achieve the aims set out in Section 1.3:

- Could MAP and their novel combinations with sanitation treatments extend the shelf life of raspberries in cold storage beyond the average of 5-7 days? (Chapter 3)
- Could MAP and their novel combinations with sanitation treatments extend the shelf life of blueberries in cold storage beyond the average of 2-4 weeks? (Chapter 4)
- Could perforated MAP as retail packaging extend the shelf life of raspberries and blueberries beyond the performances of the existing commercial clamshell punnets? (Chapter 5)
- Could MAP and ethylene scavenging maintain the quality of broccoli under suboptimal temperatures and prolonged storage, thereby, replacing top-icing in broccoli shipment? (Chapter 6)

Chapter 2: Literature review

Section A. Blueberries and raspberries

The below is modified from:

Huynh, N. K., Wilson, M. D., Eyles, A., & Stanley, R. A. (2019). Recent advances in postharvest technologies to extend the shelf life of blueberries (*Vaccinium* sp.), raspberries (*Rubus idaeus* L.) and blackberries (*Rubus* sp.). Journal of Berry Research, 9(4), 687-707.

A1. Introduction

Berries are high-value crops that are not only seasonal but also highly perishable (Mitcham, 2007; Sobekova et al., 2013). Therefore, increasing their shelf life to enhance distribution options, and to extend availability outside of peak production periods has proven to be challenging (Bower, 2007; Hancock et al., 2008). By their nature, shelf life of berries can vary considerably among cultivars, but in general, is still limited due to their high respiration rates (52-245 mg CO_2 kg^{-1} h^{-1} at 20 ºC), fragile structures and high susceptibility to fungal development (Mitcham, 2007; Perkins-Veazie, 2016a, 2016b). The shelf life of raspberries can be as short as 2-5 days, even under optimum storage conditions (-0.5-0 ºC, 90-95% RH) (Perkins-Veazie, 2016b).

Blueberries have a relatively longer shelf life which has been attributed to the presence of a protective layer of epicuticular wax comprising triterpenoids and β-diketone (Chu et al., 2017) that forms a natural barrier against moisture loss and pathogenic attacks (Chu et al., 2018a). The major species of blueberries grown commercially are highbush blueberry (*Vaccinium corymbosum* L.), rabbiteye blueberry (*Vaccinium ashei* Reade) and lowbush blueberry (*Vaccinium angustifolium* Ait) (Retamales & Hancock, 2018). Depending on chilling requirements and winter hardiness, highbush cultivars are further classified as northern (800-1000 h of chilling and adapting to cold mid-winter temperatures < -20 ºC), southern (< 550 h of

chilling and not tolerating < 0 ºC) and intermediate (400-800 h of chilling). Northern highbush blueberries, which are the main cultivars in Tasmania, can last to 2 months under current best commercial practice conditions (-0.5-0 ºC, above 90% RH, 10% CO_2), especially if the initial quality is high (Hancock et al., 2008; Paniagua et al., 2014). On average, however, the shelf life is typically up to 2 weeks for lowbush, northern highbush and southern highbush cultivars (Jackson et al., 1999; Perkins-Veazie et al., 1994) and up to 4 weeks for rabbiteye cultivars (Miller et al., 1988).

A1.1 Common symptoms of quality deterioration

A1.1.1 Fungal development

Bruising can occur at any stage along the supply chain from the field to the packhouse or during distribution and retail. It can markedly increase the susceptibility of berries to postharvest fungal infection resulting in decay. A visible decay incidence as low as 1-2% is considered enough to reduce the marketability of blueberries and raspberries (USDA, 2002, 2004). For blueberries, the most common pathogens causing spoilage are *Botrytis cinerea*, *Alternaria alternata* and *Alternaria tenuissim* (Alternaria rot), and *Colletotrichum gloeosporioides* (anthracnose fruit rot) (Polashock et al., 2017). For raspberries, common pathogens are *B. cinerea, Cladosporium sp.*, *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. (Tournas & Katsoudas, 2005). Latent infections of fruit occur as the fungal spores are dispersed in the air, and favoured by a cool, wet environment then infect the flower or growing fruit (Funt, 2013; Retamales & Hancook, 2018). The control of these fungal diseases is mainly based on preharvest management, including cultivar selections, preharvest spraying, removal of the infected canes/wood, careful handling at harvest and avoiding harvesting overripe fruit. For postharvest, rapid cooling is recommended to reduce disease levels, but proactive management is hindered by a current lack of practical sanitisation treatments (Horvitz, 2017).

A1.1.2 Changes in sensorial attributes

Sensorial indicators of the shelf life of berries include colour changes, dehydration and softening. Blueberries become darker with storage, turning from bright purplish blue to dark blue due to the loss of the waxy bloom (do Nascimento Nunes et al., 2004), although the amounts of wax, and changes in compositions during fruit ripening and storage, may vary with cultivars (Chu et al., 2017; Chu et al., 2018b). Damage to the wax layer can also occur during harvesting and postharvest handling. Removal of the cuticular wax has been shown to accelerate the accumulation of reactive oxygen species (ROS), which consequently decreases the activities of antioxidant enzymes that were a part of the plant natural defence mechanisms (Chu et al., 2018b).

Raspberries darken quickly, turning from pink/light red to dark red after harvest, due to increases in the anthocyanin contents and the pH (Sjulin & Robbins, 1987). The biosynthesis pathways of anthocyanins in fruit have been mostly discovered as reviewed by Paliyath et al. (2012). The formation of anthocyanins requires the metabolic precursors *p*-coumaryl-CoA, which is converted from phenylalanine via the phenylpropanoid pathway, and malonyl-CoA, which is formed from acetyl-CoA after glycolysis. One *p*-courmaryl-CoA and three malonyl-CoA molecules then enter a condensation reaction to produce chalcone, which would be further converted to anthocyanins via the flavonoid pathway. Picking raspberries at early maturity, that is pink and firm, was suggested to improve storability (Sjulin & Robbins, 1987) but this may compromise the eating quality (do Nascimento Nunes, 2009b).

Postharvest loss of moisture alters fruit appearance, texture, and flavour, and reduces marketable weight (do Nascimento Nunes & Emond, 2007). Moisture loss can also cause the concentrating effects that lead to the increases in anthocyanin contents and TSS (Krüger et al., 2011). Raspberries are more prone than blueberries to dehydration due to the lack of epicuticular wax, with a maximum acceptable moisture loss of 6% (Horvitz, 2017). In

blueberries, moisture loss of > 2-8% (depending on the cultivars) reduces the waxy bloom (do Nascimento Nunes & Emond, 2007), causes loss of firmness and leads to shrivelling (Paniagua et al., 2013).

Softening also occurs in all berries owing to the solubilisation and depolymerisation of cell wall compounds due to enzymatic activities (H. Chen et al., 2015; Talcott, 2007). An increase in water-soluble pectin and a decrease in sodium-carbonate-soluble pectin, hemicellulose and cellulose were observed during the softening of blueberry (H. Chen et al., 2015). In raspberries, their quick postharvest softening was correlated to the activities of polygalacturonase and pectin methylesterase (Iannetta et al., 1999).

A1.2 Ethylene effects

Raspberries are non-climacteric (Iannetta et al., 1999), but the climacteric response of blueberries varies depending on cultivars (V. Paul et al., 2012; Retamales & Hancock, 2018). However, all of them are low-ethylene producers (0.1-1.0 µL kg^{-1} h^{-1}) (Kader, 1985). Raspberries are highly sensitive to ethylene at pre-harvest (Iannetta et al., 1999), although the role of this plant hormone on postharvest quality and storability of raspberries, as well as of blueberries, is still not well understood. In raspberries, ethylene increased the incidence of grey mould (*B. cinerea*) and darkened fruit colour from red to purple-red (Bushway et al., 2008). The maturation of 'Jersey' highbush blueberries was also suggested to be stimulated by ethylene (Watanabe et al., 2021). However, a recent study of five primocane raspberry genotypes ('BP1', 'Crimson Treasure', 'Heritage', 'Nantahala', and NY 10-24-a breeding selection from the Cornell raspberry breeding program) found no correlation between ethylene production rates and fruit colour stability or anthocyanin content or shelf life (Palonen & Weber, 2019). In blueberries, the inhibition of ethylene action by its antagonist, 1-methylcyclopropene (1-MCP) had no effects on shelf life and quality of highbush cultivars

(DeLong et al., 2003), but accelerated loss of firmness in rabbiteye cultivars (MacLean & Scott NeSmith, 2011).

Other important plant hormones that contribute to fruit development and ripening are abscisic acid (ABA), auxins, gibberellins, cytokinins, brassinosteroids and jasmonic acid, but little research has been done on their effects. A recent study suggested that auxin and jasmonates modulate the biosynthesis of red raspberries (Moro et al., 2017), but the roles of other plant regulators in raspberry ripening still need to be discovered (Fuentes et al., 2019). The activities of these phytohormones have been reviewed for a broader category of soft fruits (Vicente & Sozzi, 2007) and for non-climacteric fruit (Fuentes et al., 2019).

A2. Physico-chemical methods for shelf life extension of berries

A2.1 Heat treatments

Heat treatments improve the shelf life of fresh produce by reducing physiological changes, eliminating insects of phytosanitary concern and controlling microorganisms and decay on produce surface (Mahajan et al., 2014). While there has been no study applying heat treatments to raspberries, possibly because of their delicate structures, beneficial effects were observed in blueberry treated by vapour heat and hot air (> 30 ºC, RH > 90%) (Zhao et al., 2013), and hot water (> 40 ºC) dipping (L. Fan et al., 2008). For example, incubation at 50 ºC for 30 min during storage reduced the respiration rate, malondialdehyde content and decay of southern highbush blueberries (‘Misty’, ‘O’Neal’ and ‘Sharpblue’) (Zhao et al., 2013). Similarly, dipping ‘Burlington’ blueberries in hot water (45-60 ºC) for 15-30 s lowered weight loss, shrivelling, fruit split, and decay caused by *B. cinerea* and *Collectotrichum* spp. after 4 weeks at 0 ºC and 2 days at 20 ºC (L. Fan et al., 2008). However, the same study showed that the heat-treated fruits exhibited lower titratable acidity and total soluble solid contents and thinner wax bloom. Heat treatment might also trigger the production of stress-induced volatiles such as ethanol and ethyl acetate (L. Fan et al., 2008). Such responses indicated that the benefits of

heat treatment in blueberries would particularly require further optimisation of time-temperature combinations, as effective heat treatments are often near to the limits that the fruit can tolerate (Wszelaki, 2003).

A2.2 Ultraviolet (UV) irradiation

UV radiation refers to a broad band of wavelengths comprised of short-wave UV-C (200-280 nm), medium-wave UV-B (280-320 nm), and long-wave UV-A (320-400 nm). Although all wavelengths have microbicidal effects, UV-A is considered to have little practical value to shelf life extension of fresh produce because of the low absorption by living cells. UV-C has stronger biocidal effects than UV-A and B, due to its high-energy state (Bintsis et al., 2000), but might not be effective against internal rot fungi due to its low penetration depth (Ramos et al., 2013).

A2.2.1 UV-C (short-wave)

Several studies have shown reductions in microbial load and improvements in produce shelf life treated with UV-C (1-8 kJ m^{-2}). UV-C can alter microbial DNA or stimulate the production of photoproducts that suppress the germination of microbial spores (Bintsis et al., 2000; Sastry et al., 2000). However, most of these studies examined blueberries whilst only one study was found for raspberries. In blueberries inoculated with *Escherichia coli* O157:H7, 1-10 min irradiation using UV-C at 200 J m^{-2} s^{-1} reduced the microbial counts on the calyx by 1.5-2.1 log CFU g^{-1} and on the skin by 3.1-5.5 log CFU g^{-1} (C. Kim & Hung, 2012). Irradiation with UV-C at 1-4 kJ m^{-2} reduced ripe rot incidence caused by *C. acutatum* by 10% after storage for 7 days at 5 ºC followed by 2 days at 20 ºC in northern highbush 'Bluecrop' and 'Collins' cultivars (Perkins-Veazie et al., 2008). Although showing some promise for blueberries, caution is required as higher doses (8 kJ m^{-2}) of UV-C have been reported to increase decay incidence (Perkins-Veazie et al., 2008).

The effectiveness of UV-C in inactivating microorganisms has shown to be affected by the physical structure of the produce and the targeted microorganisms. The inactivation kinetics and E90 (the amount of energy required to kill 90% of the target microorganisms) of UV-C was influenced by surface roughness and spreading coefficients of the commodity being treated (Adhikari et al., 2015). In that study, a 12-min treatment of UV-C at 10.5 kJ m^{-2} only reduced *E. coli* O157:H7 by 1.1 log CFU g^{-1} on inoculated raspberry.

Reduced softening by UV-C was observed in boysenberries (*Rubus ursinus* × *Rubus idaeus*), primarily by disrupting cell-wall degrading enzymes (Vicente et al., 2004), but there has been no study showing if the same effects could be achieved for raspberries.

Additionally, the stress from the exposure to irradiation may induce the production of antioxidant compounds as part of the fruits' natural defence mechanism (S.Y. Wang et al., 2009). At 2-4 kJ m^{-2}, UV-C increased anthocyanins by 10% in 'Bluecrop' blueberries (Perkins-Veazie et al., 2008) and increased flavonoids by 10% in 'Duke' (Nguyen et al., 2014; S.Y. Wang et al., 2009), but not in 'Collins' cultivar (Perkins-Veazie et al., 2008). Notably, the levels of antioxidants were particularly high immediately after the radiation treatment, but dropped sharply during storage (Nguyen et al., 2014; S.Y. Wang et al., 2009).

A2.2.2 UV-B (medium-wave)

UV-B is of interest as an alternative to UV-C because it offers comparable efficacy while being less harmful to overall fruit quality (Eichholz et al., 2011). However, this option might have limited commercial applications on berries. Although irradiation with UV-B at 6 kJ m^{-2} reduced weight loss, decay and delayed increase in the soluble solid-to-titratable acidity ratio in 'Duke' blueberries during 28 days of cold storage (Nguyen et al., 2014), observed changes in volatiles and phenolics in 'Bluecrop' blueberries irradiated by UV-B at different radiation intensities and durations could imply changes in sensory characteristics (Eichholz et al., 2011).

A2.2.3 Pulsed UV-light

Pulsed UV-light refers to the release of intense broad-spectrum electromagnetic radiation (100-1100 nm) energy in short bursts, which may allow greater decontamination potential than conventional UV-light radiation (Miller et al., 1988; Oms-Oliu et al., 2010). In inoculated blueberries, treatment with 226 kJ m^{-2} reduced *E. coli* O157:H7 and *Salmonella* by 2.9 and 4.3 log CFU g^{-1}, respectively (Bialka & Demirci, 2007). In inoculated raspberries, pulsed UV-light at 720 kJ m^{-2} reduced *E. coli* O157:H7 by 3.9 log CFU g^{-1}, and reduced *Salmonella* by 3.4 log CFU g^{-1} at 594 kJ m^{-2} (Bialka & Demirci, 2008). Although pulsed UV-light affords good microbial control, its commercial viability may be discouraged by its effects on fruit sensory properties. High surface temperature due to the heat generated during treatments adversely affected blueberry appearance, including serious discolouration and loss of wax bloom (Huang & Chen, 2014). A system voltage of 3800 kg m^2 s^{-3} A^{-1} was noted for causing a cooked appearance and loss of integrity in blueberries (Bialka & Demirci, 2007). Severe darkening and softening over 10 days of refrigerated storage was reported on raspberries treated at 282 kJ m^{-2} for 30 s (W. Xu & Wu, 2016).

A water-assisted pulsed UV-light system (wet pulsed light) immersing the produce in agitated water was proposed to minimise the temperature increase and allow fruit movement and rotation for better energy distribution (Huang & Chen, 2014). Indeed, 60 s of water-assisted pulsed light reduced *E. coli* O157:H7 and a 4-strain cocktail of *Salmonella* by > 5.8 log CFU g^{-1} in inoculated blueberries while fruit appearance remained unchanged (Huang & Chen, 2014). Similarly, the same treatment reduced *Salmonella* by 3 log CFU g^{-1} in inoculated raspberries (Huang et al., 2015). The feasibility of this technology, however, could be limited by the residual surface moisture left on berries, which can encourage microbial growth (Horvitz, 2017).

A2.3 Sanitisation

In general, sanitisation by washing is not recommended for berries as it can promote mechanical damage and residual surface moisture, particularly in raspberries owing to their hollow structure (Horvitz, 2017). The few studies that have reported the efficacy of sanitisers mostly examined blueberries with a focus on food safety rather than fruit quality and control of fungal development. Washing with 50-100 mg L^{-1} chlorinated water failed to reduce microbial load in blueberries (Popa et al., 2007). In contrast, sulphur dioxide (SO_2) fumigation reduced the incidences of mould development in eight blueberry cultivars ('Emerald', 'Jewel', 'Legacy', 'Misty', 'Reveille', 'Snow', 'South Moon', and 'Star') over 28 and 35 days of cold storage at 1 ºC (Cantín et al., 2012), and for six cultivars ('Brigitta', 'O'Neal', 'Duke', 'Legacy', 'Elliott', and 'Aurora') over 45 days at 0-1 ºC followed by 3 days at 20 ºC (Rivera et al., 2013). This was achieved without altering fruit quality even at an applied gas level as high as 194 nL L^{-1} s^{-1} (Cantín et al., 2012). The SO_2-treated blueberries also had less decay when stored in ambient atmosphere compared to the untreated fruits stored in controlled atmosphere (3% O_2 & 3-12% CO_2). A major obstacle of sulphite use is the consumer opposition to additives (Tarnavölgyi, 2003) and possible allergic reactions, particularly for asthmatics (Santos et al., 2012). The use of SO_2/sulphite must be declared on package labelling. Consumer concerns around possible health effects of sodium hypochlorite and sulphur dioxide have encouraged researchers to look for alternatives, with chlorine dioxide (ClO_2) and ozone being the most studied for berries.

A2.3.1 Chlorine dioxide (ClO_2)

ClO_2 is a powerful oxidiser, 2.5 times stronger than chlorine in oxidation capacity and is capable of penetrating microbial cell walls and altering cellular metabolism (Joshi et al., 2013). ClO_2 can be used as an antimicrobial agent in both gaseous and aqueous forms. Aqueous ClO_2 is stable at pH 6.0-10.0 (Wu, 2016), but less effective than its gaseous state which provides

better penetration into small areas where water cannot penetrate due to surface tension (Joshi et al., 2013). In the USA, the highest level of aqueous ClO_2 allowed for whole fresh produce is 3 mg L^{-1} and the treated produce must be subsequently washed with clean water (Food and Drug, 21 C.F.R §173.300, 2019).

Dipping blueberries in aqueous ClO_2 (2 mg L^{-1}, 2 min) maintained fruit firmness and resulted in significant but small reductions of decay incidence after an 8-day storage at 4 ± 1 ºC (19% decay, vs. 22% in the untreated) (Xu et al., 2016). Similarly, treatment with 1 and 3 mg L^{-1} for 10s to 1 h reduced five foodborne pathogens (*Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Staphylococcus aureus* and *Yersinia enterocolitica*), yeasts and moulds in inoculated blueberries by < 2 log CFU g^{-1} (Wu & Kim, 2007). The effectiveness of aqueous ClO_2 increased when higher concentrations and/or longer treatment times were applied. For example, increasing to 15 mg L^{-1} of ClO_2 for 1 h reduced microbial load by up to 2.43-4.25 log CFU g^{-1} (Wu & Kim, 2007). Likewise, dipping blueberries at a much higher concentration of ClO_2 (100 mg L^{-1}) for 10 min with agitation resulted in nearly 1-1.5 log CFU g^{-1} less total aerobic bacteria and fungi counts than wash water, without affecting sensory quality and overall acceptability over 12 days of storage at 4 and 20 ºC (Chun et al., 2013).

Gaseous ClO_2 (4.1 mg L^{-1}, 30 min at 23 ºC and 75-83% RH) reduced *S. enterica* and fungi by 0.52 log CFU g^{-1} and 3.02 log CFU g^{-1}, respectively, in inoculated raspberries, and by up to 2.70 log CFU g^{-1} in blueberries (Sy et al., 2005). Increasing treatment times to 60 and 120 min, and ClO2 concentrations to 6.2 and 8.0 mg L^{-1}, however, provided no additional benefits. Treatment with ClO_2 gas at 4 mg L^{-1}, but over a longer duration i.e. 12 h was also effective in reducing microbial growth of blueberries inoculated with *Listeria monocytogenes*, *Salmonella*, *E. coli* O:157:H7 as well as yeasts and moulds (Popa et al., 2007). Sensorial attributes were unaltered in both studies (Popa et al., 2007; Sy et al., 2005). ClO_2 gas released from pads embedded in packaging was found to lower yeasts and mould counts of raspberries as well as

reduce fruit weight loss and improve fruit redness intensity compared to the control after 8 days at 1 ºC (Maghenzani et al., 2017).

A2.3.2 Ozone

Ozone is a strong oxidising agent that has shown potential as a sanitiser for blueberries (Chiabrando et al., 2006) and raspberries (Giuggioli et al., 2015). It is typically applied in gaseous form. The ozone decomposes to O_2 and is recognised as GRAS (Generally Regarded as Safe) by the US Food and Drug Administration (21 CFR§184.1563, 2019).

Treatment of blueberries with 700 µL L^{-1} ozone for 2 or 4 days prior to controlled atmosphere storage (15% O_2 & 10% CO_2) increased marketable yield by 4% and 7%, respectively, compared to the control (Song et al., 2003). Ozone storage also maintained firmness of 'Ozark Blue' blueberries after 10 days (Concha-Meyer et al., 2015) and 'Brigitta' blueberries after 5 weeks, although this effect was not consistent across the three cultivars ('Bluecrop', 'Coville' and 'Brigitta') (Chiabrando et al., 2006). -

A further benefit of ozone is its ability to oxidise ethylene, thereby delaying ripening and senescence in ethylene-sensitive fruits including berries (Wills & Golding, 2015). However, exposure to ozone can trigger a stress response altering the fruit metabolism. Respiration rates of 'Coville' blueberries increased immediately after 1-2 day(s) of constant treatments of 200 µL L^{-1} ozone (Song et al., 2003). In addition, the concentration of stress-indicating compounds such as methanol, ethanol and 2-nonanone (methyl heptyl ketone) increased markedly in 'Coville' blueberries stored in 700 µL L^{-1} ozone for 2-4 days, suggesting changes in flavours. Similarly, 'Grandeur' raspberries had better overall sensory quality than the control when stored for 9 and 15 days under continuous ozone flushing at either of 500 µL L^{-1} or a combination of 12 h of 200 µL L^{-1} + 12 h of 50 µL L^{-1}. However, the higher concentrations resulted in lower scores for taste and flavours (Giuggioli et al., 2015).

Industrial applications of gaseous ozone have been hindered by the risk of explosion and toxicity to the operators (Guzel-Seydim et al., 2004). Alternatively, ozonated water, which is generated by passing ozone through water, could be a solution as shown in apple (Achen and Yousef, 2001) and strawberries (Alexandre et al., 2002). However, the residual moisture may restrict the practical feasibility of this option for berries (Furlan et al., 2011) .

A2.4 Edible coatings

Edible coatings are thin layers of edible biomaterials that can maintain fruit freshness and increase shelf life by acting as protective barriers preventing moisture loss and microbial growth, as well as limiting O_2 and CO_2 exchange (Table 2.1). Findings from the literature indicate that chitosan-based edible coatings may be most promising at improving the shelf life of blueberries and raspberries (Table 2.1). Chitosan (poly β-(1-4)-N-acetyl-D-glucosamine) is a polysaccharide derived from chitins that has been extensively studied to extend the shelf life of fresh produce (Romanazzi et al., 2017). In the context of berries, fruit weights were maintained, and large reductions of fungal development were observed in various blueberry cultivars (5-30%) (Duan et al., 2011; Jiang et al., 2016; Vieira et al., 2016; Yang et al., 2014) and 'Tullmeen' raspberries (93%) (Han et al., 2004). However, while improving firmness of blueberries (Duan et al., 2011; Jiang et al., 2016; Vieira et al., 2016; Yang et al., 2014, chitosan coatings resulted in greater loss of firmness in 'Tullmeen' raspberries (Giuggioli et al., 2015). Additional technical concerns that need to be addressed before edible coatings have wide commercial applications include the maintenance of the natural wax bloom which affects the desirable perceived colour of blueberries (Abugoch et al., 2016; Mannozzi et al., 2018) and overcoming the difficulty in completely drying out any residual coating materials accumulating inside the fruit hollow of picked raspberries (Han et al., 2004). Furthermore, the extra costs for coating materials might also limit their commercial feasibility.

Table 2.1 Recent studies of edible coatings on blueberries and raspberries

Coating materials	Cultivars	Storage conditions	Comments on effects	References
BLUEBERRIES				
Acid-soluble chitosan	'Duke'	2 °C, 7 days + 20 °C, 12 days	↓ 15% decay rate and ↓ weight loss Retained firmness close to day 0	Duan et al., 2011
Calcium caseinate	'Elliot'	2 °C, 7 days + 20 °C, 12 days	↑ 25% decay rate	
Water-soluble chitosan	'Elliot'	2 °C, 7 days + 20 °C, 15 days	↓ 5% decay rate ↑ firmness	
Chitosan Chitosan + blueberry leaf and fruit extracts	'Langfeng'	2 °C, 35 days	↓ 15-30% decay rate and ↓ weight loss ↑ firmness Retained TA close to day 0	Yang et al., 2014
Chitosan + *Aloe vera* fractions	'Duke'	5 °C, 25 days	↑ blueberry shelf life by approximately 5 days Maintained TSS close to day 0 and ↓ losses of weight, TA and pH	Vieira et al., 2016
Chitosan	Rabbiteye	2 °C, 35 days	Inhibited *Botryotinia fuckeliana* isolated from decayed berries *in vitro* ↓ losses of weight and firmness ↑ phenolic and anthocyanin contents and slowed their losses	Jiang et al., 2016
Pullulan	'Bluecrop'	16 °C, 14 days or 4 °C, 28 days	↓ decay by 31% (14 days at 16 °C) and 20% (28 days at 4 °C) ↓ 7% weight loss at 16 °C	Kraśniewska et al., 2017

Coating materials	Cultivars	Storage conditions	Comments on effects	References
Quinoa Protein /Chitosan /Sunflower oil	Not mentioned	4 °C, 35 days	↓ fungal growth by 3 log CFU g^{-1} and ↓ 5% weight loss ↓ 32% firmness and altered wax bloom and colours	Abugoch et al., 2016
Sodium alginate Pectin	Not mentioned	4 °C, 10 days	↓ yeast counts by 1.5-1.8 log CFU g^{-1} ↓ mesophilic aerobic bacteria count by 2 log CFU g^{-1} ↑ firmness but caused glossy appearance	Mannozzi et al., 2018
Limonene Limonene + liposomes	Not mentioned	4 °C, 63 days	24-33% decay incidence ↓ 32-40% total fruit loss	Umagiliyage et al., 2017
RASPBERRIES				
Chitosan Chitosan + calcium gluconate	'Tullmeen'	2 °C, 21 days in dark	↓ 93% decay incidence ↓ weight loss and delayed colour changes ↓ firmness	Han et al., 2004
Chitosan + Vitamin E	'Tullmeen'	2 °C, 21 days in dark	Altered colour (due to yellowish and less transparent coating solution) ↓ firmness	
Aloe vera gel	Iranian native species	4 °C, 8 days in dark	↓ 8.5-12% decay incidence	Hassanpour, 2015
Sodium alginate + eugenol Sodium alginate + citral Pectin + eugenol Pectin + eugenol + citral	Not mentioned	0.5 °C, 15 days	Inhibited growths of aerobic mesophilic microorganisms Failed to maintain weights, phenolic and anthocyanin contents	Guerreiro et al., 2015 Guerreiro et al., 2016

A3. Packaging-based solutions for shelf life extension of berries

Berries sold in the fresh market are commonly packed in clamshells with opening ratios of 3-10% (Bautista & Yang, 2012). This design is widely used in industry as it allows rapid and effective cooling of fresh produce (de Castro et al., 2005), the escape of heat generated by produce respiration (Pathare et al., 2012), as well as preventing ethylene accumulation (Van der Steen et al., 2002) and moisture condensation. However, for berries with large surface area-to-volume ratios, the vents could make them more susceptible to freezing, chilling and drying damage (Pathare et al., 2012). For example, clamshells of raspberries packed in macro-perforated film with 200 holes of 3 mm-diameter per m^2 (no modified atmosphere formed) exhibited 20% higher weight loss after 14 days at 7 ºC, compared to those packed in modified atmosphere films with low water vapour permeability (Van der Steen et al., 2002). The control was unsaleable by day 3 because of fungal growth. To address the limitations of clamshells, modified atmosphere packaging (MAP) and active packaging have been studied, both as alternative and complementary solutions. In addition, considering the high quantity of packaging needed versus berry weights, applications of eco-friendly packaging materials would make significant reductions in plastic waste and be key in meeting consumer concerns for sustainable packaging (Terry et al., 2011; Wilson et al., 2019a). An early study suggested that replacing polypropylene trays with cardboard was also beneficial in removing moisture and raspberry juice leakage (Seglina et al., 2010).

A3.1 Modified atmosphere packaging (MAP)

The biological basis of MAP effectiveness is firstly on the suppression of oxidation reactions by lowering the substrate (O_2) availability (Saltveit, 2020). Aerobic respiration of living cells to produce energy (ATP) includes three processes: glycolysis to convert hexoses to pyruvates, tricarboxylic acid cycle (TCA cycle) to convert pyruvates to CO_2, and electron transport. NADH is produced at the end of TCA cycle and its electrons would be transferred to O_2 in

the electron transport pathway, to regenerate NAD^+ and produce ATP. The regeneration of NAD^+ is necessary for the glycolysis process to continue. Therefore, decrease in O_2 in the environment would first slow down the electron transport and TCA cycle, and lead to the accumulation of NADH and pyruvate. When O_2 becomes insufficient, plants need to use fermentative pathways to reproduce NAD^+ and provide ATP. However, the induction of fermentation was suggested to be independent to the reduction of respiration rates (Geigenberger, 2003), and high CO_2 can modulate the respiratory enzymes (Saltveit, 2020). Consequently, the reduction of O_2 availability might not fully explain the suppression of respiration rates in MAP. The low O_2, elevated CO_2 conditions also regulate other metabolic pathways and enzymatic reactions that could help preserve the produce quality postharvest. Examples of those pathways are the production and activity of ethylene (Saltveit, 1999), and discoloration caused by polyphenol oxidases.

In berries, MAP has been used to suppress mould growth (Forney, 2009; Forney et al., 2015), reduce respiration rate (Haffner et al., 2002), alter ethylene metabolism (Watkins & Zhang, 1998) and reduce weight loss (Table 2.2). Typical storage conditions recommended for blueberries range from 5-10% O_2 & 10-12% CO_2 at 0 ºC (Mitcham, 2007; Paniagua et al., 2014), while for raspberries, they range from 5-10% O_2 & 10-20% CO_2 (Bower, 2007). Softening, decolouration and development of off-flavours particularly occurred in blueberries when CO_2 was higher than 15% (Harb & Streif, 2004). An average loss of firmness of 24 N m^{-1} was also reported in nine raspberry cultivars stored under elevated CO_2 atmospheres (12.5% CO_2 & 7.5% O_2) for 4 weeks, compared to those kept in air (Forney et al., 2015). In contrast, fermentation and formation of off-odours (acetaldehyde, ethanol and ethyl acetate) in raspberries were induced by low O_2. An O_2 level over 4% at 0 ºC, 6% at 10 ºC or 8% at 20 ºC was suggested as being needed to prevent fermentative induction (Joles et al., 1994).

The major concern for using MAP for berries is that inappropriate designs could lead to high concentrations of CO_2 and depletion of O_2 over time when subject to non-optimal temperature conditions during storage. This can result in atmospheres with less oxygen than those needed to maintain basal aerobic respiration. Most commercially available plastic films are unable to maintain appropriate ratios of O_2 and CO_2 permeability (Mangaraj et al., 2009). This explains why non-perforated films often resulted in softening in both blueberries (Rodriguez & Zoffoli, 2016) and raspberries (Adobati et al., 2015; Giovanelli et al., 2014; Peano et al., 2013). In addition, the resistance of plastic packaging to water vapour permeation prevents water vapour due to produce transpiration escaping from the packaging and leads to moisture condensation that favours mould growth (Horvitz, 2017). Perforated MAP could aid by accelerating gas exchange and increasing the permeability to water vapour. When LDPE films of the same thickness were used to pack 1.5 kg of blueberries, packages with two perforations each of 3 mm^2, resulted in lower CO_2 concentrations being accumulated (6.2% versus 9.2%) and greatly reduced the percentage of soft fruits (7.1% versus 27%), compared to the film without perforations (Rodriguez & Zoffoli, 2016). However, commercial MAP films often have higher CO_2 transmission rates than O_2 transmission rates and therefore failed to create sufficient CO_2 concentrations to control mould growth and delay quality losses (Moor et al., 2009).

Table 2.2 Available literature on MAP applications for blueberries and raspberries

Packaging specifications	Fruit variety	Storage conditions and initial atmosphere [a]	Comments on effects [b]	References
BLUEBERRIES				
PE film (100 µm) Biobased film (50 µm)	'Duke'	1 °C, 45 days 10% CO_2 + 11% O_2	↓ 10% weight loss	Peano et al., 2015
LDPE gusseted bag ViewFresh® (50 µm), 0.02 gauge 2 microperforations = 0.3 mm^2	'Brigitta'	0 °C, 30 and 45 days + 18 °C, 1 and 3 days	↑ 20-30% sound fruits Maintained firmness during storage	Moggia et al., 2014
LDPE (60 µm) No perforation	'Brigitta'	0 °C 30 and 45 days	↓ 3% weight loss after 30 days and 6.4% after 45 days ↑ 16.8% soft fruits after 30 days and 12% after 45 days ↑ 5% red berries	Rodriguez & Zoffoli, 2016
LDPE (60 µm) Two perforations (3 mm^2)	'Brigitta' 'Legacy'	0 °C 30 and 45 days	↓ 3% weight loss after 30 days and 6.4% after 45 days Maintained firmness during storage ↑ 5% and 10% red berries after 30 and 45 days, respectively	
RASPBERRIES				
PE film (76.2 µm)	'Qualicum' 'Chilliwack' 'Meeker'	1 °C, 7 days 10% CO_2 + 5% O_2	Maintained the red-ripe colour ↑ softening ↑ the accumulation of volatiles associated with off-odours	Toivonen et al., 1999

Packaging specifications	**Fruit variety**	**Storage conditions and initial atmosphere** [a]	**Comments on effects** [b]	**References**
Biodegradable and compostable film (non-commercial, 25 µm)	'Himbo Top'	1 °C, 4 days 1 °C, 2 days + 18 °C, 2 days Air or 10% O_2 + 10% CO_2	Control: macro-perforated PP film (20 µm, no modified atmosphere) Maintained fruit colour and aroma profiles close to day 0	Peano et al., 2013 Giuggioli et al., 2015
PP film (30 µm, non-perforated)	'Himbo Top'	1 °C, 4 days Air or 10% O_2 + 10% CO_2	Control: macro-perforated PP film (20 µm, no modified atmosphere) ↑ softening	Peano et al., 2013
Xtend® film (commercial MAP) LDPE bags (30 µm)	'Polka'	1.6 °C, 4 days or 1.6 °C, 3 days + 6°C, 1 day	Maintained light red colours Failed to create sufficient CO_2 levels to suppress mould growth	Moor et al., 2009
Master-bags made from LDPE (low gas barrier) LDPE/EVOH/LDPE (high gas barrier) with or without oxygen absorbers	'Erika'	4 °C, 7 days	Control: lidded PET macro-perforated rigid trays ↑ softening and anaerobic metabolisms	Giovanelli et al., 2014
Master-bags made from PLA	'Erika'	4 °C, 4 days	Control: lidded PET macro-perforated rigid trays Maintained colour and firmness close to day 0	
Master-bags made from LDPE (medium gas barrier, 500 µm)	'Erika'	5 °C, 6 days	Control: lidded PET macro-perforated rigid trays ↓ mould growth ↑ softening and drupelet breakages	Adobati et al., 2015

Packaging specifications	Fruit variety	Storage conditions and initial atmosphere [a]	Comments on effects [b]	References
Master-bags made from LDPE (low gas barrier, 25 µm)	'Erika'	5 °C, 6 days	Control: lidded PET macro-perforated rigid trays Maintained fruit firmness and colour ↑ shelf life by 2 days	
Master-bags made from LDPE (low gas barrier, 25 µm) + CO_2 emitters (BioFresh®)	'Erika'	5 °C, 8 days	Control: lidded PET macro-perforated rigid trays Maintained fruit firmness during storage ↑ shelf life by 4 days	
Cardboard boxes (145×120×80 mm) placed in PLA (polylactic acid) film pouches of 40 µm thickness	'Polana'	4 °C, 14 days	↓ weight loss Retain fruit colour as at harvest and higher ascorbic acid contents Prevented condensation and moisture accumulation	Seglina et al., 2010

[a] Initial atmosphere inside packages was air (approx. 20% O_2 and 0% CO_2) if not mentioned.

[b] Results were compared to fruits packed in vented clamshells unless stated otherwise.

For high-value products like berries, customised MAP bags based on produce respiration rates, produce weights, packaging properties and desirable atmospheres are currently commercially available. Microperforated MAP with lidding film and unvented berries trays are available in Europe, but their applications remain limited. Industrial systems combining a device measuring the respiration rate, software to reliably calculate the numbers and sizes of microperforations, and an inline laser perforation system, are also available. Examples are PerfoTec® (PerfoTec B.V., The Netherlands) and StarMAP® (LaserMicro Rofin-BaaselLasertech, Germany) (Wani et al., 2018). However, the high establishment and production costs associated with this MAP film technology may limit their commercial viability.

Alternatively, advanced mathematical modelling is available, allowing the prediction of effects of various factors on the produce quality, or the optimisation of packaging designs for a particular application (Belay et al., 2016). Commonly, the Michaelis-Menten enzymatic kinetics equation (Rennie & Tavoularis 2009); Arrhenius-type equations (Caleb et al., 2012), Fick's First Law, heat transfer equations or a combination of several methods is used. The Michaelis-Menten enzymatic kinetics equation has been shown to provide good estimations of the respiration rates of several fresh produce under different gas concentrations (Belay et al., 2016; Song et al., 2002; Torrieri et al., 2010). The Arrhenius-type equations describe the effects of temperature on biological reactions of produce such as respiration (Caleb et al., 2012). Combinations of the two models, therefore, are often used to predict a biological change as a function of gas compositions and temperature (Mangaraj & Goswami, 2011; Torrieri et al, 2010). In contrast, Fick's First Law is used to calculate the permeate flux and estimate gas and water vapour permeability of the packaging (Xanthopoulos et al., 2012). In-depth information is available in the recent comprehensive reviews on advanced MAP modelling by Belay et al. (2016) and Belay et al. (2019). However, industrial uptake for

integrative modelling might be challenged by the required interdisciplinary knowledge from bioprocess engineering, packaging materials science and engineering, and data science, for model development, verification and troubleshooting.

A3.2 Active packaging with ethylene control properties

Trials on controlling ethylene during storage of blueberries and raspberries have been conducted, although the roles of ethylene in quality and shelf life of these berry fruits are not fully understood. The most common ethylene scavenger is potassium permanganate ($KMnO_4$), which is usually embedded in silica gel at a concentration of about 4-6%, and supplied in sealed, ethylene-permeable sachets (Wyrwa & Barska, 2017) or it can also be added directly into polymeric films (Mehyar & Han, 2011). The ethylene action inhibitor 1-MCP can be released as a gas from sachets (Lee et al., 2006) and polymeric films when activated under high relative humidity conditions (Hotchkiss et al., 2007). Alternatively, 1-MCP can be applied as a liquid formulation (SmartFreshTM, AgroFresh, Inc.).

There has been no work solely evaluating ethylene control for shelf life extension of raspberries. The effectiveness of ethylene control on the postharvest storability of blueberries seems to depend on the cultivar and storage durations. In 'Lanfeng' blueberries, the addition of $KMnO_4$ sachets reduced fruit weight loss and decay during both cold storage (60 days, 0 ºC) and on-shelf display periods (8 days, 20 ºC) (S. Wang et al., 2018). Fruit firmness was also retained by suppressing the activity of cell wall enzymes (pectin methyl esterase, polygalacturonase, cellulase and β-galactosidase). Likewise, 'Berkeley' blueberries exposed to 1-MCP (1.0 μL L^{-1}, 18 h) showed less softening (12.3%) than the control fruits during 8 days of storage at 4 ºC (Xu et al., 2016). The treated fruits also showed a slower decrease in titratable acidity and soluble solid contents. Similar findings were reported for 'Lateblue' blueberries stored in air for 21 days after being treated with 1-MCP (0.3 μL L^{-1}, 24 h) at 20 ºC, but the advantages were not found after 28 days, or among samples stored under

controlled atmosphere (3% O_2 & 11% CO_2) for 60 days (Chiabrando & Giacalone, 2011). 1-MCP up to 0.4 µL L^{-1} for 24 h at 20 ºC had no influence on the shelf life quality of two other highbush cultivars ('Burlington' and 'Coville') (DeLong et al., 2003). 'Mistý' and 'Blue Cuinéx' blueberries treated with 1-MCP (1.0 µL L^{-1}, 12h) had similar respiration rates and quality attributes as the control after 14-day storage at 4 ºC, except for the firmness of 'Mistý', which was about 1.2 times higher than the untreated fruits (Grozeff et al., 2017).

Combinations of ethylene control with other methods could be beneficial through synergistic effects. For example, a permeable packaging film with an ethylene transmission rate of 1.98 mL m-2 h^{-1} kPa^{-1} in combination with high-oxygen MA (95% O_2 & 5% N_2) reduced ethylene accumulation inside raspberry packages to 37 µL L^{-1}, compared to 60 µL L^{-1} in samples in high oxygen MA and high barrier film. This combination of ethylene permeable film and high oxygen MA limited mould growth after 14 days at 7 ºC with only a single mouldy fruit being found in a 150 g raspberry sample (approximately 40 fruits) (Jacxsens et al., 2003). Spraying 'Blue Cuinéx' blueberries with 10 mL of 1 mmol L^{-1} S-nitrosoglutathione (GSNO, a NO donor) upon 1-MCP exposure had better retention of fruit firmness and ascorbic acid concentrations than either treatment alone (Grozeff et al., 2017). Also, 1-MCP combined with UV-C irradiation reduced respiration rate, ethylene production, decay incidence, softening, changes in colour, titratable acid and soluble solid contents of 'Berkeley' blueberries over 8 days at 4 ºC (Xu & Liu, 2017). These studies using MA stores, and with scrubbers to control ethylene, could be taken forward to studies of control at the package levels.

A4. Additional approaches for shelf life extension of berries

A range of alternative approaches have been trialled and have shown some success for shelf life extension of blueberries and raspberries (Table 2.3). These technologies include sanitisation using electrolysed water (EW) and hydrogen peroxide, and high O_2 (superatmospheric O_2, 70-100%) and/or high CO_2 (> 20%).

Electrolysed water (EW) is generated from the electrolysis of NaCl solutions producing mainly hypochlorous acid and has shown to be a safe, effective and inexpensive sanitiser for a range of fruits and vegetables (reviewed by Rahman et al., 2016). Acidic electrolysed water (AEW, pH 2.3-2.7) has been shown to efficiently sanitise blueberries (Table 2.3), but the application of AEW to fresh sensitive produce is limited by its acidic property (L. Ma et al., 2017). These concerns have shifted industry focus toward using neutral electrolysed water (pH 6-8), which has similar antimicrobial effects to AEW but with fewer associated risks to produce physiology (Joshi et al., 2013; L. Ma et al., 2017).

Hydrogen peroxide (H_2O_2) is also a strong oxidiser that can be used in either aqueous or vapour form at concentrations between 1-5%. H_2O_2 is safe as the compound quickly decomposes to water and oxygen (De Corato, 2020). Although only a few successes have been reported for the use of H_2O_2 as a postharvest sanitiser for fresh produce, positive results were obtained in blueberries (Crowe et al., 2005) (Table 2.3). However, changes in fruit colour and decrease in antioxidant properties, could be a critical drawback of H_2O_2 technology in berries (Alexandre et al., 2012).

Table 2.3 Additional approaches for shelf life extension of berries

Technology	Studied commodities	Storage conditions	Beneficial effects	References
Electrolysed water (EW) washing				
pH 2.3- 2.7 31.1 mg free chlorine L^{-1} 1 min	Blueberry (unspecified cultivar)	4 °C, 1 day	↓ inoculated *E.coli* O157:H7 by nearly 4 log CFU g^{-1}	Pangloli & Hung, 2013
pH 2.8 48 mg free chlorine L^{-1} 5 min	Blueberry 'Brightwell'	4 °C, 15 days	Delayed fruit softening Delayed decay and cell membrane permeabilities Maintained anthocyanins, total phenolic contents and antioxidant activities	Y. Chen et al., 2017 Y. Chen et al., 2019
Hydrogen peroxide				
Spraying, 1%, 2 min	Blueberry (lowbush, unspecified cultivar)	Not studied	↓ total anaerobe, yeast and mould populations by 2 log CFU g^{-1} right after the treatment Had no adverse effects on fruit colour	Crowe et al., 2005
Superatmospheric (high O_2) treatments-N_2 balance				
95 kPa O_2	Raspberry (unspecified cultivar)	7 °C, 5 days	Inhibited mould growth	Van der Steen et al., 2002

Technology	Studied commodities	Storage conditions	Beneficial effects	References
Superatmospheric (high O_2) treatments-N_2 balance				
60-100 kPa O_2 (continuous flow during storage)	Blueberry 'Duke'	5 °C, 35 days	↓ 14-19% decay compared to fruits stored in air	Zheng et al., 2008
Short-time high CO_2 99.9kPa CO_2, 2 and 3 days at 1 ºC	Blueberry 'Bluegold'	1 °C, 50 days	↓ 15% fruit rots after 40 days ↑ shelf life to 50 days	Jiang et al., 2011

Section B. Broccoli

B1. Introduction

Broccoli is a cool-climate, slow-maturing, cruciferous vegetable. Each broccoli unit is comprised of an edible stalk holding branchlets of green floral buds (Bhattacharjee et al., 2011). The flower heads are highly perishable with a high respiration rate of 280-320 mg CO_2 kg^{-1} h^{-1} at 20 ºC (Toivonen & Forney, 2016). The storage life of broccoli can be as short as 2-3 days in air at 20 ºC (Costa et al., 2005; Fukasawa et al., 2010). The plant suffers accelerated senescence when nutrients and hormone supplies are suddenly stopped, as the green floral buds (florets) were immature and in a rapid growing state at the time of being harvested (Page et al., 2001). Quality losses are mainly characterised by wilting, yellowing and formation of off-odours (Toivonen & Forney, 2016) resulting from dehydration, respiration, ethylene production, lipid peroxidation (King & Morris, 1994; Zhuang et al., 1995) and enzymatic reactions (Rai et al., 2009).

Controlling temperature (near 0 ºC), humidity (> 95%), and ethylene are three important strategies to improve the storability of broccoli (Ekman, 2014; X. Fan & Mattheis, 2000; Watkins, 2008). Under cold storage, the quality of broccoli can be retained for up to 3-4 weeks (Toivonen & Forney, 2016). Low temperature and high humidity conditions, however, are difficult to obtain and maintain throughout the supply chain and marketing chain. A great variation between 2 and 12 ºC was observed during broccoli transport, storage, and display in Sweden (Jacobsson et al., 2004b). Temperature fluctuations cause stresses and cellular disruption that particularly affect the texture of broccoli (Deschene et al., 1991; Jacobsson et al., 2004a).

B2. Physico-chemical methods for shelf life extension of broccoli

B2.1 Pre-storage temperature treatments for shelf life extension of broccoli

Cooling broccoli soon after harvest is important to quickly reduce respiration and maintain tissue turgidity (Brennan & Shewfelt, 1989; Toivonen, 1997). Fast cooling lowers the vapour pressure deficit between the product and the surrounding air; thereby, reducing moisture loss during the beginning of cold storage (Gillies & Toivonen, 1995). The two most common cooling methods are top-icing with crushed or flaked ice, and hydro-cooling (Ekman, 2017). Top-icing has been reported to maintain the greenness and extend the shelf life of broccoli to 10 weeks at 0-1 ºC (Felt et al., 1983). The meltwater was believed to help rehydrate and retain firmness of broccoli during transportation and storage (Gillies & Toivonen, 1995). However, top-icing has several disadvantages, including the development of rots, stem splits and discolouration, extra handling costs, and the uses of non-recyclable polystyrene boxes (Ekman, 2014, 2017). In addition, the meltwater can create favourable conditions for microbial growth and spreading contamination (Felt et al., 1983), and potentially pose a safe handling hazard if it accumulates on vehicle floors (Brecht et al., 2019). Hydro-cooling has been proposed as an alternative to top-icing, but would only be effective if followed by packaging (Gillies & Toivonen, 1995). Current industrial practice often combines both methods, i.e, using hydro-cooling to rapidly lower the broccoli temperature after harvest, and top-icing in packing to maintain the low temperatures (Toivonen & Forney, 2016).

Heat treatments exposing broccoli to hot water or hot air have been shown to improve broccoli shelf life. The benefits of heat treatments include the suppression of respiration rates and ethylene production (Tian et al., 1997), reduction of fungal infection (Dong et al., 2004), and increases in firmness (Liu et al., 2017). However, optimising the temperature and the heat-exposure time is crucial to the outcomes of heat treatments, and can vary between varieties (Costa et al., 2005), seasons, and pre-harvest conditions (P. Wu & Li, 2001). For hot

air treatment, a combination of 48 ºC and 3h was recommended as best to retain chlorophyll reflecting the greenness of broccoli during storage at 0 ºC (Lemoine et al., 2009) and at 20 ºC (Costa et al., 2005). Dipping in water at 45 ºC for 4 min (Dong et al., 2004), or at 42-46 ºC for 10 or 30 min (P. Wu & Li, 2001), or at 50 ºC for 2 min (Forney, 1995), reduced the losses of chlorophyll and delaying yellowing during storage, compared to the untreated broccoli. Higher temperatures and longer treatment periods generally reduced the rates of yellowing (Forney, 1995; P. Wu & Li, 2001), but developed off-odours and increased fungal spotting and bacterial rots on florets (Forney, 1995). Combinations of temperatures > 48 ºC and durations > 10 min caused heat injury and the formation of off-odours (P. Wu & Li, 2001). Offensive smells also appeared immediately in broccoli heated at 52 ºC for 3 min (Forney, 1995). It should also be noted that the warm broccoli must be cooled down rapidly after heat treatments to remove the residual heat and prevent consequential adverse effects.

B2.2 Sanitisation

There has been little scientific literature about sanitisation of whole broccoli, possibly because it is rarely a part of broccoli pre-storage treatments. Studies on fresh-cut broccoli suggested that reduction of initial microbial loads should also improve the storability and quality of whole broccoli. Washing fresh-cut broccoli in chlorinated water (100 µL L^{-1}, pH 7), electrolysed water (100 µL free chlorine L^{-1}, pH 7.2) and ozonated water (2 µL L^{-1}) for 1.5 or 3 min lowered the aerobic plate counts up to 1 log CFU g^{-1} during 9 days of storage, compared to using tap water (Das & Kim, 2010). Similarly, mesophilic counts, yeasts and moulds were reduced about 1-2 log CFU g^{-1} broccoli fresh weight by dipping fresh-cut 'Parthenon' broccoli (Navarro-Rico et al., 2014) and broccoli sprouts (Liu et al., 2017) in electrolysed water. The effectiveness of electrolysed water increased with temperatures but extending dipping time from 5 to 10 min made no differences (Liu et al., 2017). However, sanitisers could be stress agents that increase reactive oxygen species (ROS) and damage the

plant cells (Martínez-Hernández et al., 2013). Slight increases in yellowing were reported in sanitised broccoli (Das & Kim, 2010), but effects on other attributes remain unknown.

B2.3 UV-irradiation and light treatments

Postharvest exposures to UV radiation and visible light have been demonstrated to delay yellowing in broccoli. The chlorophyll retention resulted from suppressing the expression of genes that encoded enzymes involved in chlorophyll catabolism, particularly pheophytinase *BoPPH* and pheophorbide a oxygenase *BoPaO* (Aiamla-or et al., 2019; Büchert et al., 2011a). Short exposure of broccoli to UV-C at 4-10 kJ m^{-2} delayed chlorophyll degradation and pheophytins accumulation during storage at 20 ºC (Costa et al., 2005). Compared to UV-C, UV-B is more advantageous as it involves less harmful wavelengths, while providing similar effects of chlorophyll retention at doses > 8.8 kJ m^{-2} (Aiamla-or et al., 2009). In contrast, UV-A was found to be ineffective at the same intensities.

Illumination treatments using light emitting-diodes (LED) have been proposed as a novel and economic technology to preserve the quality of vegetables (Morrow, 2008). Different light spectra have been trialled in broccoli, but the results remain inconsistent. Low-intensity green light (6.54 W m^{-2}) was found to increase chlorophyll and ascorbic acid contents in broccoli in 20 days at 4 ºC (Loi et al., 2019). Continuous exposure of broccoli to red light at 16.35 W m^{-2} reduced yellowing, loss of ascorbate and the production of ethylene during 4 days at 20 ºC (G. Ma et al., 2014). No effects were found for blue light in the same study, explained by different photoreceptors and subsequent morphogenetic and photosynthetic responses of the plant to the two light spectra. In contrast, broccoli exposed to low-mid intensity (6.54-9.5 W m^{-2}) white-light continuously or for 2-3h per day retained higher chlorophyll contents than untreated broccoli during storage (Favre et al., 2018; Hasperué et al., 2016; Pintos et al., 2020). White light is also more feasible for retail display, but the treatment could stimulate stomata opening, resulting in increased weight loss (Favre et al., 2018; Hasperué et al., 2016).

B3. Packaging-based solutions for shelf life extension of broccoli

B3.1 CA and MAP

Early applications of CA have shown potential for improving the storability of broccoli. Overall, the main benefits of CA were to reduce respiration rates, yellowing, ethylene production and microbial growth (Table 2.4). Available literature generally recommended 1-2% O_2 & 5-10% CO_2. The success of CA for broccoli storage suggested the potential of using MAP for broccoli, which could offer similar advantages without requiring continuous gas modification. In addition, packaging was considered as the most significant factor in reducing weight loss and maintaining broccoli firmness (Gillies & Toivonen, 1995). A summary of available literature on MAP for broccoli is presented in Table 2.5.

A major benefit of MAP is to create a high RH environment within the package, thereby reducing weight loss in broccoli (Serrano et al., 2006). Weight loss during storage and retail mainly results from moisture loss and can be detrimental to the marketability of broccoli (Jia et al., 2009; Serrano et al., 2006). Moisture loss causes surface dehydration and wilting and reduces the crispness and chewing resistance of broccoli (Jacobsson et al., 2004b). Under prolonged storage, moisture loss could harden the broccoli tissues due to the production of lignin and suberin (Jacobsson et al., 2004a). Unpacked broccoli lost up to 12% of weight after 7 days at 10 ºC (Jacobsson et al., 2004c) and 46.36% after 20 days at 1 ºC (Serrano et al., 2006). In contrast, most of the MAP conditions trialled reduced weight loss to < 4%, although the values depended on the WVTR and perforated areas of the packaging materials (Jacobsson et al., 2004; Jia et al., 2009; Paulsen et al., 2018; Serrano et al., 2006).

Similar to CA, MAP also delays yellowing in broccoli florets, especially at suboptimal temperatures (Fernández-León et al., 2013; Jacobsson et al., 2004b; Jia et al., 2009; Paulsen et al., 2018; Rai et al., 2009). Yellowing is a key quality measure of broccoli and is associated with the degradation of chlorophylls (Martínez-Hernández et al., 2013). Broccoli stored in air

experienced rapid yellowing within 3 days at 20 ºC (Costa et al., 2005; Fukasawa et al., 2010) or 8 days at 10 ºC (Cai et al., 2019). In contrast, MAP was found to maintain broccoli greenness 2-3 times longer under similar conditions (Jia et al., 2009; Rai et al., 2009). The mechanisms of MA in delaying chlorophyll degradation in broccoli are still under investigation, but have been linked to the reduced production of ethylene and enzymes involving in chlorophyll catabolism (Büchert et al., 2011a; Büchert et al., 2011b; Deschene et al., 1991; Gómez-Lobato et al., 2012).

The major challenge of CA/MAP applications for broccoli is to prevent the formation of offensive odours. The persistent undesirable smells produced by broccoli mainly come from volatile sulphur compounds, such as methanethiol, hydrogen sulphide, dimethyl disulphide, and dimethyl trisulphide (Forney et al., 1991; Hansen et al., 1993). These volatiles are produced by the enzymatic reactions during the deterioration of lipid membranes and cell disruption (Chin & Lindsay, 1994; Dan et al., 1997). In addition, extreme atmospheres (< 1% O_2 or > 15% CO_2) were found to stimulate anaerobic respiration and the development of off-odours such as ethanol, acetaldehyde, methyl acetate and acetone (Forney et al., 1991; Gillies et al., 1997). At the same time, the appearance of broccoli remained unchanged (Gillies et al., 1997). Therefore, it would be hard to detect over-modified packages without opening them.

Existing literature also suggested that MAP could mitigate the adverse effects caused by storage at suboptimal temperatures in the short-term, but good temperature management will be important for broccoli quality in prolonged storage (Hagen & Larsen, 2020; Jia et al., 2009; Paulsen et al., 2018). For example, broccoli stored at 8 ºC in perforated PP bags had acceptable sensory quality after 21 days, but significantly weaker characteristic aroma compared to samples at 4 ºC (Paulsen et al., 2018). At 15 ºC, all broccoli was spoilt after 14 days. Similarly, Hagen & Larsen (2020) compared the effectiveness of two MAP bags with high and low perforation levels in maintaining broccoli quality under ‘cold storage’ (16 days

at 4 ºC) and 'realistic storage' (4 days at 4 ºC + 3 days at 19 ºC + 9 days at 4 ºC). It was found that the colours of broccoli remained good in 'cold storage', but were poor in 'realistic storage', regardless of the use of MAP and the levels of perforations (Hagen & Larsen, 2020). Furthermore, elevated temperatures enhance broccoli respiration rates and change film permeability (Sandhya, 2010); thereby, increasing the risks of O_2 depletion and excessive CO_2 accumulation. For this reason, commercial microperforated MAP often targets 10% O_2 & 10% CO_2 to compensate for temperature fluctuations, instead of the recommended levels of 1-2% O_2 & 5-10% CO_2 (Lucera et al., 2011; Paulsen et al., 2018).

B3.2 Ethylene control

The presence of ethylene, either from endogenous or exogenous sources, has been linked to the acceleration of chlorophyll loss and yellowing in broccoli (Cai et al., 2019). Ethylene control could be performed by inhibition using a hormone antagonist (Watkins, 2008), or by removal using a scavenger (Mehyar & Han, 2011). 1-Methylcyclopropene (1-MCP) is the most widely applied ethylene antagonist that has been shown to benefit broccoli storability (Able et al., 2002; de Beer & Crouch, 2015; X. Fan & Mattheis, 2000; Watkins, 2008). 1-MCP treatment, followed by wrapping in shrinkable films, extended the storage life of broccoli at 5 ºC to 27 days (Kasim et al., 2007), which was comparable to 3-4 weeks at 0 ºC (Toivonen & Forney, 2016). Likewise, a novel in-box 1-MCP treatment (SmartFresh^{TM}) in addition to MAP prolonged the storability of broccoli for a week, achieving 28 days at 0 ºC (Sabir, 2012) or 15 days at 7.5 ºC + 3 days at 10 ºC (de Beer & Crouch, 2015). However, the efficiency of 1-MCP is limited as new ethylene binding sites develop after the treatment (Able et al., 2002). This observation suggested that continuous exposure to 1-MCP by using a slow-releasing sachet as designed by Lee et al., (2006) or continuous removal of ethylene by scavengers would better extend the storage life of broccoli.

Potassium permanganate ($KMnO_4$) is the most common ethylene scavenger that has been studied in many fresh horticultural produce types, particularly climacteric fruit (Álvarez-Hernández et al., 2018, 2019). $KMnO_4$ oxidises ethylene to acetaldehyde, then to acetate, and further to CO_2 and water. However, there has not been sufficient evidence to conclude on the efficacy of $KMnO_4$-based ethylene scavenger in delaying senescence of non-climacteric produce (Álvarez-Hernández et al., 2019). The commercial $KMnO_4$-based ethylene scavengers often have about 4-6% $KMnO_4$ embedded in silica gels, and are supplied in sealed, ethylene-permeable sachets (Wyrwa & Barska, 2017). $KMnO_4$ could also be added into polymeric films during manufacturing (Mehyar & Han, 2011). In broccoli, a mixture of $KMnO_4$ and natural clays (Ryan® Ethylene Absorption Sachets) was found to better retain chlorophyll and its derivatives after 11 days at 10 ºC (Caleb et al., 2016).

Other ethylene scavenging technologies use adsorption surfaces with activated carbon, zeolite or clays; and catalysts such as palladium (Pd) and titanium oxide (TiO_2) (Álvarez-Hernández et al., 2019). In broccoli, only zeolite (Esturk et al., 2014) and palladium (Pd) (Abe & Watada, 1991; Cao et al., 2015) have been trialled. A LDPE film blended with 8% of Tazetut® (an inorganic ethylene adsorber containing 50% zeolite), extended the storage life of broccoli at 4 ºC to 20 days. In comparison, broccoli only lasted for 5 days when unpacked, and 15 days in MAP without Tazetut® (Esturk et al., 2014). Contrasting findings for the efficacy of Pd-based ethylene scavengers have been reported. Treating broccoli florets with 30 g kg^{-1} of a $PdCl_2$-$CuSO_4$-based product supported by acidified activated carbon powder delayed chlorophyll degradation and yellowing (Cao et al., 2015). In contrast, Abe & Watada (1991) found that charcoals with palladium chloride ($PdCl_2$) were unable to reduce chlorophyll loss. Also, the costs of Pd make this technology economically unviable for most commercial applications (Álvarez-Hernández et al., 2019).

Table 2.4 Studies of controlled atmosphere on broccoli

% O_2	% CO_2	Storage temperature and duration	Comments on the effects	References
0.125-0.5	0	10 ºC, 7 days	↓ respiration rates ↓ yellowing Induced fermented and decay-type off-odours Moderate to high *Erwinia carotovora* decay incidences (17-75%)	Hansen et al., 2001
0-0.5	20	10 ºC, 7 days	↓ respiration rates ↓ yellowing Controlled microbial growth Induced sour/fermented off-odours	
0.5	10	0 ºC, 28 days	↓ respiration rates ↓ weight loss Controlled microbial growth	Izumi et al., 1996
0.5	10	5 ºC, 21 days	Controlled microbial growth	
1	10	10 ºC, 7 days	↓ yellowing ↓ ethylene production Controlled microbial growth	
1	10	15 ºC, 7 days	↓ ethylene production	Yamauchi and Watada, 1998
2	6	4 ºC, 49 days	↓ yellowing Controlled microbial growth	Bastrash et al., 1993

% O_2	% CO_2	Storage temperature and duration	Comments on the effects	References
2	6	4 ºC, 35 days	↓ yellowing ↓ stem turgor Browning at the cut surface	Paradis et al., 1996
3	5	5 ºC, 60 days	↓ yellowing	Deschene et al., 1991
5	10	1-2 ºC, 21 days	↓ yellowing ↓ weight loss Maintain firmness	Fernández-Léon et al., 2012
10	5	1-2 ºC, 27 days	↓ weight loss Maintain firmness	Fernández-Léon et al., 2013
10	11	4 ºC, 21 days	↓ ethylene production	Berrang et al., 1990

Table 2.5 Main findings from recent studies on MAP for broccoli

Packaging	**Storage conditions**	**Comments on the effects ***	**References**
Oriented PP bag, 35 µm Laser perforated Designed for broccoli	10 ºC, 7 days	↓ weight loss, yellowing and browning ↓ crispness and chewing resistance ↑ DMDS and DMTS concentrations	Jacobsson et al., 2004a Jacobsson et al., 2004b
PP bag, 20 µm Non-perforated	1 ºC, 28 days	↓ weight loss to < 1.5% Maintained chlorophyll contents	Serrano et al., 2006
PP bag, 20 µm Macro-perforated		High weight loss (13.33%) and yellowing	
PP bag, 25 µm Micro-perforated		↓ weight loss to < 1.5% Maintained chlorophyll contents	
PP bag, 35 µm 4 perforations (d = 0.3 mm)	20 ºC, 10 days	↓ yellowing	Rai et al., 2009
PP bag, 30 µm Injected with 5% O_2 & 10% CO_2 Microperforated to attain 10% O_2 & 5% CO_2	5 ºC, 12 days	↓ respiration rate ↓ weight loss, yellowing/chlorophyll loss ↓ Maintained β-carotenoid content	Fernández-León et al., 2013
Perforated PP bags, 30 µm	4 ºC, 21 days	↓ weight loss to < 1% Maintained green colour, aroma and stem hardness	Paulsen et al, 2016
	8 ºC, 21 days	↓ weight loss to < 1% and maintain green colour ↓ characteristic odours	
	15 ºC, 14 days	↓ weight loss to < 3% and maintain green colour ↓ characteristic odours and stem hardness	

Packaging	Storage conditions	Comments on the effects *	References
PP bag, 35 µm 40 microperforations (200 x 2-3 µm)	4 ºC, 4 days + 19 ºC, 3 days + 4 ºC, 9 days	↓ weight loss Maintained firmness Unable to prevent yellowing	Hagen et al., 2020
BOPP bag, 40 µm 560 perforations (90 x 13 µm)			
PE bag, 40 µm No perforation 2 microperforations (d = 750 µm) 4 macroperforations (d = 8.8 mm)	4 ºC, 23 days 20 ºC, 5 days	↑ shelf life (30% yellow) of broccoli florets by 2-3 times ↓ weight loss to < 4%	Jia et al., 2009
Polyvinylidene fluoride - sulfonated poly ether ether ketone blended film (1:1 w/w)	1 ºC, 30 days	↓ yellowing ↓ loss of ascorbic acid Maintain texture and overall sensorial quality	He and Xiao, 2018
PBAT, 0.1-0.15 mm No perforation	12 ºC, 7 days	Maintained green colour High weight loss (9.88% per day)	Marzano-Barreda et al., 2020
* results were compared to unpacked broccoli unless stated otherwise			

Section C. Conclusion

It would be possible to extend the shelf life of blueberries, raspberries, and broccoli by applying novel postharvest technologies together with good temperature and RH control. For raspberries, the application of novel technologies is limited by their fragile drupelet structures and high respiration rates. Therefore, gaseous sanitisation and UV-B irradiation treatments that avoid the use of water will offer the most benefits in terms of shelf life extension. In contrast, blueberries, characterised by their more robust structure and lower respiration rates, will benefit from additional options such as aqueous sanitisers, mild heat treatments and edible coatings. The latter two, however, are often limited by their adverse effects on the appearance of blueberry wax blooms. For broccoli, visible light treatments, optimisation of perforated MAP and optimised uses of ethylene scavengers would potentially be cost-effective solutions to replace top-icing shipment.

Combinations of technologies as hurdle treatments could enhance their effectiveness for shelf life extension, compared to individual technologies alone. For example, MAP pallet liners combined with sanitisation methods might reduce weight loss, retain sensorial quality, and actively prevent mould growth for berries. As raspberries and broccoli are ethylene sensitive, MAP combined with ethylene control could be considered to delay senescence. Other new technologies, such as packaging that can minimise mechanical damage induced by vibrational effects, will arguably become more important as supply chains lengthen. While there are current packaging options that mitigate vibration damage, their efficacy for extending the shelf life of berries will need to be evaluated.

Despite the positive findings in scientific trials, some of the reviewed technologies such as UV-B and fumigation might be commercially impractical due to the high capital investment and safety hazards involved. Several of the other potential treatments would encounter technical difficulties in current industrial settings, such as mild heat treatment, aqueous

washing of blueberries and edible coatings. The easiness to be incorporated to the current industrial practice has been identified as a key factor for successful technology adoptions (Madrid, 2019).

There are still knowledge gaps and challenges that will need to be addressed. Most of the available studies were performed in the laboratories under well-controlled environments. In addition, experiments using small experiment units, such as a punnet of berries, a broccoli head or 100g broccoli florets, with stricter material selection criteria than commercial produce, might not be able to guarantee the repeatability of the findings on industrial scale. Therefore, this study was directed towards commercial trials on technologies that could potentially improve the shelf life of berries and broccoli, as well as easily be adapted into the existing production. They were (i) MAP as liners for bulk-pack of raspberries, blueberries, and broccoli, (ii) MAP liners in combination with vapour-releasing sanitisation products for berries and (iii) MAP with ethylene scavengers for broccoli. In addition, the project also studied a novel approach for a retail MAP at lab-scale for raspberries and blueberries, using unvented trays sealed with perforated lid films. This approach of retail MAP presently has only limited applications commercially. This research also explored more explicitly how the respiration rates and aroma profiles of berries and broccoli change as a function of storage conditions. These measurements could indicate the visual and eating quality when the produce reaches the consumers, which subsequently will determine consumers' acceptance and repeat purchasing behaviour (Chironi et al., 2017).

Section D. Thesis structure

As reviewed in Chapter 1 and 2, raspberries, blueberries and broccoli represents high-value, perishable fresh horticultural products of which shelf life improvements would be beneficial to expand market reach, improve consumers satisfaction and reduce food waste. The three produce are also examples of fruit and vegetables having different characteristics that

challenge the applications of postharvest technologies. They are: (i) the fragile, hollow structures and rapid respiration of raspberries, (ii) the short seasonality (in Tasmania) and high susceptibility to mould growth and quality losses under extended storage of blueberries and (iii) the high respiration and ethylene sensitivity of broccoli. Available literature (Chapter 2) suggested that MAP and their novel combinations with vapour-releasing sanitisation treatments or ethylene scavengers were promising for extending the shelf life of raspberries, blueberries and broccoli. Chapters 3-6 of this thesis, therefore, were conducted to experimentally investigate the effects of those prospective packaging techniques on the quality of the three produce, against current commercial practices. The key results and analyses from the four experiments were discussed in chapter 7 to draw implications of the research for the industry and the contributions of this work to the scientific knowledge.

The outline of chapters below is to describe the individual trials within this research.

Chapter 3: Effects of modified atmosphere packaging and sanitisation on the shelf life and quality of raspberries under commercial settings

The effects of MAP and their novel combinations with sanitation treatments on the shelf life and quality of 'Maravilla' raspberries in commercial settings were studied over two harvests of fruit. The trials were conducted using a cardboard tray of 12 x 125 g punnets of raspberries (1.5kg fruit equivalent) as an experiment unit for the potential to scale up the technology for use in pallets. In the first harvest, two types of MAP (a mineral-impregnated bag with low WVTR and a microperforated bag with high WVTR) were studied as single treatments or in combinations with a vapour-releasing sanitisation products (SO_2 or H_2O_2). The second trial examined two types of microperforated bags with different levels of perforations and their combinations with H_2O_2-releasing sachets. The raspberries were stored at 1.5 ºC in a commercial berry cold room. Fruit quality including the weight percentages of mouldy fruit,

respiration rates, weight losses, chemical quality attributes and aroma profiles were assessed after 10 and 20 days in trial 1, and after 10, 16 and 21 days in trial 2.

Chapter 4. Effects of modified atmosphere packaging and sanitisation on the shelf life and quality of blueberries under commercial settings

The effects of MAP and their novel combinations with sanitation treatments on the shelf life and quality of blueberries were studied over two harvests, one of 'Legacy' and one of 'Powder Blue' cultivar. The trials used a cardboard tray of 12 x 125 g punnets of blueberries (1.5kg fruit equivalent) as an experiment unit for the potential to scale up the technology for use in pallets. MAP (a mineral-impregnated bag with low WVTR or microperforated bag with high WVTR designed for blueberries) was trialled as single treatments or in combinations with a vapour-releasing sanitisation products (SO_2 or H_2O_2). The blueberries were stored at 1.5 ℃ in a commercial berry cold room. Fruit quality including the weight percentages of mouldy fruit, respiration rates, weight losses, chemical quality attributes and aroma profiles were assessed after 6, 8 and 10 weeks.

Chapter 5. Perforated modified atmosphere packaging to extend shelf life of blueberries and raspberries

This experiment trialled perforated MAP as a novel approach for retail packaging to extend the shelf life of raspberries and blueberries, compared to the performances of the current clamshell punnets. The proposed packaging used 1 L non-vented trays sealed with perforated lid films (perforation diameter d = 70 µm) to pack 125 g berries. Current commercial vented clamshell punnets were used as the control. In total, 15 MAP designs with different combinations of perforations (2, 4 and 6) and initial MAP conditions (10% O_2 & 9% CO_2, 17% O_2 & 0% CO_2, 14.5% O_2 & 0% CO_2, 8.5% O_2 & 0% CO_2 and 20.7% O_2 & 0% CO_2) were studied for blueberries. Seven passive MAP designs with different perforations (0-6) were studied for raspberries. Changes with storage were assessed by visible mould growth,

respiration rates, fruit chemical quality and aroma profiles after 8 weeks for blueberries and after 11 days for raspberries.

Chapter 6. Modified atmosphere packaging for iceless broccoli shipment

The experiment in this chapter assessed the potentials of using MAP systems as alternatives to top-icing in broccoli shipment, to maintain the quality of broccoli under suboptimal temperatures and prolonged storage. Four MAP systems (HDPE liners with ethylene scavenging sachets, LDPE mineral-impregnated MAP, BOPP microperforated MAP and PA microperforated MAP) were compared with top-icing under three simulated shipping conditions. The scenarios being studied were high-value, long distance domestic routes (7 days) with good temperature management (2 ºC) or a broken cool chain (13 ºC), and sea freight with refrigeration (42 days at 2 ºC). Quality assessment at the end of the storage periods included respiration rates, chlorophyll and carotenoid contents, L-ascorbic acid contents and GC-MS aroma profiles.

Chapter 7. General discussion and conclusion

From analysing the key results obtained in the four experiments on the applications of MAP to extend the shelf life of raspberries, blueberries and broccoli, this chapter drew implications of the research for the industry and recommendations for further research. The effects of MAP when used alone and in combinations with a supplementary treatment (vapour-releasing sanitisation or ethylene scavenging products) on the quality of the stored produce were considered. The potential approach to design and optimise a retail MAP was included as demonstrated in blueberries and raspberries. The limitations of this work and suggestions for improvements were also covered.

Chapter 3: Effects of modified atmosphere packaging and sanitisation on the shelf life and quality of raspberries

3.1 Introduction

Raspberry (*Rubus idaeus* L.) is a high-value fruit crop with increasing production worldwide (Sobekova et al., 2013). The fruit are appreciated for their taste and nutritional values (Aprea et al., 2009). Fresh raspberries, however, are extremely perishable owing to their fragile structures, high respiration rates and susceptibility to fungal infections. Deterioration of fruit quality is characterised by dehydration, fruit darkening, softening and collapse, and mould growth (Huynh et al., 2019). Current industrial practices rely mainly on cold storage (0-2 °C) resulting in a maximum shelf life of 7-10 days after harvest. The perishability of raspberries has been limiting their market expansion and is a cause of food waste at retail stores and in households (do Nascimento Nunes, 2009b).

Presently, raspberries for the fresh market are picked and packed in the field without any sanitisation treatments (Horvitz, 2017). This approach limits options for postharvest management of fungal disease. Sanitisation using ozone (O_3), sulphur dioxide (SO_2) and chlorine dioxide (ClO_2) have been studied, mostly for managing microbial safety of raspberries rather than for prevention of fungal spoilage that appears towards the end of the shelf life. The hollow structures of the fruit are susceptible to damage by aqueous sanitation; hence, the use of gaseous sanitisers has been suggested as a more promising approach (Huynh et al., 2019). In addition, there are now safer and simpler alternatives to conventional fumigation. Options include vapour-releasing SO_2 sheets and H_2O_2 pouches applied in storage facilities or included in packaging.

CA and MAP elevate CO_2 and lower O_2 concentrations and have been shown to extend shelf life of fresh produce by suppressing fruit respiration rates and microbial growth (M.D.

Wilson et al., 2019a). For raspberries, atmospheres of 5-10% O_2 and 10-15% CO_2 are recommended and have been demonstrated to reduce respiration rate and delay loss of fruit quality (Haffner et al., 2002; Siro et al., 2006). Pallets of products, enclosed in plastic shrouds flushed with 10-15% CO_2 to generate MAP, is now used as a standard procedure for the distribution of raspberries around the United States (Madrid & Beaudry, 2020). While this practice is considered unnecessary for other regions due to the closer proximity of farms to markets, MAP in pallets could benefit the storability of raspberries at the production sites.

Despite of some benefits shown in maintaining the fruit colours during storage, the conditions in early studies of MAP for raspberries were either not able to control mould growth or induced softening and formations of off-odours (Table 2.2). Rapid quality deteriorations, particularly the presence of visible mould even at low levels, would stop consumers from buying the raspberries. These limitations suggested the needs to improve control against mould growth by optimising the MAP or using sanitisers as in-package supplementary treatments to MA. In addition, only limited research has been conducted under commercial settings that involve broader ranges of initial fruit quality and would allow for better industrial implications. This experiment, therefore, aimed to investigate the potential of shelf life extension for ‘Maravilla’ raspberries in industrial settings using bulk-pack modified humidity MAP technologies in combination with vapour-releasing SO_2 and H_2O_2 sanitising treatments. The packaging types studied were mineral-clay impregnated MAP with low WVTR and microperforated MAP with high WVTR given that these materials enable in-package variation of gas compositions and humidity. The high humidity condition created by packaging with low WVTR could improve the effectiveness of SO_2 against fungal development (Saito et al., 2020) and maintain the active water vapour for optimal performance of the H_2O_2-releasing product. In contrast, low humidity could prevent moisture condensation that favours mould growth (Horvitz, 2017).

3.2 Materials and methods

3.2.1 Plant materials, packaging, and storage

This study used raspberries (*Rubus idaeus* cv. 'Maravilla') that were manually harvested from a commercial farm in East Devonport, Tasmania, Australia, in May 2019 (first harvest) and Dec 2019 (second harvest). Corresponding to the two trials, 60 and 64 cardboard trays of fruit respectively were taken from a pallet that had passed quality controls on-farm and at the packing shed. The fruit were precooled to 2 °C and packed within 4 h in MAP sealed by a heat impulse sealer (Mercier Corp., Taipei, Taiwan). There were four replicates for each treatment for each assessment day. One replicate was a cardboard tray of 12 punnets x 125 g of raspberries reflecting current commercial practice. The types of packaging and sanitisers evaluated in the two seasons are listed in Table 3.1. "Unpacked" fruit referred to raspberries in clamshell punnets placed in cardboard trays without any extra external packaging. Due to the lower decay incidences found in the first trial with high WVTR packaging (B), another high WVTR packaging (C) was used in Trial 2 to replace packaging (A).

SO_2-releasing sheets (BerryGuard^{TM}, OSKU S.A., Santiago, Chile, supplied by Biopac, West Burleigh, Australia) of 15 cm x 21 cm were placed between two layers of the punnets within a tray. H_2O_2-releasing pouches were injected with 40 mL of distilled water to activate the product and were placed along the longer side of the trays when packed. The release of H_2O_2 was verified by peroxide pH test strips (measuring range 1-100 µL L^{-1}) placed in the packages (Precision Laboratories, AZ, USA). At the end of each trial (day 20 for trial 1 and day 21 for trial 2), H_2O_2 and SO_2 levels were measured using Kitagawa Gas Detector tubes (Air-Met Pty Ltd., Nunawading, Australia).

After packing, trays were randomly stacked into layers of 12 trays, ensuring that each treatment had at least a tray at the bottom, in the middle, on the top surface and at the corners. The pallet was stored at 1.3-1.6 °C in an industrial cool room with forced-air cooling for 20

days in May 2019 and 21 days in Dec 2019. The humidity of the storage room was uncontrolled, being 70 ± 3% RH in the first trial and 85 ± 2% RH in the second one as measured by a humidity logger (Jaycar Electronics, NSW, AU). The tray locations in the stacks were randomised after every day of headspace measurement of % O_2 and % CO_2. Fruit quality assessment was performed on day 0 (also referred as "fresh fruit"), 10 and 20 in Trial 1, and on day 0, 10, 16 and 21 in Trial 2. The addition of an assessment day (day 16) in Trial 2 was due to the absence of mould growth on day 10 and high mould incidences on day 20 in Trial 1.

3.2.2 Headspace % O_2 and % CO_2 and respiration rates

The evolution of O_2 and CO_2 inside the MAP-packed raspberry trays was measured by a portable gas analyser (Dansensor CheckPoint 3, Mocon, Ringsted, Denmark) on day 5, 10, 14 and 20 in the first trial and on day 5, 10, 16 and 21 in the second trial. The Dansensor is equipped with a zirconia O_2 sensor and an infrared dual beam CO_2 sensor that allow the detection of O_2 and CO_2 with ± 0.01% and ± 0.8% accuracies, respectively (as measured at 23 ºC, low O_2 MAP and steady state). Sampling was performed by inserting a Terumo needle (0.60 x 32 mm) through rubberised gas sealing strips (Septum, Novatech Controls Pty. Ltd., Cheltenham, Australia) into the packages, and gas extraction started manually. The machine was operated in continuous sampling mode. The O_2 and CO_2 levels were recorded when the numbers shown became stable, which was within 10 s on average.

Respiration rates of raspberries were measured using closed system experiments following the method of Haffner et al. (2002) with minor modifications. For each replicate, about 40 g of raspberries (6-8 fruit) from three punnets were weighed into Mason jars (Ball®, South Kempsey, Australia) of 236.6 mL capacity. The jars were tightly sealed with metal lids with sampling holes covered by the rubberised gas sealing strips and immediately stored in a refrigerator at 2 ± 0.5 °C. The headspace O_2 and CO_2 concentrations were measured

periodically using a gas analyser (Dansensor CheckMate 3, Mocon, Ringsted, Denmark) and plotted against time to calculate O_2 consumption and CO_2 production rates (mg kg^{-1} h^{-1}).

3.2.3 Weight loss and fruit mould assessment

Weight loss was determined as the average of weight differences of two punnets (one from the upper and one from the lower layer) on the days the experiments were set up (day 0) and on the day that tray was assessed.

Fruit with visible mould, regardless to the severity, were separated and weighed. The weight of mouldy fruit was calculated as a percentage against the total fruit weight. In Trial 1, three random punnets of raspberries from each tray were examined for visible mould growth on each assessment day. Due to the low incidences of mould in the second trial, all 12 punnets in one tray were evaluated. On day 21, three mould-free punnets were further stored at 4 °C up to 6 days to simulate retail display periods and to observe changes in mould development.

3.2.4 Analysis sample preparation

Sampling was performed by randomly picking 50 g of raspberries (approximately 11-13 fruit) from 11 punnets of each tray. The fruit were blended with 100 mL of distilled water for 30 s. The blended samples were then centrifuged at 3000 *g* for 5 min (Centrifuge 5702 R, Eppendorf, Macquarie Park, Australia) to obtain clear juices.

3.2.5 Chemical quality attributes

Total anthocyanin contents of berries were measured by the differential pH method as described by Denev et al. (2010) with minor modifications. Briefly, two aliquots (2 mL each) of juice were diluted to 25 mL, one with a pH 1.0 solution and another with a buffered pH 4.5 solution. The total anthocyanin content, expressed as cyanidin-3-glucoside equivalents, was calculated by the following formula:

$$\text{Anthocyanin content (mg kg}^{-1}\text{ FW)} = \frac{\text{A x MW x DF x 1000}}{\varepsilon \text{ x L}}$$

where $A = (A_{510nm}$ pH 1.0 – A_{700nm} pH 1.0) – (A_{510nm} pH 4.5 – A_{700nm} pH 4.5),

MW= cyanidin-3-glucoside molecular weight (449.2 g mol^{-1}),

DF = dilution factor,

ε = cyanidin-3-glucoside molar absorptivity (26 900 L mol^{-1} cm^{-1}),

and L = cell pathlength (1 cm).

pH and total soluble solid contents of the raspberry juices were measured by using a portable pH meter (pH 22 LAQUAtwin, Horiba Ltd., Kyoto, Japan) and a bench-top refractometer (HI-96801, Hanna Instruments, Leighton Buzzard, UK). Titratable acidity (TA) was determined by titrating 10 mL of raspberry juices with NaOH 0.1M to pH 8.2 (AOAC 942.15). The percentages (%) of acid in berries were calculated as grams of citric acid (predominant acid) per 100 g of fruit:

$$citric\ acid\ (\%) = \frac{0.1 \text{ x } V_{NaOH} \text{ x } 64}{DF \text{ x } 10}$$

where V_{NaOH} (mL) = volume of NaOH used to titrate the sample,

M = 64 (g mol^{-1}) = molecular weight of citric acid

and DF = dilution factor.

The L-ascorbic acid contents of raspberry juices were determined by the iodine titration method (AOAC 967.22) against L-fascorbic acid standards (Merck PTY Ltd., Bayswater, Australia).

3.2.6 Aroma profiles by SPME/GC-MS

Aroma profiles of raspberry juice were analysed by SPME GC-MS following the method of Aprea et al. (2009) with minor modifications. Briefly, 5 mL of raspberry mash was immediately frozen in 20 mL sealed glass vials after blending of fruit, stored at -18 °C and analysed within one week. Thawed samples were kept at 3.5 ± 0.5 °C in the chilled rack of the GC-MS and analysed within 12 h.

The vials were incubated at 40 °C for 5 min prior to sampling by a carbon wide range/polydimethylsiloxane fibre (120 µm) (PAL SPME Arrow, CTC Analytics AG, Zwingen, Switzerland) for 10 min at the same temperature. The fibre was conditioned at 180 °C for 30 min before runs and for 5 min between runs. Desorption of adsorbed volatile compounds was carried out in splitless mode in the injector port for 2 min at 220 °C (Shimadzu AOC-6000 autosampler, Shimadzu Corp., Kyoto, Japan). The machine was coupled with an SH-Rxi-624Sil MS column (30 m, 0.25 mm ID, 1.4 µm df). The temperature of the GC oven was held at 60 °C for 3 min, ramped up to 220 °C at 8 °C min^{-1} and held for 5 min, then increased to 250 °C at 10 °C min^{-1} and held for another 5 min. The carrier gas was helium at a total flow rate of 20 mL min^{-1}. The GC-MS interface temperature was 250 °C.

Identification of headspace raspberry volatiles was carried out by a mass spectrometer (Shimadzu GCMS-QP2020, Shimadzu Corp.) operated in electron ionisation mode scanning in the range of *m*/*z* 20-500. Chromatograms were analysed by GCMS Postrun Analysis (GCMS Solution Ver. 4.50©, Shimadzu Corp.) and compounds were matched with NIST17, FFNSC 3 and Wiley9 libraries for identifications. Data were reported as mean of peak areas processed with the probabilistic quotient normalisation (PQN) method.

3.2.8 Data analysis

Minitab 19.1.1 (Minitab, LLC., Pennsylvania, USA) was used to perform one-way ANOVA with Tukey post-hoc test for analysing treatment effects on fruit quality, and Pearson's correlation coefficient of the means for O_2 consumption and CO_2 production rates. Kruskal-Wallis test followed by uncorrected Dunn's post-hoc test was applied for the percentage of mouldy fruit on day 20 of the first trial using GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, USA). The significance level for all statistical tests was $p = 0.05$.

Table 3.1 Packaging and sanitisation types that were evaluated in the two trials

Treatment	MA at equilibrium *		Trial 1	Trial 2
	O_2 (%)	CO_2 (%)		
Mineral-clay impregnated MAP bags (A)	18.1	3.1	X	
A + H_2O_2-releasing pouches	18.5	3.1	X	
A + SO_2-releasing sheets	16.7	4.5	X	
Microperforated MAP bags (B)	14.8	7.5	X	X
B + H_2O_2-releasing pouches	16.3	6.6	X	
B + SO_2-releasing sheets	15.8	6.8	X	X
Microperforated MAP bags (C)	17.9	3.9		X
C + H_2O_2-releasing pouches	18.6	3.5		X
Control/Current commercial packaging (clamshells in cardboard trays)	–		X	X

* When measured in the current study for 'Maravilla' raspberries (1.5 kg / bag) stored at 2 ^oC, after 5 days in trial 1 and 4 days in trial 2. The headspace compositions only varied slightly on the following assessment days.

Material sources: Mineral-clay impregnated MAP bags: PEAKFresh®, Netley, Australia

Microperforated MAP bags: Xtend®, Stepac L.A. Ltd, Tefen, Israel

SO_2-releasing pad BerryGuard^{TM}: OSKU S.A., Santiago, Chile, supplied by Biopac, West Burleigh, Australia

3.3 Results

3.3.1 Headspace gas compositions and respiration rates

The headspace O_2 and CO_2 concentrations of the raspberry packages reached equilibrium after 4-5 days and varied among packaging types (Table 3.1). Packaging treatments A and C resulted in higher O_2 and lower CO_2 concentrations compared to treatment B, regardless of the presence of sanitisers. The O_2 consumption and CO_2 production rates of fresh raspberries measured at 2 °C varied slightly between the two trials, ranging from 15.07-19.50 mg O_2 kg^{-1} h^{-1} and 17.63-23.81 mg CO_2 kg^{-1} h^{-1}. In the first trial, all raspberries packed in microperforated bags had a lower CO_2 production rate on day 10 with a mean depression of 8.23 mg kg^{-1} h^{-1}, compared to the fruit packed clamshells (Figure 3.1A). Differences in respiration between the treatments, however, were not observed on day 20. In the second trial, there were no differences in raspberry respiration rates between day 0 and the treatments on day 10 and 16, but all MA-packed samples had lower respiration rates on day 21 than those in clamshell punnets (Figure 3.1B). There was a strong positive correlation between the means for O_2 uptake and CO_2 production rates ($r > 0.80$, n = 4).

The presence of H_2O_2 was verified by the colour reading of the peroxide strips. On day 5 of both trials, the readings indicated approximately 10 µL H_2O_2 L^{-1}. The release of SO_2 was verified by the manufacturer (OSKU S.A) but was not able to be detected in this experiment due to the technical unavailability. Residual levels measured by Kitagawa tubes were below the detection limits of 0.1 µL L^{-1} for SO_2 and 0.2 µL L^{-1} for H_2O_2.

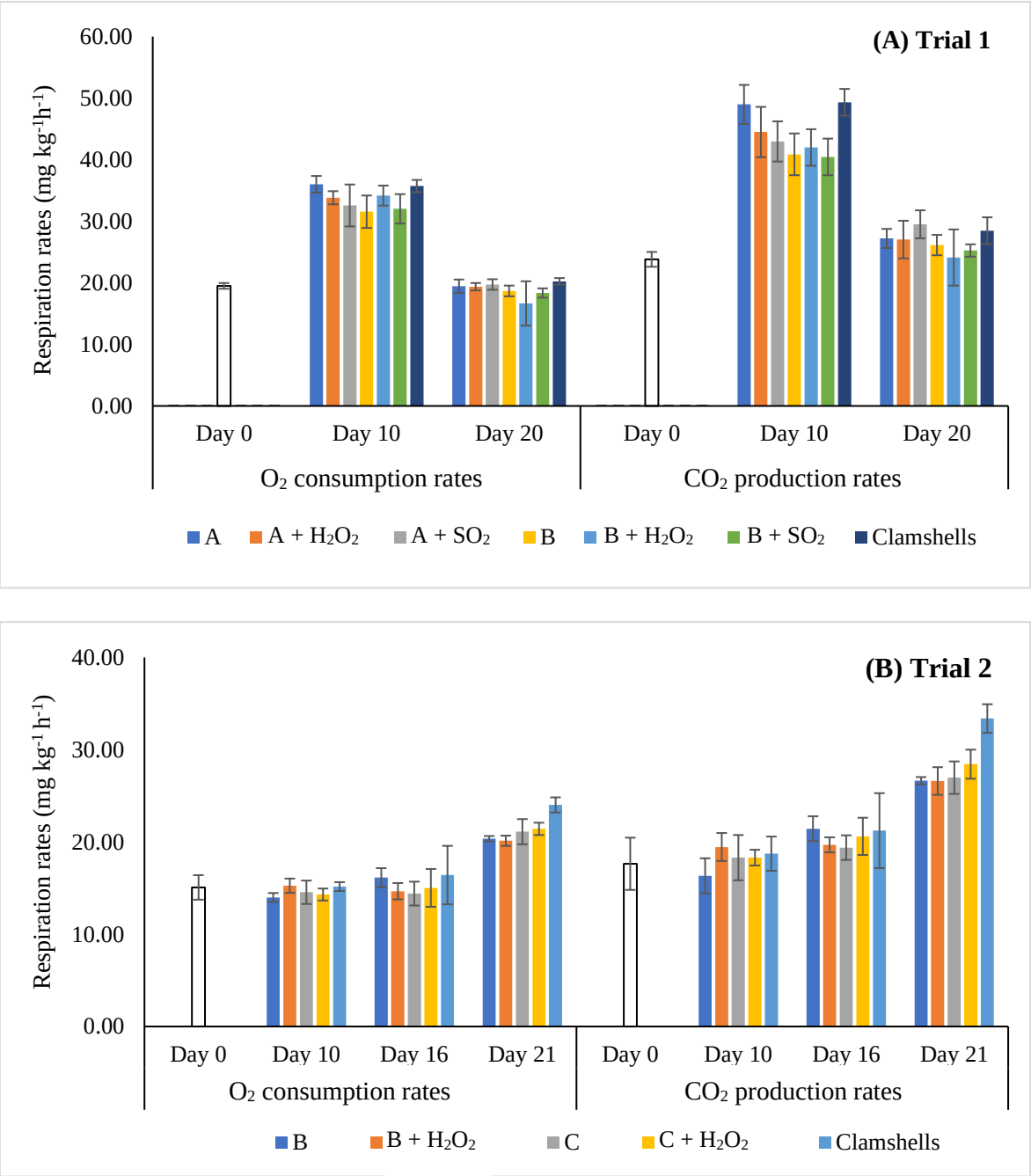


Figure 3.1. Respiration rates of fresh raspberries and of fruit after cold storage (n = 4, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR (18.1% O_2 & 3.1% CO_2), B: microperforateed MAP with high WVTR (14.8% O_2 & 7.5% CO_2) and C: microperforated MAP with high WVTR (17.9% O_2 & 3.9% CO_2)

3.3.2 Fruit mould

Overall, both trials showed that mould growth was more influenced by the conditions developed in different packaging than by sanitisation. In the first trial, visible fungal

development was not observed on day 10, but in all treatments on day 20 with smaller weight percentages of mouldy fruit (0.67-1.4%) found in microperforated MAP bags B (Figure 3.2). The moulds appeared to be *B. cinerea* (grey mould), *Mucor* sp. (fluffy white with black tips) and *Penicillium* sp. (green mould). There were noticeable variations among replicates, particularly in the unpacked raspberries and those packed in mineral-clay MAP with low water permeability. For examples, two clamshell replicates had 17-20% mouldy fruit (by weight) while the other two had 4-8%. In four trays packed in MAP A with H_2O_2 pouches, one had approximately 18% mouldy fruit, two had 5-10% and one had no mould.

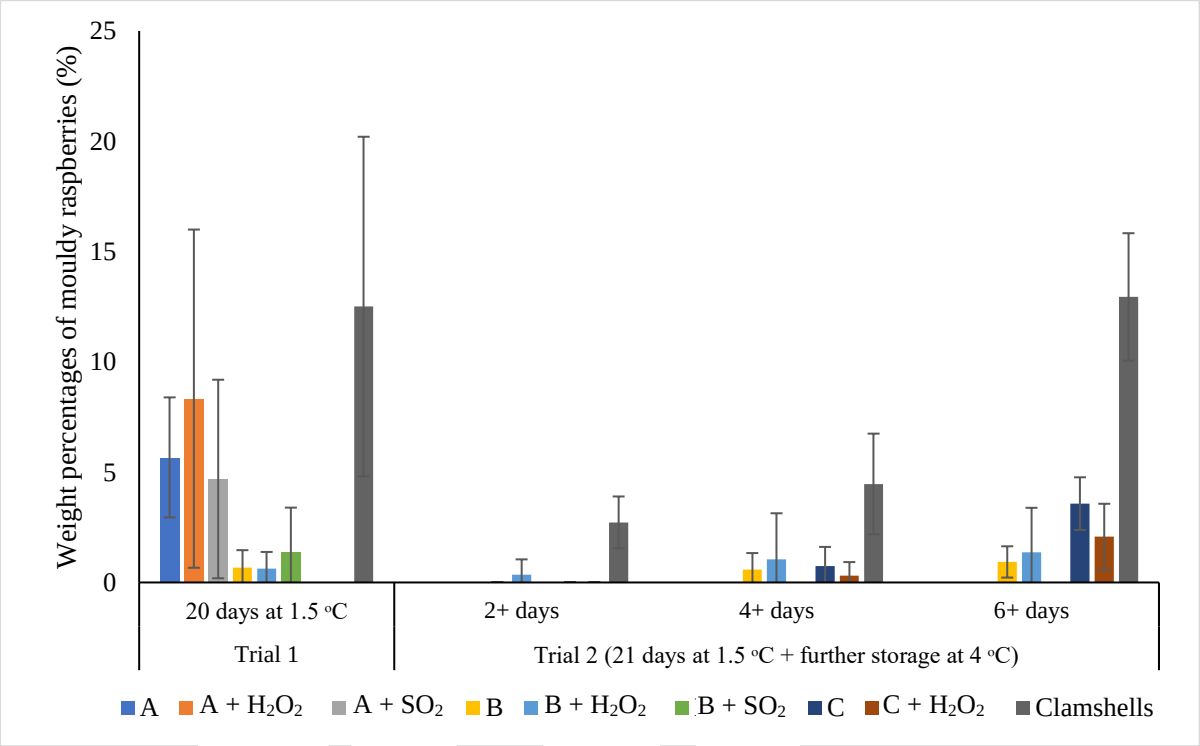


Figure 3.2 Weight percentages of mouldy raspberries (n = 4. p = 0.05)

20 days at 1.5 ºC (trial 1) and during further storage at 4 ºC after 21 days at 1.5 ºC (trial 2)

A: mineral-clay impregnated MAP with low WVTR (18.1% O_2 & 3.1% CO_2), B: microperforateed MAP with high WVTR (14.8% O_2 & 7.5% CO_2) and C: microperforated MAP with high WVTR (17.9% O_2 & 3.9% CO_2)

Raspberries in trial 2 had lower weight percentages of mouldy fruit than trial 1. By day 21, only one berry with visible mould growth was found in 12 punnets of each replicate of

unpacked fruit, and none was found in all MA-packed samples. Mould developed during further storage of the mould-free sub-samples (three punnets/cardboard tray) at 4°C (Figure 3.2). From the appearances, the dominant moulds were also *B. cinerea, Mucor* and *Penicillium.* Although there were also noticeable variations among replicates as in the first trial, overall, the weight percentages of mouldy fruit in MA-packed raspberries were 9.5-11% lower than the fruit in clamshells (12.9%) ($p < 0.05$), after 6+ days at 4 °C.

3.3.3 Weight loss

In both trials, weight loss increased in all samples over the 20-21 days of storage with the highest loss observed in the clamshells not protected by packaging. These unpacked raspberries were dull and lost approximately 3-4% of the initial weights by day 10 and > 6% on day 20-21. The three MAP treatments studied significantly reduced weight loss ($p < 0.05$) to below 2% after 20-21 days. There were no significant weight loss differences between the types of packaging, with and without sanitisers ($p > 0.05$).

3.3.4 Chemical quality attributes

In both seasons, total anthocyanin contents of raspberries increased in all samples during storage and appeared to be associated with fruit darkening ($p < 0.05$). Compared to fruit at harvest, the largest changes in anthocyanin levels were found in the unpacked raspberries which had increased by >100 mg kg^{-1} on day 10 and by 137-192 mg kg^{-1} on day 20-21 (Figure 3.3). MA-packed raspberries had a mean of 17-27% lower total anthocyanin contents than those in clamshells. The anthocyanin levels of MA-packed raspberries were not affected by the packaging types on day 10 but were lower in microperforated MAP B than in MAP A or C by day 20-21 ($p < 0.05$). The use of sanitisers did not affect the total anthocyanin contents of raspberries in the current trials.

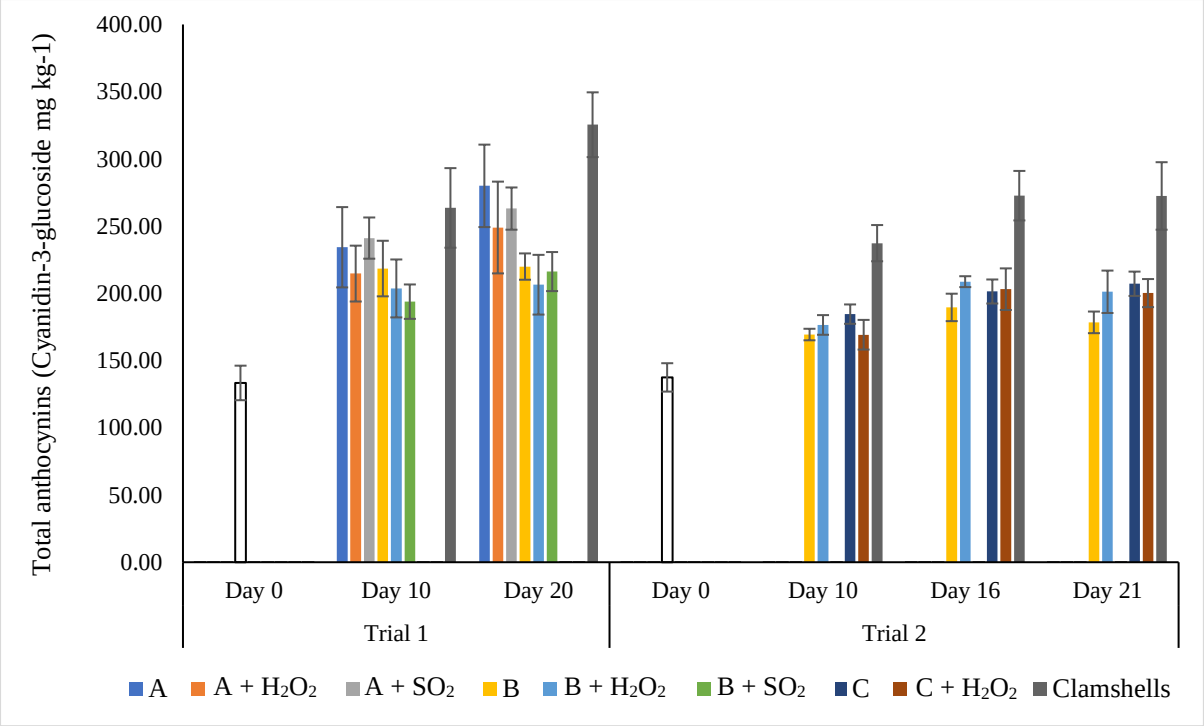


Figure 3.3 Total anthocyanin contents in raspberries in different storage conditions (n = 4, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR (18.1% O_2 & 3.1% CO_2), B: microperforated MAP with high WVTR (14.8% O_2 & 7.5% CO_2) and C: microperforated MAP with high WVTR (17.9% O_2 & 3.9% CO_2)

Overall, the two trials showed benefits of MAP in the storability of raspberries by delaying the changes in the chemical quality attributes of raspberries including pH, TSS and TA. Across two seasons, fresh raspberries had average values of 3.29-3.36 for pH, 8.7-11.3% TSS and 1.7% TA, giving TSS/TA ratios range of 5.2-7.0. Compared to fresh raspberries, the TSS/TA ratio increased by 1.4-2 times in clamshell fruit during storage ($p < 0.05$), while it remained unchanged in MAP-packed fruit ($p > 0.05$). These changes in clamshell raspberries mainly resulted from the decrease in TA, by 0.4-0.9%, which was in line with increases in pH to 3.44-3.78. Meanwhile, after storage, no differences in TSS were found in the first trial ($p > 0.05$), while all packed fruit had 1-1.5% lower TSS than the clamshell fruit in the second one.

Raspberries in these trials had an average ascorbic acid content of 274.25-308.80 mg kg^{-1} at harvest. The benefits of using MAP in preserving ascorbic acid in raspberries were

inconsistent between the two trials of this study. In the first trial, no loss in MAP packed raspberries was found after 20 days ($p > 0.05$), compared to 34.13 mg kg^{-1} decrease in fruit in clamshells. In the second season trial, however, ascorbic acid contents in all samples dropped 106.33-141.43 mg kg^{-1} by day 16 and 21 ($p < 0.05$).

3.3.5 Aroma profiles

The major volatile groups in 'Maravilla' raspberries were C6-aldehydes (hexanal and 2-hexenal), esters (ethyl acetate, 2- and 3-methyl buten-1-ol acetate and *cis*-3-hexen-1-ol acetate), C13-isoprenoids (α-ionone, β-ionone, α-dihydroionone and β-ionol) and terpenes/terpenoids. Compared to fresh fruit, the complexity, and peak areas of esters and terpenes/terpenoids increased after storage, and noticeably in clamshell raspberries (Figure 3.4 and Figure 3.5). The peak areas of these volatile groups were 5-10 times higher in clamshell raspberries than on day 0. The effects of packaging were most pronounced in the fruit stored for >10 days. Overall, the peak areas of C6-aldehydes, esters and terpenes/terpenoids were higher in raspberries packed in loose clamshells and in MAP with 17-18% O_2 & 3-4% CO_2 (packaging A and C), compared to MAP with 14-15% O_2 & 7-8% CO_2.

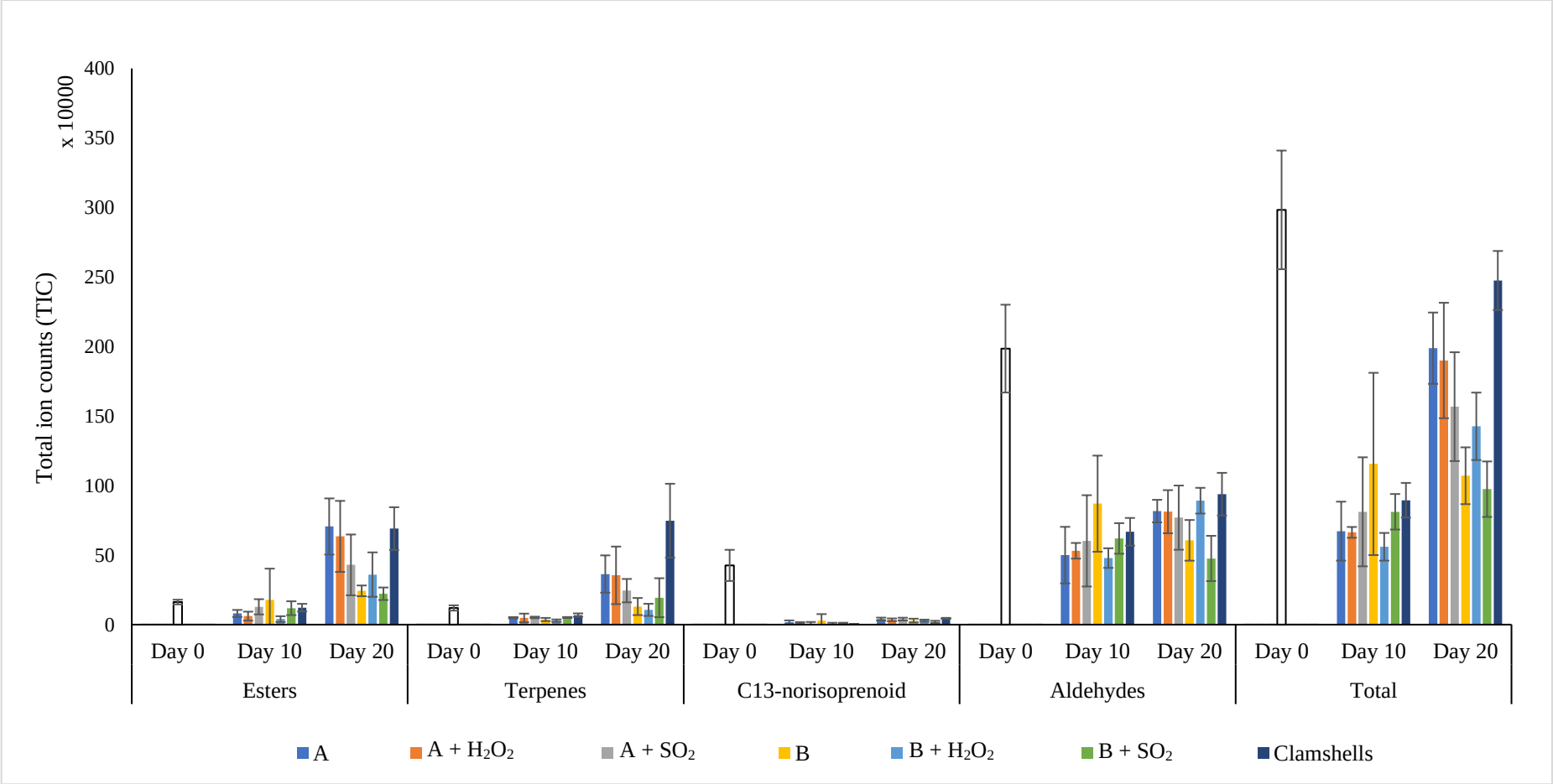


Figure 3.4 Peak areas of volatile groups in raspberries stored in different packaging (Trial 1) (n = 4, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR (18.1% O_2 & 3.1% CO_2), B: microperforateed MAP with high WVTR (14.8% O_2 & 7.5% CO_2) and C: microperforated MAP with high WVTR (17.9% O_2 & 3.9% CO_2)

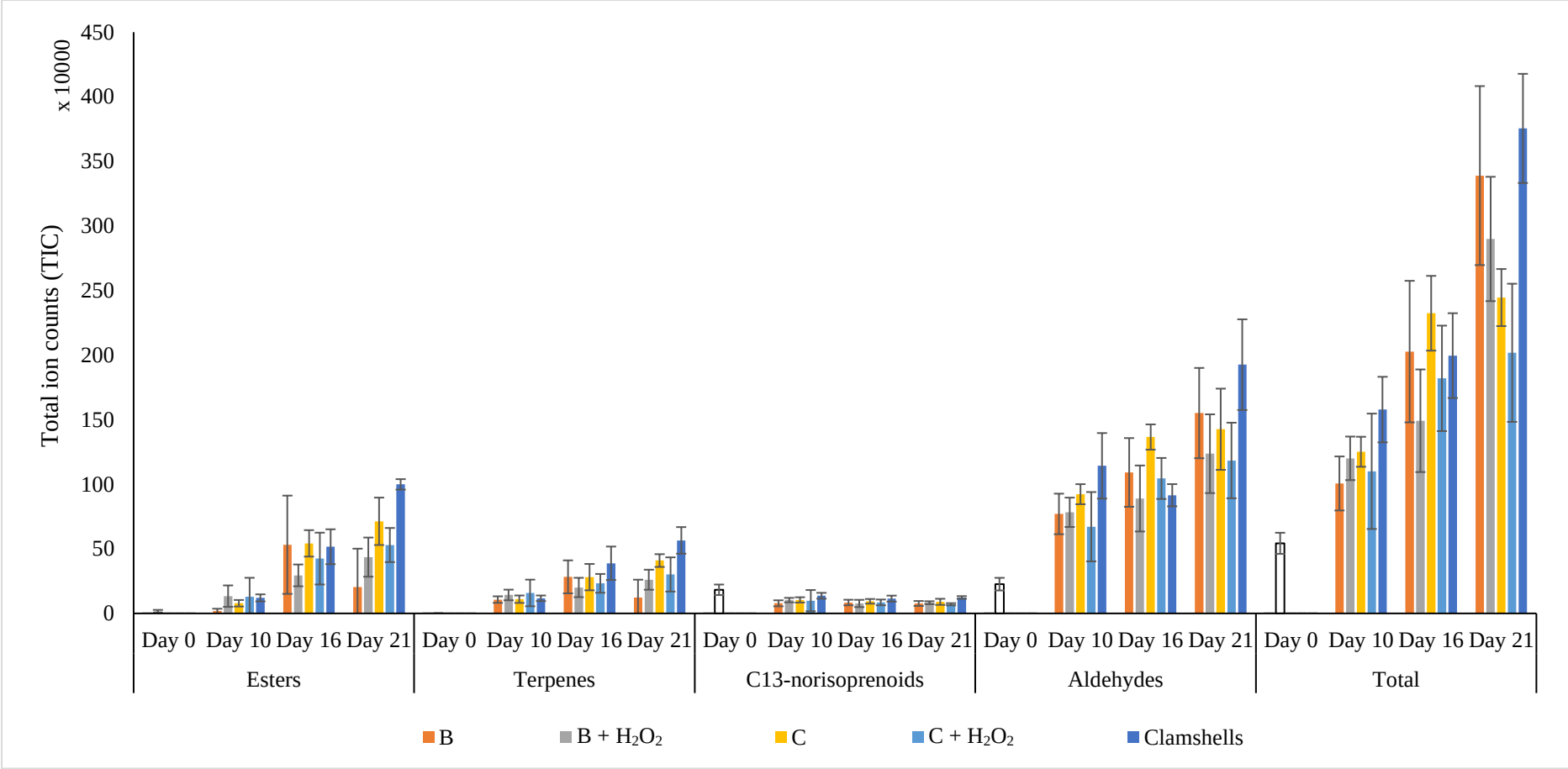


Figure 3.5 Peak areas of volatile groups in raspberries stored in different packaging (Trial 2) (n = 4, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR (18.1% O_2 & 3.1% CO_2), B: microperforateed MAP with high WVTR (14.8% O_2 & 7.5% CO_2) and C: microperforated MAP with high WVTR (17.9% O_2 & 3.9% CO_2)

3.4 Discussion

The high respiration rates, fragile structures and high susceptibility to fungal decay have been the major causes limiting the storability of raspberries (Huynh et al., 2019). This study was to investigate the effects of MH/MA packaging and their combinations with vapour-releasing sanitisation technologies on shelf life of 'Maravilla' raspberries in industrial settings. The experiment trialled MH/MA packaging in the form cardboard liners for 12-punnet trays for the potential to scale the technology for use in pallets. The benefits of MAP in extending shelf life of raspberries under commercial settings have been demonstrated by the suppression of mould growth, the reduction of weight loss, and the retention of fruit quality attributes.

3.4.1 MAP conditions to minimise incidence of mould

Among the conditions tested in this study, a modified atmosphere of 14-15% O_2 & 7-8% CO_2 with high WVTR packaging (packaging B) was best at controlling mould growth (< 1%) in 'Maravilla' raspberries stored at 1.5 °C for 20-21 days. The change of packaging materials in the second trial, from low WVTR (A) to high WVTR (C), was because of higher decay incidences found in the first trial with low WVTR packaging (A). The higher O_2 (17-18%) and lower CO_2 levels (3-4%), as developed in the mineral-clay impregnated bag A and microperforated bag C, could be the reason for their limited control over mould growth in this study, compared to packaging treatment B. In addition, packaging there was temperature fluctuation between the packing room (10 °C) and storage room (1.5 °C) in the first trial, leading to moisture condensation in the packaging A (low WVTR) that could favour mould growth (Horvitz, 2017). Moreover, the lower ethyl acetate contents, as parts of lower peak areas of esters observed in raspberries in packaging B (Figure 3.4 and Figure 3.5), could also have contributed to the smaller weights of mouldy fruit. This could be from the presence of ethyl acetate in the headspace which was suggested to stimulate the germination of *Botrytis*

cinerea spores in apples (Brown, 1922). There were considerable variations among replicates and between the two seasons, which could result from pre-harvest factors and the temperature fluctuation between the packing and the storage rooms (only in the first trial). Furthermore, it was shown that early season raspberries were often less susceptible to mould growth than late season fruit (Dennis & Mountford, 1975).

3.4.2 Impacts of vaporous sanitisers on the incidence of mould

The absence of pronounced effects from gaseous sanitisers on controlling mould growth in raspberries in this study was possibly caused by packaging properties and the losses of sanitiser effectiveness over prolonged storage. The mineral-clay impregnated bags have known absorption properties that might have limited the effects of the vapour sanitisers. According to the product datasheet of the SO_2-releasing sheets, SO_2 concentrations of 3-6 µL L^{-1} were detected in a pallet of blueberries covered with five sheets of 520 cm x 38 cm packed in LDPE pallet shroud with 0.1% ventilated area (OSKU S.A, 2018). In this study, the SO_2-releasing sheets at the dose that provided positive results for raspberries from preliminary trials in punnet scales. However. this available amount of SO_2 could have been bound and become less effective over time. Indeed, SO_2 generated by a dual-release system was better at controlling mould growth in 'Meeker' raspberries than the fast-release generator, when the fruits were stored for more than three days (Spayd et al., 1984). Because of no additional benefits observed in the first trial, as well as the risks of bleaching when increasing the SO_2 levels (Spayd et al., 1984) and preliminary trials), SO_2 was not trialled in the second study. Meanwhile, sufficient active water vapour is important to effectively activate and release H_2O_2 from the pouches. In this sense, the water initially added to the pouches could have desorbed during excessive storage, explaining the lack of effectiveness of the sanitiser.

3.4.3 Effects of treatments on respiration

On average across two seasons, the respiration rates of fresh raspberries (17.29 mg O_2 kg^{-1} h^{-1} and 20.72 mg CO_2 kg^{-1} h^{-1} at 2 °C) were slightly higher than the values reported in the literature (16-18 mg CO_2 kg^{-1} h^{-1} at 2 °C; (Perkins-Veazie, 2016b). The changes in respiration rates over time and storage conditions were inconsistent between the two seasons (Figure 3.1) with a mean increase by 1.8 times on day 10 in the first trial and 1.5 times on day 21 in the second trial. In addition, the effects of MAP on suppressing respiration were only observed on these assessment dates. The causes for these changes remained unclear. In 'Cambridge Favourite' strawberries, marked increases in respiration were also observed in fruit stored for 5-8 days, regardless to the storage atmospheres (air versus CA), and was explained by the fungal development (Woodward & Topping, 1972). This circumstance, however, might not apply to the present study because no visible mould growth was found on the dates that increased respiration were observed (day 10 in trial 1 and 21 in trial 2), as well as up to 4 additional days when further stored at 4.2 ± 0.8 °C. A possible explanation was the natural response of fruit to the changes in energy levels due to harvest and subsequent senescence processes that will decrease respiration.

3.4.4 Relationship of weight loss to packaging conditions

Raspberries in clamshells are subjected to high weight loss, as > 6% found in the current trials, caused by the fruit respiration and transpiration processes (Giovanelli et al., 2014) and dehydration by forced-air cooling. Weight loss itself was not considered as a limiting factor to raspberry shelf life (Briano et al., 2015; Haffner et al., 2002), but the associated shrivelling and dull appearances could affect the raspberries marketability (do Nascimento Nunes & Emond, 2007). By suppressing the fruit metabolisms and acting as a protective layer against the cooling air, MAP, therefore, maintained the raspberries weights (< 2% after 20-21 days)

and benefited storage. Depending on the film WVTR and perforations, higher values of 3.0-6.5% were also reported (Bounous et al., 1996; Briano et al., 2015; Siro et al., 2006).

3.4.5 Impacts of packaging on raspberry anthocyanins and TSS changes

Raspberries are rich in anthocyanins with an average level of 135.42 mg of Cyanidin-3-glucoside equivalent per kg at harvest. This anthocyanin level was generally lower than those found in the literature, possibly because of substantial variations between fruit at different maturities (Krüger et al., 2011; Stavang et al., 2015) and across cultivars. For example, 190-510 mg anthocyanins kg^{-1} was reported in 12 red raspberry cultivars (Anttonen & Karjalainen, 2005), and 172-503 mg kg^{-1} in ten other genotypes (Mazur et al., 2014). Postharvest increases of anthocyanins in raspberries leading to darker fruit has been reported in several studies (Kalt et al., 1999; Krüger et al., 2011; Palonen & Weber, 2019; Stavang et al., 2015) and can negatively affect visual attractiveness (Stavang et al., 2015). For the fresh market, bright red, shiny raspberries are generally preferred by consumers and would appear fresher under store lighting (Weber, 2013). In contrast, dark-red fruit could be perceived as riper, overripe, and sweeter (Clydesdale, 1993). For this reason, delaying the accumulation in anthocyanins (by 17-27% on average) as in raspberries in packaging B and C was advantageous for the fruit marketability. The increases in anthocyanins were linked to fruit metabolisms that synthesise phenolic compounds from carbon skeletons of organic acids, which was in line with decreases in TA (Kalt et al., 1999), and the concentrating effects because of high moisture loss (Krüger et al., 2011). The observations in TA and weight loss found in this study matched with both explanations, particularly in the unpacked samples. Meanwhile, no effects from sanitisers on anthocyanin levels were observed, although bleaching in raspberries stored with SO_2 generators (Spayd et al., 1984) and degradation of anthocyanins in strawberries washed with 5% H_2O_2 (Alexandre et al., 2012) have been documented.

The measurement of TSS/TA ratios has been shown as a good indicator for the sweetness, acidity, and astringency of raspberries (Aaby et al., 2019). As shown in this study, packing raspberries in MAP could be favourable for maintaining the tasting quality of the fruit, especially in long-term storage, by delaying the changes in TSS/TA. In clamshells, TSS/TA ratios of raspberries increased by 1.4-2 times during storage as TSS increased and TA dropped when the organic acids were used for energy (Biale, 1950) and fruit lost moisture (concentrating effects).

3.4.6 Influences of packaging on volatiles

The distinctive raspberry aroma is an important quality attribute to consumer acceptability (Giuggioli et al., 2015). This study confirmed the major compounds in raspberries as in literature including C13-norisoprenoids, terpenes/terpenoids, C6 aldehydes and esters (Aaby et al., 2019; Aprea et al., 2015; Forney et al., 2015). Even so, fruit flavour is a complex quality attribute influenced by several factors such as genetics, environment, harvest maturity and postharvest handling (Baldwin, 2002). This explains the diversity of volatiles and aroma evolutions during storage observed between two current trials, as well as in comparison with previous studies.

Overall, raspberries in clamshells had higher peak areas for total volatiles as well as terpenes, esters, and aldehydes mainly because these are products of oxidation and membrane degradation processes (Aprea et al., 2015). Terpenes/terpenoids and esters were the groups most influenced by packaging types in both trials. In raspberries, terpenes are responsible for floral, herbal notes. Their diversity and intensity increase as raspberries ripen (Robertson, 1995) and during postharvest (Forney et al., 2015; Giuggioli et al., 2015; Morales et al., 2014). Plant terpenes are formed from isopentenyl diphosphate and dimethylallyl diphosphate via the mevalonate and methylerythritol 4-phosphate pathways (Aprea et al., 2015). The carbon skeletons used in the syntheses of terpenes are generated from TCA cycle which

requires sufficient O_2 to oxidate pyruvate (Mir & Beaudry, 2002). Consequently, raspberries stored in air and in less modified atmospheres (packaging A and C) could have had more precusors for the formations of terpenes, resulting in the higher peak areas of these compounds compared to fruit in packaging B.

Ethyl acetate, a fermentative product associated with a "cloying" flavour (Aaby et al., 2019), was the major ester found in the current trials. Esters, in general, are produced from alcohols and acyl-CoAs via β-oxidation and amino acid pathways (El Hadi et al., 2013; Giuggioli et al., 2015). As the highest peak areas of ethyl acetate were found in clamshell raspberries stored for > 10 days, it could be suggested that the accummulation of ethyl acetate by postharvest ageing could happen more quickly than that caused by the accumulation and esterifications of ethanol in MAP (Giuggioli et al., 2015). Indeed, a sharp increase in ethyl acetate, by > 2000 ppb, was reported in postharvest ageing of raspberries after 4 days in air at 4 ºC (Boschetti et al., 1999). Similarly, C6 aldehydes including hexanal were known as products of fatty acid degradations and stress responses (El Hadi et al., 2013). Although the low O_2 high CO_2 conditions in MAP can be stress factors to fresh produce (Watkins, 2000), the lower peak areas of C6 aldehydes in raspberries packed in MAP B on day 20 and 21 suggested that the technology could alleviate membrane degradation by suppressing the fruit's respiration rates.

3.5. Conclusions

The current study demonstrated the possibility to extend the shelf life of 'Maravilla' raspberries under commercial pre-retail conditions by using MH/MA packaging. An MA of 14-15% O_2 & 7-8% CO_2 and low humidity condition, as developed in a microperforated bag with high WVTR, extended the berry shelf life to 20-21 days. This was achieved by limiting fruit decay by fungi, reducing weight loss and maintaining quality attributes close to those at harvest. The aroma profiles of raspberries were modified with storage time and MAP

conditions, particularly for terpenes/terpenoids. The presence of sanitisers, H_2O_2 and SO_2, did not provide additional benefits to the shelf life of raspberries in this study. There were considerable variations in fruit postharvest behaviour among raspberries from one pallet and across two seasons. It would be critical to further study the sensorial properties and consumer acceptability improvements to justify the use of MAP, along with verification trials in different growing locations and on other varieties.

Chapter 4: Effects of MAP and sanitisation on shelf life of blueberries

4.1 Introduction

The production of 'superfruit' blueberries (*Vaccinium*) has been increasing rapidly in recent decades as a result of marketing strategies and increased consumer awareness about healthy diets (FAOSTAT, 2018; Retamales & Hancock, 2018). Compared to raspberries studied in Chapter 3, blueberries have a relatively longer shelf life, typically between 2-4 weeks at 0-2 ºC, depending on pre-harvest factors and fruit quality at harvest (Perkins-Veazie, 2016b, Retamales & Hancock, 2018). This is related to the slower respiration rates of 2-10 mg CO_2 kg^{-1} h^{-1} (Perkins-Veazie, 2016b), and the presence of a natural, protective layer of epicuticular wax that prevents the fruit from moisture loss and pathogenic attacks (Chu et al., 2018a). However, blueberries in long-term cold storage are still highly susceptible to mould growth, weight loss leading to softening and shrivelling, as well as external and internal darkening (Mitcham, 2007; Retamales & Hancock, 2018). These quality deteriorations have been limiting both market expansion, and the extension of in-store availability of blueberries during off-season periods, as well as consumer satisfaction (Chironi et al., 2017).

CA and MAP have been studied extensively for blueberries, but their practical applications are only widely used for export (Madrid & Beaudry, 2020). High CO_2 concentrations of 10-12% have been recommended to suppress mould growth in berries. The CO_2 level of 15% is often considered as the upper limit as higher concentrations caused softening, discolouration, and development of off-flavours (Forney et al., 2003; Harb & Streif, 2004). In addition, a level of > 2% O_2 is required to prevent hypoxia and fermentation (Retamales & Hancock, 2018). However, CA requires external systems for frequent gas monitoring, and sudden modifications of the surrounding atmospheres could pose abiotic stress and negative effects on fruit quality (Falagán & Terry, 2018). Mismatches between MAP designs and berry

respiration would either fail to reach the recommended CO_2 levels to suppress mould growth or result in too high CO_2 (> 15%) and O_2 depletion during long storage (Huynh et al., 2019).

Combinations of MAP and sanitisation treatments, therefore, have been suggested to be more efficient at extending the storability of blueberries (Huynh et al., 2019). Technologies that have low risks and require low capital investments would be particularly advantageous in commercial settings. In this sense, innovative gaseous sanitisation technologies such as vapour-releasing SO_2 sheets and H_2O_2-releasing pouches allow the products to be placed in liners/MAP for active control against mould growth (de Siqueira Oliveira et al., 2018; Saito et al., 2020). Sulphur dioxide (SO_2) is one of the most powerful antifungal agents in the food industry (Santos et al., 2012), and has been shown to effectively control mould growth in blueberries (Cantín et al., 2012; Rivera et al., 2013; Saito et al., 2020). Hydrogen peroxide (H_2O_2) is a safe and environment friendly sanitiser with higher antimicrobial activity than chlorate, but mainly studied for washing (de Siqueira Oliveira et al., 2018; Yoon & Lee, 2018). Furthermore, microperforation technology and the availability of a range of WVTRs offer superior control of the in-package humidity. Low humidity could prevent moisture condensation that favours mould growth (Horvitz, 2017). Equally, high humidity was shown to enhance the effectiveness of SO_2 against mould growth (Saito et al., 2020), and is essential to maintain the active water vapour for optimal performance of the H_2O_2-releasing product.

Extending the industrially achievable storage periods of blueberries would provide opportunities to expand reach for national and international markets, as well as partly offset the period of fruit unavailability between the blueberry seasons of different production regions. This experiment aimed to evaluate the potential of extending storability of ‘Legacy’ and ‘Powder Blue’ blueberries beyond the average 2-4 weeks in commercial settings, by applying modified humidity MAP technologies in combination with SO_2 or H_2O_2 vapour-releasing sanitisation technologies. The MAP studied were mineral-clay impregnated MAP

with low WVTR and microperforated MAP with high WVTR. These technologies were novel to the industries and have not been trialled on commercial scales.

4.2 Materials and methods

4.2.1 Plant materials, packaging, and storage

This study used 'Legacy' highbush blueberry (*Vaccinium corynbosum*) and 'Powder Blue' rabbiteye blueberry (*Vaccinium ashei*) from a commercial farm in East Devonport in January and February 2020, respectively. The blueberries were manually harvested at market-grade maturity, optically sorted for colours, sizes, and rots, and packed in 125 g vented clamshell punnets. Cardboard trays of blueberries, each made up of 12 punnets, were then precooled at 1.5 ± 0.2 °C. In both trials, 66 cardboard trays of fruit were taken from a pallet that passed quality control at the farm distribution centre and packed on the same date within 4 h. The types of packaging and sanitisers evaluated were listed in Table 4.1. "Unpacked" referred to cardboard trays without an extra external packaging. One cardboard tray was used as a replicate, and there were three replicates for each treatment for each assessment day.

SO_2-releasing sheets (BerryGuard^{TM}, OSKU S.A., Santiago, Chile) were used as recommended by the manufacturer (OSKU S.A, 2018). A sheet of 21 cm x 31 cm was placed between two layers of the punnets within a tray of 1.5 kg blueberries. For H_2O_2-releasing pouches (ChillSafe®), 40 mL of distilled water was injected into the sachets to activate the product. The pouches were placed on top of the punnets when packed. The release of H_2O_2 was verified by peroxide pH test strips (measuring range 1-100 µL L^{-1}) placing in the packages (Precision Laboratories, AZ, USA).

After packing, trays were randomly stacked into one pallet with a base of 12 trays and stored at 1.5 ± 0.2 °C in a commercially used berry cool room with forced-air cooling for up to 10 weeks. The humidity of the storage room (uncontrolled) was 85 ± 2 % RH.

4.2.2 Headspace % O_2 and % CO_2 and respiration rates

The evolution of O_2 and CO_2 inside the MAP-packed blueberry packages were measured by a gas analyser (Dansensor CheckMate 3, Mocon, Ringsted, Denmark) at 1.5 °C in week 2, 4, 6, 8 and 10. Respiration rates of blueberries were measured using closed system experiments slightly modified from the method of Haffner et al. (2002). Approximately 50 g of blueberries from three of the 12 punnets of each replicate were weighed into Mason jars of 236.6 mL volume. The jars were sealed with metal caps with sampling holes covered by rubberised gas sealing strips (Novatech Controls Pty. Ltd., Cheltenham, Australia) and stored at 2 ± 0.5 °C in a pharmaceutical refrigerator. The headspace O_2 and CO_2 concentrations were measured periodically using the gas analyser and plotted against time to calculate O_2 consumption and CO_2 production rates (mg kg^{-1} h^{-1}).

4.2.3 Weight loss and fruit mould assessment

Weight loss was determined as the average of weight differences of two marked punnets between day 0 and the day that cardboard tray was assessed. During storage, one punnet was placed on the upper layer and another on the lower layer. Fruit with visible mould growth, regardless to the severity, were separated from four of the 12 blueberries punnets from each cardboard tray in week 6, 8 and 10. The weight percentages of mouldy fruit were calculated against the total fruit weight.

4.2.4 Sample preparation

Sampling was performed by picking 40 g of blueberries with no visible moulds from six random punnets of each tray. The fruit were blended with 100 mL of distilled water for 30 s. The blended samples were then centrifuged at 3000 *g* for 10 min at 20 °C (Centrifuge 5702 R, Eppendorf, Macquarie Park, Australia) to obtain clear juices.

Table 4.1 Packaging and sanitisation types evaluated in the current study

Treatment	MA at equilibrium *			
	Trial 1 ('Legacy')		Trial 2 ('Powder Blue')	
	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)
Mineral-clay impregnated MAP bags (A)	16.9 ± 1.5	2.0 ± 0.4	18.3 ± 1.0	1.8 ± 0.5
A + H_2O_2-releasing pouches	18.0 ± 1.8	1.8 ± 0.9	18.8 ± 1.7	1.7 ± 0.5
A + SO_2-releasing sheets	16.2 ± 1.7	2.0 ± 0.4	18.8 ± 1.4	1.6 ± 0.9
Microperforated MAP bags (B)	17.0 ± 0.4	4.1 ± 0.4	17.8 ± 0.8	3.4 ± 0.9
B + H_2O_2-releasing pouches	17.9 ± 0.7	3.9 ± 0.9	18.2 ± 0.5	3.4 ± 0.6
B + SO_2-releasing sheets	17.0 ± 1.4	3.4 ± 0.3	17.5 ± 0.5	3.7 ± 0.5
Unpacked = cardboard trays of 12 clamshells	–		–	

* When measured in the current study with 1.5 kg blueberries / bag stored at 1.5 °C, after 4 weeks. The headspace compositions only varied slightly on the following assessment points.

Material sources:

Mineral-clay impregnated MAP bags: PEAKFresh®, Netley, Australia

Microperforated MAP bags: Xtend®, Stepac L.A. Ltd, Tefen, Israel

SO_2-releasing pad BerryGuard™: OSKU S.A., Santiago, Chile, supplied by Biopac, West Burleigh, Australia

4.2.5 Chemical quality attributes

Total anthocyanin contents of blueberries were measured by the differential pH method slightly modified from Denev et al. (2010). Briefly, two portions of the clear blueberry juices, 2 mL each, were diluted to 25 mL, one with a pH 1.0 solution and another with a buffered pH 4.5 solution. The total anthocyanin content expressed as cyanidin-3-glucoside equivalents was calculated using the formula:

$$\text{Anthocyanin content (mg kg}^{-1}\text{ FW)} = \frac{\text{A x MW x DF x 1000}}{\varepsilon \text{ x L}}$$

where $A = (A_{510nm}\text{ pH 1.0} - A_{700nm}\text{ pH 1.0}) - (A_{510nm}\text{ pH 4.5} - A_{700nm}\text{ pH 4.5})$,

MW= cyanidin-3-glucoside molecular weight (449.2 g mol^{-1}),

DF = dilution factor,

ε = cyanidin-3-glucoside molar absorptivity (26 900 L mol^{-1} cm^{-1}),

and L = cell pathlength (1 cm).

pH and total soluble solid contents of the clear blueberry juices were measured by using a portable pH meter (pH 22 LAQUAtwin, Horiba Ltd., Kyoto, Japan) and refractometer (Ade Advanced Optics, Oregon City, US).

Titratable acidity (TA) was determined by titrating 10 mL of clear blueberry juices with NaOH 0.1 M to pH 8.2 (AOAC 942.15). The concentrations of acid in blueberries were calculated as grams of citric acid (predominant acid) per 100 g of fruit.

The ascorbic acid contents of blueberry juices were determined by the iodine titration method (AOAC 967.22) against L-ascorbic acid standards (Merck PTY Ltd., Bayswater, Australia).

4.2.6 Aroma profiles by SPME Arrow/GC-MS

Aroma profiles of blueberry mash were analysed by SPME GC-MS with a method slightly modified from that of Aprea et al. (2009). A 5 mL sample of blueberry mash was

immediately frozen at -18 °C, in 20 mL sealed glass vials after blending of fruit and analysed within one week. Samples were thawed on the date of analysis and kept in the chilled rack (3.5 ± 0.5 °C) of the GC-MS system until analysis within 12 h. The vials were incubated at 40 °C for 5 min, then sampled by a carbon wide range/polydimethylsiloxane fibre (120 µm) (PAL SPME Arrow, CTC Analytics AG, Zwingen, Switzerland) for 30 min at the same temperature. Fibre conditioning was performed at 180 °C for 30 min before runs and for 5 min between runs. Adsorbed volatile compounds were desorbed as the fibre exposed to 220 °C for 2 min in splitless mode (Shimadzu AOC-6000 autosampler, Shimadzu Corp., Kyoto, Japan). GC analysis was carried out with an SH-Rxi-624Sil MS column (30 m, 0.25 mm ID, 1.4 µm df) held at 60 °C for 3 min, ramped up to 220 °C at 8 °C min^{-1} and held for 5 min, then increased to 250 °C at 10 °C min^{-1} and held for another 5 min. Helium was used as the carrier gas at a total flow rate of 20mL min^{-1}. The temperature of GC-MS interface was 250 °C.

Identification of headspace blueberry volatiles was carried out by a mass spectrometer (Shimadzu GCMS-QP2020, Shimadzu Corp.) operating in electron ionisation mode with *m*/*z* range of 20-500. Chromatograms were analysed by GCMS Postrun Analysis (GCMS Solution Ver. 4.50©, Shimadzu Corp.) and compound identifications were performed against NIST17, FFNSC 3 and Wiley9 libraries. Data were reported as mean of peak areas processed with probabilistic quotient normalisation (PQN) method.

4.2.7 Data analysis

Statistical analyses were performed using Minitab 19.1.1 (Minitab, LLC., Pennsylvania, USA) and GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, USA). One-way ANOVA and Tukey's test for multiple pairwise comparisons were used to analyse the treatment differences between unpacked and different MAP applications. Within packed fruit, the

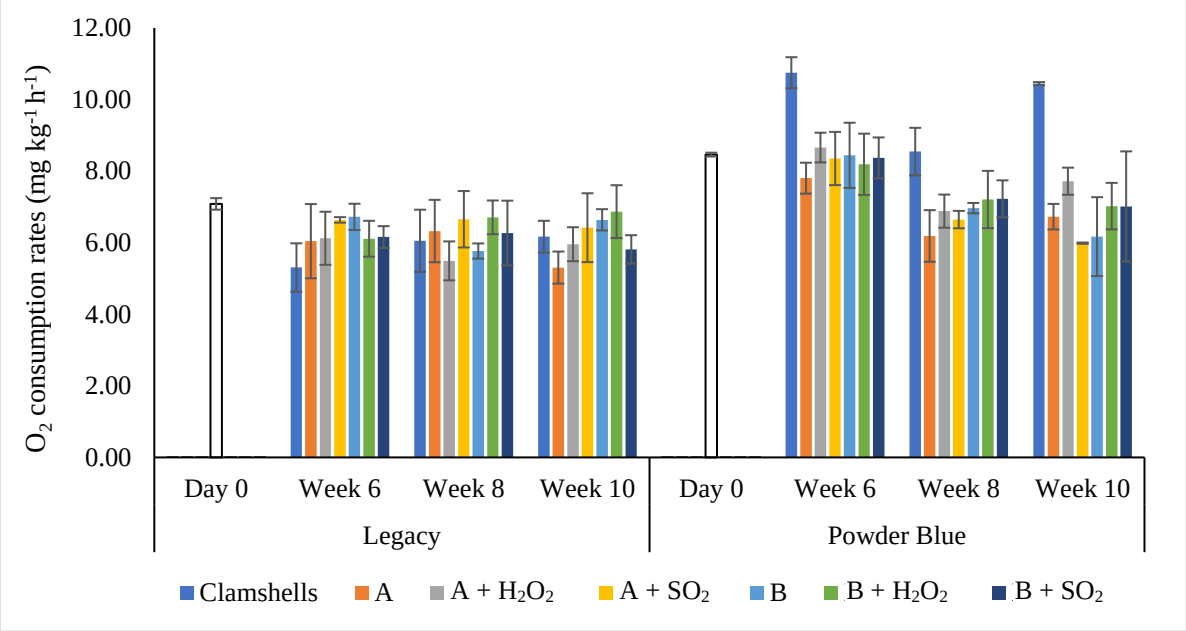
interactions between packaging types and sanitisers were analysed by two-way ANOVA. The significance level for all statistical tests was $p = 0.05$.

4.3 Results

4.3.1 Headspace % O_2 and % CO_2 and respiration rates

The headspace atmospheres of packed blueberry trays were not highly modified and reached equilibria after 4 weeks. Both MAP types resulted in 16-18% O_2, while the CO_2 levels were higher in microperforated bags, being 3.4-4.1%, compared to 1.6-2.0% in the mineral-clay impregnated packaging (Table 4.1). The O_2 and CO_2 compositions did not differ between 'Legacy' and 'Powder Blue' blueberries, although the respiration rates of 'Powder Blue' (8.46 ± 0.05 mg O_2 kg^{-1} h^{-1} and 9.23 ± 0.09 mg CO_2 kg^{-1} h^{-1}) were higher than 'Legacy' (7.08 ± 0.16 mg O_2 kg^{-1} h^{-1} and 6.98 ± 0.05 mg CO_2 kg^{-1} h^{-1}) at harvest.

The effects of packaging and sanitisation on respiration rates differed between the two blueberry cultivars. There was no difference found between packed and unpacked 'Legacy' blueberries ($p > 0.05$). The grand mean values of 'Legacy' respiration rates were 6.16-6.18 mg O_2 kg^{-1} h^{-1} and 6.31-6.94 mg CO_2 kg^{-1} h^{-1} across all samples at three assessment points. In contrast, MAP-packed 'Powder Blue' blueberries had significant lower respiration than unpacked fruit ($p < 0.05$), ranging between 1.69-3.67 mg O_2 kg^{-1} h^{-1} and 2.08-5.29 mg CO_2 kg^{-1} h^{-1} (Figure 4.1). There was no effects resulting from the different types of MAP and sanitisers used ($p > 0.05$).



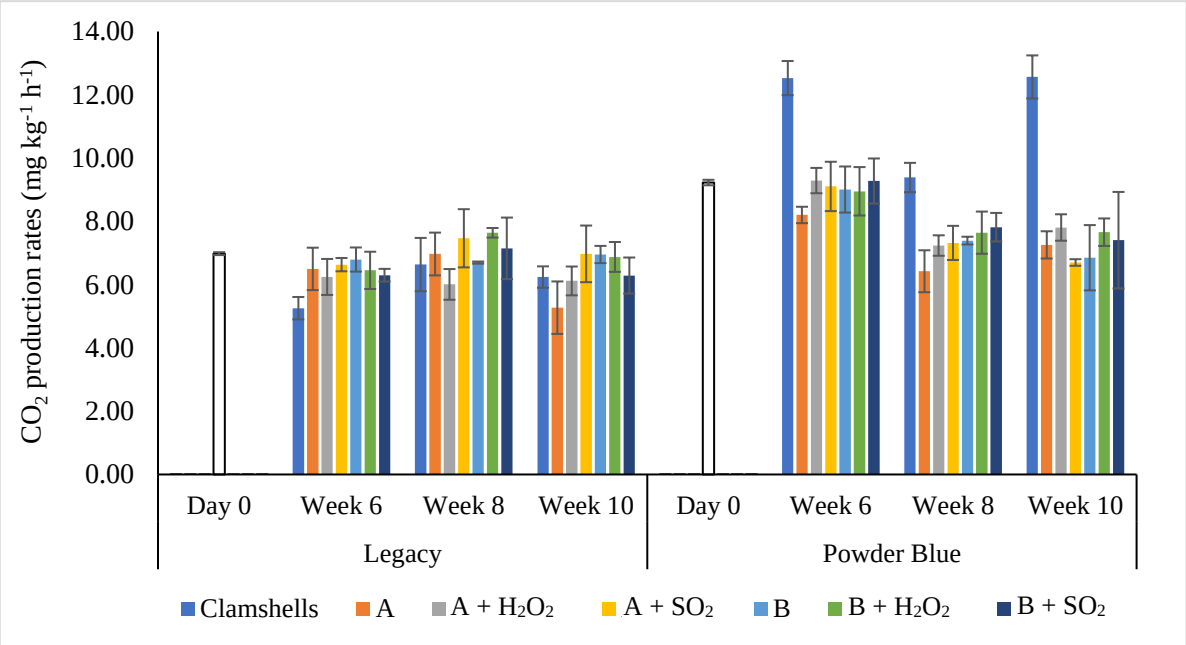


Figure 4.1 Respiration rates at 2 ºC of 'Legacy' and 'Powder Blue' blueberries after storage in different packaging at 1.5 ºC (n = 3, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR, B: microperforateed MAP with high WVTR

4.3.2 Weight loss

Under commercial settings, unpacked blueberries generally lost approximately 2-3% of weight every two weeks, while MAP reduced weight loss by > 4 times during storage (p <

0.05) (Figure 4.2). Within packed blueberries, the choice of packaging and sanitisers individually affected weight loss. Specifically, weight loss was around 1-2.5% lower in mineral-clay impregnated MAP bags, compared to microperforated MAP; and up to 1% higher in samples with H_2O_2-releasing pouches, compared to those with no sanitisation treatment or with SO_2-releasing sheets.

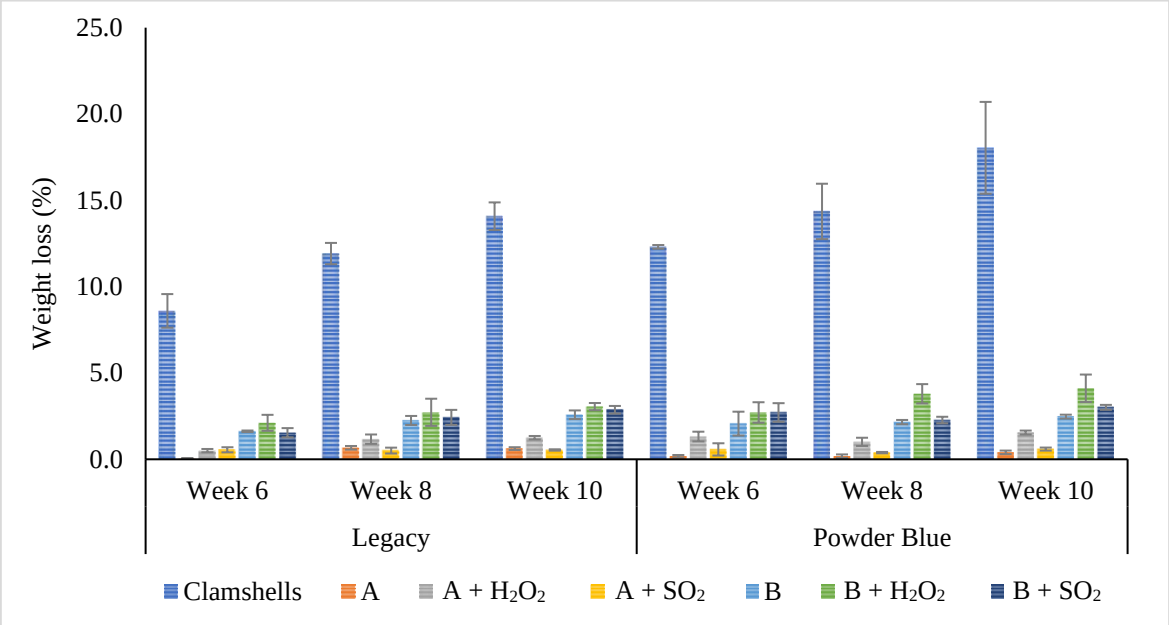


Figure 4.2 Weight loss of 'Legacy' and 'Powder Blue' blueberries after storage in different packaging applications at 1.5 ºC (n = 3, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR, B: microperforateed MAP with high WVTR

4.3.3 Fruit mould

The applications of MAP and sanitisation treatments greatly reduced the weight portions of mouldy fruit in 'Legacy' and 'Powder Blue' blueberries over 6-10 weeks of cold storages ($p < 0.05$) (Figure 4.3). Visible mouldy fruit accounted for nearly 9-14% unpacked blueberries by weights after 6 weeks and reached 25-35% after 10 weeks. In contrast, the weight percentages of mouldy fruit in MAP-packed blueberries remained lower than 5% for 'Legacy' after 6 weeks, and lower than 2% for 'Powder Blue' after 6 and 8 weeks. From the appearances of mouldy fruit, the moulds appeared to be *Alternaria* sp. (greenish grey),

Botrytis (grey) and *Epicoccum* sp. (dense, orange-yellow mycelium at the stem scars). SO_2 was best at controlling mould growth with none found in both trials.

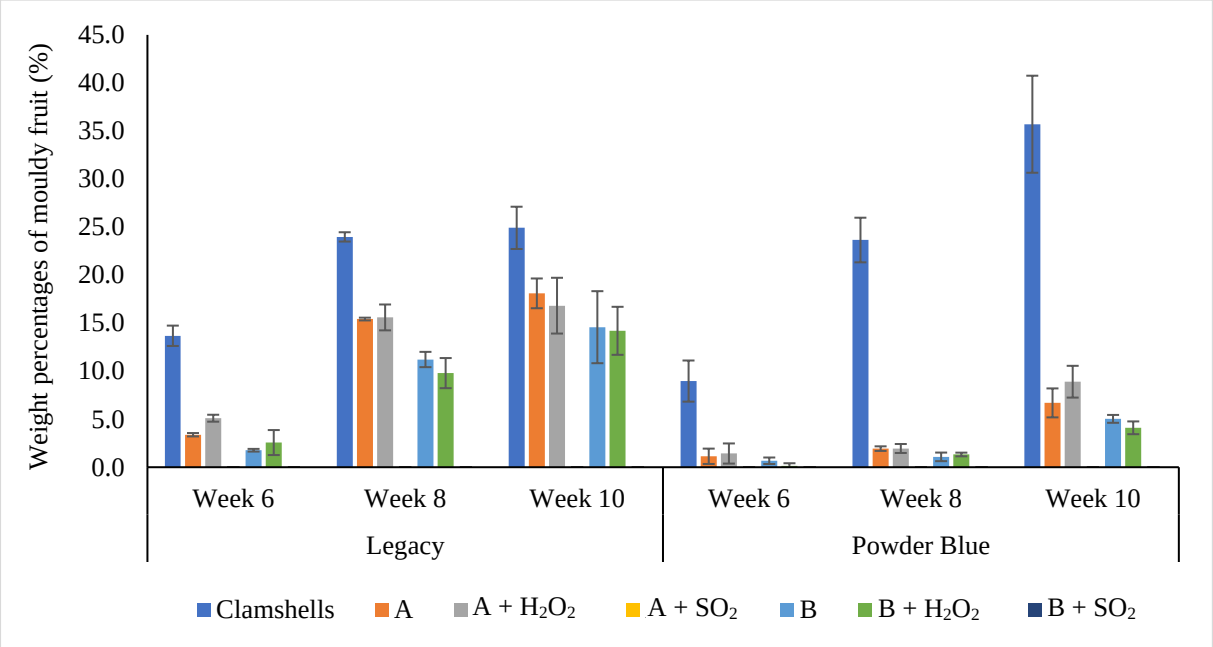


Figure 4.3 Percentages of mouldy fruit by weights in 'Legacy' and 'Powder Blue' blueberries after storage in different packaging applications at 1.5 ºC (n = 3, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR, B: microperforateed MAP with high WVTR

However, there was discolouration at the stem scars, particularly obvious in 'Legacy' (Figure 4.4). The severity of discolouration, indicated by the areas being bleached, varied within one punnet and among 12 punnets from one cardboard tray replicate. There was no difference between the two packaging types ($p > 0.05$), as well as no effects of applying vaporous H_2O_2 in suppressing fungal development ($p > 0.05$). The colour readings of peroxide test strips verified the presence of approximately 10 µL H_2O_2 L^{-1} on day 7 of both trials. The residual levels of SO_2 and H_2O_2 measured on week 6 were below the detection limits of Kitagawa Gas Detector tubes (Air-Met Pty Ltd., Nunawading, Australia), which were 0.1 µL L^{-1} for SO_2 and 0.2 µL L^{-1} for H_2O_2.

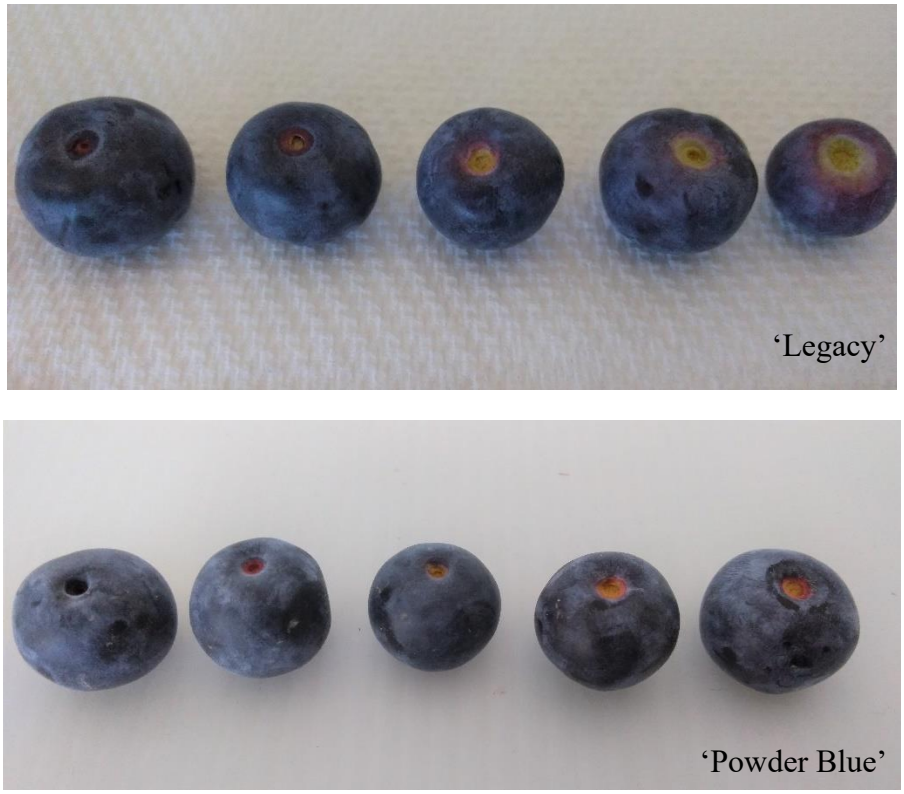


Figure 4.4 Variations in degrees of SO_2 injuries in ‘Legacy’ and ‘Powder Blue’ blueberries (severity increased from left to right)

4.3.4 Chemical quality attributes

Overall, the total anthocyanin contents in blueberries increased during storage ($p < 0.05$) but were not affected by packaging and sanitisation treatments ($p > 0.05$). In fresh ‘Legacy’ and ‘Powder Blue’ blueberries, the total anthocyanin levels measured were 38.70 ± 8.34 and 156.19 ± 6.88 cyanidin-3-glucoside mg kg^{-1}, respectively. Microperforated MAP maintained the anthocyanin contents of ‘Legacy’ blueberries as fresh after 6 weeks, but this effect was not seen in ‘Powder Blue’ blueberries (Figure 4.5). After 10 weeks, all samples had the same anthocyanin contents with grand means of 119.27 mg kg^{-1} (‘Legacy’) and 201.64 mg kg^{-1} (‘Powder Blue’) across seven treatments.

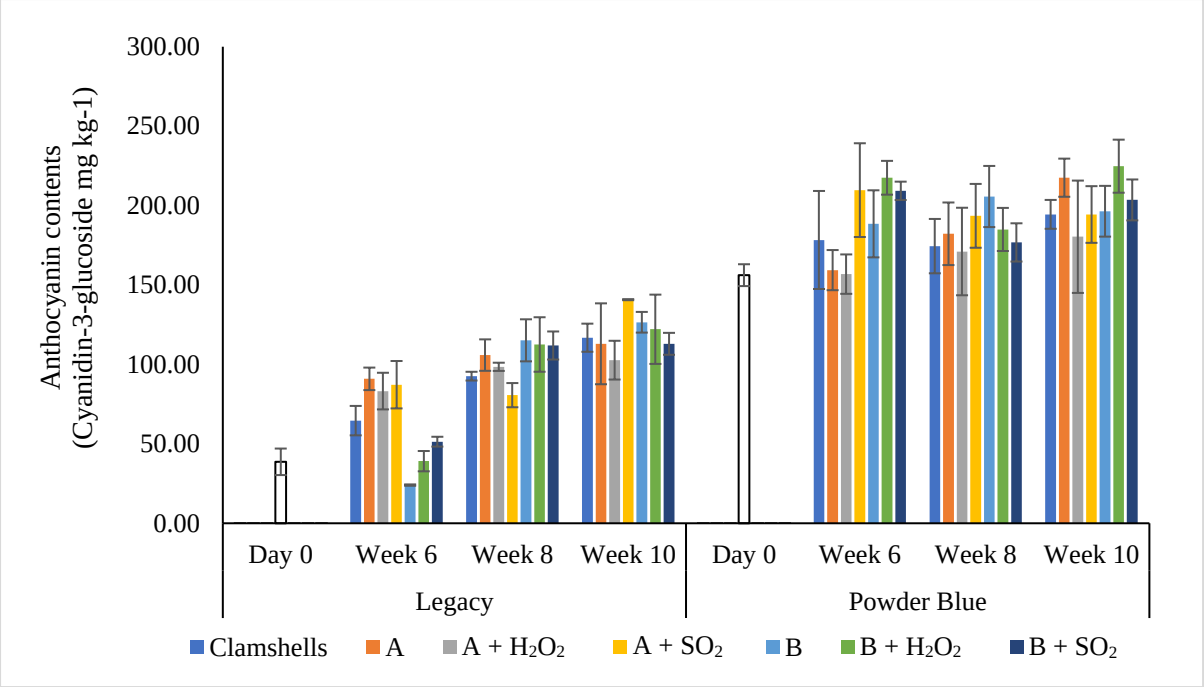


Figure 4.5 Total anthocyanin contents in 'Legacy' and 'Powder Blue' blueberries after storage in different packaging applications at 1.5 ºC (n = 3, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR, B: microperforateed MAP with high WVTR

In contrast, packing blueberries preserved other quality measurements, including pH, TA, and TSS, closest to fresh fruit ($p > 0.05$). This meant a pH 3.68, 0.64% TA and 10.43% TSS in 'Legacy', and a pH 3.30, 0.71% TA and 10.44% TSS in 'Powder Blue' blueberries. In unpacked fruit, TSS increased by 2-4% after storage, possibly resulting from dehydration. Consequently, the TSS/TA ratio also increased in unpacked fruit (Figure 4.6), although the change in TA was minor (up to 0.1%). The complete data of pH, TA and TSS for both trials can be found in Table A2.3 and Table A2.4 of Appendix 2.

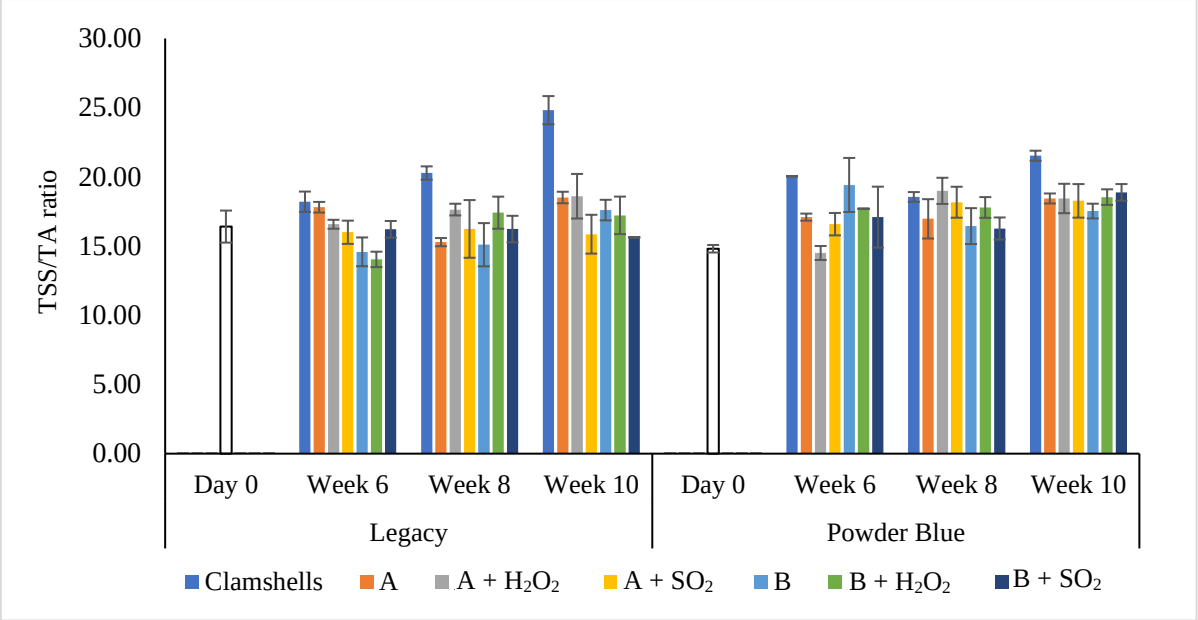


Figure 4.6 TSS/TA ratios in 'Legacy' and 'Powder Blue' blueberries after storage in different packaging applications at 1.5 ºC (n = 3, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR, B: microperforated MAP with high WVTR

4.3.5 Aroma profiles

The main volatile classes found in 'Legacy' and 'Powder Blue' blueberries in this study were aldehydes, ketones and terpenes/terpenoids. The mean values of peak areas of the aroma compounds in 'Legacy' and 'Powder Blue' can be found in Table A2.5 and Table A2.6 of Appendix 2. Overall, the two cultivars shared similarities in the major compounds identified and trends of aroma evolutions. Packaging and sanitisation treatments only had significant effects on terpenes/terpenoids of which peak areas were lower in MA-packed samples, compared to the unpacked ($p < 0.05$) (Figure 4.7). C6 aldehydes including hexanal and 2-hexenal were the most abundant compounds both varieties. Some ketone and terpenes/terpenoids were only detected in the later stages of storage such as neral, 2-undecanone and (Z)-6,10-dimethyl-5,9-undecadien-2-one. There were volatiles only identified in one variety, including caryophyllene oxide in 'Legacy', and 2-heptanone, 2-nonanone, eucalyptol and α-Terpineol in 'Powder Blue'. Ethyl 3-methyl butanoate was only

found in SO_2-treated ‘Legacy’ blueberries stored for 6 and 10 weeks, and in unpacked fruit after 10 weeks, but at ~ 5-8 times smaller peak areas (Table A2.5, Appendix 2). For all treatments, there were high variations between aroma profiles of replicates.

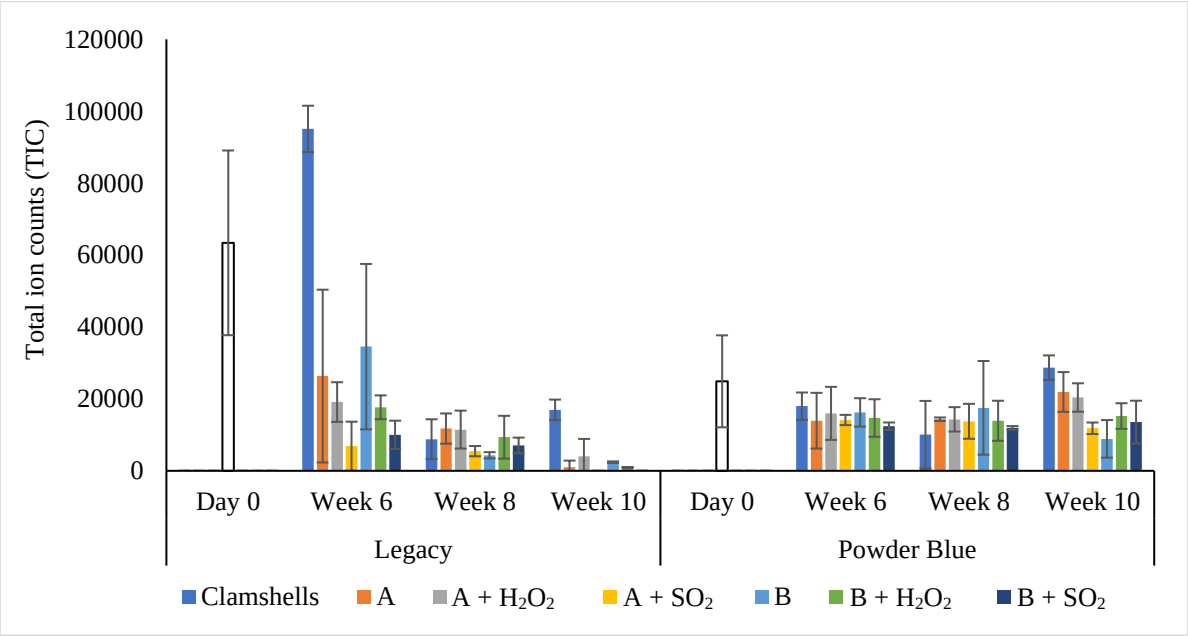


Figure 4.7 Total peak areas of terpenes/terpenoids in ‘Legacy’ and ‘Powder Blue’ blueberries after storage in different packaging applications at 1.5 ºC (n = 3, p = 0.05)

A: mineral-clay impregnated MAP with low WVTR, B: microperforated MAP with high WVTR

4.4 Discussion

Postharvest fungal development, respiration and the associated quality losses have been the major challenges to extend storage life of blueberries and expand market reach (Huynh et al., 2019). This study demonstrated the possibility of MAP applications with moderately modified conditions (16-18% O_2 & 2-4% CO_2) to improve the storability of ‘Legacy’ and ‘Powder Blue’ blueberries in commercial settings to 6 and 8 weeks, as compared to the average 2-4 weeks. The major benefits of MAP in this study were to minimise weight loss and suppress mould growth in blueberries.

The recommended MA condition of 5-10% O_2 & 10-12% CO_2 has been difficult to achieve and maintain throughout storage with most commercial MAP products (Huynh et al., 2019; Madrid & Beaudry, 2020). Low-permeability and non-microperforated packaging would possibly cause O_2 depletion and high CO_2 accumulation (> 15%) that lead to anaerobic respiration, darkening, softening and discolouration (Forney et al., 2003; Harb & Streif, 2004). In contrast, packaging with porous structure and microperforations, like those trialled in the current study, could prevent such circumstances. However they would be unlikely to reach the desirable CO_2 levels of 10-12% because of the moderate blueberry CO_2 production rate of 7.0-9.2 mg kg^{-1} h^{-1} at 2 °C (Figure 4.1) and natural gas permeation (Madrid & Beaudry, 2020).

4.4.1 Relationship of weight loss to packaging conditions

Weight loss is the main cause of softening and shrivelling in blueberries with a maximum permissible level of 5-8% of their initial weights (Paniagua et al., 2014). Blueberries in commercial storage are often subjected to dehydration by force-air cooling that is used maintain the temperature close to 0 °C (Madrid & Beaudry, 2020; Pathare et al., 2012). In this sense, MAP acts as a barrier protecting blueberries from dehydration by forced-air cooling and maintains a high RH within the packages. The extent of protection depends on the packaging's WVTR and perforations/vented areas (Saito et al., 2020), which explains the lower weight loss found for mineral-clay impregnated bag (non-vented) than the microperforated MAP. Meanwhile, blueberries in packages containing the H_2O_2-releasing pouches appeared to have higher weight loss than other packed fruit, regardless of the MAP used. This observation suggested that the moisture-removing ingredient in the H_2O_2 product could have changed the vapour pressure in the packages' headspace (Erickson, 2017).

4.4.2. Impacts of MAP on the incidence of mould

Mould growth is one of the main issues terminating the storability and marketability of blueberries (Huynh et al., 2019; Madrid & Beaudry, 2020). With > 10% reduction of visible mould growth than the unpacked in both cultivars, this study confirmed that MAP with CO_2 levels lower than recommendations still partly controlled mould growth. Packing in MA of 4.8-9.4% CO_2 extended the storage life of 'Northblue' blueberries by 9 days at 3 ºC (Koort et al., 2018). In a trial with nine highbush blueberries cultivars, CA with 6% CO_2 reduced berry decay by 5% after 8 weeks at 0 ºC, which was comparable to treatments with 12-19% CO_2 (Alsmairat et al., 2011). There were, however, great variations in decay incidences found among cultivars when stored in CO_2-enriched atmospheres, in agreement with other studies (Koort et al., 2018; Rodriguez & Zoffoli, 2016; Schotsmans et al., 2007). In this study, 'Powder Blue' blueberries appeared to benefit more from MAP than 'Legacy' with < 2% decay after 8 weeks, while storage life of 'Legacy' was ended after 6 weeks. At market-grade maturity, rabbiteye blueberries were suggested to have better storability than highbush blueberries owing to their firmer and tougher skins (Silva et al., 2005). Higher contents of cell wall components, i.e. fibres and complex polysaccharides, were found in rabbiteye blueberries 'Climax', 'Premier' and 'Tiffblue', compared to highbush cultivars 'Bluecrop' and 'Jersey'. Additionally, environmental factors such as weather and locations could also impact the fruit performance during storage (Prange & DeEll, 1997).

4.4.3 Impacts of vaporous sanitisers on the incidences of mould

Applications of multiple hurdle technologies such as MAP combined with sanitisation have been proposed to better extend berry shelf life than single treatments (Huynh et al., 2019). However, the combinations of vaporous H_2O_2 and SO_2 products with MAP in this study did not improve the storability of blueberries. The weights percentages of mouldy fruit in blueberry trays packed with H_2O_2-releasing pouches were not different from those packed in

MAP only. This was possibly because the storage periods were longer than the durations that the product remained active (~30 days). In contrast, SO_2 successfully controlled mould growth with no moulds found in both trials at all assessment points. According to the product datasheet, concentrations of 3-6 μL L^{-1} were detected in a pallet of blueberries covered with five 520 cm x 38 cm BerryGuard™ sheets packed in LDPE pallet shroud with 0.1% ventilated area (OSKU S.A, 2018). However, the dose of SO_2 used in this study caused bleaching at berry stem scars, discolouration and softening. These adverse effects have been previously linked with excessive SO_2 treatments in 'Tifblue' rabbiteye blueberries (T. Kim et al., 2010) and 'Emerald' highbush blueberries (Saito & Xiao, 2017). SO_2 has been suggested to oxidise the blueberries wax bloom, decrease pH, and increase surface acidity that changed the colour of anthocyanins from deep purple to red and gave the fruit a vivid appearance (Kim et al., 2010). These explanations were in line with the current findings that blueberries packed with SO_2-releasing sheets had lower pH and higher TA than other treatments. Furthermore, the injuries were less severe in 'Powder Blue' compared to 'Legacy', possibly because of firmer skins of rabbiteye cultivars (Silva et al., 2005). The varied severities of damage among blueberries within one replicate/variety possibly resulted from other preharvest factors that could affect the fruit properties. Examples could be cell wall compositions, pH and other factors that vary as a function of maturity (Lobos et al., 2018; Vicente & Sozzi, 2007)

4.4.4 Influences of treatments on respiration

CA/MA has been showed to extend shelf life of blueberries by suppressing their respiration rates during storage (Falagán et al., 2020), but little is known about the respiration patterns after moving out from CA/MA. Rapid increases in respiration post-CA/MA were noticed in some produce including raspberries (Harb & Streif, 2004; Zagory & Kader, 1989), and could affect subsequent storage duration and quality at retails and in households. This adverse

effect was not observed in the current study where the post-MAP respiration rates of packed blueberries were indifferent ('Legacy') or lower ('Powder Blue') than the controls (Figure 4.1). Overall, the respiration rates of blueberries in this study were close to reported values in literature (2-10 mg CO_2 kg^{-1} h^{-1}) (Perkins-Veazie, 2016b), although there were significant variations between three assessment days.

4.4.5 Impacts of packaging on blueberry anthocyanins

Blueberries are marketed and favoured for their high anthocyanin contents and sweet, intense flavours (Gilbert et al., 2015; Stevenson & Scalzo, 2012). The anthocyanin levels found in this study, 38.70-119.27 mg kg^{-1} ('Legacy') and 156.19-201.64 mg kg^{-1} ('Power Blue'), were lower than reported values on the same varieties (Stevenson & Scalzo, 2012). This observation perhaps resulted from the analytical method. In both highbush and rabbiteye blueberries, anthocyanins are concentrated in the fruit skins (S.Y. Wang et al., 2012) and can be bound to cell wall components including pectin (H. Chen et al., 2015). The method herein using water extraction and centrifugation would, therefore, mainly obtain free anthocyanins in water-soluble pectin. Several other factors in analytical procedures as well as cultivation practices, environmental growth condition, and fruit maturity could also have a role in the differences in anthocyanin contents found in different studies (Routray & Orsat, 2011).

The changes in anthocyanin contents during 6-10 weeks of cold storage differed between 'Legacy' (increased) and 'Powder Blue' (mainly unchanged) blueberries. Different trends in anthocyanins changes were found subject to cultivars and maturities at harvest (Kalt et al., 2003; Routray & Orsat, 2011). Postharvest biosynthesis of anthocyanins and other phenolic compounds were proposed to use carbon skeletons of organic acids (Kalt et al., 1999), which was consistent to the decreases in TA in 'Legacy'. Based on this mechanism, microperforated MAP with lower O_2 conditions could have suppressed fruit metabolisms, leading to slower increases in anthocyanin contents in 'Legacy' in week 6, as well as higher TA and lower pH

in both trials. Additionally, increases in water-soluble pectin were reported during storage due to the activities of cell wall degrading enzymes (H. Chen et al., 2015). Therefore, there could be more water-extractable anthocyanins from the stored blueberries using the same analytical method.

4.4.6 Influences of packaging on volatiles

Flavour intensity is one of the key determinants of consumer overall liking of blueberries, together with sweetness and texture (Gilbert et al., 2015). The volatiles identified in this study were previously reported in the same cultivars (Beaulieu et al., 2014; Cheng et al., 2020), including those most important ones to blueberry flavours such as terpenes/terpenoids, C6 aldehydes and 2-undecanone and 2-heptanone (Gilbert et al., 2015). Although the aroma profiles of blueberries have been studied extensively (Sater et al., 2020), the effects of CA/MA and storage on aroma have not been well described (Huynh et al., 2019; Sater et al., 2020). Flavours were shown to attract the highest preferences in the larger (61%) population segments in a consumer-assisted study for quality traits selection for blueberries (Gilbert & Olmstead, 2014). Therefore, optimisation of postharvest processes to better maintain the flavour of blueberries would be useful. This study found significant lower total peak areas for terpenes/terpenoids in MA-stored blueberries, compared to vented clamshell punnets. Terpenes/terpenoids have been reported to contribute to the floral, grassy, minty flavours in blueberries (Cheng et al., 2020). The syntheses of plant terpenes/terpenoids require carbon skeletons generated in the tricarboxylic acid cycle (Mir & Beaudry, 2002). Consequently, the lower peak areas of terpenes/terpenoids in packed fruit could be a result of lower O_2 conditions in MAP which reduced pyruvate oxidation. Similar observation was found in blackcurrants where terpenes syntheses were suppressed in three weeks in low O_2, high CO_2 conditions (Harb et al., 2008).

A balance of high sugars and high acids is important to achieve desirable berry flavours with TSS/TA ratios often used to predict fruit taste. High sugars, low acids were linked to a bland taste, while low sugars, high acids gave sour berries (Kader, 1991). Therefore, the higher TSS/TA ratios in unpacked fruit and the lower ratios in SO_2-treated blueberries as observed herein could imply modifications in fruit tastes. However, how the changes in TSS/TA ratios and aroma profiles link to sensory perception would require further studies, as instrumental measurements were shown to not fully reflect blueberry taste and eating quality (Saftner et al., 2008).

4.5 Conclusion

Overall, the findings from this study suggested that shelf life extension of blueberries by MAP beyond the average 2-4 weeks is possible, although the extent of improvements would be subject to cultivars and fruit quality at harvest. MA of 16-18% O_2 & 2-4% CO_2 by cardboard-tray MAP liners extended the shelf life of 'Legacy' blueberries to 6 weeks and 'Powder Blue' blueberries to 8 weeks in commercial settings. The major benefits of MAP were to minimise weight loss and partially suppress mould growth. Sanitisation by H_2O_2-releasing pouches would be more suitable for routine sanitisation of storage facilities than in long-storage MAP. While SO_2 effectively controlled mould growth, matching SO_2 levels and MAP ventilations to avoid the adverse effects would be crucial when considering using the sanitiser. In addition, expanding the use of MAP in berry industry would require further understandings in sensorial properties of the stored fruit, especially flavours which could exhibit great variability even within fields.

Chapter 5: Perforated modified atmosphere packaging to extend shelf life of blueberries and raspberries

5.1 Introduction

Blueberries (*Vaccinium*) and raspberries (*Rubus idaeus* L.) are highly valuable yet perishable crops with an increasing global demand and production (FAOSTAT, 2018; Retamales & Hancock, 2018; Sobekova et al., 2013). Their moderate to high respiration rates, delicate, fragile structures together with high susceptibility to fungal infections and rapid quality loss have limited their storability and market expansion (Huynh et al., 2019), as well as increased food waste at retail and in households (do Nascimento Nunes, 2009b). Under current best industrial practices, the average storage life achieved is still around 2-4 weeks for blueberries and 5-7 days for raspberries (Huynh et al., 2019; Madrid & Beaudry, 2020).

CA and MAP have been shown to improve the storability of blueberries (Alsmairat et al., 2011; Koort et al., 2018; Paniagua et al., 2014) and raspberries (Haffner et al., 2002; Siro et al., 2006). The major benefits of CA/MA are to reduce weight loss, suppress mould growth and delay quality losses such as darkening in raspberries. Generally, 5% O_2 and 15% CO_2 could be considered as the critical levels for both berries as lower O_2 and/or higher CO_2 caused softening, discolouration and development of off-odours (Forney et al., 2003; Harb & Streif, 2004; Joles et al., 1994). Pallets wrapped in shrouds of film to induce MA have been used commercially for exportation of blueberries and long-distance distribution of raspberries (Madrid & Beaudry, 2020). However, matching the berry respiration rates with the gas exchange that needs to be achieved has been challenging when applied with wrapped cardboard trays or pallets, and therefore, limited the extensive use of MAP. Indeed, most commercially available MAP is not able to attain and maintain the recommended levels for these berries of 5-10% O_2 and 10-15% CO_2 throughout storage (Huynh et al., 2019; Madrid & Beaudry, 2020). Low levels of CO_2 or high O_2 do not successfully control mould growth

and suppress fruit respiration. Conversely, insufficient gas exchange through the packaging leads to O_2 depletion and excessive CO_2 accumulation. Alternatively, retail MAP in the form of non-vented berry punnets sealed with perforated lid films could be a potential approach to maintain the MA at the acceptable levels. The smaller quantity of fruit and film areas would allow better estimation and optimisation of the numbers and/or sizes of perforations. In addition, MAP traysealers also provide options to replace the air (~21% O_2) with a predetermined atmosphere (active MAP) (Wandelen, 2011), which could be useful to accelerate the development of low O_2 and high CO_2 conditions.

This experiment aimed to evaluate the potential of using perforation-mediated MAP as retail packaging to extend the storability beyond the average 2-4 weeks for blueberries, and 5-7 days for raspberries. Preliminary trials showed high respiration rates of raspberries (19.50 mg O_2 kg^{-1} h^{-1} and 23.81 mg CO_2 kg^{-1} h^{-1}) resulting in rapid O_2 reduction and CO_2 accumulation in the package headspace. This observation suggested that active MAP, with an initially reduced O_2 level and/or elevated CO_2, would be unlikely to benefit the storage of raspberries. In contrast, blueberries had moderate respiration rates (6.69 mg O_2 kg^{-1} h^{-1} and 6.86 mg CO_2 kg^{-1} h^{-1}) and required a relatively longer time to establish MA via fruit respiration. Therefore, combinations of five initial atmospheres (four active and one passive MAP) and three levels of perforations per package were studied for blueberries, while air with seven levels of perforations were studied for raspberries.

5.2 Materials and methods

5.2.1 Plant materials, packaging and storage

'Legacy' highbush blueberry (*Vaccinium corynbosum*) and 'Maravilla' raspberry (*Rubus idaeus* L.) were manually harvested at market-grade maturity from a commercial farm in East Devonport, Tasmania, in January and March 2020, respectively. The blueberries were picked in buckets, then optically sorted at 10 °C (the operational temperature of the blueberry

packing room) for colour, size, and rots before being packed in 125 g clamshells. Raspberries were hand-picked and packed in the field. Both berries were pre-cooled at 1.5 ± 0.2 °C before transported to the laboratory in 1.5 h. The temperature of the fruit at arrival was 4.2 ± 0.5 °C. A total of 54 punnets of blueberries and 48 punnets of raspberries were used.

Packing was performed using a Multivac T100 MAP traysealer (Multivac, Keilor Park, Australia). Approximately 125 g of non-mouldy, non-damaged berries mixed from three punnets were packed in 1 L-polypropylene trays with polyethylene lamination (270 mm x 170 mm) (Alto Packaging Ltd., South Granville, Australia). The lidding film was made from biaxially oriented polypropylene (BOPP) with six 70-µm perforations per film at a spacing of one every 25 mm (Harden Packaging Ply Ltd., West Heidelberg, Australia). For blueberries, five initial atmospheres were studied (A: 10% O_2 & 9% CO_2, B: 17% O_2 & 0% CO_2, C: 14.5% O_2 & 0% CO_2, D: 8.5% O_2 & 0% CO_2 and E: air, 20.7% O_2 & 0% CO_2) combined with three levels of perforations (2, 4 and 6) per pack. The atmospheric conditions A-D required air removal and gas flushing by operating the traysealer at an evacuation pressure of 200 kPa and a flushing pressure of 800 kPa. These pressures were obtained from preliminary trials to effectively achieve the desirable atmospheres without damaging the fruit during the evacuation stage. For raspberries, only air (20.7% O_2 & 0% CO_2) was used as the initial atmosphere and seven levels of perforations were studied (0 to 6). The numbers of perforations were controlled by sealing with double layers of Sellophane tape. Berries in vented clamshells designed for 125 g fruit were used as negative controls. Samples were prepared in three replicates for blueberries and four replicates for raspberries due to the greater natural variations in decay incidences observed in raspberries in preliminary trials. The blueberries and raspberries were stored at 2 °C (similar to the operational temperature of the storage sites to best simulate industrial conditions) for 8 weeks and 11 days, respectively. “Fresh” berries referred to the day 0 fruit.

5.2.2 Headspace % O_2 and % CO_2 and respiration rates

The evolution of O_2 and CO_2 inside the MAP-packed blueberry packages were measured by a gas analyser (Dansensor CheckMate 3, Mocon, Ringsted, Denmark) every two days for raspberries. For blueberries, the headspace was checked every two days during week 1 and every week afterwards as equilibria were achieved.

Respiration rates of berries were measured using closed system experiments (Haffner et al., 2002). Approximately 50 g of blueberries or 40 g of raspberries were sealed in Mason jars of 236.6 mL volume with metal caps and stored at 2 ± 0.5 °C in a refrigerator. The caps had sampling holes covered by rubberised gas sealing strips (Novatech Controls Pty. Ltd., Cheltenham, Australia) to allow periodical headspace sampling for O_2 and CO_2 concentrations. The O_2 and CO_2 levels were plotted against time to calculate O_2 consumption and CO_2 production rates (mg kg^{-1} h^{-1}).

5.2.3 Weight loss and fruit mould assessment

Weight loss was determined as the weight differences between day 0 and the assessment day. Berries with visible moulds, regardless to the severity, were separated. The weight percentages of mouldy fruit were calculated against the total fruit weight.

5.2.4 Pre-analytical sample preparation

Approximately 40 g of berries, with no visible moulds, were blended with 100 mL of distilled water for 30s. The blended samples were then centrifuged at 3000 *g* for 10 min at 20 °C (Centrifuge 5702 R, Eppendorf, Macquarie Park, Australia) to obtain clear juices.

5.2.5 Chemical quality attributes

Total anthocyanin contents of berries were measured by the differential pH method from Denev et al. (2010) . A 2 mL aliquot of clear berry juice was diluted to 25 mL with a pH 1.0

solution or a buffered pH 4.5 solution. The total anthocyanin content, expressed as cyanidin-3-glucoside equivalents, was calculated using the formula:

$$\text{Anthocyanin content (mg kg}^{-1}\text{ FW)} = \frac{\text{A x MW x DF x 1000}}{\varepsilon \text{ x L}}$$

where $A = (A_{510nm}\text{ pH 1.0} - A_{700nm}\text{ pH 1.0}) - (A_{510nm}\text{ pH 4.5} - A_{700nm}\text{ pH 4.5})$,

MW= cyanidin-3-glucoside molecular weight (449.2 g mol^{-1}),

DF = dilution factor,

ε = cyanidin-3-glucoside molar absorptivity (26 900 L mol^{-1} cm^{-1}),

and L = cell pathlength (1 cm).

pH and total soluble solid contents of the clear berry juices were measured using a portable pH meter (pH 22 LAQUAtwin, Horiba Ltd., Kyoto, Japan) and a refractometer (Ade Advanced Optics, Oregon City, US).

Titratable acidity (TA) was determined following the AOAC 942.15 method. A 10 mL sample of clear berry juices was titrated with NaOH 0.1M to pH 8.2. TA values were expressed as grams of citric acid (predominant acid) per 100 g of fruit.

The ascorbic acid contents of blueberry juices were determined by the iodine titration method (AOAC 967.22) against L-ascorbic acid standards (Merck PTY Ltd., Bayswater, Australia).

5.2.6 Headspace analysis by SPME Arrow/GC-MS

A 5 mL sample of blended berries was immediately frozen at -18 °C in 20 mL sealed glass vials and analysed within three day using SPME GC-MS following the method of Aprea et al. (2009) with slight modifications. Samples were thawed on the date of analysis and kept at 3.5 ± 0.5 °C during GC-MS runs within 12h. The vials were incubated at 40 °C for 5 min then exposed to a carbon wide-range/polydimethylsiloxane fibre (120 µm) (PAL SPME Arrow, CTC Analytics AG, Zwingen, Switzerland) for 30 min at the same temperature. The

desorption of volatile compounds was carried out at 220 °C for 2 min in splitless mode following by GC analysis with an SH-Rxi-624Sil MS column (30 m, 0.25 mm ID, 1.4 µm df). The instrument was programmed to hold at 60 °C for 3 min, ramp up to 220 °C at 8 °C min^{-1} and hold for 5 min, then increase to 250 °C at 10 °C min^{-1} and hold for another 5 min. The carrier gas used was helium at a total flow rate of 20 mL min^{-1}. Fibre conditioning was performed at 180 °C for 30 min before runs and for 5 min between runs.

The berry volatiles were passed through a mass spectrometer (Shimadzu GCMS-QP2020, Shimadzu Corp.) operating in electron ionisation mode with *m*/*z* range of 20-500 for identifications. The temperature of the GC-MS interface was 250 °C. Chromatograms were analysed by GCMS Postrun Analysis (GCMS Solution Ver. 4.50©, Shimadzu Corp.) against NIST17, FFNSC 3 and Wiley9 libraries. The peak areas were processed with the probabilistic quotient normalisation (PQN) method.

5.2.7 Data analysis

Minitab 19.1.1 (Minitab, LLC., Pennsylvania, USA) was used for one-way ANOVA and Tukey's test for multiple pairwise comparisons to analyse the treatment differences. Pearson's correlation coefficient was performed between the means of the weight percentages of mouldy fruit or GC-MS peak areas and headspace O_2/CO_2 levels. Two-way ANOVA was performed in GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, USA) to study the interactions between the initial MAP atmospheres and the number of perforations on O_2/CO_2 levels and mould growth in blueberry packages. The significance level for all statistical tests was $p = 0.05$.

5.3 Results

5.3.1 Headspace % O_2 and % CO_2 and respiration rates

The levels of O_2 and CO_2 developed in the blueberry trays were greatly affected by the initial atmospheres, the numbers of perforations and their interactions (Table 5.1). Most packages reached their equilibria atmospheres after 4 days (Figure 5.1), except for D4, D6, E2 and E4, which required 8 days. No treatment resulted in extreme conditions (< 1% O_2 or > 20% CO_2) in the blueberry trays after 8 weeks at 2 °C. The combinations that achieved and maintained the atmospheres close to the targeted ranges of 5-10% O_2 & 10-15% CO_2 were A2 (10% O_2 & 9% CO_2 with 2 perforations), B2 (17% O_2 & 0% CO_2 with 2 perforations), C2 (14.5% O_2 & 0% CO_2 with 2 perforations) and D4 (8.5% O_2 & 0% CO_2 with 4 perforations).

For raspberries, the headspace O_2 reduced and CO_2 built up gradually over the 11 days of storage at rates corresponding to the numbers of perforations (Figure 5.2). Only packages with 6 perforations maintained the headspace in the target range of > 5% O_2 and < 15% CO_2, throughout 11 days at 2 °C. Other samples had CO_2 levels higher than 15% after 6-8 days, and O_2 depletion (< 1%) occurred in packages with no and 1 perforation.

Table 5.1 Headspace O_2/CO_2 levels and fungal decay in stored blueberries

Packaging designs *	Headspace % O_2	Headspace % CO_2	% Fungal decay (w/w)
A2	9.51 ± 1.80 ^{fgh}	11.23 ± 1.91 ^{bc}	5.50 ± 1.50 ^{cd}
A4	16.20 ± 0.56 ^{abcd}	4.77 ± 0.51 ^{efg}	8.02 ± 0.63 ^{bcd}
A6	17.33 ± 0.81 ^{ab}	3.83 ± 0.76 ^{fg}	6.50 ± 1.38 ^{bcd}
B2	11.93 ± 1.10 ^{defg}	8.93 ± 1.03 ^{cde}	3.73 ± 0.35 ^{d}
B4	15.17 ± 0.45 ^{bcde}	6.13 ± 0.67 ^{def}	5.81 ± 1.23 ^{bcd}
B6	14.27 ± 1.27 ^{bcdef}	6.90 ± 1.28 ^{cdef}	7.87 ± 3.61 ^{bcd}
C2	7.09 ± 2.23 ^{gh}	13.80 ± 2.00 ^{ab}	2.54 ± 0.51 ^{d}
C4	12.33 ± 2.30 ^{cdef}	8.93 ± 2.15 ^{cde}	11.64 ± 2.36 ^{abc}
C6	17.13 ± 1.58 ^{abc}	4.03 ± 1.56 ^{fg}	11.87 ± 2.69 ^{ab}
D2	4.67 ± 0.27 ^{h}	16.40 ± 0.44 ^{a}	8.35 ± 0.88 ^{bcd}
D4	10.29 ± 3.46 ^{efg}	10.87 ± 3.35 ^{bcd}	9.97 ± 0.26 ^{abc}
D6	15.33 ± 1.89 ^{bcd}	5.47 ± 1.75 ^{ef}	7.19 ± 3.03 ^{bcd}
E2	13.40 ± 1.40 ^{bcdef}	7.43 ± 1.62 ^{cdef}	7.01 ± 2.06 ^{bcd}
E4	15.54 ± 1.97 ^{bcd}	4.84 ± 2.23 ^{ef}	15.25 ± 0.17 ^{a}
E6	16.77 ± 0.31 ^{abcd}	4.40 ± 0.36 ^{efg}	11.62 ± 4.28 ^{abc}
Vented clamshell punnets	20.70 ± 0.00 ^{a}	0.00 ± 0.00 ^{g}	7.37 ± 1.48 ^{bcd}

Means followed with different superscripts are significantly different at $p < 0.05$ ($n = 3$).

Measurements were conducted in week 5 for clamshell blueberries and in week 8 for MAP-packed blueberries.

***: Initial atmospheres:**

A: 10% O_2 & 9% CO_2 C: 14.5% O_2 & 0% CO_2 E and vented clamshell punnets: 20.7% O_2 & 0% CO_2

B: 17% O_2 & 0% CO_2 D: 8.5% O_2 & 0% CO_2

The number following the letter referred to the number of perforations in the lid film.

Vented clamshell punnets:

Blueberries: made from PET, 125 mm x 120 mm x 35 mm with 28 holes of 5 mm x 10 mm (Multisteps Pty Ltd., Devonport, Australia)

Raspberries: made from PET, 125 mm x 120 mm x 45 mm with 28 holes of 5 mm x 10 mm (Multisteps Pty Ltd., Devonport, Australia)

Two-way ANOVA	**Significance**		
Interaction	**	***	**
Initial atmosphere	****	****	****
Perforations	****	****	****

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ **** $p < 0.0001$

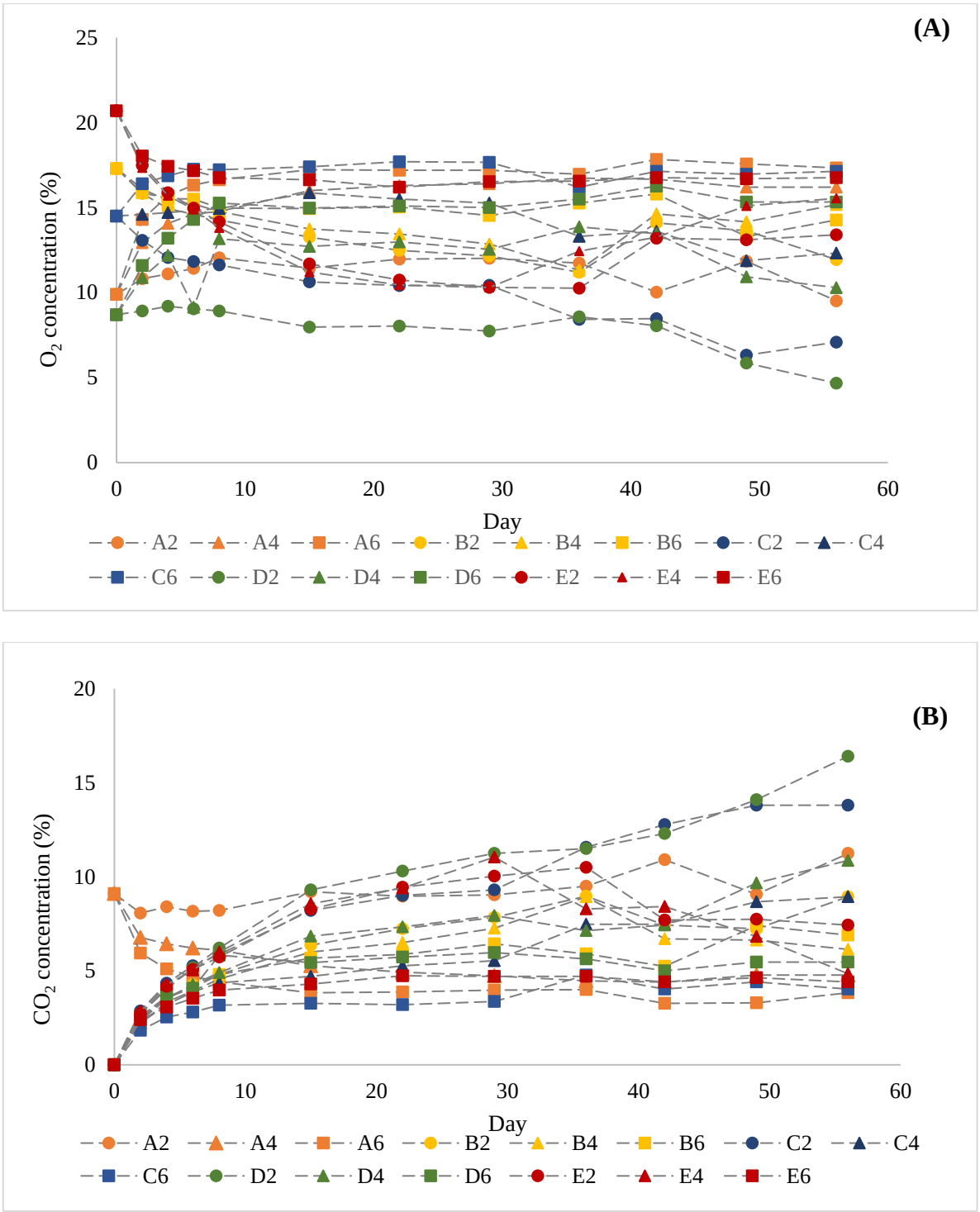
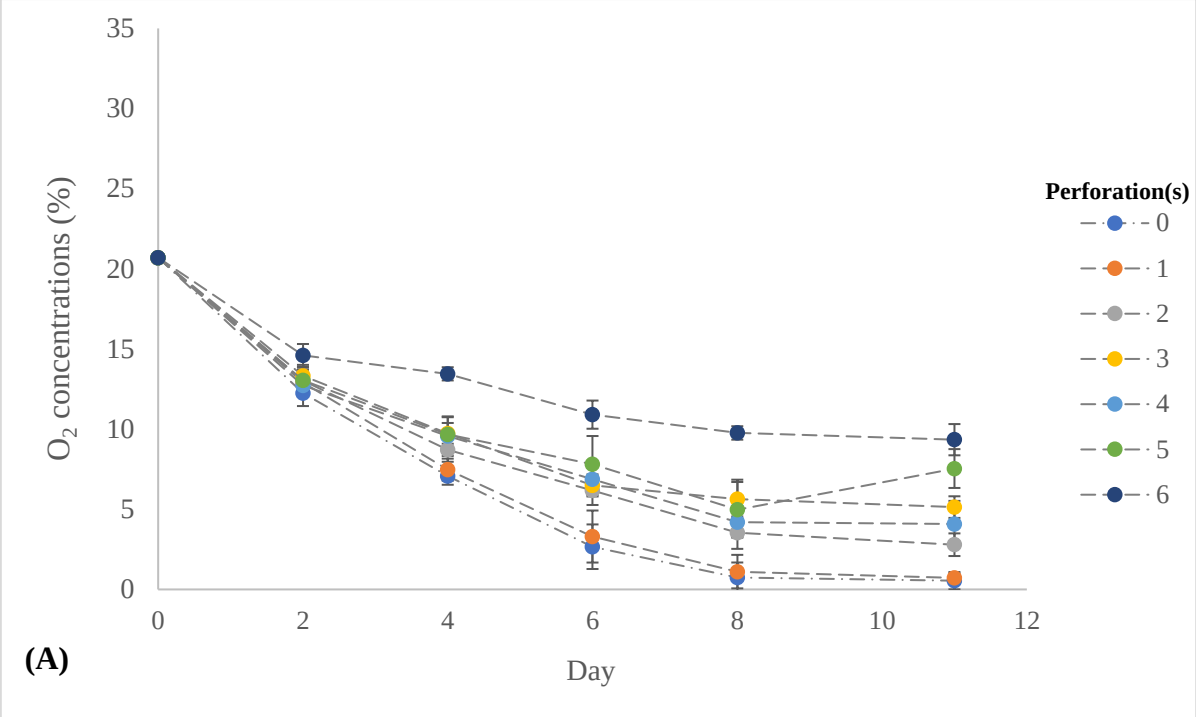


Figure 5.1 Headspace O_2 (A) and CO_2 (B) concentrations of blueberry packages during storage (n =3)

The letters in treatment name referred to initial atmospheres: A: 10% O_2 & 9% CO_2, B: 17% O_2 & 0% CO_2, C: 14.5% O_2 & 0% CO_2, D: 8.5% O_2 & 0% CO_2 and E: air, 20.7% O_2 & 0% CO_2. The following number referred to the number of perforations.



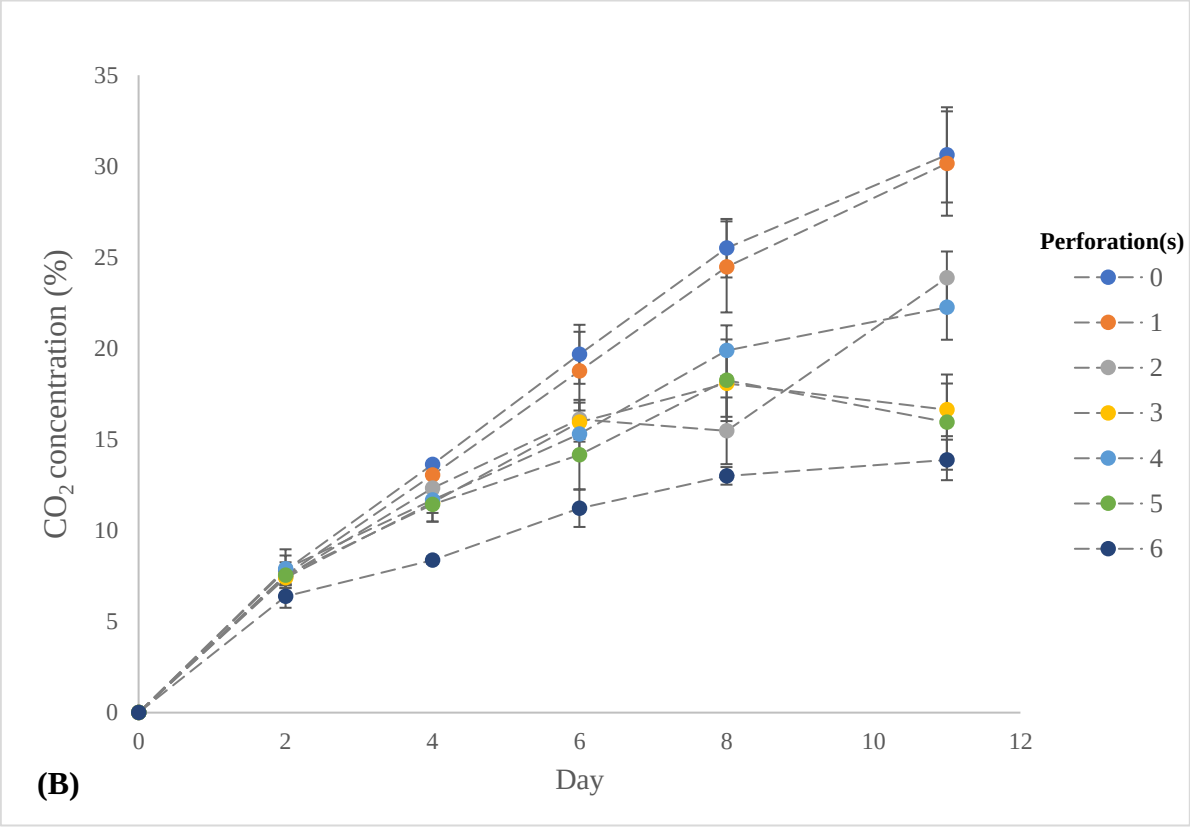


Figure 5.2 Headspace O_2 (A) and CO_2 (B) concentrations of raspberry packages during storage (n = 4)

Fresh ‘Legacy’ blueberries at the start of storage had moderate respiration rates of 7.08 ± 0.16 mg O_2 kg^{-1} h^{-1} and 6.98 ± 0.05 mg CO_2 kg^{-1} h^{-1} at 2 °C. The respiration rates of stored blueberries could not be measured as visible moulds were observed during the measurements (2-3 days at 2 °C). ‘Maravilla’ raspberries had high respiration rates of 19.09 ± 0.69 mg O_2 kg^{-1} h^{-1} and 25.85 ± 1.41 mg CO_2 kg^{-1} h^{-1} at harvest. The values were unchanged after 11 days in air storage at 2 °C (Figure 5.3). MAP packed raspberries had 6.39-11.10 mg kg^{-1} lower CO_2 production rates than those packed in clamshells, regardless of the numbers of perforations. Lower O_2 consumption rates, by 4.28 mg kg^{-1} on average, were only observed in packages with 0-2 perforations where O_2 levels were extreme (< 3%).

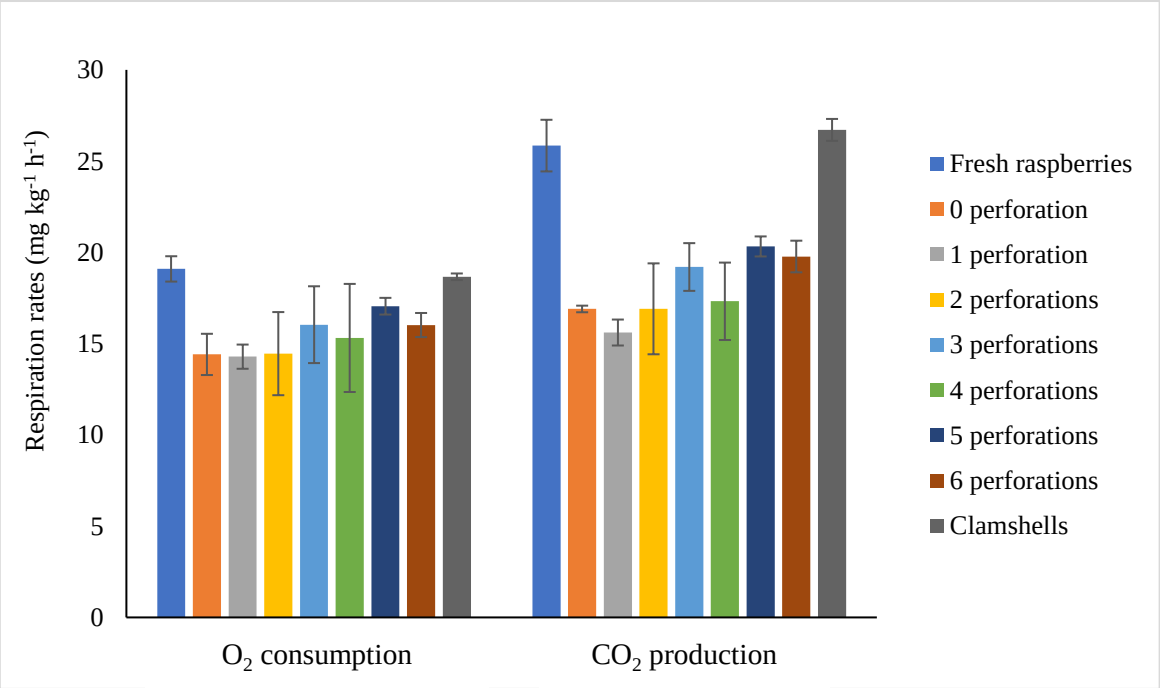
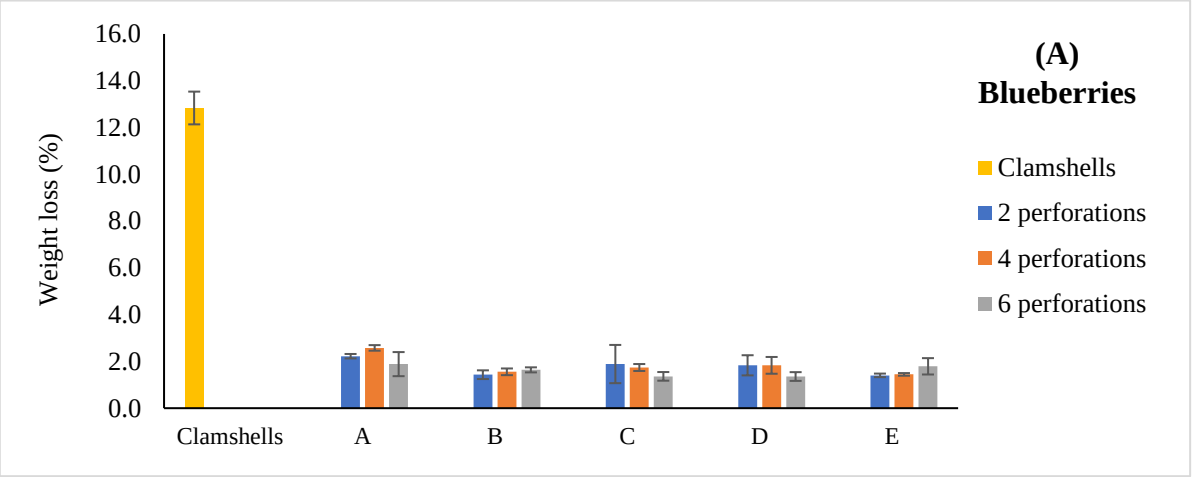


Figure 5.3 Respiration rates at 2 ºC of fresh and stored raspberries (n = 4, p = 0.05)

5.3.2 Weight loss

Packing berries in plastic trays with lid films substantially reduced weight loss, compared to clamshells ($p < 0.05$) (Figure 5.4). Blueberries in clamshells lost approximately 12.8% weight with severe shrivelling apparent after 5 weeks and, together with the development of

visible mould, this resulted in earlier shelf life termination. In comparison, blueberries in MAP lost < 3% weight after 8 weeks with no differences between packaging designs. Similarly, raspberries in MAP lost < 2 % weight after 11 days, compared to 6.3% in fruit packed in clamshells. Packages with 0-3 perforations had slightly higher weight losses, about 0.5-0.9%, compared to those with 4-6 perforations.



The letters in treatment name referred to initial atmospheres: A: 10% O_2 & 9% CO_2, B: 17% O_2 & 0% CO_2, C: 14.5% O_2 & 0% CO_2, D: 8.5% O_2 & 0% CO_2 and E: air, 20.7% O_2 & 0% CO_2. The following number referred to the number of perforations.

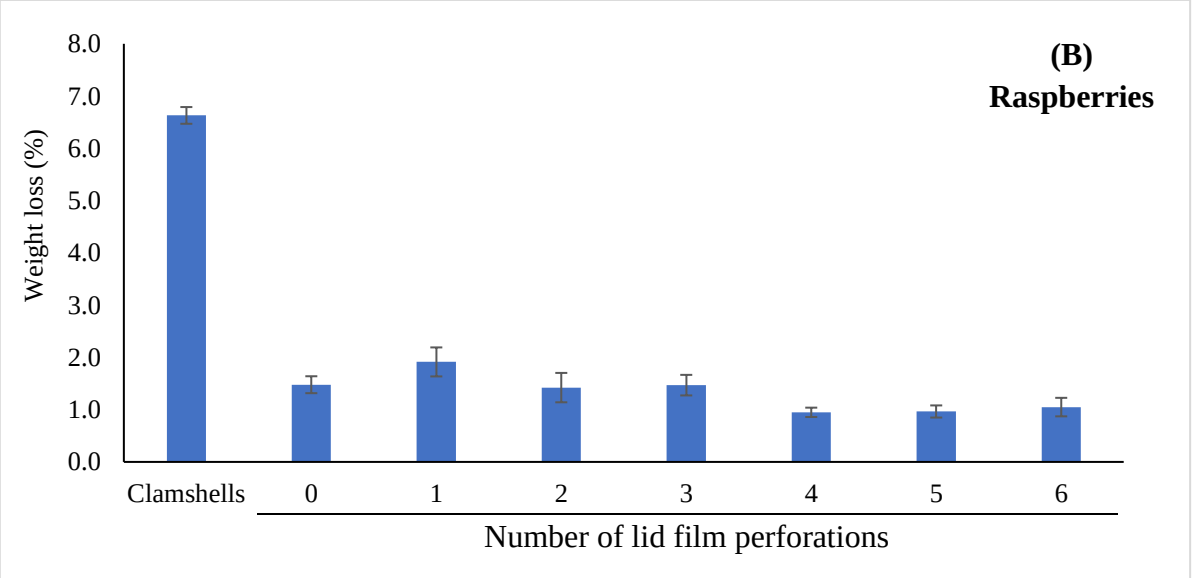


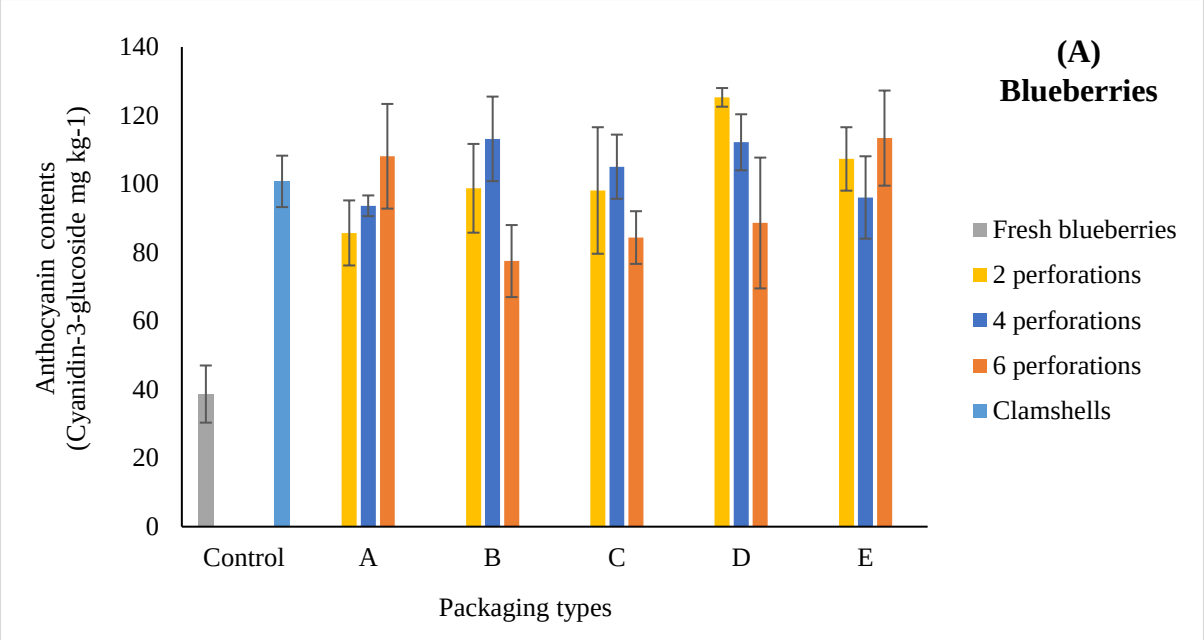
Figure 5.4 Weight loss in fresh and stored (A) blueberries (n = 3, p = 0.05) and (B) raspberries (n = 4, p = 0.05)

5.3.3 Fruit mould

MAP with appropriate designs reduced visible mould growth in blueberries and raspberries ($p < 0.05$). All packed raspberries had no moulds after 11 days at 2 °C, compared to 3.9% mouldy fruit (w/w) in clamshells. The shelf life of blueberries in clamshells was terminated in week 5, which was 3 weeks earlier than the MAP samples, due to severe weight loss and shrivelling. Even so, the weight percentage of mouldy fruit in the clamshells in week 5 (7.4%) was still as high as most of the MAP samples that were stored for 8 weeks. The weight portions of mouldy fruit for blueberries in MAP varied widely between 2.5-15.2%. It was influenced by the initial atmosphere, the numbers of perforations and their interactions (Table 5.1). The lowest weight percentages of mouldy blueberries after 8 weeks were 2.5% and 3.7%, found in C2 (14.5% O_2 & 0% CO_2 with 2 perforations) and B2 (17% O_2 & 0% CO_2 with 2 perforations), respectively. However, the correlations between headspace O_2 or CO_2 levels and the weight percentages of mouldy fruit were weak (r_{O_2} = 0.31 and r_{CO_2} = -0.31, n = 3). From the fruit appearances, the predominant mould appeared to be *B. cinerea* (grey mould) in raspberries, and *Alternaria* sp. (greenish grey) in blueberries.

5.3.4 Chemical quality attributes

At harvest, blueberries and raspberries had a mean total anthocyanin level of 38.70 ± 8.34 mg kg^{-1} and 164.85 ± 3.50 mg kg^{-1}, respectively. The anthocyanin contents increased during cold storage ($p < 0.05$), to 77.52-125.29 mg kg^{-1} in blueberries and to 182.39-288.15 mg kg^{-1} in raspberries (Figure 5.5). MAP designs had no effects on blueberry anthocyanins ($p > 0.05$). For raspberries, packages with 5 and 6 perforations maintained the anthocyanin levels of close to those at harvest but approximately 62.47-105.76 mg kg^{-1} lower than in clamshells.



The letters in treatment name referred to initial atmospheres: A: 10% O_2 & 9% CO_2, B: 17% O_2 & 0% CO_2, C: 14.5% O_2 & 0% CO_2, D: 8.5% O_2 & 0% CO_2 and E: air, 20.7% O_2 & 0% CO_2. The following number referred to the number of perforations. Blueberries in clamshells (controls) were stored for 5 weeks. MAP samples were stored for 8 weeks.

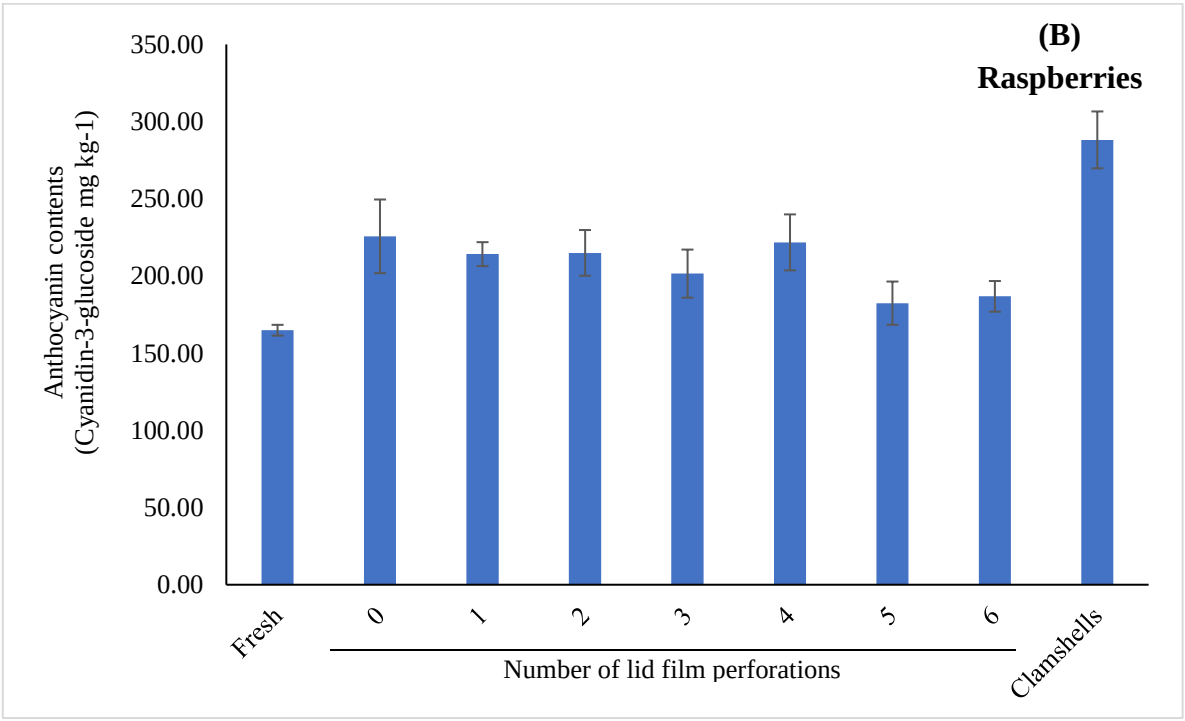


Figure 5.5 Total anthocyanin contents in fresh and stored (A) blueberries (n = 3, p = 0.05) and (B) raspberries (n = 4, p = 0.05)

The effects of storage and packaging on other quality attributes, including pH, TA, TSS and L-ascorbic acid were different between blueberries and raspberries. Fresh blueberries had a mean pH of 3.68, 0.64% TA, 10.43% TSS and approximately 123.16 ± 17.75 mg L-ascorbic acid per 100 g fruit. pH, TA, and L-ascorbic acid contents of blueberries did not change significantly after storage ($p > 0.05$) (Table 5.2). In contrast, these measurements differed between fresh and stored raspberries ($p < 0.05$) but were not affected by the packaging designs ($p > 0.05$). At harvest, raspberries had a mean pH of 3.27, 1.62% TA and 8.66% TSS. After 11 days, pH increased together with a mean 0.14% drop of TA, and L-ascorbic acid reduced by 50% from an initial level of 387.03 ± 0.38 mg per 100 g fruit (Table 5.3). Both blueberries and raspberries in clamshells had higher TSS than fresh and MA-packed fruit ($p < 0.05$), possibly resulted from dehydration. The changes in TSS and TA led to increases in TSS/TA ratios in clamshell fruit.

5.3.5 Aroma profiles

The aroma profiles of 'Legacy' blueberries in this study were mainly made up of aldehydes and terpenes/terpenoids, followed by ketones and alcohols (Figure 5.6). Fresh blueberries had the highest total peak areas of aldehydes (hexanal, 2-hexenal, benzeneacetaldehyde and nonanal) and ketones (6-methyl-5-hepten-2-one and undecane-2-one), but geraniol was the only terpenoid. Additional terpenes/terpenoids were identified in stored fruit, including linalool, α-terpineol, citral and caryophyllene oxide. Overall, the evolution of blueberry aroma profiles differed between the air and MAP storage but there were no significant differences between MAP designs. The total peak areas of each volatile group were higher in clamshell fruit than in MA-packed blueberries, particularly aldehydes (2 times) and terpenes/terpenoids (7 times) ($p < 0.05$). However, there was no strong correlation between volatile groups and the headspace O_2/CO_2 levels.

Table 5.2 Chemical quality attributes of fresh and stored ‘Legacy’ blueberries

Packaging designs*	pH (per 100g)	% TSS	% TA (g citric acid/100g fruit)	TSS/TA	L-ascorbic acid (mg/100g fruit)
Fresh blueberries	3.68 ± 0.06 ^{a}	10.43 ± 0.03 ^{b}	0.64 ± 0.05 ^{ab}	16.41 ± 1.16 ^{bc}	123.16 ± 17.75 ^{a}
A2	3.62 ± 0.03 ^{bcd}	10.31 ± 0.19 ^{b}	0.63 ± 0.01 ^{ab}	16.37 ± 0.64 ^{bc}	123.12 ± 17.80 ^{a}
A4	3.56 ± 0.03 ^{bcd}	10.39 ± 0.00 ^{b}	0.66 ± 0.00 ^{a}	15.63 ± 0.00 ^{bc}	112.33 ± 0.03 ^{a}
A6	3.67± 0.03 ^{abcd}	10.44 ± 0.04 ^{b}	0.62 ± 0.01 ^{ab}	16.95 ± 0.36 ^{b}	112.95 ± 0.38 ^{a}
B2	3.69± 0.03 ^{abcd}	9.90 ± 0.43 ^{b}	0.62 ± 0.01 ^{ab}	16.00 ± 0.32 ^{bc}	113.33 ± 0.27 ^{a}
B4	3.63 ± 0.04 ^{abcd}	10.21 ± 0.23 ^{b}	0.64 ± 0.01 ^{ab}	15.99 ± 0.32 ^{bc}	123.36 ± 18.31 ^{a}
B6	3.57 ± 0.03 ^{abcd}	10.07 ± 0.29 ^{b}	0.66 ± 0.01 ^{ab}	15.28 ± 0.60 ^{bc}	123.11 ± 18.37 ^{a}
C2	3.57 ± 0.01 ^{abcd}	10.39 ± 0.05 ^{b}	0.62 ± 0.00 ^{ab}	16.74 ± 0.00 ^{bc}	122.61 ± 17.27 ^{a}
C4	3.55 ± 0.03 ^{cd}	9.19 ± 0.50 ^{b}	0.66 ± 0.03 ^{ab}	14.06 ± 1.38 ^{c}	133.92 ± 17.75 ^{a}
C6	3.70 ± 0.06 ^{abc}	10.34 ± 0.19 ^{b}	0.58 ± 0.04 ^{b}	17.87 ± 1.03 ^{b}	113.07 ± 0.20 ^{a}
D2	3.66 ± 0.02 ^{abcd}	10.44 ± 0.04 ^{b}	0.62 ± 0.00 ^{ab}	16.74 ± 0.00 ^{bc}	112.89 ± 0.49 ^{a}
D4	3.60 ± 0.08 ^{abcd}	10.35 ± 0.18 ^{b}	0.63 ± 0.01 ^{ab}	16.37 ± 0.64 ^{bc}	123.55 ± 18.25 ^{a}
D6	3.55 ± 0.03 ^{d}	10.43 ± 0.04 ^{b}	0.65 ± 0.02 ^{ab}	16.00 ± 0.64 ^{bc}	112.77 ± 0.42 ^{a}
E2	3.70 ± 0.05 ^{ab}	10.57 ± 0.24 ^{b}	0.60 ± 0.04 ^{ab}	17.62 ± 1.52 ^{b}	113.10 ± 0.56 ^{a}
E4	3.59 ± 0.05 ^{abcd}	10.42 ± 0.03 ^{b}	0.64 ± 0.02 ^{ab}	16.18 ± 0.56 ^{bc}	133.33 ± 18.12 ^{a}
E6	3.67 ± 0.02 ^{abcd}	10.47 ± 0.01 ^{b}	0.61 ± 0.03 ^{ab}	17.17 ± 0.74 ^{b}	113.20 ± 0.10 ^{a}
Clamshells	3.71 ± 0.10 ^{a}	13.69 ± 0.36 ^{a}	0.59 ± 0.06 ^{ab}	23.19 ± 2.14 ^{a}	112.97 ± 0.34 ^{a}

Means followed with different superscripts are significantly different at $p < 0.05$ (n = 3).

Measurements were conducted in week 5 for clamshell blueberries and in week 8 for MAP-packed blueberries.

*: Initial atmospheres:

A: 10% O_2 & 9% CO_2 C: 14.5% O_2 & 0% CO_2 E and vented clamshell punnets: 20.7% O_2 & 0% CO_2

B: 17% O_2 & 0% CO_2 D: 8.5% O_2 & 0% CO_2

The number following the letter referred to the number of perforations in the lid film

Table 5.3 Chemical quality attributes of fresh and stored 'Maravilla' raspberries

Packaging designs	**pH (per 100g)**	**% TSS**	**% TA (g citric acid/100g fruit)**	**TSS/TA**	**L-ascorbic acid (mg/100g fruit)**
Fresh raspberries	3.27 ± 0.01^{d}	8.66 ± 0.01^{b}	1.62 ± 0.02^{a}	5.34 ± 0.05^{c}	387.03 ± 0.38^{a}
0 perforation	3.44 ± 0.05^{ab}	8.49 ± 0.15^{b}	1.45 ± 0.03^{b}	5.87 ± 0.11^{b}	147.14 ± 19.21^{c}
1 perforation	3.42 ± 0.09^{abc}	8.54 ± 0.04^{b}	1.48 ± 0.01^{b}	5.79 ± 0.00^{bc}	176.57 ± 44.19^{c}
2 perforations	3.37 ± 0.04^{abcd}	8.59 ± 0.06^{b}	1.49 ± 0.02^{b}	5.76 ± 0.05^{bc}	177.76 ± 45.09^{c}
3 perforations	3.39 ± 0.02^{abcd}	8.56 ± 0.08^{b}	1.48 ± 0.01^{b}	5.79 ± 0.00^{bc}	157.81 ± 36.84^{c}
4 perforations	3.34 ± 0.08^{bcd}	8.47 ± 0.12^{b}	1.48 ± 0.04^{b}	5.71 ± 0.13^{bc}	137.44 ± 1.93^{c}
5 perforations	3.32 ± 0.05^{bcd}	8.58 ± 0.09^{b}	1.51 ± 0.02^{b}	5.68 ± 0.12^{bc}	196.67 ± 38.43^{c}
6 perforations	3.31 ± 0.02^{cd}	8.56 ± 0.04^{b}	1.51 ± 0.03^{b}	5.66 ± 0.10^{bc}	224.48 ± 47.27^{c}
Vented clamshells	3.47 ± 0.04^{a}	9.84 ± 0.60^{a}	1.45 ± 0.05^{b}	6.82 ± 0.61^{a}	304.18 ± 48.03^{b}

Means followed with different superscripts are significantly different at $p < 0.05$ (n = 4).

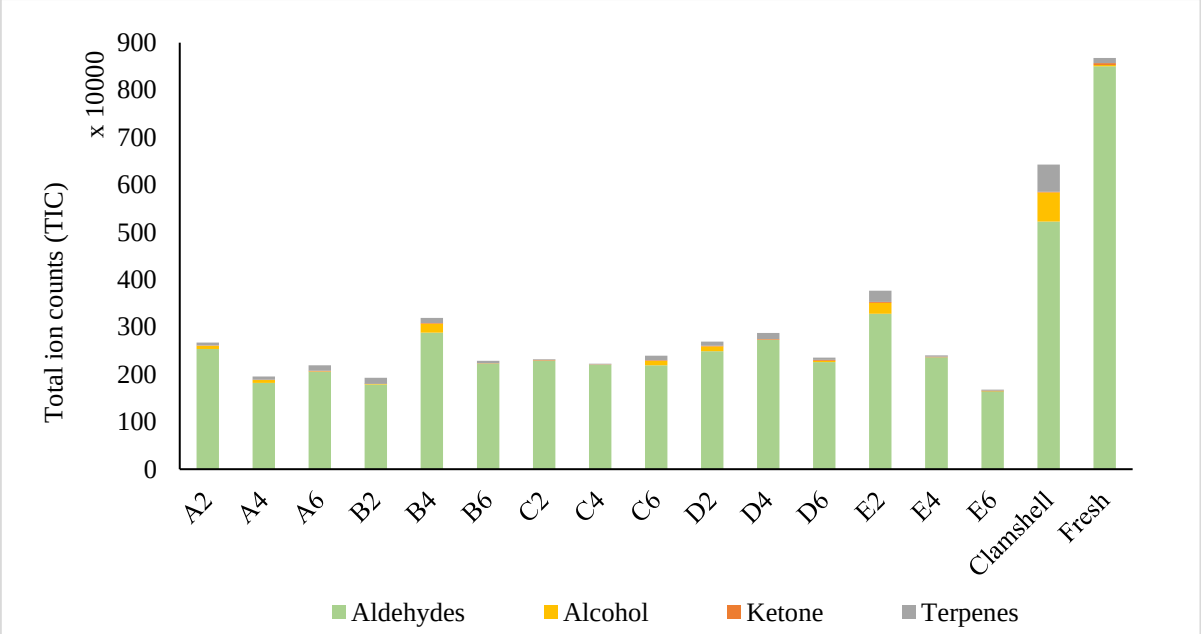


Figure 5.6 GC-MS peak areas of volatiles in fresh and stored blueberries grouped by chemical classes (n = 3)

The letters in treatment name referred to initial atmospheres: A: 10% O_2 & 9% CO_2, B: 17% O_2 & 0% CO_2, C: 14.5% O_2 & 0% CO_2, D: 8.5% O_2 & 0% CO_2 and E: air, 20.7% O_2 & 0% CO_2. The following number referred to the number of perforations.

The raspberries ('Maravilla') had relatively more complex aroma profiles compared to the blueberries, comprising of C13-norisoprenoids, aldehydes, esters, terpenes, ketones, furans, alcohols and acids (Figure 5.7). C13-norisoprenoids including α-ionone, β-ionone, α-dihydroionone and β-ionol were the most abundant volatile group in fresh raspberries. The complexity and intensities of volatiles generally increased upon storage. Raspberries in clamshells had 5-7 times higher total peak areas ($p < 0.05$) of terpenes/terpenoids (7 times), ketones (6 times) and C13-norisoprenoids (~5 times) than MAP-samples. There were strong linear correlations between the peak areas of terpenes/terpenoids and the headspace O_2/CO_2 levels (r_{O_2} = 0.874 and r_{CO_2}=-0.794, n = 4). The headspace O_2 concentrations were also positively correlated to total peak areas of ketone (r = 0.729, n = 4) and negatively to acetic acid (r = -0.702, n = 4). Acetic acid was not detected in fresh raspberries or the fruit in clamshells or in MAP with 6 perforations.

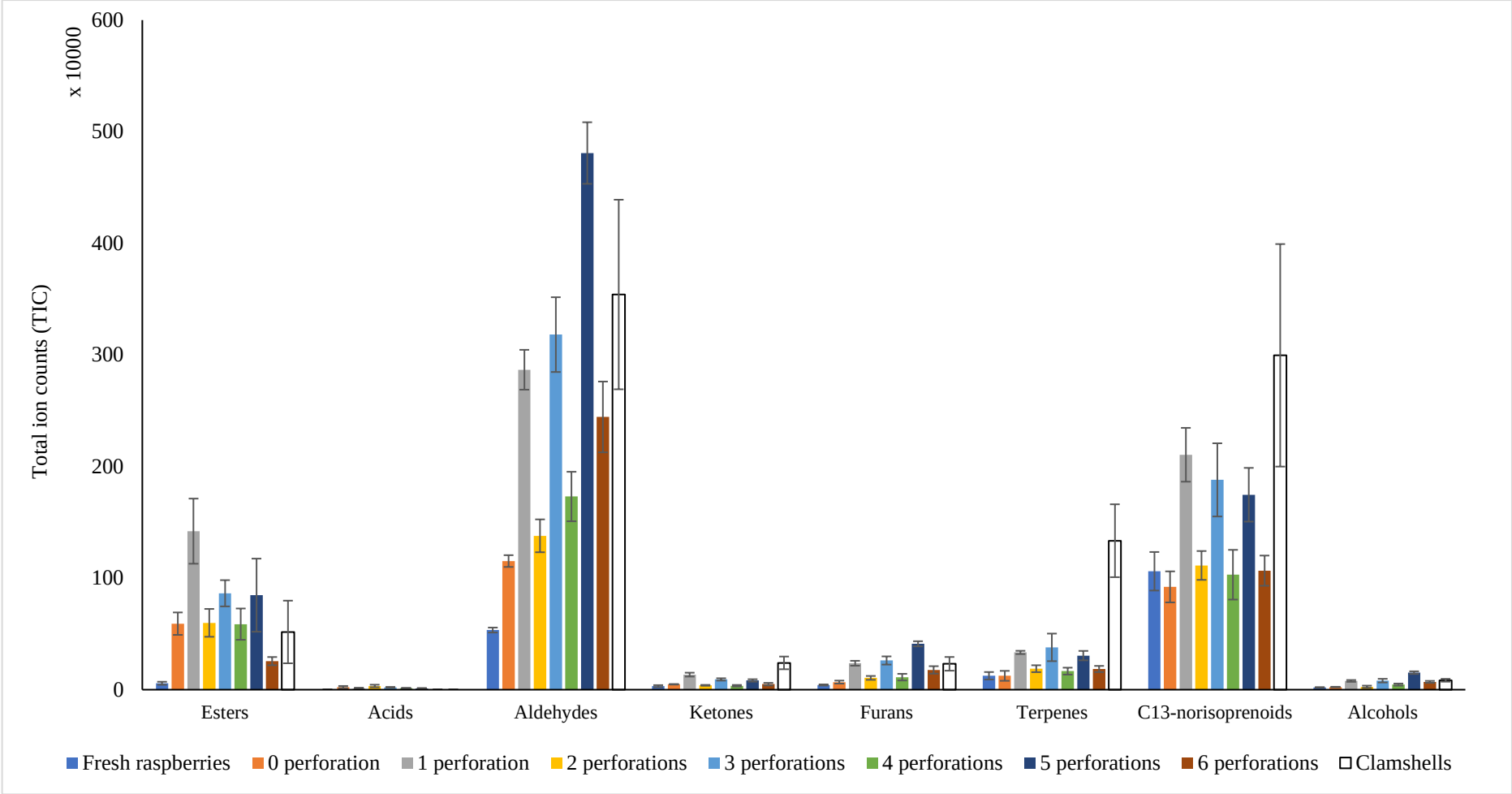


Figure 5.7 GC-MS peak areas for different volatile classes identified in fresh and stored raspberries (n = 4, p = 0.05)

5.4 Discussion

Berries are perishable and have short postharvest storability because of their delicate structures and high susceptibility to mould growth (Huynh et al., 2019). This research demonstrated the potential of using retail MAP with non-vented trays and perforated lid films to extend the storage life beyond the average 2-4 weeks for blueberries and 2-5 days for raspberries. The findings in this study confirmed the benefits of MAP on suppressing mould growth, reducing weight loss in both berries and delaying darkening in raspberries. A typical MA of 5-10% O_2 & 10-15% CO_2 has been shown to benefit berry storability (Huynh et al., 2019; Madrid & Beaudry, 2020). Perforations accelerated gas exchange through the lid films to prevent O_2 depletion and excessive CO_2 accumulation.

5.4.1 Benefits of MA for blueberries

The respiration rates of fresh blueberries (6.98 ± 0.05 mg CO_2 kg^{-1} h^{-1} at 2 °C) measured in this study were close to the range provided in literature (2-10 mg CO_2 kg^{-1} h^{-1} at 0 °C, Perkins-Veazie, 2016a), while those of raspberries (25.85 ± 1.41 mg CO_2 kg^{-1} h^{-1} at 2 °C) were higher (16-18 mg CO_2 kg^{-1} h^{-1} at 2 °C; Perkins-Veazie, 2016b). The moderate respiration rates of 'Legacy' blueberries suggested that active MAP, with an initially reduced O_2 level and/or elevated CO_2, would be more likely to successfully reach the desirable O_2/CO_2. This concept was proven by the findings in this study with the four designs, A2, B2, C2 and D4 that achieved and maintained the MA levels close to the recommended 5-10% O_2 & 10-15% CO_2 over 8 weeks of storage. However, only two of these treatments, B2 (17% O_2 & 0% CO_2 with 2 perforations) and C2 (14.5% O_2 & 0% CO_2 with 2 perforations), were also associated with low mouldy fruit weight (< 4%) in week 8. These observations suggested that the headspace of blueberry packages could have been altered by mould growth during the extensive 8 weeks storage, explaining the weak correlations between headspace O_2/CO_2 and mould growth.

5.4.2 Benefits of MA for raspberries

Fresh 'Maravilla' raspberries, in contrast, had high respiration rates that quickly lowered O_2 and built up CO_2 concentrations in the package headspaces. The low O_2, high CO_2 conditions suppressed fruit respiration rates and mould growth, as observed in all MAP samples. However, only packages with 6 perforations successfully maintained the O_2 and CO_2 in safe levels throughout 11 days of storage. Insufficient gas exchanges in packages with 0-4 perforations led to extreme conditions of $CO_2 > 15\%$ & $O_2 < 5\%$, which subsequently caused anaerobic respiration and softening. Indeed, an O_2 level above 4% at 0 °C, 6% at 10 °C and 8% at 20 °C were recommended to prevent fermentation in raspberries (Joles et al., 1994).

5.4.3 Impacts of MA on weight loss

Weight loss and its associated changes in quality attributes reduce the storability and marketability of berries (Huynh et al., 2019). In this trial, blueberries in clamshells lost 12.8% of their weight and showed severe shrivelling after 5 weeks, while raspberries lost 6.3% of their weight and their shiny appearances after 11 days. Softening and shrivelling occurred when weight loss exceeded 5-8% in blueberries (Paniagua et al., 2014), and 3.8% in raspberries, together with darkening and dull appearances (do Nascimento Nunes & Emond, 2007). Vented clamshells, although offering fast cooling to remove field heat, could make fruit more susceptible to dehydration and freezing damage during storage (Pathare et al., 2012). The enclosed MAP design as in this trial, in that sense, provided a protective layer against forced-air cooling and created a high RH condition within the packages; thereby, minimising weight loss to < 3% in blueberries and < 2% in raspberries. The number of perforations did not affects weight loss of blueberries. Meanwhile, raspberries packages with 0-3 perforations had slightly higher weight losses than those with 4-6 perforations. This result could be linked to softening and drupelet leakages observed in the trays, as a result of the exposure of fruit to extreme O_2 and CO_2 levels. In addition, it should be noted that the

moisture condensation could accumulate within the packages due to fruit transpiration and temperature fluctuations. The condensation could subsequently favour mould growth (Horvitz, 2017) and appear unattractive at retail. This issue could be mitigated by using anti-fogging films and placing absorbing pads under the berries.

5.4.4 Impacts of MA on colour

Berries are valued for their high anthocyanin contents, and sweet, pleasant flavours (Gilbert et al., 2015; Stevenson & Scalzo, 2012; Zafra-Stone et al., 2007). The anthocyanin levels of fresh 'Legacy' blueberries (38.70 ± 8.34 mg kg^{-1}) and 'Maravilla' raspberries (164.85 ± 3.50 mg kg^{-1}) in this study were lower than the reported values for the same cultivars (Golding et al., 2014; Stevenson & Scalzo, 2012). This could be due to various factors from growing conditions, berry maturity, postharvest handling to extraction and analysis methods (Krüger et al., 2011; Routray & Orsat, 2011; Stavang et al., 2015). Postharvest increases of anthocyanin contents in both berries as found herein were also described in several studies (Kalt et al., 2003; Krüger et al., 2011; Palonen & Weber, 2019; Stavang et al., 2015), but was more pronounced in raspberries because anthocyanin concentrations correspond with the degree of redness (Aaby et al., 2019), and negatively affect attractiveness (Stavang et al., 2015). Meanwhile, the darker colour perceived in stored blueberries was linked to the thinning of wax bloom (do Nascimento Nunes et al., 2004), and was hard to measure due to the great variability between individual berries and across cultivars (Chu et al., 2017).

Fresh market raspberries are often more appealing to customers when they are bright red colours with a shiny appearance. Dark-red fruit are often considered as riper, overripe, and sweeter (Clydesdale, 1993). For this purpose, MAP with 5 and 6 perforations were advantageous at delaying the darkening in raspberries. The increases in anthocyanin contents of stored raspberries in this trial could be explained by the concentrating effects as the berries dehydrated (Krüger et al., 2011), and the biosynthesis of anthocyanins and other phenolic

compounds using carbon skeletons of organic acids (Kalt et al., 1999). However, the similar observation in blueberries possibly resulted from more water-extractable anthocyanins available in stored fruit as data for TA and weight loss did not support the two proposed mechanisms. Indeed, unlike raspberries which have anthocyanins locating in drupelets, the anthocyanins of 'Legacy' blueberries are concentrated in the fruit skins (Ribera et al., 2010). The fruit cell wall is made up of water-soluble pectin, sodium carbonate-soluble pectin, chelator soluble pectin, hemicellulose, and cellulose (H. Chen et al., 2015). The three pectin fractions extracted from blueberries were shown to have different anthocyanin-binding ability across a pH range of 2.0-4.5 (Lin et al., 2016). Binding efficiency was lowest in water-soluble fractions at all pH values, while higher in chelator-soluble pectin at 2.0-3.6 and higher in sodium carbonate-soluble pectin at pH 3.6-4.5. The anthocyanin extraction procedure in this study used water and centrifugation, and therefore would mainly isolate free anthocyanins in water-soluble pectin. This explained the much lower anthocyanin contents found herein compared to other literature sources (Golding et al., 2014; Stevenson & Scalzo, 2012). Furthermore, increases in water-soluble pectin and decreases in the other two fractions were found during blueberry storage, owing to the activities of cell wall degrading enzymes (H. Chen et al., 2015). Consequently, the same procedure should be able to extract more anthocyanins from the stored blueberries.

5.4.5 Effects of MA on volatiles

Fruit flavour is a complex attribute mainly determined by the sugar/acid balance and aroma compounds (Baldwin et al., 2007). While external traits such as colours and appearance would initiate buying, it is eating quality including flavours that is needed to drive consumers' repeat purchases. The TSS/TA ratios are usually used to predict the sugar/acid balance and have been demonstrated as a good indicator for sweetness, acidity, and astringency in raspberries (Aaby et al., 2019). In contrast, instrumental measurements were

shown to be insufficient to indicate sensory scores in 12 blueberry cultivars (Saftner et al., 2008). Values of TSS/TA between 10 and 33 were generally found in blueberries with TSS/TA > 32 suggesting poor keeping quality (Beaudry, 1992). In this study, the TSS/TA ratios of both berries were not affected by MAP designs, but were higher in clamshell fruit, 1.2 times for raspberries and 1.4 times for blueberries, after storage. The changes in TSS/TA ratios could imply changes in taste perceptions, at least for raspberries. Increases in TSS/TA in the clamshell berries were results of increases in TSS and decreases in TA as fruit respired and used organic acids for energy (Biale, 1950), as well as concentrating effects. Therefore, MAP suppressed fruit respirations and reduced weight loss, thereby, alleviating the changes in TSS/TA in storage.

The pleasant, distinctive aroma of berries is an important factor towards consumer acceptability and overall liking (Gilbert et al., 2015; Giuggioli et al., 2015). The major volatiles identified in this study were also the key compounds suggested for blueberry flavours: C6 aldehydes, terpenes/terpenoids and 2-undecanone (Gilbert et al., 2015), as well as raspberry flavours: C13-norisoprenoids, C6 aldehydes and terpenes (Aaby et al., 2019; Aprea et al., 2015; Forney et al., 2015). Upon storage, the total peak areas of all volatiles in blueberries decreased while raspberries increased substantially. In addition, the aroma profiles of both berries generally became more complex. These findings suggested that there could be changes in overall flavour intensities and perceptions.

The differences in aroma evolutions were mainly observed between MAP and clamshell samples of both berries, but not between different MAP designs. The peak areas of aldehydes were significant lower in all MAP samples of blueberries than fresh fruit and in clamshells. Raspberries packages with 6 perforations, which was the only treatment not resulting in extreme MA conditions during storage, also had lower peak areas of aldehydes compared to clamshell-stored fruit. The smaller peak areas of C6 and C9 aldehydes in MA-packed fruit

could be the result of delayed senescence and be advantageous, because these compounds are products of fatty acid oxidation as in membrane degradation (El Hadi et al., 2013) and are characterised as green, grassy and pungent (Aaby et al., 2019; Hongsoongnern & Chambers Iv, 2008). However, MAP might also lead to smaller peak areas of terpenes/terpenoids which contribute to the floral, herbal aroma of berries. The syntheses of terpenes/terpenoids require the carbon skeletons from the TCA cycle (Mir & Beaudry, 2002) and therefore, would be suppressed by the low O_2 condition in MAP. Indeed, this study found total peak areas of terpenes/terpenoids were 7 times lower in MAP samples of blueberries and raspberries, compared to the fruit in clamshells. Furthermore, there were strong linear correlations between the peak areas of terpenes/terpenoids in raspberries and the headspace O_2/CO_2 levels (r_{O_2}= 0.874 and r_{CO_2}= -0.794). The similar mechanism possibly could have resulted in lower peak areas of C13-norisoprenoids in MA packed raspberries as these volatiles are products of carotenoid oxidation (Hampel et al., 2007). C13-norisoprenoids, especially α- and β-ionone, are important volatiles for the floral, fruity, and typical raspberry scent (Aaby et al., 2019; Aprea et al., 2015), which might indicate a more intense, riper raspberry aroma in clamshell fruit.

5.5 Conclusions

This study found that a model of retail MAP using enclosed trays with perforated lid films extended shelf life of 'Legacy' blueberries to 8 weeks and 'Maravilla' raspberries to 11 days at 2 °C, by suppressing mould growth, reducing weight loss and delaying quality changes. For 125 g fruit packed in 1 L non-vented trays, blueberries benefited from active MAP with initially reduced O_2 (17% or 14.5%) and 2 perforations of 70 µm, while raspberries required 6 perforations for sufficient gas exchange. The GC-MS aroma profiles of berries stored in MAP had noticeably lower peak areas for terpenes (in both berries) and C13-norisoprenoids (in raspberries) compared to berries in clamshells, and remained close to the levels observed

in fruit at harvest. Further studies are needed to confirm current findings with blueberries and raspberries from different seasons, locations, and varieties. Sensory evaluation and consumer acceptability on eating quality and the packaging format would be key determinants for wider uses in the berry industry. In addition, unlike berries packed in pallet MAP which would be moved to normal air conditions before retail display, the fruit in retail-MAP would remain in the MA condition until consumption. Therefore, research to understand the potential changes in package headspace conditions and fruit quality throughout the supply chain would be useful for optimising the packaging designs.

Chapter 6: Modified atmosphere packaging for iceless broccoli shipment

6.1 Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is a high value, nutritious vegetable with increasing production worldwide (FAOSTAT, 2018). However, fresh broccoli is extremely perishable because of its high respiration rates and sensitivity to ethylene (X. Fan & Mattheis, 2000; Gillies & Toivonen, 1995). At 15 ºC, the shelf life of broccoli can be as short as 2-3 days (Feng Xu et al., 2016). Rapid postharvest quality deterioration is mainly characterised by wilting, yellowing and the formation of off-odours (do Nascimento Nunes, 2009a).

Extending the storability of broccoli is necessary, not only to reduce food waste (Hagen & Larsen, 2020), but also to expand market reach and improve consumer satisfaction. Current commercial practice uses rapid postharvest cooling to quickly lower respiration rates of broccoli and maintain the tissue turgidity (Toivonen, 1997), followed by cold storage and refrigerated transportation. Ideally, at 0 ºC and > 95% RH, the storage life of broccoli could be extended to 3-4 weeks (Brecht et al., 2019; Toivonen & Forney, 2016). However, this condition is rarely achievable in actual supply chains. For example, temperatures between 2 and 12 ºC were observed during broccoli transport, storage, and display in Sweden (Jacobsson et al., 2004b). Consequently, top-icing (i.e, covering the broccoli with crushed or flaked ice), is often used during broccoli shipment, aiming to keep the broccoli cool and hydrated (Gillies & Toivonen, 1995). However, the meltwater could create favourable conditions for microbial growth and spreading contamination (Felt et al., 1983), as well as pose hazards to safe handling if the meltwater accumulates on the vehicle floor (Brecht et al., 2019). Furthermore, top-icing shipment of broccoli also adds extra handling cost (Ekman, 2017) and often involves the use of non-recyclable polystyrene boxes that cause environmental concerns.

MAP and ethylene control have been shown to extend the shelf life of broccoli (Esturk et al., 2014; Hagen & Larsen, 2020; Jacobsson, Nielsen, Sjöholm, et al., 2004; Marzano-Barreda et al., 2020; Serrano et al., 2006). The major benefits of MAP are to delay yellowing, reduce weight loss and ethylene production, and maintain broccoli firmness. However, commercial applications of MAP for broccoli are still limited due to the insufficient evidence to justify the investments. Also, MAP for broccoli needs to overcome the risks of O_2 depletion (< 1%), excessive CO_2 accumulation (> 10-15%) associated with inappropriate packaging choices, and temperature fluctuations (Hagen & Larsen, 2020; He & Xiao, 2018). These extreme MA conditions can induce the formations of severe off-odours and terminate the marketability of broccoli (Jacobsson et al., 2004b; Tulio, et al., 2003).

Ethylene control has most widely been studied through the inhibition of ethylene by the antagonist, 1-MCP. Applications of 1-MCP showed a buffering effect against undesirable conditions such as abuse temperatures or the presence of exogenous and endogenous ethylene (Abe & Watada, 1991; de Beer & Crouch, 2015). However, its commercial use is limited by the on-going development of binding sites after 1-MCP treatments (Abe & Watada, 1991) and it requires legal registration for use. These findings suggest that packaging systems with the ability to continuously reduce ethylene levels would be useful alternatives to 1-MCP, but available literature in this area remains scarce.

Matching the produce respiration rates and the gas permeability of MAP is the key to successfully achieve the desirable compositions of O_2 and CO_2 (Vermeulen et al., 2018). While most of polymeric films have low permeability to CO_2 and O_2, resulting in inadequate O_2 and CO_2 exchanges (Al-Ati & Hotchkiss, 2003; González-Buesa et al., 2013), the permeability of MAP could be increased by integrating inorganic fillers or creating perforations (Vermeulen et al., 2018). Tailor-made microperforated bags could also be manufactured by pre-determining produce respiration rates at designated storage temperatures and calculating the numbers/sizes of

perforations to achieve desired headspace compositions (Hussein et al., 2015). In addition, a range of WVTRs is also offered for better control of in-package humidity that affects produce weight loss and moisture condensation (Jalali et al., 2017). Besides, packaging systems with ethylene scavenging properties are available in the form of potassium permanganate ($KMnO_4$) sachets to be added to broccoli commercial liners/containers, or film materials containing ethylene adsorbers (Sadeghi et al., 2019). These products are advantageous for industrial applications owing to their ease of use and lower capital investment compared to controlled atmospheres and fumigation.

This research aimed to investigate the potential of using innovative packaging systems to replace top-icing in broccoli shipment. Removing ice from broccoli shipment is necessary to address the associated issues with broccoli quality, operational costs, safety handling and sustainability. Three simulated shipping conditions were studied including high-value longer domestic routes under good temperature management and broken cold chain (7 days at 2 ºC or 13 ºC), and seafreight with refrigeration as for exportation (42 days at 2 ºC). The MAP systems being trialled were the available technologies that provided shelf life improvements in preliminary trials of this study but were not currently used by the industry. They included a LDPE mineral-clay impregnated MAP with low WVTR, and a BOPP bag with low WVTR and microperforations optimised based on pre-determined broccoli respiration rates and storage conditions, and the addition of $KMnO_4$ sachets to the existing HDPE liner. In the 42-day trial, a PA MAP with high WVTR but having similar gas exchange properties to BOPP was also tested to examine the effects of WVTR in extensive broccoli storage.

6.2 Materials and methods

6.2.1 Designs of experiments

Lab-based trial to optimise MAP on quality of broccoli florets

Broccoli (*Brassica oleracea* var. *italica*, 'Thunderdome') were ordered from a local greengrocer in Launceston, Tasmania in November 2019. The broccoli was grown by a commercial field in Forth, harvested and hydrocooled to 3 ºC using ice-water at 0 ºC for about 20 min. The broccoli was packed into two x 8 kg-polystyrene boxes with top-icing. The entire boxes were kept at 2 ºC during the holding period and arrived at the laboratory four days after pack date. At arrival, all ice had melted, and the temperature of broccoli was 5.7 ± 0.3 ºC. There was no sign of quality deterioration. Broccoli florets from the two outermost layers were cut and then kept at 2 ºC until packing. Broccoli florets (150 g) were randomly packed as described in Table 6.1 Packaging types that were evaluated in the lab and commercial trials. There were four replicates for each treatment. Samples were stored in domestic fridges set at 2 ºC and 13 ºC for 7 days.

Iceless broccoli shipment in commercial settings

Broccoli of 'Ironman' cultivar was supplied by from the same grower. The broccoli was harvested, hydrocooled to 3 ºC using ice-water at 0 ºC for about 20 min and kept at 2 ºC until packing at the farm processing centre two days later. The broccoli passed quality control at the farm to supply for a major supermarket chain in Australia. Broccoli heads from the pallet were randomly packed as described in Table 6.1. Samples were prepared in four replicates, each included three broccoli heads (~1 kg). The packed broccoli was stored at 2 ºC after packing and sent to a supermarket's distribution centre (117.7 km away) the following day for further storage. The temperature was maintained at 2 ± 0.4 ºC during transportation. The storage conditions studied included (i) 7 days at 2 ºC to simulate longer domestic routes with good temperature management, (ii) 7 days at 13 ºC to simulate longer domestic routes in a broken cool chain, and (iii) 42 days at 2 ºC to simulate sea freight with refrigeration for exportation.

Table 6.1 Packaging types that were evaluated in the lab and commercial trials

Packaging type	Lab trials	Commercial trials
	'Thunderdome' broccoli florets (150 g)	**'Ironman' broccoli heads (1 kg)**
Control	220 mm x 220 mm commercial sandwich bags Unsealed	590mm x 390 mm x 720mm HDPE black liners, 15 µm with top-icing (750 g), unsealed
Ethylene scavenging	2 x 5g BiOn® sachets added to sandwich bags Half close	4 x 5g BiOn® sachets added to HDPE liners Gusted with rubber bands to minimise volume
LDPE mineral-clay impregnated MAP	250 mm x 300 mm, 25 µm, low WVTR Heat sealed to enclose	300 mm x 400 mm, 25 µm, low WVTR Gusted with rubber bands to minimise volume for the 7-day trials and to the packaging size for the 42-day trials to provide more O_2 from ambient air for broccoli respiration in prolonged storage
Broccoli-targeted BOPP microperforated MAP bags	250 mm x 300 mm designed for 150g broccoli florets to achieve 10% O_2 & 10% CO_2 at 13 ºC, low WVTR Heat sealed to enclose	320 mm x 330mm, designed for 1 kg broccoli to achieve > 1% O_2 & 10% CO_2 at 2 ºC, low WVTR Heat sealed to enclose
Broccoli-targeted PA microperforated MAP bags	–	300 mm x 400 mm, designed for 1 kg broccoli to achieve > 1% O_2 & 10% CO_2 at 2 ºC, high WVTR Heat sealed to enclose
Material sources: Sandwich bags: Hercules® TwinZip ®, Thailand Ethylene scavenging sachets - Bi-on®, Bioconservacion S.A, Barcelona, Spain Mineral-clay impregnated MAP bags: PEAKFresh®, Netley, Australia Microperforated MAP bags: Xtend®, Stepac L.A. Ltd, Tefen, Israel		

6.2.2 Headspace % O_2 and % CO_2 and respiration rates

The evolution of O_2 and CO_2 were measured using a gas analyser (Dansensor CheckMate 3, Mocon, Denmark) every two days for the 7-day trials and every seven days for the 42-day trial.

Respiration rates of broccoli were measured using closed system experiments following the method of Haffner et al. (2002) with minor modifications. Approximately 35 g of broccoli florets were weighed into glass mason jars of 236.6 mL capacity that were tightly sealed with metal caps and immediately stored in a refrigerator at 2 ± 0.5 °C. Both sides of the sampling holes were covered by rubberised gas sealing strips (Septum, Novatech Controls Pty. Ltd., Cheltenham, Australia). The concentrations of O_2 and CO_2 in the headspace were measured periodically using a gas analyser (Dansensor CheckMate 3, Mocon, Denmark) and plotted against time to calculate O_2 consumption and CO_2 production rates (mg kg^{-1} h^{-1}).

6.2.3 Weight changes

Weight changes of broccoli were calculated by the weight differences between day 0 and the assessment date as percentages.

6.2.4 Chemical quality attributes

Extractions of chlorophylls (a and b) and carotenoids were performed following the method described by M.D. Wilson et al. (2019b) with minor modifications. In brief, 20 g of broccoli buds were sampled from all broccoli florets or heads within a replicate and blended with 100 mL distilled water for 1 min. 4 mL subsample was mixed with 16 mL acetone (Merck KGaA, Darmstadt, Germany) and centrifuged at 20 °C and 3000 *g* for 10 min. The supernatants were read by a spectrophotometer (Shimadzu Corp., Kyoto, Japan) at 663, 646 and 470 nm. Chlorophyll and carotenoid contents were calculated based on the formulas from Lichtenthaler and Wellburn (1983).

The L-ascorbic acid contents of broccoli were determined by the iodine titration method (AOAC 967.22) against L-ascorbic acid standards (Merck KGaA, Darmstadt, Germany).

6.2.5 Aroma profiles by SPME/GC-MS

Aroma profiles were assessed for samples in commercial trials. Immediately after blending, 5 mL of the broccoli mashes were transferred to 20 mL sealed glass vials, kept at 2-4 ºC and analysed within 24 h. The aroma profile analysis by SPME/GC-MS were performed based on the method of Aprea et al. (2009) with slight modifications. Briefly, the vials were incubated at 40 ºC for 5 min, followed by headspace sampling by a carbon wide range polydimethylsiloxane fibre (120 µm) (PAL SPME Arrow, CTC Analytics AG, Zwingen, Switzerland) for 30 min at the same temperature. The fibre was conditioned at 180 °C for 30 min before runs and for 5 min between runs.

The adsorbed volatile compounds were desorbed in 2 min at 220 ºC in splitless mode (Shimadzu AOC-6000 autosampler, Shimadzu Corp.). GC analysis was carried out on an SH-Rxi-624Sil MS column (30m, 0.25 mm ID, 1.4 µm df). The GC oven was held at 60 ºC for 3 min, ramped up to 220 ºC at 8 ºC min^{-1} and held for 5 min, then increased to 250 ºC at 10 ºC min^{-1} and held for another 5 min. The carrier gas was helium at a total flow rate of 20 mL min^{-1}. The temperature of the GC-MS interface was 250 ºC.

Identifications of broccoli aroma compounds were performed by a mass spectrometer (Shimadzu GCMS-QP2020, Shimadzu Corp.) operated in electron ionisation mode scanning in the range of *m*/*z* 20-500. Identifications were made by matching chromatograms with NIST17, FFNSC 3 and Wiley9 libraries (GCMS Postrun Analysis, GCMSsolution Ver. 4.50©, Shimadzu Corp.). Data were reported as means of peak areas processed using the probabilistic quotient normalisation (PQN) method.

6.2.6 Data analysis

Statistical analyses by one-way ANOVA and Tukey's test for multiple pairwise comparisons were performed in Minitab 19.1.1 (Minitab, LLC., Pennsylvania, USA) at a significance level of $p < 0.05$.

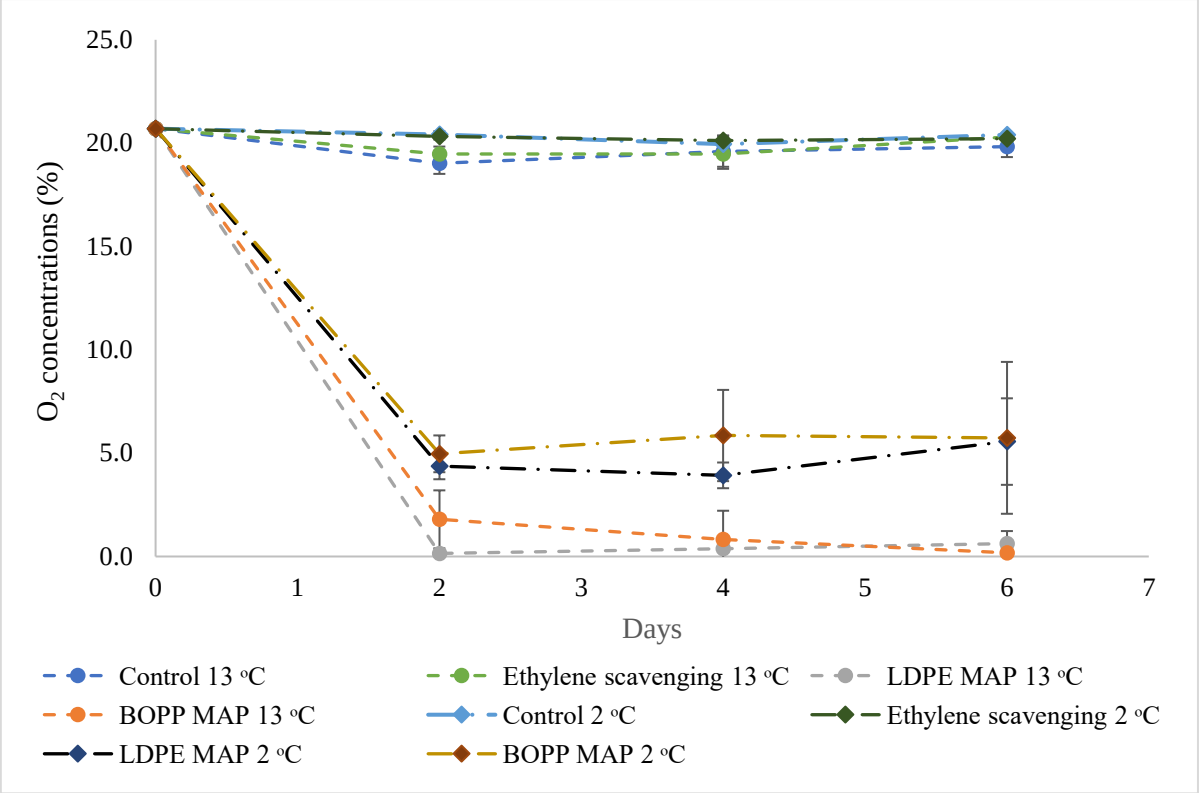
6.3 Results

6.3.1 Packaging tests in lab-based trials using broccoli florets

6.3.1.1 Headspace compositions and broccoli respiration rates

The headspace O_2 and CO_2 concentrations of packages differed between packaging types and storage temperatures. There was no MA developed in sandwich bags with and without ethylene scavengers as the packages were unsealed. LDPE and BOPP MAP resulted in 3.9-5.74% O_2 at 2 ºC and 0.18-1.79% O_2 at 13 ºC after 2 days (Figure 6.1). The CO_2 levels developed were about 4.4-7% in LDPE MAP at both temperatures but reached 14-19% in BOPP MAP. The headspace of both packaging types became anaerobic after 2 days at 13 ºC, but off-odours were only detected in BOPP packages (19% CO_2) when opened.

Respiration rates of broccoli florets changed with storage temperatures and duration ($p < 0.05$) but did not differ significantly between packaging treatments ($p > 0.05$). After 7-day storage at both temperatures, the O_2 consumption rates of 'Thunderdome' broccoli generally reduced by half, from an initial value of 82.67 ± 12.57 mg O_2 kg^{-1} h^{-1} (Figure 6.2). The CO_2 production rates also dropped from 65.20 ± 1.42 mg kg^{-1} h^{-1} to 17.77-30.47 mg kg^{-1} h^{-1} in florets stored at 13 ºC but were unaffected after storage at 2 ºC.



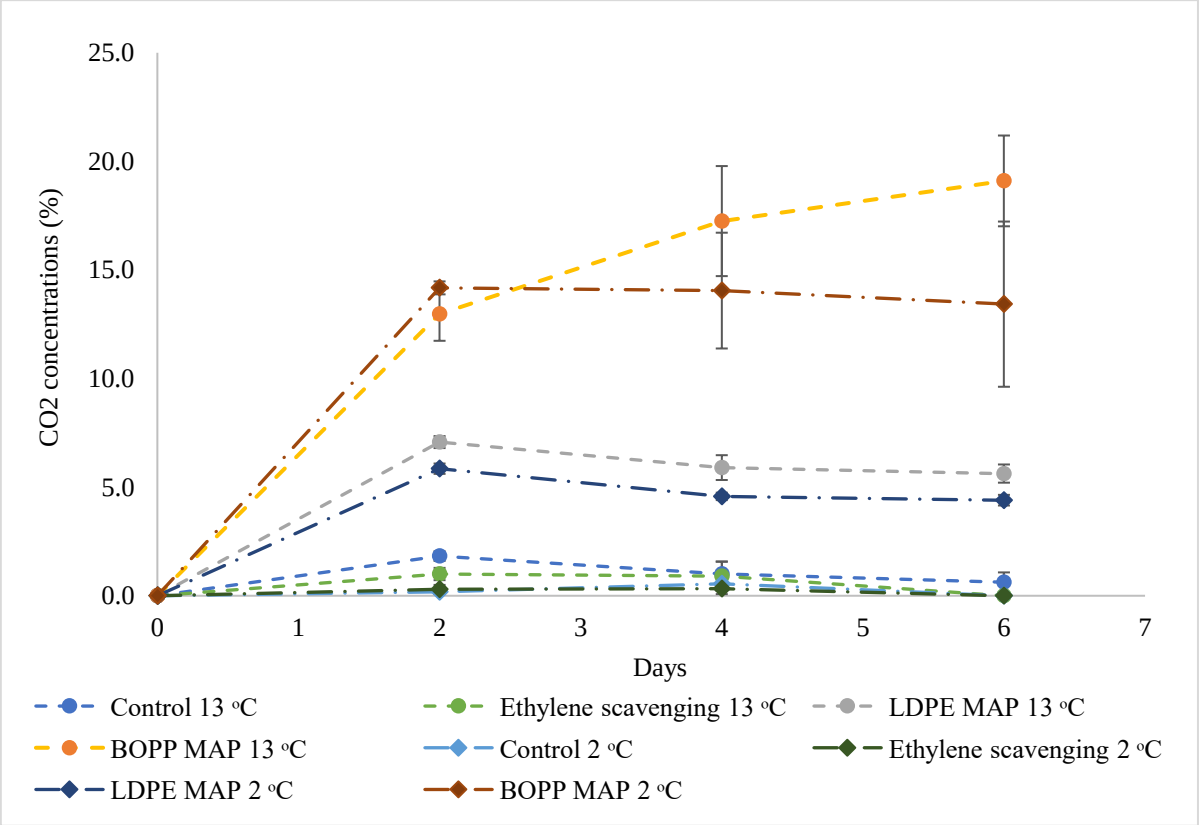


Figure 6.1 Headspace O_2 and CO_2 concentrations of 'Thunderdome' broccoli florets packages during 7 days at 2 ºC and 13 ºC (n = 4)

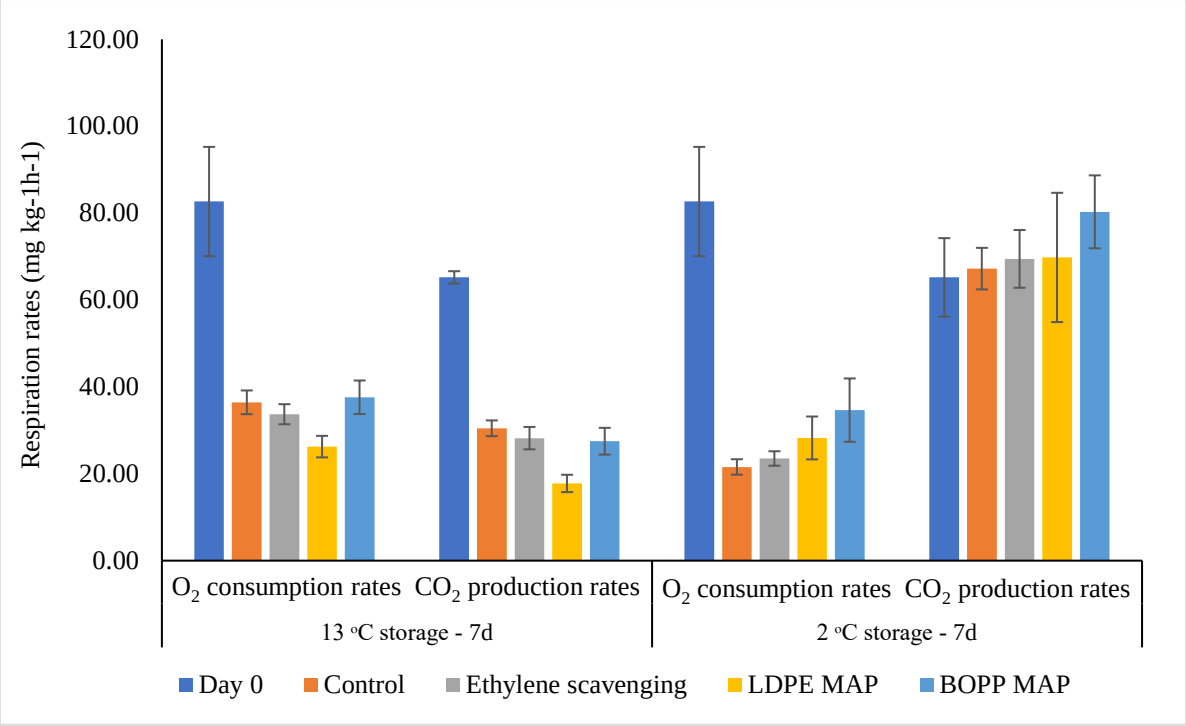


Figure 6.2 Respiration rates of 'Thunderdome' broccoli florets after 7-day storage in different packaging (n = 4)

6.3.1.2 Quality measurements

Different trends were observed for the measured quality attributes in broccoli florets after storage. The chlorophyll a contents were preserved to the same levels as day 0 (49.41 ± 2.48 mg kg^{-1}), by low-temperature storage (2 ºC) and by MAP at an abuse temperature (13 ºC) ($p > 0.05$) (Figure 6.3). In contrast, broccoli stored in normal atmosphere (with and without ethylene scavenging sachets) lost 23.30-28.51 mg kg^{-1} chlorophyll a after 7 days ($p < 0.05$). The contents of chlorophyll b and carotenoids were mostly unchanged ($p > 0.05$). In addition, broccoli weights and L-ascorbic acid contents reduced after storage, regardless to the packaging and storage temperatures. Overall, weight loss of the broccoli florets ranged from 2.5-5.2% and L-ascorbic acid reduced from 539.63 mg kg^{-1} to 117.82-281.13 mg kg^{-1}.

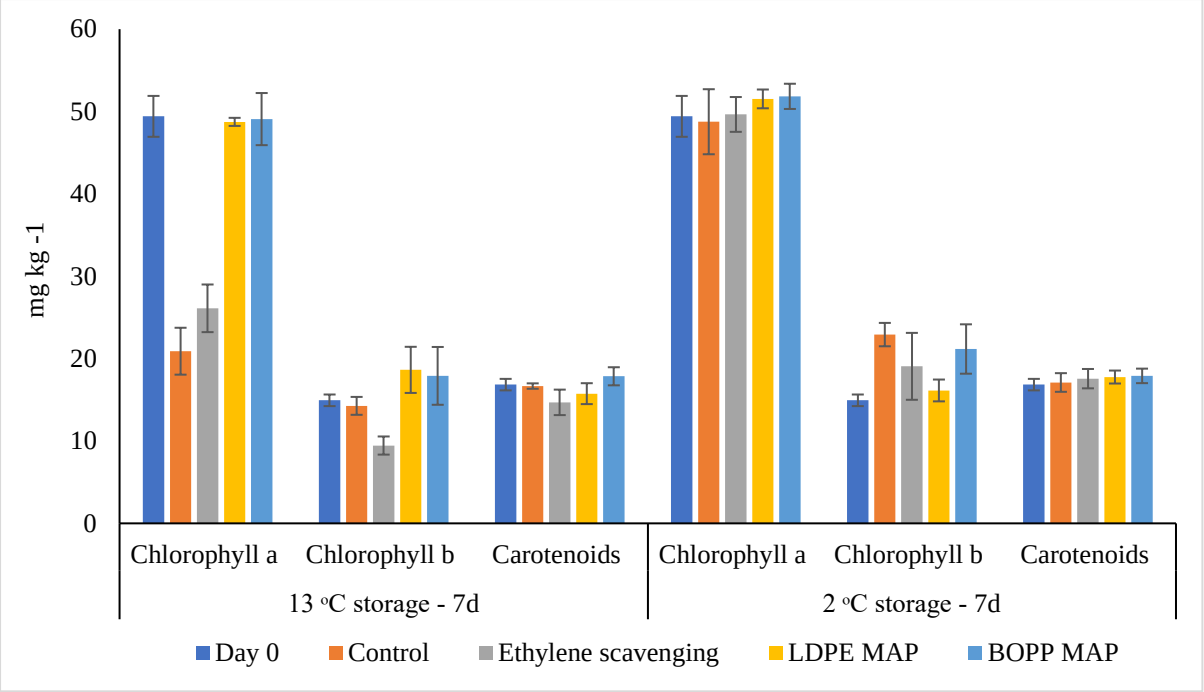
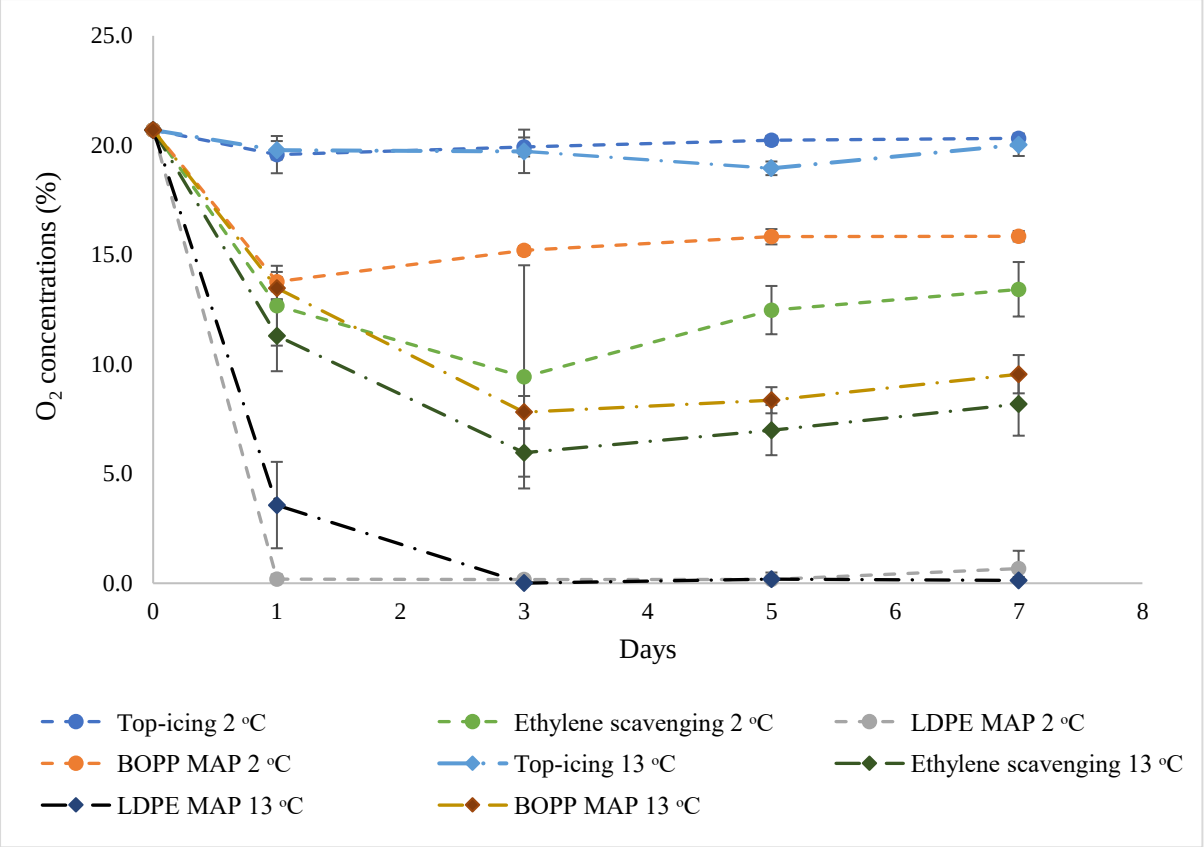


Figure 6.3 Quality measurements of 'Thunderdome' broccoli florets in different packaging after 7-day storage at 13 or 2 ºC (n = 4, p = 0.05)

6.3.2 Commercial trials with whole broccoli in industrial settings

6.3.2.1 Headspace compositions and broccoli respiration rates

The MA conditions in enclosed broccoli packages developed after one day and only slightly fluctuated during storage (Figure 6.4 and 6.5). The O_2 levels were lowest in LDPE MAP in all scenarios, < 1% in 7-day trials and ~ 5% in the 42-day trial. The accompanied CO_2 levels, however, did not exceed 13%. The highest CO_2 levels (11.5-13.8%) were found in BOPP MAP stored at 13 ºC, but O_2 remained > 7.8% during 7-day storage. Liners with ethylene scavenging sachets had comparable O_2 levels to BOPP and PA MAP (13-15% at 2 ºC and 8-9.5% at 13 ºC), but CO_2 concentrations were always low, ranging from 1.5-4%.



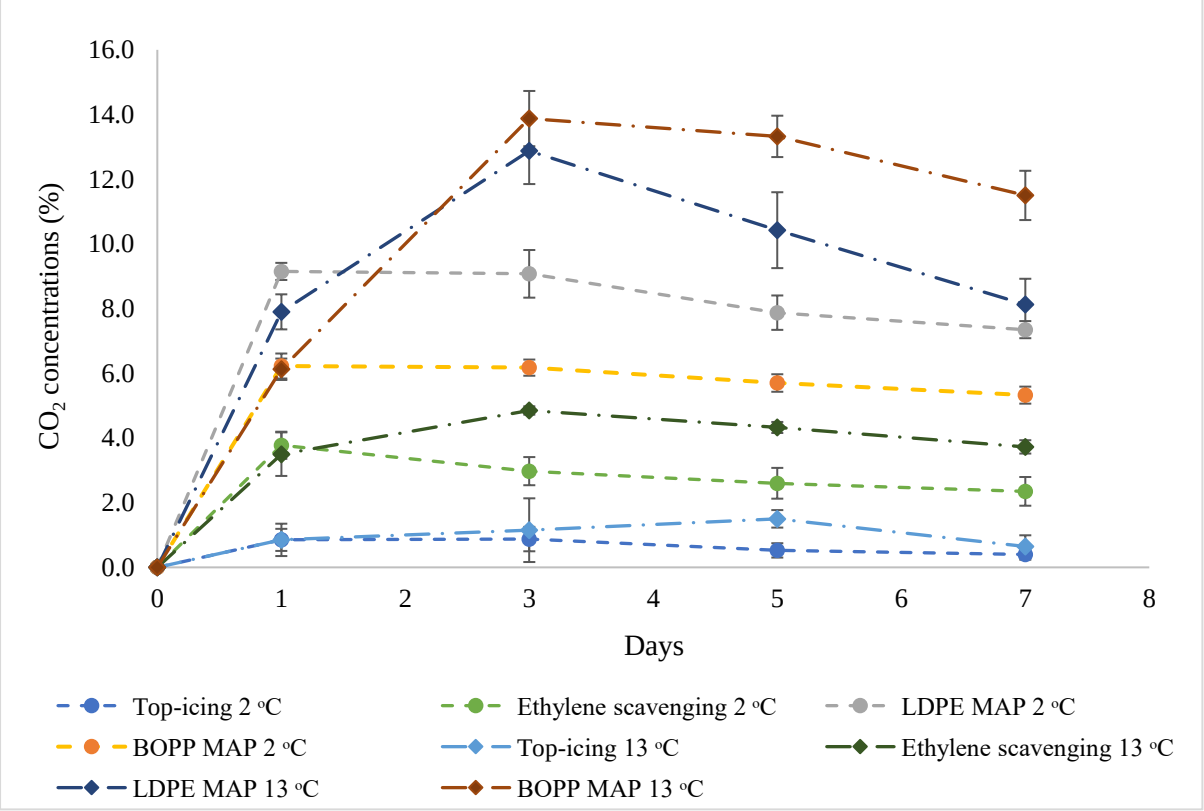
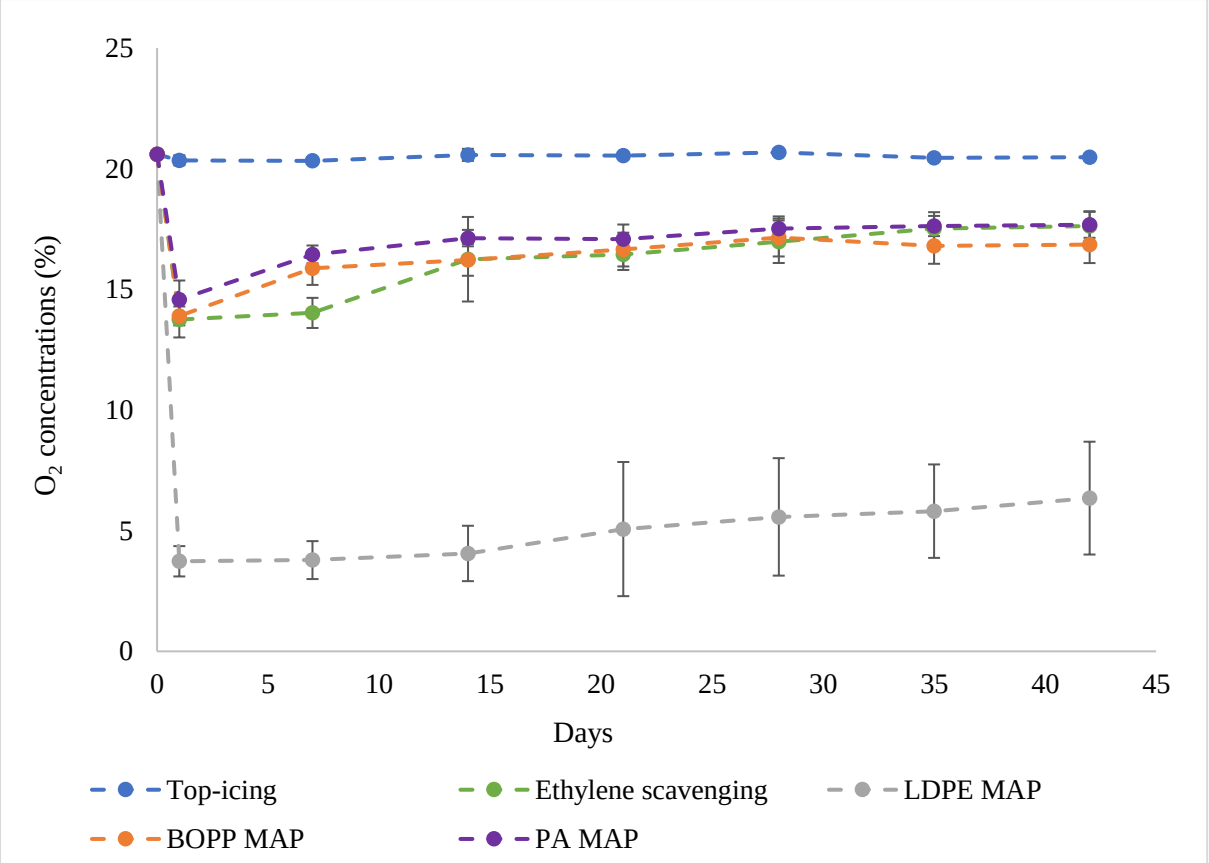


Figure 6.4 Headspace O_2 and CO_2 of 'Ironman' broccoli packages during 7 days at 2 ºC and 13 ºC (n = 4)



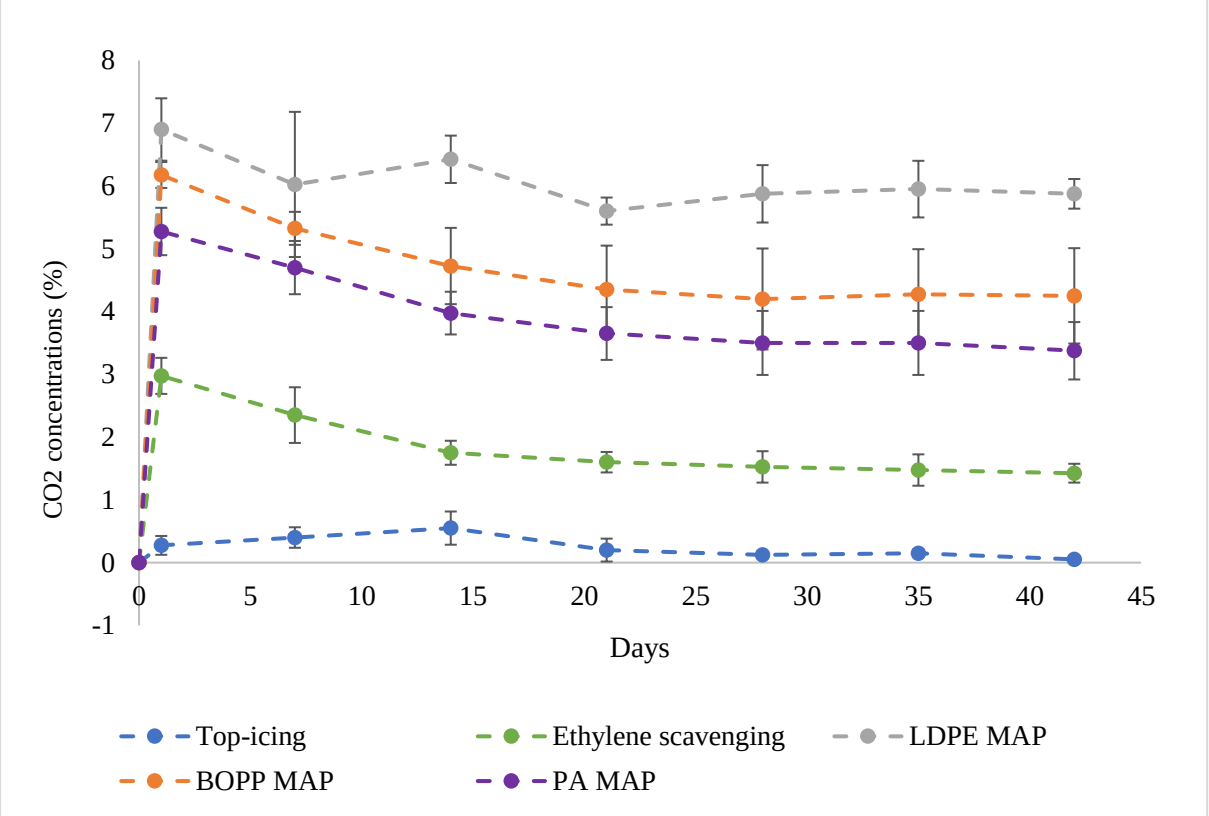


Figure 6.5 Headspace O_2 and CO_2 concentrations of 'Ironman' broccoli packages during 42 days at 2 ºC (n = 4)

The respiration rates of 'Ironman' broccoli were affected by storage conditions and durations ($p < 0.05$). Fresh broccoli had high respiration rates of 36.57 ± 1.05 mg O_2 kg^{-1} h^{-1} and 33.88 ± 1.42 mg CO_2 kg^{-1} h^{-1} at 2 ºC. After 7-day storage in abused temperature (13 ºC), MAP maintained the respiration rates closer to day 0, while the values increased to 58.66 ± 5.87 mg O_2 kg^{-1} h^{-1} and 48.91 ± 8.30 mg CO_2 kg^{-1} h^{-1} for ice-topped broccoli (Figure 6.6). In cold storage (2 ºC), the respiration rates were not affected by packaging treatments, but halved after 42 days.

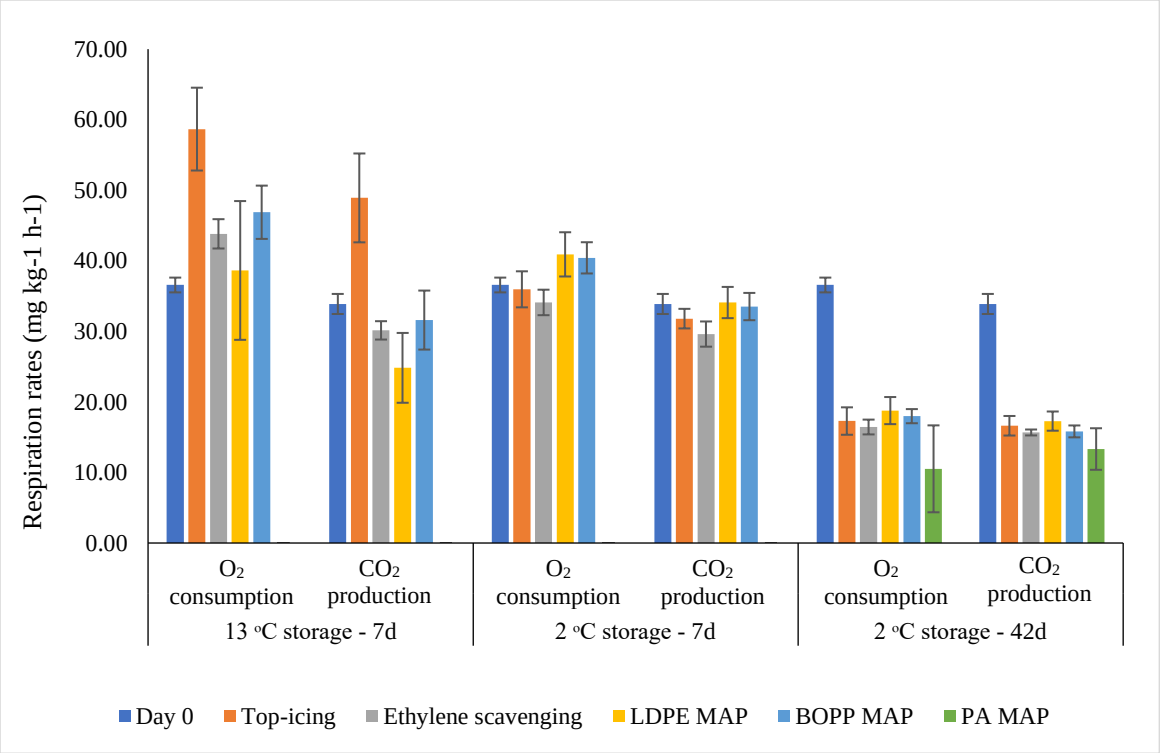


Figure 6.6 Respiration rates of fresh and stored 'Ironman' broccoli (n = 4, p = 0.05)

6.3.2.2 Weight changes

Packaging types significantly affected the weight changes of broccoli in all storage conditions ($p < 0.05$). Ice-topped broccoli had weights increased by 6-12% mainly due to the uptake of meltwater, which adversely affected the broccoli appearance, especially at 13 ºC. In contrast, broccoli in MAP lost 0.5-0.9% weight after 7 days and 0.5-1.9% after 42 days (Figure 6.7). Weight losses of broccoli in MAP increased with storage duration ($p < 0.05$) but were not

affected by temperatures and packaging types in 7-day trials ($p > 0.05$). In the 42-day trial, broccoli in PA MAP had highest weight loss of 1.87% with dehydrated appearance, followed by HDPE liners + ethylene scavengers, and LDPE and BOPP MAP.

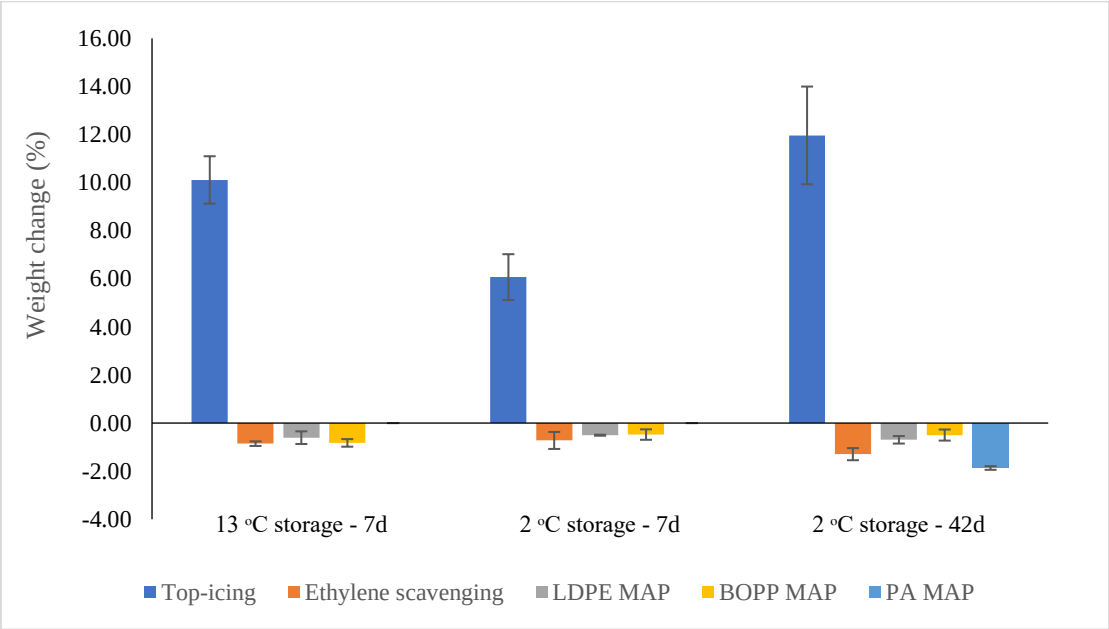


Figure 6.7 Weight changes of 'Ironman' broccoli in different packaging after storage (n = 4, p = 0.05)

6.3.2.3 Chlorophyll, carotenoid, and L-ascorbic acid contents

Storage temperatures and packaging types significantly affected the retention of chlorophyll contents in broccoli ($p < 0.05$), while carotenoid contents did not change in all conditions ($p > 0.05$). Current extraction and analytical method found on average 43.35 mg chlorophyll a, 15.84 mg chlorophyll b and 15.28 mg carotenoids per kg fresh 'Ironman' broccoli. There was no change in chlorophyll contents in all samples after 7 days at 2 ºC, and in all MA-packed broccoli after 42 days at 2 ºC or 7 days at 13 ºC ($p > 0.05$). In contrast, chlorophyll a level of ice-topped broccoli was reduced to 34.27 mg kg^{-1} after 42 days at 2 ºC. At 13 ºC, ice-topped broccoli started turning yellow on day 5 and became completely yellow on day 7 with a loss of 78% chlorophyll a and 63% chlorophyll b ($p < 0.05$) (Figure 6.8 and Figure A3.1, Appendix 3).

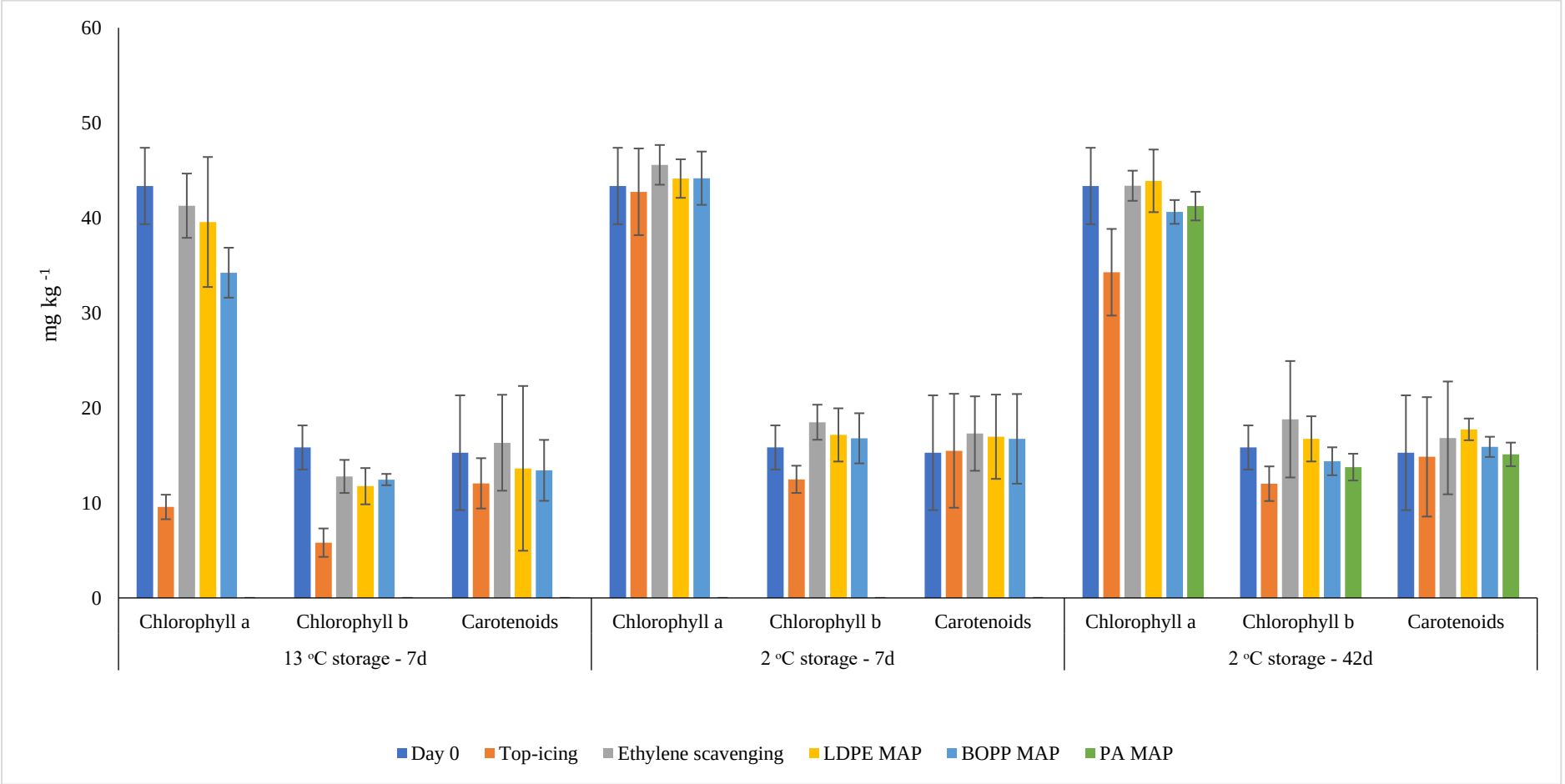


Figure 6.8 Quality measurements of 'Ironman' broccoli in different packaging after storage (n = 4, p = 0.05)

The L -ascorbic acid contents in broccoli were affected by storage duration ($p < 0.05$), but not packaging type and temperature ($p > 0.05$). From an initial value of 466.44 ± 37.28 mg kg^{-1}, the L-ascorbic acid contents reduced to by > 50% after 7 days and > 80% after 42 days.

6.3.2.4 Aroma profiles

The major broccoli volatiles identified by SPME GC-MS in these trials were C6 aldehydes (hexanal and 2-hexenal), followed by sulphur compounds (dimethyl disulphide DMDS, dimethyl trisulphide DMTS and 2-methylbutyl isothiocyanate), (E)-3-hexen-1-ol and acetone. The evolution of broccoli aroma profile in different packaging types differed between the three storage scenarios. After 7 days at both temperatures, top-icing broccoli had the lowest peak areas for C6 aldehydes and acetone was not detected in samples using HDPE liners with permanganate ethylene scavenging sachets. At 13 ºC, 2-methylbutyl isothiocyanate was not found in any treatments. The peak areas of DMDS and DMTS in BOPP and LDPE MAP packed broccoli were about 8.5 times higher than in top-icing (Figure 6.9). These values were insignificantly different from fresh broccoli, except that DMDS area in LDPE MAP broccoli was 4 times higher than day 0. In contrast, DMDS and DMTS peak areas in ice-topped broccoli were 3 times higher than fresh and other packaging types on average after 42 days at 2 ºC (Figure 6.10).

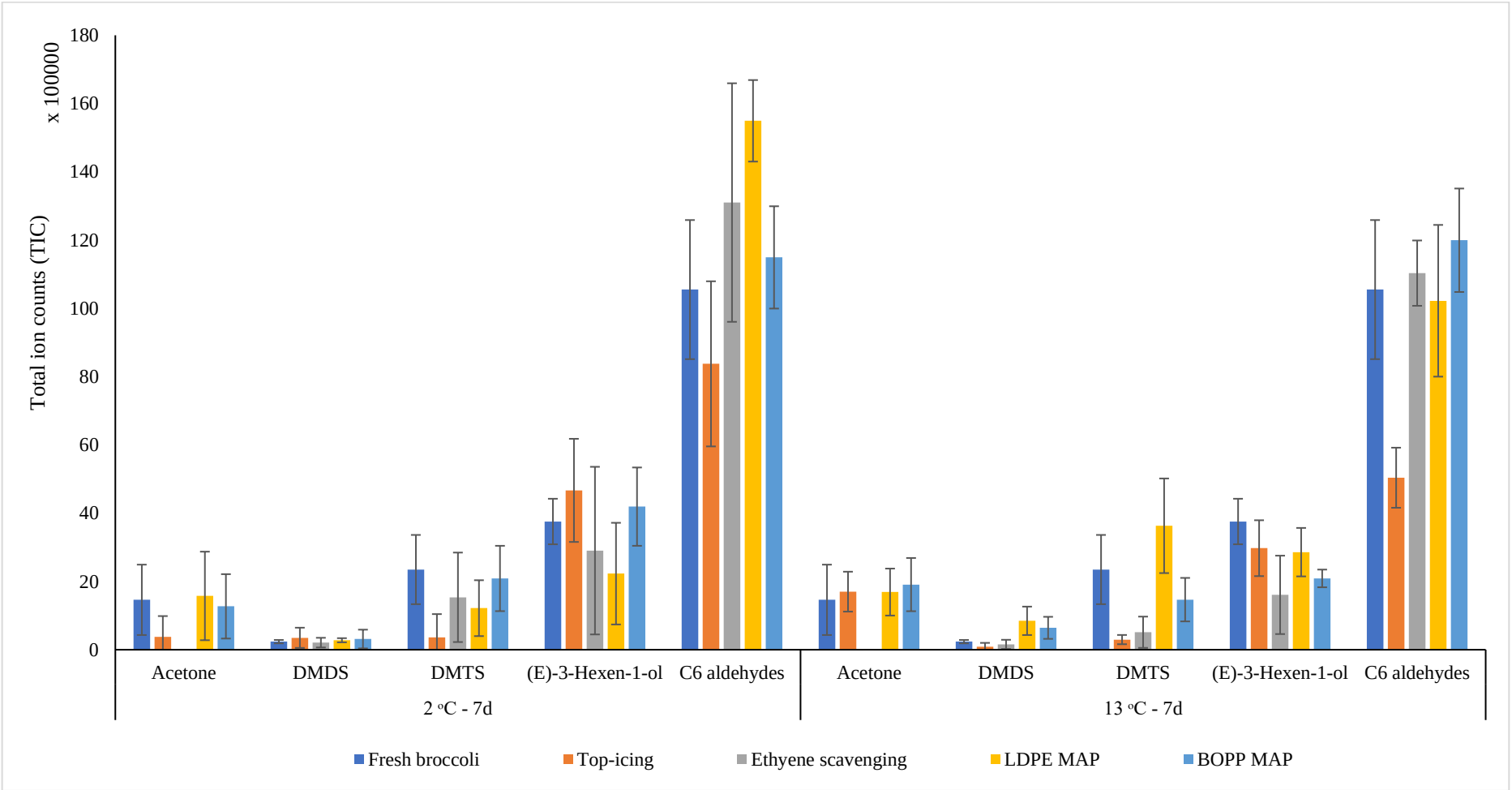


Figure 6.9 Peak areas of major volatile compounds in 'Ironman' broccoli in different packaging after 7-day storage (n = 4, p = 0.05)

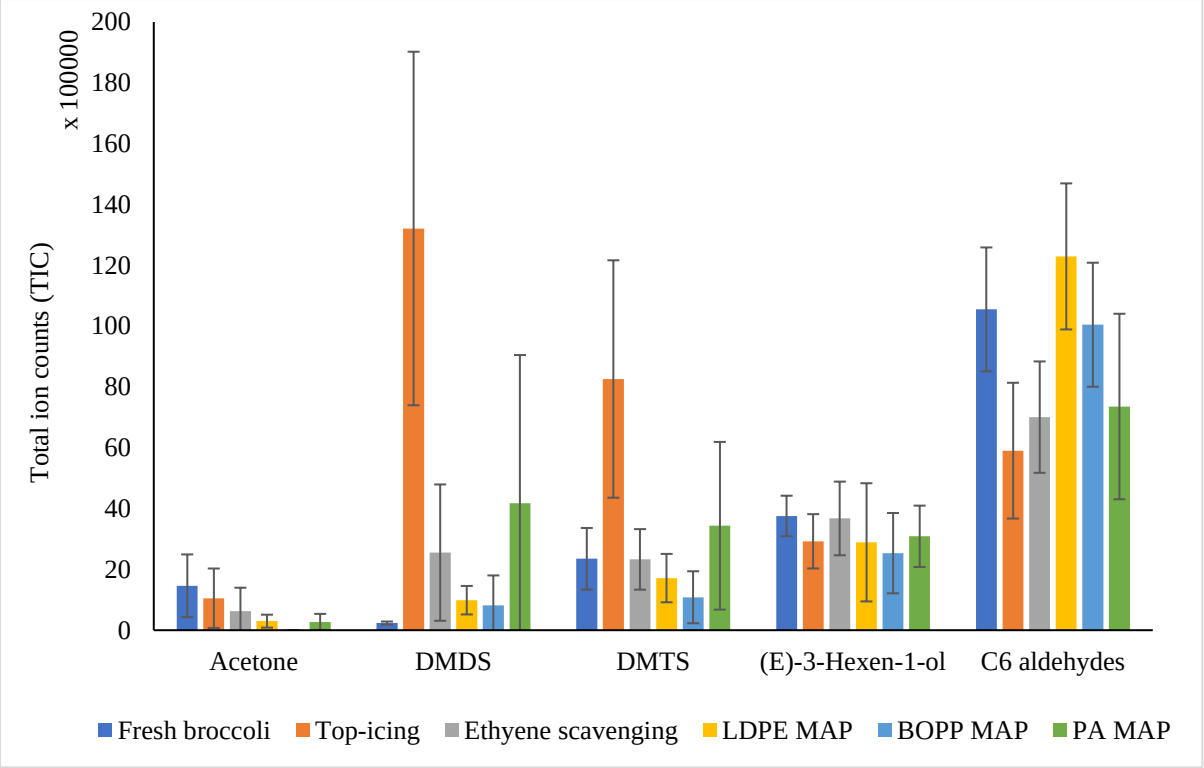


Figure 6.10 Peak areas of major volatile compounds in 'Ironman' broccoli in different packaging after 42-day storage (n = 4, p = 0.05)

6.4 Discussion

The current study demonstrated that MAP technologies were better options than top-icing at maintaining broccoli quality in three simulated broccoli shipping conditions. The major benefits of the packaging trialled in this study were to suppress broccoli respiration rates, minimise weight loss and reduce exposure to ethylene.

6.4.1 Relationship of weight loss to packaging conditions

Moisture loss is the major cause of wilting and alters broccoli texture, making the stem elastic and more fibrous (Jacobsson et al., 2004c; Serrano et al., 2006). Top-icing was proposed to alleviate moisture loss as the meltwater would help rehydrate the broccoli and retain firmness (Gillies & Toivonen, 1995). Even so, the water absorption leading to 6-12% increases in weight adversely affected broccoli appearance in this study. Meanwhile, MAP aided the prevention of moisture loss by creating a high RH condition within the packages and

prevented the dehydration by forced-air cooling in the distribution centre. The extent of moisture exchange between the packaging and the external environment was determined by its WVTR and perforations if present. Among the packaging trialled, LDPE and BOPP MAP were best at minimising broccoli weight losses. However, their low WVTRs led to the accumulation of moisture condensation inside the packages that favoured rots of the fallen buds, as observed in the 42-day trial. In contrast, PA MAP was designed to achieve similar gas exchange performance as the BOPP packaging, but with higher WVTR. Consequently, there was no moisture condensation in PA MAP after 42 days, but the broccoli appeared dehydrated with a mean weight loss of 1.9%. Similar observations were noticed in the process of optimising MAP for broccoli branchlets (Caleb et al., 2016). In that study, a WVTR of 21.6 mg h^{-1} (a BOPP film) was considered as too low and led to the built-up of moisture condensation, while 216 mg h^{-1} (a cellulose-based film) caused shrivelling and excessively reduced marketable weights.

6.4.2 Impacts of packaging on broccoli respiration

Fresh broccoli is extremely perishable, with a high respiration rate and high sensitivity to ethylene, leading to rapid postharvest quality deterioration (Cai et al., 2019). While allowing quick establishment of MA conditions in the packages, their high respiration rates (36.57-82.67 mg O_2 kg^{-1} h^{-1} and 33.88-65.20 mg CO_2 kg^{-1} h^{-1} as found in the two cultivars trialled) also increase the risks of O_2 depletion and CO_2 accumulation that subsequently induce the formation of off-odours.

The benefits of MAP in delaying broccoli senescence were particularly noticeable under abuse temperature conditions (13 ºC) and in long-term cold storage. The respiration rates of ice-topped broccoli increased by 12-15 mg kg^{-1} h^{-1} after 7 days at 13 ºC, compared to day 0, while those of MA-packed broccoli were more stable. L. Li et al. (2016) found the rapid increases in respiration rates during 8 days at 10 ºC accompanied with decreases in ATP

associated with the senescence that limited the shelf life of broccoli stored in air to 12 days. The reduced energy levels due to postharvest aging probably also explained the 50% reduction in respiration rates that was observed in all samples stored for 42 days at 2 ºC in this study, compared to the initial broccoli.

6.4.3 Impacts of packaging on broccoli colour

Yellowing is a critical quality deterioration of broccoli which is associated with the reductions of chlorophyll contents (Luo et al., 2020). The floral buds of broccoli are harvested when still immature and in a rapid growth stage (Hasperué et al., 2015). The stress from the sudden cut of nutrients and hormone supplies triggers senescence leading to rapid degradation of chlorophyll (Martínez-Hernández et al., 2013) to prevent the generation of free radicals from photochemical reactions (Matile et al., 1999). Chlorophyll degradation during broccoli senescence has been linked to the increased expression of pheophytinase (*BoPPH*), which is regulated by the hormone ethylene and cytokinins (Büchert et al., 2011a; Büchert et al., 2011b). This process explained the severe chlorophyll losses (up to 78%) and yellowing in air-stored (lab trial) and ice-topped broccoli (commercial trial), particularly at 13 ºC. In contrast, MA had no inhibition effects on the expression of *BoPPH* (Büchert et al., 2011a), although MA-stored broccoli in this study retained the chlorophyll contents closest to the initial values. This observation suggested that the chlorophyll retention in MA could relate to the expressions of other genes involving in chlorophyll catabolism including pheophorbide *a* oxygenase (*BoPaO*) (Gómez-Lobato et al., 2012). Furthermore, the high CO_2, low O_2 conditions of MA were suggested to inhibit the biosynthesis and activity of ethylene in broccoli (Deschene et al., 1991), which could have had a role in delaying chlorophyll degradation. The LDPE mineral-clay impregnated MAP trialled in this experiment also has known ethylene-absorbing properties. Due to the complex interactions between MA, ethylene and the enzymes related to chlorophyll catabolic pathways, it remains

unclear if the developed MA or ethylene scavengers or both had contributed to the retention of chlorophyll contents in the cases of HDPE liners with ethylene scavenging sachets and LDPE MAP in this study.

6.4.4 Influences of packaging on aroma

Ethanol was suggested to be the most important compound responsible for the off-odours developed under high CO_2 conditions (> 10-15% CO_2) (Toivonen & DeEll, 2001), while pungent sulphurous compounds were linked to the low O_2 atmospheres (0.5% O_2) (Forney et al., 1991). In this study, however, off-odours were only detected in packages that had both low O_2 and high CO_2, typically < 1% O_2 & > 11% CO_2. In contrast, no offensive odours were detected in the samples with < 1% O_2 & 7-9% CO_2, nor in the packages with 8% O_2 & 13-15% CO_2.

Sulphurous compounds are important to the aroma of broccoli. While isothiocyanates are responsible for the distinctive broccoli flavours, DMDS and DMTS were associated with the offensive odours as in overcooked and spoiled broccoli (Hansen et al., 1993). The formations of sulphurous volatiles are linked to enzymatic reactions that occur when cells are injured (Chin & Lindsay, 1994), as in membrane degradation during storage and in maceration. The GC-MS peak areas of volatiles from MA-packed broccoli were mostly close to the initial broccoli in all trialled scenarios, possibly because MA suppressed respiration rates and slowed cell deterioration. An exception was broccoli packed in LDPE MAP stored at 13 ºC for 7 days which had 4 times higher DMDS than fresh broccoli. This observation could be a result of membrane and cellular compartmentalisation breakdown due to the extreme MA conditions (< 1% O_2 & 8-13% CO_2) (Dan et al., 1997). Similarly, the peak areas of DMDS and DMTS in ice-topped broccoli stored at 2 ºC for 42 days were 3-times higher than fresh and MA-packed broccoli, suggesting more severe membrane degradation in these samples. Meanwhile, storage at 13 ºC for 7 days greatly accelerated senescence in ice-topped broccoli,

as evidenced by serious yellowing. DMDS and DMTS in these samples possibly had been further metabolised, resulting in 8.5-times lower peak areas compared to other treatments and freshly harvested broccoli. Furthermore, the absence of volatile isothiocyanates in all samples stored at 13 ºC could indicate changes in overall flavour perceptions of the broccoli as the remaining compounds are towards green notes (C6 aldehydes and (E)-3-hexen-1-ol) and off-odours (DMDS, DMTS and acetone) (Hansen et al., 1993; Jacobsson et al., 2004a).

Lastly, the different observations between the lab and commercial trials in this study also highlighted the challenges and requirements when upscaling MAP technologies. The respiration rates of fresh broccoli could vary greatly with pre-harvest factors such as cultivars as observed in the 'Thunderdome' and 'Ironman' broccoli. Therefore, optimisation of MAP would need to be considered for specific cultivars and for growing or harvest environmental conditions. For consistent development of headspace O_2/CO_2 levels, microperforated bags must be used at their full sizes, while non-perforated MAP require optimised produce weight-to-packaging volume ratios.

6.5 Conclusion

This study found novel MAP technologies were better than conventional top-icing at maintaining broccoli quality in three simulated shipping conditions. The benefits of MAP, including lowering broccoli weight loss, suppression of respiration rates and delaying chlorophyll degradation, were manifest under abuse temperature (7 days at 13 ºC) and extensive storage (42 days at 2 ºC) conditions. The optimisation of packaging design, or of the ratio of produce weights versus packaging volume to maintain an MA condition of > 1% O_2 and < 15% CO_2, is important to prevent the formation of off-odours in package headspaces. Packaging with low WVTR were better at maintaining broccoli weight and fresh appearance after 42 days but had high moisture condensation. Meanwhile, MAP systems with ethylene scavenging properties aided broccoli storability, but the contribution of individual

factors remains unclear. Further studies are needed to confirm current findings with broccoli across different seasons, varieties, harvest time and delayed packing. In addition, greater understanding of the influence of packaging on microbial quality and sensory evaluation would be useful in justifying and tuning of MAP for wider industrial application.

Chapter 7: General discussion

Fresh fruit and vegetables are labile products that suffer rapid quality deterioration and senescence after harvest. MAP has been proven to extend the shelf life of several types of fresh produce by protecting them from handling damage and moisture loss, and through suppressing metabolism (M.D. Wilson et al., 2019a; X. Chen et al., 2020). MAP is also available in various forms from retail packaging to bulk storage bags, and as pallet wrapping for shrouds. However, MAP has had a slow adoption speed with relatively few commercial applications in fresh horticultural produce (Madrid, 2019; Mahajan et al., 2017). This observation has been explained by the lack of strong evidence of shelf life improvements failing to justify the extra costs of the packaging. MAP is product-specific, and some produce can respond negatively to the MA developed (X. Chen et al., 2020). The effectiveness of the systems to attain and maintain the desirable high CO_2 and low O_2 conditions has also been a challenge, particularly under varying produce temperatures and respirations (Mahajan et al., 2017; Zhang et al., 2016). Microperforations and porous materials offer the potential to increase packaging permeability, and therefore, allow better control on the atmosphere developed inside the packages.

Optimisations of the packaging systems by combining MAP with other technologies have been identified as a research need for future applications of MAP in fresh foods (X. Chen et al., 2020; Zhang et al., 2016). The use of supplementary technologies that are able to overcome the limitations of MAP should allow more moderated and effective MA conditions in which O_2/CO_2 levels would not exceed the tolerance of the produce. Examples of such preservation techniques are sanitisation, heat treatments, ethylene inhibitors and scavengers, for the active control against microbial growth, ethylene and enzyme activities (Watkins, 2018). These technologies are also available in the formats of sachets or sheets that are easy and safe to use, without requiring large capital investments (Álvarez-Hernández et al., 2019; Erickson, 2017; Saito et al., 2020). The research challenge was to understand the potential for optimising MA in

conjunction with related commercial packaging and other postharvest treatments, to achieve a more effective, consistent and robust shelf life extension than individual applications of each technology.

This objective was approached by first studying the efficacy of MAP and their combinations with other postharvest technologies on higher-value, perishable produce. This would allow industry to more easily gain experience and provide a pathway to better estimation of margins of benefits for further applications. Therefore, this research has trialled MAP, and their combinations with sanitisation and ethylene scavenging treatments, for effectiveness in extending the shelf life of raspberries, blueberries, and broccoli. These are three Tasmanian high-value products with known commercial shelf life issues for shipment to the Australian mainland and further export.

7.1 The effects of MAP systems on the quality of raspberries, blueberries and broccoli in commercial settings

This work has demonstrated that MAP is able to extend the shelf life of raspberries and blueberries in cold storage, as well as better maintain broccoli quality under three simulated shipping conditions compared to conventional top-icing. Specifically, at 1.5-2 ºC, as in commercial cold storage, an MA of 14-15% O_2 & 7-8% CO_2 extended storability of raspberries by 1-2 weeks (Chapter 3), while an MA of 16-18% O_2 & 2-4% CO_2 extended storability of blueberries by 4-6 weeks (Chapter 4). MA with > 1% O_2 and < 15% CO_2 maintained the quality of broccoli under an abuse temperature condition (7 days at 13 ºC) and extensive storage (42 days at 2 ºC) where conventional top-icing practice led to severe quality losses (Chapter 6). These atmospheric conditions are different to the recommended levels in literature, of 5-10% O_2 & 10-20% CO_2 for raspberries (Bower, 2007), 5-10% O_2 & 10-12% CO_2 for blueberries (Mitcham, 2007; Paniagua et al., 2014) and 1-2% O_2 & 5-10% CO_2 for broccoli (Toivonen & Forney, 2016). However, for the prolonged storage needed for export

purposes they would be more industrially achievable without risking exceeding the low O_2 and high CO_2 tolerance limits of the produce.

The main benefits of optimised MAP found in this study were similar to previous reports on the same produce. These are maintaining produce weight and delaying quality deterioration including mould growth in berries (Adobati et al., 2015; Alsmairat et al., 2011; Koort et al., 2018), darkening in raspberries (Giovanelli et al., 2014; Moor et al., 2009) and yellowing in broccoli (Esturk et al., 2014; Hagen & Larsen, 2020; Marzano-Barreda et al., 2020). In addition, the availability of a range of packaging with different WVTR allowed trials to optimise the packaging for different applications. For example, MAP with high WVTR prevented moisture accumulation in berries that could subsequently favour mould growth (Horvitz, 2017), while low WVTR was better at minimising weight loss in broccoli during extensive storage (Chapter 6). The delay in quality deterioration was attributed to the low O_2 and high CO_2 conditions in MAP that suppressed produce respiration rates and microbial growth (M.D. Wilson et al., 2019a).

Matching the respiration rates of the produce and the gas transmission rates of the packaging is the key to the successful application of MA (Mangaraj et al., 2009; M.D. Wilson et al., 2019a). However, this is also a technical challenge for wider applications of MAP in the industry (Bishop et al., 2020; Brandenburg, 2020). This study trialled two categories of commercial packaging with different methods for optimisation. The mineral-clay impregnated bag currently requires a trial-error approach to determine the suitability and optimise applications to match the existing film permeability. In contrast, the gas exchange properties of microperforated bags were tailor-made for each application, based on pre-determined respiration rates, known storage conditions, durations and desirable MA (Hussein et al., 2015). However, neither types of packaging were able to attain the ranges of recommended MA for raspberries, blueberries, and broccoli (Huynh et al., 2019; Madrid &

Beaudry, 2020; Paulsen et al., 2018). This observation could be explained by the significant effects of the ratios between bag volume and produce weight on the final MA developed in non-perforated MAP (Rodriguez & Zoffoli, 2016). This was also seen in the mineral-clay MAP in broccoli trials (Chapter 6). For microperforated MAP, the manufacturing requires information on respiration rates of the target produce at the designated storage temperatures, which could be obtained by experimental measurement beforehand or from scientific literature. However, as shown in this study, the respiration rates of the actual experimental materials could still vary greatly from those determined in preliminary trials and from available literature (Perkins-Veazie, 2016b; Toivonen & Forney, 2016). These variations could be the results of varying preharvest factors (cultivation practices, cultivars, maturities, etc.) and postharvest handling such as delay cooling and holding periods and conditions before the start of experiments (King & Morris, 1994; Mitcham, 2007). In addition, microperforated MAP often compromises the targeted optimum MA to avoid the risk of O_2 depletion and excessive CO_2 accumulation (Lucera et al., 2011).

Additional postharvest technologies, such as sanitisation and ethylene control, have been proposed as complementary treatments to offset the limitations of MAP, particularly for long-term storage (Huynh et al., 2019). However, trials using MAP in combination with H_2O_2-releasing pouches or SO_2-releasing sheets in berries, and with $KMnO_4$ ethylene scavenging sachets in broccoli, did not show superior storability and quality. The lack of effectiveness of sanitisation in berries was possibly because the sanitisers had become inactive during the long trials (Spayd et al., 1984). The developed MA and the natural plant defence mechanisms could also have been sufficient to control fungal decay in the early stages of cold storage but not for extended periods (Tournas & Katsoudas, 2005). Therefore, these findings emphasise the importance of starting with high quality of berries, as technologies were not able to compensate the quality loss caused by extensive storage (Madrid & Beaudry, 2020).

Increasing the concentrations of sanitisers, particularly SO_2, is not a viable solution as it could cause bleaching and softening (Spayd et al., 1984) as observed in SO_2-treated blueberries (Chapter 4) and in preliminary trials for raspberries. The uses of SO_2 would also need to meet legislation requirements, as it could trigger allergic responses in sensitive people (Freedman, 1980). Meanwhile, H_2O_2 could benefit routine sanitisation of storage areas where humidity is adequate, and the sachets could be replaced periodically. In broccoli, the benefits of using ethylene scavengers in a MAP system remained unclear. This observation was in line with suggestions that the high CO_2, low O_2 conditions in MAP could inhibit ethylene biosynthesis and activity (Deschene et al., 1991), and interfere with the expression of genes involving in chlorophyll catabolism (Gómez-Lobato et al., 2012).

7.2 The effects of perforated MA as a retail packaging on the quality of raspberries and blueberries

Conventional passive MAP for storage and large volume shipments relies on the produce respiration to build up MA, which might not reach the desirable O_2 and CO_2 levels, or is only capable of being achieved over an extended storage period (Sousa-Gallagher et al., 2016). MA in a retail packaging, therefore, could be easier to optimise owing to smaller produce volumes and packaging sizes and thus faster equilibration of the gas atmospheres. In addition, produce with moderate respiration rates could benefit from the accelerated establishment of MA by replacing the air (~21% O_2) in the package headspace with a desirable atmosphere (Wandelen, 2011). The current results demonstrated the advantages of this new approach in maintaining the MA levels and quality of raspberries and blueberries during storage (Chapter 5). A model of retail MAP using a non-vented 1L tray sealed with a perforated BOPP lid film was trialled to pack 125 g of berries. Raspberries had high respiration rates that rapidly built up MA in the trays. A percentage of vented area of 5×10^{-9} formed by six microperforations

of 70-µm diameter combined with air as the initial atmosphere was found to be optimal for raspberries. Compared to commercial vented clamshell punnets, the design resulted in 4 days extra shelf life at 2 ºC with a headspace of 9.3% O_2 & 13.9% CO_2 after 11 days. This MA condition was closer to the recommended levels of 5-10% O_2 & 10-20% CO_2 for raspberries (Bower, 2007) than the 14-15% O_2 & 7-8% CO_2 obtained from the bulk-storage MAP trialled in Chapter 3. Similarly, the model of retail MAP was able to maintain the headspace close to 10% O_2 & 10% CO_2 (Mitcham, 2007; Paniagua et al., 2014), which was not achievable with the bulk-storage MAP trialled in Chapter 4 due to the moderate respiration rates of blueberries. The optimal design that extended the storability of blueberries for 3 weeks at 2 ºC comprised of an initially reduced O_2 (17% or 14.5%) atmosphere with a lid film having two microperforations of 70-µm that formed a percentage of vented area of 1.68×10^{-9}. Because of the limited produce seasonality versus long storage time, the research was not able to perform a verification study for this trial.

7.3 Suggestions for future research

This work represents a comprehensive overview of the potential for optimising MAP in combination with related post-harvest technologies to significantly extend the shelf life and quality preservation of three perishable produce examples. However, time and resource pressures limited further investigation into other factors important for commercial applications, such as variations due to preharvest factors, as well as sensory properties of the stored products. Several preharvest factors, such as genetics, environments, and cultivation practices, could greatly affect the postharvest quality and subsequent storability of fresh produce (Madrid & Beaudry, 2020; Yahia et al., 2019). Therefore, more evidence from industry-based research need to be gained across different harvest seasons, locations, and cultivars to validate improvements. Most of the available publications in MAP for berries and broccoli are lab-based research (Chapter 2) that were conducted under well-controlled

environments and had better material screening. In contrast, market-grade fresh produce often has broader ranges of quality criteria, and thus, higher variations, particularly when quality control was performed by sub-sampling. For example, in the first raspberry commercial trial (Chapter 3), the fungal decay in control samples stored in clamshells punnets varied between 4-20% after 20 days of storage. Variations were noticeably smaller in blueberries and broccoli where the individual produce items were mechanically or manually sorted (Chapter 4 and 6). This issue could be mitigated, as was done in the trials reported in this work, by increasing the numbers of replicates, increasing the size of experimental units and randomising the berry trays with in one pallet. However, repeating the trials with optimisation would allow for more reliable results and confidence in the repeatability of the packaging performance.

The early findings on perforated MA as a retail packaging for raspberries and blueberries (Chapter 5) could provide some insights for upcoming research using the same approach for berries as well as other soft fruit and fresh-cut produce. These datasets could be used to verify and might help improve the existing predictive models on O_2 and CO_2 diffusion rates through microperforated packaging films (González-Buesa et al., 2009; D.R. Paul & Clarke, 2002; Renault et al., 1997; Zanderighi, 2001). However, future research on modelling would need to consider the changes in respiration rates with produce aging as observed in this work (Chapter 3, 4 and 6), which would lead to changes in physiological requirements of the produce during storage. The selectivity of different plastic materials to O_2 and CO_2 adsorption and diffusion (Exama et al., 1993) and particularly, the effects of temperature fluctuation must be further studied, as it is a significant issue in commercial practice, and causes complex changes to the parameters of the models. Furthermore, while modelling is valuable for understanding the sciences, empirical research that is less time-consuming and

potentially provides faster and reliable packaging solutions to the industrial problems might be of greater interest from the industry perspective.

Further studies on the sensorial properties of the stored products would also be critical because eating quality determines consumer satisfaction and encourage repeated purchases (Baldwin et al., 2007). This research found significant changes in the GC-MS aroma profiles of berries and broccoli after storage in MAP, compared to fresh and control samples, noticeably the lower amounts of terpenes in MA-packed berries. The biosynthesis of terpenes requires precursors from pyruvate oxidation process, which is interfered with in the low O_2 condition of MA. Similar observations were reported in strawberries (Peano et al., 2014) and blackcurrants (Harb et al., 2008) and could be expected in other produce that also has the formations and evolutions of terpenes during storage. Since terpenes are responsible for the herbal, floral notes in berries (Aprea et al., 2015), it would be important to evaluate the sensorial impacts of this change and if terpene synthesis could be recovered after the fruit is removed from MAP, as found in blackcurrants (Harb et al., 2008). Traditional sensory evaluation requires either a large number of untrained participants or training the panellists and adds to the costs of research (W. Wang & Liu, 2019). E-tongues, innovative analytical instruments that replicate human taste perception, offer opportunities to measure taste attributes without having panellists. The sensor array of an e-tongue analyses non-volatile compounds in liquid matrices to evaluate sweetness, saltiness, bitterness, sourness, umami and astringency (Tan & Xu, 2020). Combining the information from GC-MS, e-tongue analysis, and TSS/TA values, therefore, would provide a more complete understanding of sensory properties of the stored products. Furthermore, this study was not able to quantify texture of berries due to the unavailability of an objective, reliable measurement method (C. Li et al., 2011). While an instrument specifically developed to measure the firmness of soft

fruit is available (e.g. Biowork Firmtech II) (NeSmith et al., 2000), development of low-cost, reliable methods would be helpful for improved industry and research uptake.

Other potential research themes for the shelf life extension of fresh horticultural produce include the trialling of temperature-responsive packaging that changes in gas permeability to counter the increases in produce respiration rates when temperatures increase (Clarke, 2001), and active packaging with antimicrobial properties (Shinde et al., 2018). Development of new cultivars that are bred for slow respiratory patterns, optimisation of postharvest processes for flavour retention, and monitoring the aroma markers for early detection of spoilage, are also promising approaches.

7.4 Recommendations for potential industry applications

Overall, the results in this study suggested that MAP optimisation in combination with selected postharvest treatments represents a low-cost investment to extend the storability of raspberries, blueberries and broccoli in term of infrastructures and training. However, beside the need of further research on the variations due to preharvest factors and sensory evaluations, as detailed in Section 7.3, understanding consumer acceptance and cost-benefit analyses would help to justify the wider applications of MAP for berries and broccoli, and for fresh produce generally.

It is also necessary to communicate with consumers about the sustainability impacts of packaging. While marine pollution caused by plastic packaging is a growing concern in recent years, and consumers particularly want to see a reduction in the use of plastic packaging (White & Lockyer, 2020), governments, as well as the packaging and food sectors, to reduce plastics in packaging, targeting the reduction of single-use plastic would be a more practical option than the entire elimination of plastics (Berriman, 2020). In that sense, recyclable MAP could be a more sustainable alternative to replace top-icing and non-

recyclable polystyrene boxes in broccoli shipment. This approach could also benefit other produce that are also currently ice-topped, such as Brussel sprouts and green beans (Brecht et al., 2019). In addition, the extended shelf life resulted from MAP would reduce the amounts of food being wasted, which also represents a serious environmental and social problem (Berriman, 2020; Evans, 2011; White & Lockyer, 2020). Recently, biodegradable packaging has been attracting the industries as a solution to the backlash of consumers to plastic packaging. However, it should be emphasised that changes in packaging should be carefully trialled to avoid the risks of increasing food waste. For examples, commercially available sustainable packaging was trialled in the preliminary experiments of this project, including unvented clamshells made from polylactic acid (PLA), a lab-made MAP by sealing cellophane film, and a commercial biodegradable MAP made from cellulose-based materials. The PLA clamshell did not establish MA or improve the storability of berries, while the latter two packaging examples rapidly resulted in anaerobic conditions that caused early termination of the shelf life of berries and broccoli. In addition, the proposed replacement packaging would need proper assessments for recyclability and carbon footprint, and the potential of forming microplastics (Berriman, 2020).

Cost-benefit analyses would be of interest for business operators. The extended shelf life would open opportunities for these Tasmanian products to reach further domestic and premium foreign markets at good quality. This would avoid food waste from being rejected or marked down, while improving consumer satisfaction for repeated purchases. It would also partly extend the in-store availability for berries during the 2-3 month off-production periods between the end of the availability of Tasmanian fruit and the beginning of production elsewhere in Australia. Furthermore, while this thesis was placed in the context of Tasmania, the technologies could be applicable for other parts of Australia and the world, and for other fresh produce. Extending the storability of fresh produce to generate resilience in the supply

chain has become even more important for countering disruptions such as that caused by the COVID-19 global pandemic. Restrictions with unknown resolution dates minimise human movement and physical contact, resulting in decreases in labour availability throughout the supply chain (Stephens et al., 2020). Stay-at-home orders have led to less frequent shopping trips and thus, requiring fresh produce to be stored longer at farms, retailers, and households. In addition, flights have been reduced significantly, making sea-freight the preferable and most feasible method.

7.5 General conclusion

The present work has demonstrated the potential advantages of optimised MAP technologies over the current commercial practice in extending the storability of raspberries, blueberries, and broccoli as examples of higher value cool-climate produce. The benefits of MAP as bulk packaging were shown over two harvests of raspberries, and for two cultivars of blueberries under industrial settings. Similar effects were achieved with the novel model of berry retail packaging using unvented trays and perforated lid films. Furthermore, the research demonstrated the possibility to successfully replace top-icing in broccoli shipment by MAP in three shipping scenarios. The results of this work should help provide scientific insights for the berry and broccoli industries to consider the applications of MAP for long-term storage and shipments. The proposed model of retail MAP with unvented trays and perforated lid film could possibly be used to obtain data for verifying and improving the existing optimisation mathematical models. Applications of such packaging could be to replace bags or vented containers for minimally processed fruit and vegetables. The research also suggested directions for future studies that would be important to aid in applying MAP commercially. These include better understanding about the sensorial properties of the stored products with the potential of using advanced instrumental analyses, cost-benefit evaluations, and sustainability assessments.

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Appendix 1. Supplementary data for Chapter 3

Table A1.1 Respiration rates of fresh and stored 'Maravilla' raspberries in Trial 1

Treatment	O_2 consumption rate at 2 ºC (mg kg^{-1} h^{-1})			CO_2 production rate at 2 ºC (mg kg^{-1} h^{-1})		
	Fresh	**Day 10**	**Day 20**	**Fresh**	**Day 10**	**Day 20**
Loose clamshell	19.50 ± 0.47^{B}	35.71 ± 1.00^{aA}	20.24 ± 0.53^{aB}	23.81 ± 1.21^{B}	49.32 ± 2.17^{aA}	28.47 ± 2.17^{aB}
Mineral-clay impregnated MAP bags (A)	19.50 ± 0.47^{B}	36.00 ± 1.35^{aA}	19.44 ± 1.09^{aB}	23.81 ± 1.21^{B}	48.97 ± 3.18^{abA}	27.20 ± 1.55^{abB}
A + H_2O_2	19.50 ± 0.47^{B}	33.82 ± 1.06^{aA}	19.37 ± 1.60^{aB}	23.81 ± 1.21^{B}	44.49 ± 4.08^{bcA}	27.02 ± 3.06^{abB}
A + SO_2	19.50 ± 0.47^{B}	32.56 ± 3.40^{abA}	19.72 ± 0.86^{aB}	23.81 ± 1.21^{B}	42.96 ± 3.27^{bcA}	29.50 ± 2.27^{aB}
Microperforated MAP bags (B)	19.50 ± 0.47^{B}	31.55 ± 2.63^{bA}	18.68 ± 0.87^{abB}	23.81 ± 1.21^{B}	40.87 ± 3.39^{cA}	26.13 ± 1.65^{abB}
B + H_2O_2	19.50 ± 0.47^{B}	34.17 ± 1.63^{abA}	16.65 ± 3.60^{bB}	23.81 ± 1.21^{B}	41.98 ± 2.97^{cA}	24.11 ± 4.57^{cB}
B + SO_2	19.50 ± 0.47^{B}	32.01 ± 2.39^{bA}	18.35 ± 0.75^{abB}	23.81 ± 1.21^{B}	40.44 ± 2.97^{cA}	25.25 ± 1.01^{bcB}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 4

Table A1.2 Respiration rates of fresh and stored ‘Maravilla’ raspberries in Trial 2

Treatment	O_2 consumption rate at 2 ºC (mg kg^{-1} h^{-1})				CO_2 production rate at 2 ºC (mg kg^{-1} h^{-1})			
	Fresh	**Day 10**	**Day 16**	**Day 21**	**Fresh**	**Day 10**	**Day 16**	**Day 21**
Loose clamshell	15.07 ± 1.33^{B}	15.16 ± 0.48^{abB}	16.41 ± 3.17^{aB}	24.01 ± 0.82^{aA}	17.63 ± 2.83^{B}	18.73 ± 1.85^{abB}	21.23 ± 4.06^{aB}	33.37 ± 1.55^{aA}
Microperforated MAP bags (B)	15.07 ± 1.33^{B}	13.99 ± 0.47^{bC}	16.13 ± 1.03^{aB}	20.35 ± 0.30^{bcA}	17.63 ± 2.83^{B}	16.33 ± 0.47^{bC}	21.43 ± 4.35^{aB}	26.64 ± 0.39^{bA}
B + H_2O_2	15.07 ± 1.33^{B}	15.26 ± 0.77^{aB}	14.66 ± 0.89^{aB}	20.13 ± 0.56^{cA}	17.63 ± 2.83^{B}	19.45 ± 0.77^{aB}	19.68 ± 0.82^{aB}	26.61 ± 1.50^{bA}
Microperforated MAP bags (C)	15.07 ± 1.33^{B}	14.55 ± 1.27^{abB}	14.40 ± 1.29^{aB}	21.11 ± 1.36^{bcA}	17.63 ± 2.83^{B}	18.30 ± 2.45^{abB}	19.39 ± 1.33^{aB}	26.97 ± 1.76^{bA}
C + H_2O_2	15.07 ± 1.33^{B}	14.30 ± 0.65^{abB}	15.02 ± 2.05^{aB}	21.42 ± 0.67^{bA}	17.63 ± 2.83^{B}	18.30 ± 0.85^{abB}	20.61 ± 2.02^{aB}	28.43 ± 1.58^{bA}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 4

Table A1.3 Quality measurements of fresh and stored raspberries in Trial 1

Treatment	Weight loss (%)		Total anthocyanins (mg kg^{-1})			Ascorbic acid (mg kg^{-1})		
	Day 10	Day 20	Fresh	Day 10	Day 20	Fresh	Day 10	Day 20
Loose clamshell	3.21 ± 0.66^{aB}	7.33 ± 1.51^{aA}	133.35 ± 12.85^{A}	263.55 ± 12.79^{aB}	325.43 ± 14.52^{aB}	308.80 ± 24.20^{AB}	366.79 ± 15.42^{aA}	274.67 ± 27.83^{bB}
Mineral-clay impregnated MAP bags (A)	0.73 ± 0.07^{cB}	1.75 ± 0.32^{bA}	133.35 ± 12.85^{A}	234.29 ± 29.59^{abB}	279.96 ± 24.09^{bB}	308.80 ± 24.20^{B}	325.85 ± 20.88^{bAB}	338.60 ± 8.45^{aA}
A + H_2O_2	0.54 ± 0.04^{dB}	1.41 ± 0.08^{bA}	133.35 ± 12.85^{A}	214.71 ± 29.86^{bcB}	248.97 ± 3.66^{bcB}	308.80 ± 24.20^{A}	340.15 ± 15.17^{abA}	315.03 ± 28.05^{abA}
A + SO_2	0.74 ± 0.19^{bcB}	1.90 ± 0.70^{bA}	133.35 ± 12.85^{A}	241.11 ± 20.77^{aB}	263.06 ± 34.13^{bB}	308.80 ± 24.20^{B}	372.02 ± 21.08^{aA}	318.68 ± 29.16^{abB}
Microperforated MAP bags (B)	1.22 ± 0.32^{bB}	1.93 ± 0.55^{bA}	133.35 ± 12.85^{A}	218.45 ± 15.33^{bcB}	219.92 ± 15.71^{cdB}	308.80 ± 24.20^{A}	326.53 ± 31.00^{bA}	307.89 ± 22.40^{abA}
B + H_2O_2	0.67 ± 0.04^{cB}	1.44 ± 0.39^{bA}	133.35 ± 12.85^{A}	203.66 ± 20.67^{bB}	206.42 ± 9.80^{dB}	308.80 ± 24.20^{B}	351.05 ± 18.60^{abA}	307.73 ± 23.71^{abB}
B + SO_2	0.79 ± 0.19^{cB}	1.61 ± 0.29^{bA}	133.35 ± 12.85^{A}	193.82 ± 21.55^{cA}	216.19 ± 22.21^{cA}	308.80 ± 24.20^{A}	327.32 ± 29.80^{bA}	333.82 ± 15.25^{aA}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 4

Table A1.4 (continued)

Treatment	pH			TSS (%)			TA (% citric acid)			TSS/TA		
	Fresh	**Day 10**	**Day 20**	**Fresh**	**Day 10**	**Day 20**	**Fresh**	**Day 10**	**Day 20**	**Fresh**	**Day 10**	**Day 20**
Loose clamshell	3.29 ± 0.09^{B}	3.44 ± 0.11^{aB}	3.78 ± 0.10^{aA}	11.83 ± 0.17^{A}	11.33 ± 0.91^{bA}	11.55 ± 0.68^{aA}	1.68 ± 0.08^{A}	1.09 ± 0.09^{aB}	0.80 ± 0.05^{cC}	7.03 ± 0.25^{C}	10.48 ± 1.09^{abB}	14.52 ± 1.37^{aA}
Mineral-clay impregnated MAP bags (A)	3.29 ± 0.09^{B}	3.31 ± 0.05^{bB}	3.62 ± 0.11^{bA}	11.83 ± 0.17^{A}	11.20 ± 0.91^{bA}	10.58 ± 0.56^{aA}	1.68 ± 0.08^{A}	1.16 ± 0.07^{aB}	1.02 ± 0.14^{bC}	7.03 ± 0.25^{C}	9.67 ± 0.47^{bcA}	10.52 ± 1.28^{bA}
A + H_2O_2	3.29 ± 0.09^{B}	3.32 ± 0.03^{bB}	3.56 ± 0.11^{bcA}	11.83 ± 0.17^{A}	11.38 ± 1.31^{bA}	11.30 ± 1.43^{aA}	1.68 ± 0.08^{A}	1.17 ± 0.11^{aB}	1.05 ± 0.13^{abB}	7.03 ± 0.25^{C}	9.75 ± 1.41^{b}	11.00 ± 2.60^{bA}
A + SO_2	3.29 ± 0.09^{B}	3.40 ± 0.05^{abB}	3.59 ± 0.09^{bA}	11.83 ± 0.17^{A}	8.93 ± 0.85^{cB}	11.45 ± 0.83^{aA}	1.68 ± 0.08^{A}	1.17 ± 0.08^{aB}	1.05 ± 0.12^{abB}	7.03 ± 0.25^{B}	7.62 ± 0.67^{dB}	10.96 ± 0.64^{bA}
Microperforated MAP bags (B)	3.29 ± 0.09^{B}	3.36 ± 0.07^{abB}	3.48 ± 0.02^{cA}	11.83 ± 0.17^{A}	11.93 ± 1.34^{abA}	10.88 ± 1.95^{aA}	1.68 ± 0.08^{A}	1.10 ± 0.03^{aB}	1.10 ± 0.04^{abB}	7.03 ± 0.25^{C}	10.82 ± 1.48^{abA}	9.83 ± 1.43^{bA}
B + H_2O_2	3.29 ± 0.09^{B}	3.31 ± 0.06^{bA}	3.41 ± 0.07^{cA}	11.83 ± 0.17^{A}	9.45 ± 0.19^{cB}	11.30 ± 1.77^{aA}	1.68 ± 0.08^{A}	1.18 ± 0.07^{aB}	1.19 ± 0.09^{aB}	7.03 ± 0.25^{A}	8.01 ± 0.43^{cdA}	9.61 ± 2.10^{bA}
B + SO_2	3.29 ± 0.09^{B}	3.45 ± 0.07^{aA}	3.43 ± 0.06^{cA}	11.83 ± 0.17^{A}	12.93 ± 0.86^{aA}	11.25 ± 1.48^{aA}	1.68 ± 0.08^{A}	1.09 ± 0.10^{aB}	1.15 ± 0.10^{abB}	7.03 ± 0.25^{B}	11.95 ± 1.65^{aA}	9.88 ± 1.67^{bB}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 4

Table A1.5 Quality measurements of fresh and stored raspberries in Trial 2

Treatment	Weight loss (%)			Total anthocyanins (mg kg^{-1})				Ascorbic acid (mg kg^{-1})			
	Day 10	Day 16	Day 21	Fresh	Day 10	Day 16	Day 21	Fresh	Day 10	Day 16	Day 21
Loose clamshell	4.15 ± 0.73^{aA}	5.67 ± 1.49^{aA}	6.34 ± 0.89^{aA}	137.48 ± 10.56^{C}	237.33 ± 13.47^{aB}	272.66 ± 18.41^{aA}	272.45 ± 25.10^{aA}	274.25 ± 32.66^{A}	207.95 ± 3.89^{aA}	109.22 ± 21.32^{aB}	123.37 ± 18.76^{aB}
Microperforated MAP bags (B)	0.49 ± 0.23^{bA}	0.65 ± 0.20^{bA}	0.85 ± 0.31^{bA}	137.48 ± 10.56^{C}	169.36 ± 7.21^{cB}	189.54 ± 8.94^{cA}	178.43 ± 9.04^{cA}	274.25 ± 32.66^{A}	216.36 ± 21.90^{aA}	132.88 ± 22.01^{aB}	128.92 ± 21.42^{aB}
B + H_2O_2	0.56 ± 0.18^{bA}	0.68 ± 0.20^{bA}	1.08 ± 0.25^{bA}	137.48 ± 10.56^{C}	176.49 ± 11.04^{bcB}	208.69 ± 15.48^{bA}	201.17 ± 10.45^{bA}	274.25 ± 32.66^{A}	223.64 ± 22.34^{aA}	108.73 ± 20.31^{aB}	118.05 ± 11.29^{aB}
Microperforated MAP bags (C)	0.55 ± 0.32^{bA}	0.65 ± 0.31^{bA}	0.88 ± 0.13^{bA}	137.48 ± 10.56^{C}	184.54 ± 4.28^{bB}	201.38 ± 10.21^{bcA}	207.13 ± 8.04^{bA}	274.25 ± 32.66^{A}	222.84 ± 22.20^{aA}	141.43 ± 24.59^{aB}	106.33 ± 21.43^{aB}
C + H_2O_2	0.64 ± 0.20^{bB}	0.76 ± 0.27^{bB}	1.35 ± 0.18^{bA}	137.48 ± 10.56^{C}	169.21 ± 7.31^{cB}	203.09 ± 4.04^{bcA}	200.19 ± 15.77^{bcA}	274.25 ± 32.66^{A}	219.87 ± 19.3^{aA}	114.74 ± 27.68^{aB}	112.34 ± 23.08^{aB}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 4

Table A1.4 (cont.)

Treatment	pH				TSS (%)				TA (% citric acid)				TSS/TA			
	Fresh	**Day 10**	**Day 16**	**Day 21**	**Fresh**	**Day 10**	**Day 16**	**Day 21**	**Fresh**	**Day 10**	**Day 16**	**Day 21**	**Fresh**	**Day 10**	**Day 16**	**Day 21**
Loose clamshell	3.36 ± 0.04^{B}	3.30 ± 0.03^{aB}	3.39 ± 0.07^{aB}	3.49 ± 0.04^{aA}	8.77 ± 0.10^{B}	10.19 ± 0.34^{aA}	10.19 ± 0.13^{aA}	10.62 ± 0.43^{aA}	1.70 ± 0.10^{A}	1.42 ± 0.03^{cB}	1.33 ± 0.02^{bC}	1.38 ± 0.03^{cB}	5.18 ± 0.25^{C}	7.20 ± 0.15^{aB}	7.65 ± 0.06^{aA}	7.68 ± 0.41^{aA}
Micro-perforated MAP bags (B)	3.36 ± 0.04^{A}	3.15 ± 0.01^{cB}	3.20 ± 0.05^{bB}	3.16 ± 0.03^{cB}	8.77 ± 0.10^{C}	9.10 ± 0.17^{bB}	9.64 ± 0.48^{abA}	9.09 ± 0.28^{bB}	1.70 ± 0.10^{A}	1.61 ± 0.01^{aA}	1.54 ± 0.04^{abB}	1.60 ± 0.02^{aA}	5.18 ± 0.25^{B}	5.67 ± 0.06^{bB}	6.27 ± 0.47^{bA}	5.69 ± 0.18^{bB}
B + H_2O_2	3.36 ± 0.04^{A}	3.21 ± 0.06^{bA}	3.19 ± 0.08^{bA}	3.23 ± 0.05^{bA}	8.77 ± 0.10^{B}	8.92 ± 0.17^{bB}	9.36 ± 0.63^{bA}	9.12 ± 0.27^{bAB}	1.70 ± 0.10^{A}	1.57 ± 0.03^{abA}	1.53 ± 0.04^{aA}	1.56 ± 0.04^{abA}	5.18 ± 0.25^{B}	5.68 ± 0.17^{bB}	6.13 ± 0.38^{bA}	5.85 ± 0.21^{bAB}
Micro-perforated MAP bags (C)	3.36 ± 0.04^{A}	3.17 ± 0.04^{bcB}	3.15 ± 0.03^{bB}	3.23 ± 0.02^{bA}	8.77 ± 0.10^{B}	8.91 ± 0.12^{bAB}	9.47 ± 0.46^{abA}	9.09 ± 0.29^{bA}	1.70 ± 0.10^{A}	1.56 ± 0.02^{bAB}	1.52 ± 0.02^{aB}	1.58 ± 0.40^{abA}	5.18 ± 0.25^{B}	5.72 ± 0.13^{bB}	6.24 ± 0.25^{bA}	5.76 ± 0.15^{bB}
C + H_2O_2	3.36 ± 0.04^{A}	3.09 ± 0.02^{dC}	3.16 ± 0.02^{bB}	3.26 ± 0.04^{bAB}	8.77 ± 0.10^{B}	9.16 ± 0.09^{bA}	9.59 ± 0.67^{abA}	9.11 ± 0.26^{bA}	1.70 ± 0.10^{A}	1.57 ± 0.02^{abA}	1.58 ± 0.02^{aA}	1.54 ± 0.05^{bA}	5.18 ± 0.25^{B}	5.83 ± 0.03^{bA}	6.09 ± 0.47^{bA}	5.93 ± 0.35^{bA}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 4

Table A1.6 Peak areas of volatile compounds in 'Maravilla' raspberries with significant treatment differences (Trial 1)

Day	Volatile compound	Unpacked		Mineral-clay MAP A		Mineral-clay MAP A + H_2O_2		Mineral-clay MAP A + SO_2		Microperforated MAP B		Microperforated MAP B + H_2O_2		Microperforated MAP B + SO_2	
		Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median
10	β-Pinene	12627	12863^{A}	5683	6019^{AB}	5896	4830^{ABC}	5970	6360^{AB}	462	0^{C}	1247	0^{BC}	4481	3082^{ABC}
20	Ethyl acetate	376106	333531^{A}	255009	256006^{AB}	290621	246377^{AB}	125685	98818^{BC}	77587	83636^{C}	113900	100703^{BC}	82188	83505^{C}
	Acetic acid	3835	3583^{BC}	2651	3054^{C}	8831	8277^{ABC}	7352	7825^{ABC}	28952	32956^{A}	13800	12637^{AB}	23134	19605^{A}
	Hexanal	926193^{a}	911210	805805^{ab}	808045	802014^{ab}	807196	755647^{ab}	811529	593948^{b}	634381	871316^{a}	879933	464258^{c}	405842
	3-Methyl-3-buten-1-ol acetate	196037^{bc}	185405	319921^{a}	356167	319921^{ab}	356167	216705^{abc}	201967	120478^{bc}	101129	185415^{bc}	242901	103736^{c}	101126
	2-Heptanone	49940	48193^{A}	37192	35417^{AB}	26232	26231^{ABC}	33877	30098^{AB}	20354	22239^{BC}	22133	23723^{BC}	15241	15069^{C}
	3-Methyl-2-buten-1-ol acetate	59079^{ab}	55955	91344^{a}	98429	57743^{ab}	60454	60178^{ab}	52906	31706^{bc}	27182	41913^{bc}	48086	41913^{c}	48086
	α-Pinene	483118	470546^{A}	102645	131866^{AB}	77398	57109^{B}	40117	35091^{B}	32611	26321^{B}	12385	11707^{B}	55710	65996^{B}
	β-Pinene	61749	71430^{A}	38024	38472^{AB}	38754	36752^{A}	38119	37236^{A}	9268	9768^{C}	11717	12049^{BC}	11717	12049^{BC}
	(Z)-3-Hexen-1-ol acetate	58103	56194^{A}	38512	43716^{A}	41350	37628^{A}	25597	27105^{AB}	11269	13212^{B}	16248	15724^{B}	10907	12405^{B}
	β-Phellandrene	8782^{a}	9085	5104^{b}	4833	5649^{b}	5530	4659^{b}	4638	758^{c}	0	936^{c}	846	2359^{c}	2104
	D-limonene	39609^{a}	38158	19167^{b}	22222	23215^{b}	28322	23215^{b}	28322	12537^{b}	12446	9850^{b}	12221	12166^{b}	11589
	Linalool	47888	46451^{A}	36558	33645^{A}	32834	27329^{AB}	20972	19537^{AB}	10447	9216^{B}	10447	9216^{B}	12516	10619^{B}
	Longicyclene	17508	14732^{A}	11512	12019^{AB}	14285	15031^{AB}	6910	4288^{AB}	1519	0^{C}	2137	0^{BC}	1350	0^{C}

Medians with different capital letters are significantly different by Kruskal-Wallis test followed by Dunn's post-hoc test ($p < 0.05$).

Means with different lowercase letters are significantly different by ANOVA test followed by LSD post-hoc test ($p < 0.05$).

n = 4

Table A1.7 Peak areas of volatile compounds in 'Maravilla' raspberries with significant treatment differences (Trial 2)

Day	Volatile compound	Unpacked		Microperforated MAP B		Microperforated MAP B + H_2O_2		Microperforated MAP C		Microperforated MAP C + H_2O_2	
		Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median
10	o-Xylene	30752 ^{a}	30808	13259 ^{b}	12978	19040 ^{b}	17265	5246 ^{c}	4637	2627 ^{c}	0
	2-Heptanone	78234 ^{a}	77964	37087 ^{b}	34190	47720 ^{b}	48038	43117 ^{b}	42142	39812 ^{b}	39388
	Linalool	55244	55615 ^{A}	15580	12571 ^{AB}	28320	20583 ^{AB}	6925	9039 ^{B}	10664	0 ^{B}
	Nonanal	26954	26191 ^{A}	0	0 ^{B}	0	0 ^{B}	0	0 ^{B}	6891	0 ^{B}
16	Ethyl acetate	166593	165599 ^{A}	53869	63213 ^{B}	33829	26103 ^{B}	33004	32769 ^{B}	39560	37894 ^{B}
	o-Xylene	23004	22489 ^{A}	0	0 ^{B}	2841	1780 ^{B}	1185	0 ^{B}	835	0 ^{B}
	2-Heptanone	41648 ^{a}	44083	28587 ^{ab}	29704	21522 ^{b}	20540	33134 ^{a}	32328	26088 ^{ab}	26035
	α-Pinene	20243	19096 ^{A}	7822	8470 ^{B}	2580	3018 ^{B}	4862	5346 ^{B}	2842	3728 ^{B}
	β-Ocimene	5794 ^{ab}	6019	9967 ^{ab}	9354	3874 ^{b}	4279	10534 ^{a}	10453	6148 ^{ab}	7923
21	Ethyl acetate	296207	304036 ^{A}	24458	22568 ^{C}	64691	52736 ^{BC}	93968	95610 ^{AB}	67408	74826 ^{BC}
	Hexanal	1825999 ^{a}	1780672	1351782 ^{b}	1298629	1176761 ^{b}	1088651	1341228 ^{b}	1342405	1133431 ^{b}	1141626
	3-Methyl-3-buten-1-ol acetate	88544 ^{a}	82785	24800 ^{b}	23925	29791 ^{b}	28810	31927 ^{b}	32945	28303 ^{b}	26831
	2-pentyl furan	22362	17940 ^{A}	1255	0 ^{B}	625	0 ^{B}	0	0 ^{B}	1184	0 ^{B}
	trans-β-Ocimene	42243	41818 ^{A}	27171	24194 ^{AB}	11916	9888 ^{BC}	5564	0 ^{BC}	0	0 ^{C}
	β-Ocimene	18026 ^{a}	18864	6468 ^{b}	5301	8820 ^{ab}	9404	17006 ^{a}	16954	11200 ^{ab}	12598
	Geraniol	224127 ^{a}	236425	68109 ^{b}	67726	155581 ^{ab}	170975	259206 ^{a}	276376	190532 ^{a}	195527
	Geranyl acetate	6650 ^{a}	8018	1559 ^{b}	1720	5061 ^{a}	4798	9067 ^{a}	9853	7569 ^{a}	7702
	β-Dihydroionone	34296 ^{a}	33685	30872 ^{ab}	27800	26175 ^{bc}	26508	25911 ^{bc}	25315	22130 ^{c}	21965

Medians with different capital letters are significantly different by Kruskal-Wallis test followed by Dunn's post-hoc test ($p < 0.05$).

Means with different lowercase letters are significantly different by ANOVA test followed by LSD post-hoc test ($p < 0.05$).

n = 4

Appendix 2. Supplementary data for Chapter 4

Table A2.1 Respiration rates of fresh and stored 'Legacy' blueberries (Trial 1)

Treatment	O_2 consumption rate at 2 ºC (mg kg^{-1} h^{-1})				CO_2 production rate at 2 ºC (mg kg^{-1} h^{-1})			
	Fresh	**Week 6**	**Week 8**	**Week 10**	**Fresh**	**Week 6**	**Week 8**	**Week 10**
Loose clamshell	7.08 ± 0.16^{A}	5.31 ± 0.68^{aB}	6.05 ± 0.87^{aB}	6.17 ± 0.44^{aB}	6.98 ± 0.05^{A}	5.26 ± 0.35^{bB}	6.63 ± 0.84^{aAB}	6.24 ± 0.34^{abAB}
Mineral-clay impregnated MAP bags (A)	7.08 ± 0.16^{A}	6.04 ± 1.04^{aA}	6.32 ± 0.87^{aA}	5.30 ± 0.45^{aA}	6.98 ± 0.05^{A}	6.50 ± 0.67^{abAB}	6.97 ± 0.68^{aAB}	5.27 ± 0.83^{bB}
A + H_2O_2	7.08 ± 0.16^{A}	6.12 ± 0.74^{aAB}	5.49 ± 0.54^{aB}	5.96 ± 0.47^{aAB}	6.98 ± 0.05^{A}	6.24 ± 0.57^{abAB}	6.01 ± 0.49^{aB}	6.12 ± 0.46^{abB}
A + SO_2	7.08 ± 0.16^{A}	6.64 ± 0.08^{aA}	6.65 ± 0.79^{aA}	6.42 ± 0.96^{aA}	6.98 ± 0.05^{A}	6.63 ± 0.21^{aA}	7.47 ± 0.92^{aA}	6.97 ± 0.90^{aA}
Microperforated MAP bags (B)	7.08 ± 0.16^{A}	6.72 ± 0.37^{aA}	5.77 ± 0.21^{aB}	6.64 ± 0.30^{aA}	6.98 ± 0.05^{A}	6.79 ± 0.38^{aA}	6.70 ± 0.03^{aA}	6.95 ± 0.27^{aA}
B + H_2O_2	7.08 ± 0.16^{A}	6.11 ± 0.50^{aA}	6.71 ± 0.47^{aA}	6.87 ± 0.74^{aA}	6.98 ± 0.05^{A}	6.45 ± 0.59^{abA}	7.64 ± 0.15^{a}	6.88 ± 0.47^{ab}
B + SO_2	7.08 ± 0.16^{A}	6.16 ± 0.30^{aAB}	6.27 ± 0.90^{aAB}	5.81 ± 0.40^{aB}	6.98 ± 0.05	6.30 ± 0.20^{ab}	7.15 ± 0.97^{a}	6.29 ± 0.57^{ab}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 3

Table A2.2 Respiration rates of fresh and stored 'Power Blue' blueberries (Trial 2)

Treatment	O_2 consumption rate at 2 ºC (mg kg^{-1} h^{-1})				CO_2 production rate at 2 ºC (mg kg^{-1} h^{-1})			
	Fresh	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10
Loose clamshell	8.46 ± 0.05^{B}	10.75 ± 0.43^{aA}	8.54 ± 0.66^{aB}	10.44 ± 0.04^{aA}	9.23 ± 0.09^{B}	12.53 ± 0.54^{aA}	9.39 ± 0.46^{aB}	12.56 ± 0.68^{aA}
Mineral-clay impregnated MAP bags (A)	8.46 ± 0.05^{A}	7.80 ± 0.43^{bA}	6.19 ± 0.72^{bB}	6.73 ± 0.36^{bB}	9.23 ± 0.09^{A}	8.20 ± 0.26^{bA}	6.42 ± 0.66^{cB}	7.26 ± 0.43^{bA}
A + H_2O_2	8.46 ± 0.05^{A}	8.66 ± 0.42^{bA}	6.88 ± 0.46^{bB}	7.72 ± 0.38^{bAB}	9.23 ± 0.09^{A}	9.29 ± 0.40^{bA}	7.24 ± 0.32^{bcB}	7.81 ± 0.42^{bB}
A + SO_2	8.46 ± 0.05^{A}	8.35 ± 0.74^{bA}	6.64 ± 0.24^{bB}	5.99 ± 0.02^{bB}	9.23 ± 0.09^{A}	9.10 ± 0.78^{bA}	7.32 ± 0.54^{bcB}	6.70 ± 0.10^{bB}
Microperforated MAP bags (B)	8.46 ± 0.05^{A}	8.44 ± 0.91^{bA}	6.96 ± 0.15^{bB}	6.17 ± 1.10^{bB}	9.23 ± 0.09^{A}	9.01 ± 0.73^{bA}	7.39 ± 0.12^{bcB}	6.85 ± 1.03^{bB}
B + H_2O_2	8.46 ± 0.05^{A}	8.19 ± 0.86^{bA}	7.21 ± 0.80^{abA}	7.02 ± 0.65^{bA}	9.23 ± 0.09^{A}	8.95 ± 0.76^{bAB}	7.64 ± 0.67^{bcB}	7.66 ± 0.43^{bB}
B + SO_2	8.46 ± 0.05^{A}	8.37 ± 0.57^{bA}	7.22 ± 0.52^{abA}	7.01 ± 1.54^{bA}	9.23 ± 0.09^{A}	9.28 ± 0.71^{bA}	7.82 ± 0.45^{bB}	7.41 ± 1.52^{bB}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 3

Table A2.3. Quality measurements of 'Legacy' blueberries after MA and sanitisation treatments (Trial 1)

Treatment	Weight loss (%)			Total anthocyanins (mg kg^{-1})				Ascorbic acid (mg kg^{-1})			
	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10
Loose clamshell	8.60 ± 0.97^{aC}	11.92 ± 0.62^{aB}	14.08 ± 0.80^{aA}	38.70 ± 8.34^{D}	64.61 ± 9.26^{bcC}	92.62 ± 2.75^{abB}	116.82 ± 8.84^{aA}	123.09 ± 17.81^{A}	112.74 ± 0.53^{aA}	122.99 ± 17.35^{aA}	113.12 ± 0.33^{aA}
Mineral-clay impregnated MAP bags (A)	0.05 ± 0.01^{dB}	0.67 ± 0.10^{dA}	0.62 ± 0.08^{cA}	38.70 ± 8.34^{B}	90.93 ± 7.09^{aA}	115.18 ± 13.23^{aA}	112.99 ± 25.45^{aA}	123.09 ± 17.81^{A}	92.29 ± 17.34^{aA}	111.81 ± 17.32^{aA}	112.50 ± 0.81^{aA}
A + H_2O_2	0.50 ± 0.10^{cdB}	1.17 ± 0.27^{cd}	1.25 ± 0.10^{c}	38.70 ± 8.34^{C}	83.22 ± 11.53^{abA}	112.54 ± 17.15^{aA}	120.69 ± 12.20^{aA}	123.09 ± 17.81^{A}	112.87 ± 0.53^{aA}	112.80 ± 0.63^{aA}	112.31 ± 0.25^{aA}
A + SO_2	0.55 ± 0.15^{cdA}	0.51 ± 0.17^{dA}	0.54 ± 0.04^{cA}	38.70 ± 8.34^{C}	87.24 ± 14.93^{abB}	111.91 ± 8.84^{aB}	140.76 ± 0.34^{aA}	123.09 ± 17.81^{A}	112.71 ± 0.50^{aA}	113.01 ± 0.40^{aA}	102.26 ± 17.87^{aA}
Microperforated MAP bags (B)	1.62 ± 0.05^{bcB}	2.25 ± 0.26^{bcA}	2.58 ± 0.25^{bA}	38.70 ± 8.34^{B}	24.04 ± 0.43^{eB}	105.87 ± 9.90^{abA}	126.55 ± 6.51^{aA}	123.09 ± 17.81^{A}	113.03 ± 0.44^{a}	102.25 ± 17.31^{a}	112.97 ± 0.66^{aA}
B + H_2O_2	2.11 ± 0.46^{bB}	2.72 ± 0.79^{bAB}	3.05 ± 0.21^{bA}	38.70 ± 8.34^{B}	39.11 ± 6.39^{deB}	98.49 ± 2.58^{abA}	122.13 ± 21.80^{aA}	123.09 ± 17.81^{A}	92.14 ± 17.44^{aA}	122.83 ± 18.37^{aA}	102.57 ± 18.09^{aA}
B + SO_2	1.54 ± 0.27^{bcB}	2.43 ± 0.43^{bA}	2.87 ± 0.22^{bA}	38.70 ± 8.34^{C}	51.37 ± 3.09^{cdC}	80.66 ± 7.62^{bB}	112.98 ± 6.90^{aA}	123.09 ± 17.81^{A}	102.19 ± 17.28^{aA}	102.33 ± 17.41^{aA}	112.97 ± 0.21^{aA}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 3

Table A2.3 (cont.)

Treatment	pH				TSS (%)				TA (% citric acid)				TSS/TA			
	Fresh	**Week 6**	**Week 8**	**Week 10**	**Fresh**	**Week 6**	**Week 8**	**Week 10**	**Fresh**	**Week 6**	**Week 8**	**Week 10**	**Fresh**	**Week 6**	**Week 8**	**Week 10**
Loose clamshell	3.68 ± 0.06^{A}	3.80 ± 0.06^{aA}	3.78 ± 0.04^{aA}	3.80 ± 0.06^{aA}	10.43 ± 0.02^{C}	10.69 ± 0.20^{aC}	11.12 ± 0.05^{aB}	12.55 ± 0.57^{aA}	0.64 ± 0.05^{A}	0.59 ± 0.01^{cA}	0.55 ± 0.01^{bAB}	0.51 ± 0.01^{cB}	19.01 ± 0.45^{B}	18.21 ± 0.73^{aB}	20.28 ± 0.48^{aB}	24.82 ± 1.02^{aA}
Mineral-clay impregnated MAP bags (A)	3.68 ± 0.06^{A}	3.80 ± 0.03^{aA}	3.68 ± 0.05^{aA}	3.79 ± 0.03^{aA}	10.43 ± 0.02^{A}	10.45 ± 0.06^{abA}	10.22 ± 0.17^{abA}	10.40 ± 0.07^{bA}	0.64 ± 0.05^{A}	0.59 ± 0.01^{cA}	0.59 ± 0.04^{aA}	0.56 ± 0.01^{bcA}	19.01 ± 0.45^{A}	17.81 ± 0.39^{abB}	15.28 ± 0.30^{bC}	18.51 ± 0.42^{bB}
A + H_2O_2	3.68 ± 0.06^{A}	3.71 ± 0.05^{abA}	3.66 ± 0.06^{abA}	3.71 ± 0.05^{abA}	10.43 ± 0.02^{A}	10.09 ± 0.40^{abcA}	10.20 ± 0.17^{abA}	10.38 ± 0.02^{bA}	0.64 ± 0.05^{A}	0.61 ± 0.02^{bcA}	0.60 ± 0.04^{abA}	0.56 ± 0.05^{bcA}	19.01 ± 0.45^{A}	16.58 ± 0.32^{abB}	17.64 ± 0.42^{abB}	18.60 ± 1.61^{bA}
A + SO_2	3.68 ± 0.06^{A}	3.62 ± 0.07^{bcA}	3.59 ± 0.14^{abA}	3.62 ± 0.07^{bcA}	10.43 ± 0.02^{A}	10.19 ± 0.41^{abcA}	9.87 ± 1.02^{bcA}	10.18 ± 0.41^{bA}	0.64 ± 0.05^{A}	0.64 ± 0.02^{bcA}	0.63 ± 0.05^{abA}	0.64 ± 0.04^{abA}	19.01 ± 0.45^{A}	16.00 ± 0.84^{bcB}	16.24 ± 2.08^{bB}	15.86 ± 1.40^{bB}
Microperforated MAP bags (B)	3.68 ± 0.06^{A}	3.73 ± 0.04^{abA}	3.50 ± 0.04^{bA}	3.72 ± 0.04^{abA}	10.43 ± 0.02^{A}	9.52 ± 0.41^{bcAB}	8.91 ± 0.35^{cB}	10.44 ± 0.06^{bA}	0.64 ± 0.05^{A}	0.65 ± 0.02^{abA}	0.67 ± 0.01^{abA}	0.59 ± 0.03^{abcA}	19.01 ± 0.45^{A}	14.58 ± 1.04^{cdB}	15.10 ± 1.56^{bB}	17.60 ± 0.74^{bAB}
B + H_2O_2	3.68 ± 0.06^{A}	3.69 ± 0.05^{bcA}	3.68 ± 0.03^{abA}	3.69 ± 0.05^{abcA}	10.43 ± 0.02^{A}	9.27 ± 0.49^{cB}	10.40 ± 0.05^{abA}	10.44 ± 0.05^{bA}	0.64 ± 0.05^{A}	0.66 ± 0.01^{aA}	0.58 ± 0.02^{abA}	0.61 ± 0.05^{abA}	19.01 ± 0.45^{A}	14.04 ± 0.56^{dB}	17.4 ± 1.16^{abA}	17.22 ± 1.36^{bA}
B + SO_2	3.68 ± 0.06^{A}	3.55 ± 0.05^{cA}	3.64 ± 0.06^{abA}	3.55 ± 0.05^{cA}	10.43 ± 0.02^{A}	9.94 ± 0.15^{abcA}	10.19 ± 0.25^{abA}	10.44 ± 0.02^{bA}	0.64 ± 0.05^{A}	0.61 ± 0.01^{abcA}	0.61 ± 0.02^{abA}	0.67 ± 0.00^{aA}	19.01 ± 0.45^{A}	16.21 ± 0.60^{bcB}	16.23 ± 0.96^{bB}	15.63 ± 0.00^{bB}

Table A2.4 Quality measurements of 'Powder Blue' blueberries after MA and sanitisation treatments (Trial 2)

Treatment	Weight loss (%)			Total anthocyanins (mg kg^{-1})				Ascorbic acid (mg kg^{-1})			
	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10
Loose clamshell	12.30 ± 0.11^{aB}	14.36 ± 1.60^{aAB}	18.04 ± 2.66^{aA}	156.19 ± 6.88^{B}	209.28 ± 5.78^{abA}	176.80 ± 12.05^{aA}	203.54 ± 12.88^{aA}	123.25 ± 17.47^{A}	123.21 ± 18.16^{aA}	102.78 ± 17.89^{aA}	112.90 ± 0.90^{aA}
Mineral-clay impregnated MAP bags (A)	0.18 ± 0.07^{dB}	0.18 ± 0.10^{eB}	0.40 ± 0.10^{cA}	156.19 ± 6.88^{A}	178.34 ± 30.86^{abA}	174.47 ± 17.11^{aA}	194.46 ± 9.09^{aA}	123.25 ± 17.47^{A}	112.52 ± 0.59^{aA}	102.25 ± 17.97^{aA}	112.79 ± 0.33^{aA}
A + H_2O_2	1.26 ± 0.37^{cdA}	1.01 ± 0.24^{cdeA}	1.55 ± 0.11^{bcA}	156.19 ± 6.88^{B}	159.36 ± 12.64^{bB}	182.24 ± 19.69^{aAB}	217.54 ± 12.00^{aA}	123.25 ± 17.47^{A}	102.40 ± 17.63^{aA}	102.66 ± 17.86^{aA}	112.64 ± 0.65^{aA}
A + SO_2	0.57 ± 0.36^{dA}	0.39 ± 0.04^{deA}	0.59 ± 0.09^{cA}	156.19 ± 6.88^{A}	156.85 ± 12.40^{bA}	171.09 ± 27.55^{aA}	180.37 ± 35.35^{aA}	123.25 ± 17.47^{A}	112.48 ± 0.29^{aA}	101.93 ± 17.90^{aA}	112.41 ± 0.63^{aA}
Microperforated MAP bags (B)	2.07 ± 0.69^{bcA}	2.15 ± 0.12^{bcdA}	2.47 ± 0.11^{bcA}	156.19 ± 6.88^{B}	209.71 ± 29.51^{abA}	193.53 ± 20.09^{aAB}	194.38 ± 17.84^{aAB}	123.25 ± 17.47^{A}	102.49 ± 18.02^{aA}	102.68 ± 17.63^{aA}	113.19 ± 0.31^{aA}
B + H_2O_2	2.71 ± 0.59^{bB}	3.79 ± 0.56^{bAB}	4.11 ± 0.80^{bA}	156.19 ± 6.88^{B}	188.50 ± 21.11^{abAB}	205.71 ± 19.24^{aA}	196.43 ± 15.97^{aAB}	123.25 ± 17.47^{A}	123.48 ± 17.54^{aA}	102.60 ± 18.17^{aA}	112.30 ± 0.64^{aA}
B + SO_2	2.71 ± 0.54^{bAB}	2.27 ± 0.19^{bcB}	3.01 ± 0.14^{bcA}	156.19 ± 6.88^{B}	217.50 ± 10.64^{aA}	184.96 ± 13.57^{aAB}	224.79 ± 16.70^{aA}	123.25 ± 17.47^{A}	112.41 ± 30.92^{aA}	102.34 ± 18.33^{aA}	112.67 ± 0.26^{aA}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 3

Table A2.4 (cont.)

Treatment	pH				TSS (%)				TA (% citric acid)				TSS/TA			
	Fresh	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10	Fresh	Week 6	Week 8	Week 10
Loose clamshell	3.30 ± 0.03^{B}	3.32 ± 0.03^{aB}	3.34 ± 0.02^{aAB}	3.42 ± 0.05^{aA}	10.44 ± 0.04^{B}	14.15 ± 1.82^{aA}	12.55 ± 0.57^{aA}	14.38 ± 0.51^{aA}	0.71 ± 0.01^{A}	0.70 ± 0.01^{aA}	0.68 ± 0.02^{abA}	0.67 ± 0.01^{cA}	14.81 ± 0.27^{C}	20.04 ± 2.20^{aAB}	18.55 ± 0.80^{aB}	21.53 ± 0.60^{aA}
Mineral-clay impregnated MAP bags (A)	3.30 ± 0.03^{B}	3.31 ± 0.01^{aB}	3.31 ± 0.04^{aB}	3.43 ± 0.04^{aA}	10.44 ± 0.04^{B}	12.14 ± 0.06^{abA}	11.79 ± 0.57^{aA}	12.17 ± 0.04^{cA}	0.71 ± 0.01^{A}	0.71 ± 0.01^{aA}	0.70 ± 0.04^{abA}	0.66 ± 0.01^{cA}	14.81 ± 0.27^{C}	17.09 ± 0.01^{abcAB}	16.97 ± 0.35^{aB}	18.44 ± 0.36^{bA}
A + H_2O_2	3.30 ± 0.03^{B}	3.26 ± 0.02^{aB}	3.35 ± 0.02^{aB}	3.42 ± 0.03^{aA}	10.44 ± 0.04^{B}	10.42 ± 0.03^{bB}	12.54 ± 0.56^{aA}	12.15 ± 0.07^{cA}	0.71 ± 0.01^{A}	0.72 ± 0.01^{aA}	0.66 ± 0.03^{bA}	0.66 ± 0.02^{cA}	14.81 ± 0.27^{B}	14.50 ± 0.26^{cB}	18.99 ± 1.42^{aA}	18.44 ± 0.36^{bA}
A + SO_2	3.30 ± 0.03^{A}	3.22 ± 0.07^{aA}	3.31 ± 0.04^{abA}	3.27 ± 0.02^{bcA}	10.44 ± 0.04^{B}	12.13 ± 0.03^{abA}	12.45 ± 0.58^{aA}	12.82 ± 0.57^{bcA}	0.71 ± 0.01^{A}	0.73 ± 0.02^{aA}	0.69 ± 0.04^{abA}	0.70 ± 0.01^{abA}	14.81 ± 0.27^{C}	16.58 ± 0.50^{bcB}	18.17 ± 0.95^{aA}	18.27 ± 1.06^{bA}
Microperforated MAP bags (B)	3.30 ± 0.03^{AB}	3.31 ± 0.03^{aAB}	3.29 ± 0.01^{abB}	3.38 ± 0.04^{abA}	10.44 ± 0.04^{C}	13.67 ± 0.35^{aA}	11.85 ± 0.57^{aB}	11.86 ± 0.58^{cB}	0.71 ± 0.01^{A}	0.70 ± 0.01^{aA}	0.72 ± 0.02^{abA}	0.68 ± 0.01^{bcA}	14.81 ± 0.27^{C}	19.42 ± 0.81^{abA}	16.44 ± 1.12^{aBC}	17.53 ± 1.21^{bAB}
B + H_2O_2	3.30 ± 0.03^{A}	3.28 ± 0.07^{aA}	3.32 ± 0.02^{aA}	3.39 ± 0.07^{aA}	10.44 ± 0.04^{B}	12.67 ± 1.80^{abA}	12.52 ± 0.57^{aA}	12.46 ± 0.53^{bcA}	0.71 ± 0.01^{A}	0.71 ± 0.02^{aA}	0.71 ± 0.03^{abA}	0.67 ± 0.01^{bcA}	14.81 ± 0.27^{B}	17.70 ± 1.95^{abcA}	17.79 ± 1.30^{aA}	18.88 ± 0.57^{bA}
B + SO_2	3.30 ± 0.03^{A}	3.28 ± 0.03^{aA}	3.22 ± 0.05^{bA}	3.23 ± 0.04^{cA}	10.44 ± 0.04^{s}	12.12 ± 0.01^{abB}	12.14 ± 0.08^{aB}	13.43 ± 0.41^{abA}	0.71 ± 0.01^{A}	0.71 ± 0.01^{aA}	0.75 ± 0.03^{aA}	0.71 ± 0.00^{aA}	14.81 ± 0.27^{C}	17.09 ± 0.01^{abcB}	16.27 ± 0.75^{aB}	19.86 ± 0.56^{bA}

On each assessment day, means with different lowercase letters within one column are significantly different ($p < 0.05$).

For each measurement, means with different capital letters within one treatment are significantly different ($p < 0.05$).

n = 3

Table A2.5 Means of peak areas (total ion counts) of aroma compounds in 'Legacy' blueberries at harvest and after cold storage

Compounds	At harvest	Unpacked clamshells	Mineral-clay impregnated MAP (A)	A + H_2O_2	A + SO_2	Microperforated MAP (B)	B + H_2O_2	B + SO_2
Aldehydes								
Hexanal	1195549	1144628 [a]	684407 [b]	565588 [b]	747844 [b]	663488 [b]	618937 [b]	716108 [b]
		644311 [a]	291093 [ab]	492365 [ab]	299571 [ab]	324994 [ab]	312571 [ab]	227257 [b]
		365822 [a]	284159 [a]	319694 [a]	258085 [a]	262256 [a]	375236 [a]	284981 [a]
(Z)-2-Hexenal	19364	23361 [a]	11351 [ab]	14078 [ab]	4899 [b]	14690 [ab]	14279 [ab]	19724 [a]
		9309 [a]	5887 [a]	9147 [a]	6700 [a]	7138 [a]	5314 [a]	4290 [a]
		8109 [a]	5120 [b]	5323 [b]	3779 [b]	4809 [b]	5598 [b]	3923 [b]
(E)-2-Hexenal	3911305	4708226 [a]	3794143 [ab]	3366865 [b]	3286028 [b]	3526579 [b]	3380822 [b]	3466002 [b]
		1912501 [a]	1435289 [a]	1805226 [a]	1455025 [a]	1582256 [a]	1367098 [a]	1197235 [a]
		1356624 [a]	1384488 [a]	1212224 [a]	926403 [a]	1057747 [a]	1391183 [a]	1013159 [a]
Benzaldehyde	–	–	–	–	–	–	–	–
		7481 [a]	4585 [a]	4505 [a]	5092 [a]	–	3657 [a]	3692 [a]
		3594 [ab]	2263 [a]	4333 [a]	2091 [a]	1181 [ab]	3057 [ab]	530 [b]
Nonanal	12087	10262 [a]	–	6868 [a]	–	11417 [a]	–	10821 [a]
		39201 [a]	9464 [b]	8416 [b]	8878 [b]	6504 [b]	11227 [b]	10190 [b]
		6934 [a]	3059 [b]	4000 [ab]	3865 [ab]	5024 [ab]	3282 [ab]	4867 [ab]
Ketones								
6-methyl-5-hepten-2-one	9699	–	–	1611 [a]	–	3848 [a]	–	7077 [a]
		1152 [a]	3160 [a]	4683 [a]	3539 [a]	4371 [a]	3883 [a]	2519 [a]
		3848 [a]	2088 [ab]	2971 [ab]	1270 [b]	2726 [ab]	2912 [ab]	2512 [ab]
2-undecanone	–	–	–	–	–	–	–	–
		15831 [a]	7320 [a]	9944 [a]	4002 [a]	2683 [a]	7203 [a]	7874 [a]
		4232 [ab]	2945 [ab]	4958 [ab]	1944 [ab]	4054 [ab]	5667 [a]	1524 [b]

Compounds	At harvest	Unpacked clamshells	Mineral-clay impregnated MAP (A)	A + H_2O_2	A + SO_2	Microperforated MAP (B)	B + H_2O_2	B + SO_2
(Z)-6,10-dimethyl-	–	–	–	–	–	–	–	–
5,9-undecadien-2-one		27186 [a]	13111 [ab]	14760 [ab]	8583 [ab]	6752 [b]	15331 [ab]	14138 [ab]
		4049 [a]	3962 [a]	4014 [a]	2509 [a]	5627 [a]	5506 [a]	3688 [a]
Terpenes/Terpenoids								
Linalool	-	9838 [a]	–	1825 [b]	–	3834 [b]	–	–
		–	–	–	–	–	–	–
		2372 [a]	–	1167 [ab]	–	788 [b]	–	–
Geraniol	63402	71053 [a]	26346 [b]	17302 [b]	6846 [b]	28637 [b]	17665 [b]	10009 [b]
		–	–	–	–	–	–	–
		–	–	–	–	–	–	–
Neral	–	–	–	–	–	–	–	–
		–	5606 [a]	5111 [a]	1932 [a]	–	2928 [a]	3917 [a]
		14570 [a]	1039 [b]	2838 [b]	–	1614 [b]	901 [b]	–
Caryophyllene oxide	–	–	–	–	–	–	–	–
		1946 [a]	6141 [a]	6348 [a]	3538 [a]	1632 [a]	6407 [a]	7060 [a]
		–	–	–	–	–	–	–
Esters								
Ethyl 3-methyl-	–	–	–	–	29423 [a]	–	–	19896 [a]
butanoate		–	–	–	–	–	–	–
		7923 [b]	–	–	39699 [a]	–	–	66624 [a]

For each treatment (column), values presented on the 1st, 2nd and 3rd row are the mean peak areas determined for the volatile compound (row) in 'Legacy' blueberries stored for 6, 8 and 10 weeks in that order.

Means with different lowercase letters within one row are significantly different ($p < 0.05$, $n = 3$).

– refers to "not detected"

Table A2.6 Means of peak areas (total ion counts) of aroma compounds in 'Powder Blue' blueberries at harvest and after cold storage

Compounds	At harvest	Unpacked clamshells	Mineral-clay impregnated MAP (A)	A + H_2O_2	A + SO_2	Microperforated MAP (B)	B + H_2O_2	B + SO_2
Aldehydes								
Hexanal	530570	345273 [a]	292888 [a]	312289 [a]	368011 [a]	348824 [a]	374883 [a]	360765 [a]
		478772 [a]	398003 [a]	477493 [a]	430634 [a]	431508 [a]	479149 [a]	417138 [a]
		527123 [a]	485875 [a]	497871 [a]	454054 [a]	474469 [a]	472902 [a]	428893 [a]
(Z)-2-Hexenal	7158	8075 [a]	6383 [a]	6655 [a]	6569 [a]	8063 [a]	7576 [a]	8023 [a]
		21094 [ab]	17550 [b]	23805 [a]	19342 [b]	21238 [ab]	18958 [b]	17920 [b]
		10884 [a]	13731 [a]	13583 [a]	8644 [a]	12146 [a]	10289 [a]	7691 [a]
(E)-2-Hexenal	1516615	1522558 [a]	1349142 [a]	1420374 [a]	1462185 [a]	1484773 [a]	1460935 [a]	1391232 [a]
		1799940 [ab]	1570054 [b]	1976838 [a]	1675937 [ab]	1511555 [b]	1628511 [ab]	1427625 [b]
		1892328 [a]	1751385 [a]	1856373 [a]	1657256 [a]	1617969 [a]	1534682 [a]	1421097 [a]
Benzaldehyde	2660	3306 [a]	–	–	3250 [a]	4293 [a]	2366 [a]	–
		5446 [a]	4455 [a]	6111 [a]	5007 [a]	5287 [a]	4927 [a]	4895 [a]
		6117 [a]	5712 [a]	6020 [a]	4215 [a]	5278 [a]	4645 [a]	4083 [a]
Nonanal	4302	5325 [ab]	4549 [ab]	5767 [ab]	4511 [ab]	6383 [a]	4812 [ab]	4110 [b]
		9174 [a]	8827 [a]	11173 [a]	10139 [a]	9544 [a]	10306 [a]	3310 [a]
		12521 [a]	6653 [b]	7183 [b]	7742 [b]	7650 [b]	8272 [ab]	8310 [ab]
Ketones								
2-Heptanone	443987	18290 [a]	14933 [a]	15667 [a]	17058 [a]	17959 [a]	18739 [a]	18503 [a]
		–	–	–	–	–	–	–
		20483 [a]	22316 [a]	21718 [a]	17998	20566 [a]	19339 [a]	19434 [a]
6-methyl-5-Hepten-2-one	154705	2632 [a]	–	1500 [a]	–	2984 [a]	2504 [a]	–
		–	–	–	–	–	–	8752
		3867 [a]	2133 [a]	1021 [a]	3566 [a]	2029 [a]	2298 [a]	1824 [a]

Compounds	At harvest	Unpacked clamshells	Mineral-clay impregnated MAP (A)	A + H_2O_2	A + SO_2	Microperforated MAP (B)	B + H_2O_2	B + SO_2
2-Nonanone	–	2807 [a]	1598 [a]	1852 [a]	1794 [a]	3471 [a]	2714 [a]	2350 [a]
		–	–	–	–	–	–	–
		3135 [a]	977 [a]	1226 [a]	1251 [a]	1067 [a]	2754 [a]	1626 [a]
2-Undecanone	–	–	–	–	–	–	–	–
		7130 [a]	1937 [a]	2966 [a]	5774 [a]	–	7909 [a]	–
		–	–	–	–	–	–	–
(Z)-6,10-dimethyl-5,9-Undecadien-2-one	–	2812 [a]	1226 [a]	1563 [a]	2986 [a]	3558 [a]	3075 [a]	1837 [a]
		2758 [a]	3421 [a]	3969 [a]	3040 [a]	2252 [a]	2242 [a]	4464 [a]
		8429 [a]	–	–	5540 [a]	2445 [a]	3541 [a]	7477 [a]
Terpenes/Terpenoids								
Eucalyptol	3057	4758 [a]	3605 [a]	4336 [a]	4636 [a]	4751 [a]	4538 [a]	4355 [a]
		–	–	–	–	–	–	–
		9390 [a]	2825 [b]	–	–	–	–	–
Linalool	16175	11500 [a]	7164 [a]	9481 [a]	8172 [a]	11492 [a]	8781 [a]	8055 [a]
		3397 [a]	3457 [a]	4009 [a]	2617 [a]	3147 [a]	2401 [a]	3198 [a]
		10032 [ab]	12081 [ab]	13315 [a]	6659 [ab]	6532 [b]	8779 [ab]	6969 [ab]
α-Terpineol	4383	1719 [a]	3169 [a]	2153 [a]	1321 [a]	–	1367 [a]	–
		1616 [a]	4385 [a]	3592 [a]	4613 [a]	2720 [a]	4206 [a]	6131 [a]
		7166 [a]	7019 [a]	7073 [a]	2901 [a]	1592 [a]	5567 [a]	5255 [a]
Geraniol	1275	–	–	–	–	–	–	–
		3896 [a]	5671 [a]	4320 [a]	5233 [a]	11645 [a]	7306 [a]	–
		–	–	–	–	–	–	–
Neral	–	–	–	–	–	–	–	–
		1143 [a]	837 [a]	2391 [a]	1311 [a]	–	–	2604 [a]
		–	–	–	–	–	–	–

Compounds	At harvest	Unpacked clamshells	Mineral-clay impregnated MAP (A)	A + H_2O_2	A + SO_2	Microperforated MAP (B)	B + H_2O_2	B + SO_2
Esters								
Ethyl 3-methyl-	-	–	–	–	–	–	–	–
butanoate		10112 ^{ab}	8837 ^{ab}	12181 ^{a}	9707 ^{ab}	9744 ^{ab}	9963 ^{ab}	6151 ^{b}
		–	–	–	–	–	–	–

For each treatment (column), values presented on the 1^{st,} 2nd and 3rd row are the mean peak areas determined for the volatile compound (row) in 'Powder Blue' blueberries stored for 6, 8 and 10 weeks in that order.

Means with different lowercase letters within one row are significantly different ($p < 0.05$, $n = 3$).

– refers to "not detected"

Appendix 3. Supplementary data for Chapter 6

Day 0 | Top-icing (control) | HDPE liners + $KMnO_4$ sachets

BOPP MAP | LDPE MAP

Figure A3.1 Quality of 'Ironman' broccoli on the experiment start date and after 7 days at 13 ºC (commercial trials)

Day 0

Top-icing

HDPE liners + $KMnO_4$ sachets

BOPP MAP

LDPE MAP

Figure A3.2 Quality of 'Ironman' broccoli on the experiment start date and after 7 days at 2 ºC (commercial trials)

Day 0

Top-icing (control)

Outgrowth

HDPE liners + $KMnO_4$ sachets

BOPP MAP

LDPE MAP

PA MAP

Figure A3.3 Quality of 'Ironman' broccoli on the experiment start date and after 42 days at 2 ºC (commercial trials)

Table A3.1 Respiration rates of 'Thunderdome' and 'Ironman' broccoli on experiment starting dates and after storage

Treatment / Storage conditions	O_2 consumption rate at 2 ºC (mg kg^{-1} h^{-1})			CO_2 production rate at 2 ºC (mg kg^{-1} h^{-1})		
	7 days at 13 ºC	7 days at 2 ºC	42 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC	42 days at 2 ºC
'Thunderdome' branchlets (laboratory trials)						
Initial broccoli	82.67 ± 12.57^{a}	82.67 ± 12.57^{a}		65.20 ± 9.04^{a}	65.20 ± 9.04^{ab}	
Sandwich bags (control)	36.45 ± 2.73^{b}	21.56 ± 1.78^{c}		30.47 ± 1.82^{a}	67.22 ± 4.79^{a}	
Sandwich bags + $KMnO_4$ sachets	33.71 ± 2.31^{b}	23.53 ± 1.67^{bc}	Not trialled	28.20 ± 2.58^{a}	69.46 ± 6.65^{a}	Not trialled
LDPE MAP	26.25 ± 2.48^{c}	28.25 ± 4.93^{b}		17.77 ± 1.99^{b}	69.80 ± 14.88^{ab}	
BOPP MAP	37.62 ± 3.86^{b}	34.65 ± 7.29^{b}		27.49 ± 3.08^{ab}	80.29 ± 8.40^{a}	
Whole 'Ironman' broccoli (commercial trials)						
Initial broccoli	36.57 ± 1.05^{c}	36.57 ± 1.05^{ab}	36.57 ± 1.05^{a}	33.88 ± 1.42^{b}	33.88 ± 1.42^{a}	33.88 ± 1.42^{a}
Top-icing	58.66 ± 5.87^{a}	35.96 ± 2.56^{ab}	17.3 ± 1.94^{b}	48.91 ± 6.30^{a}	31.80 ± 1.39^{a}	16.62 ± 1.39^{b}
HDPE liners + $KMnO_4$ sachets	43.82 ± 2.08^{b}	34.10 ± 1.81^{b}	16.4 ± 1.05^{b}	30.14 ± 1.30^{c}	29.62 ± 1.79^{a}	15.67 ± 0.42^{b}
LDPE MAP	38.63 ± 9.84^{bc}	40.91 ± 3.13^{a}	18.8 ± 1.92^{b}	24.83 ± 4.95^{c}	34.08 ± 2.21^{a}	17.28 ±1.36^{b}
BOPP MAP	46.88 ± 3.77^{b}	40.92 ± 2.21^{a}	18.0 ± 1.00^{b}	31.60 ± 4.18^{bc}	33.51 ± 1.93^{a}	15.82 ± 0.84^{b}
PA MAP	Not trialled		10.5 ± 6.16^{b}	Not trialled		13.32 ± 2.95^{b}

For each storage condition, means with different lowercase letters within one column are significantly different ($p < 0.05$).

n = 4

Table A3.2 Quality of the initial and stored 'Thunderdome' broccoli (laboratory trials)

Treatment / Storage conditions	Chlorophyll a (mg kg^{-1})		Chlorophyll b (mg kg^{-1})		Carotenoids (mg kg^{-1})		L-ascorbic acid (mg kg^{-1})	
	7 days at 13 ºC	7 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC
Initial broccoli	49.41 ± 2.48^{a}	49.41 ± 2.48^{a}	14.94 ± 0.71^{b}	14.94 ± 0.71^{b}	16.84 ± 0.70^{ab}	16.84 ± 0.70^{a}	539.63 ± 44.70^{a}	539.63 ± 44.70^{a}
Sandwich bags (control)	20.90 ± 2.85^{c}	49.05 ± 3.50^{a}	14.27 ± 1.09^{b}	22.92 ± 4.82^{a}	16.67 ± 0.33^{ab}	17.10 ± 1.13^{a}	117.82 ± 16.52^{b}	186.85 ± 16.52^{c}
Sandwich bags + $KMnO_4$ sachets	26.11 ± 2.89^{b}	49.65 ± 2.11^{a}	9.45 ± 1.10^{c}	19.07 ± 4.06^{ab}	14.69 ± 1.55^{c}	17.57 ± 1.18^{a}	127.62 ± 17.18^{b}	223.64 ± 17.18^{b}
LDPE MAP	48.74 ± 0.49^{a}	51.53 ± 1.14^{a}	18.64 ± 2.80^{a}	16.14 ± 1.32^{b}	15.75 ± 1.27^{bc}	17.76 ± 0.79^{a}	128.47 ± 38.11^{b}	234.27 ± 38.11^{b}
BOPP MAP	49.08 ± 3.16^{a}	51.84 ± 1.53^{a}	17.91 ± 3.51^{ab}	21.17 ± 3.00^{a}	17.86 ± 1.10^{a}	17.90 ± 0.88^{a}	156.05 ± 47.20^{b}	281.13 ± 47.20^{b}

For each storage condition, means with different lowercase letters within one column are significantly different ($p < 0.05$).

n = 4

Table A3.3 Quality of the initial and stored 'Ironman' broccoli (commercial trials)

Treatment / Storage conditions	Chlorophyll a (mg kg^{-1})			Chlorophyll b (mg kg^{-1})			Carotenoids (mg kg^{-1})			L-ascorbic acid (mg kg^{-1})		
	7 days at 13 ºC	7 days at 2 ºC	42 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC	42 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC	42 days at 2 ºC	7 days at 13 ºC	7 days at 2 ºC	42 days at 2 ºC
Initial broccoli	43.35 ± 4.02^{a}	43.35 ± 4.02^{a}	43.35 ± 4.02^{a}	15.84 ± 2.32^{a}	15.84 ± 2.32^{a}	15.84 ± 2.32^{a}	15.28 ± 6.04^{a}	15.28 ± 6.04^{a}	15.28 ± 6.04^{a}	466.45 ± 37.28^{a}	466.45 ± 37.28^{a}	466.45 ± 37.28^{a}
Top-icing	9.57 ± 1.29^{c}	42.74 ± 4.56^{a}	34.27 ± 4.56^{a}	5.82 ± 1.49^{b}	12.48 ± 1.43^{a}	12.02 ± 1.82^{a}	12.06 ± 2.65^{a}	15.49 ± 5.99^{a}	14.85 ± 6.28^{a}	224.41 ± 32.43^{b}	223.04 ± 23.^{61bc}	72.41 ± 20.91^{b}
HDPE liners + $KMnO_4$ sachets	41.28 ± 3.38^{a}	45.57 ± 2.09^{a}	43.37 ± 1.59^{a}	12.79 ± 1.74^{a}	18.49 ± 1.84^{a}	18.80 ± 6.13^{a}	16.33 ± 5.05^{a}	17.30 ± 3.92^{a}	16.84 ± 5.94^{a}	240.07 ± 0.58^{b}	212.55 ± 18.10^{c}	63.18 ± 17.79^{b}
LDPE MAP	39.56 ± 6.84^{ab}	44.13 ± 2.03^{a}	43.89 ± 3.30^{a}	11.76 ± 1.91^{a}	17.15 ± 2.80^{a}	16.74 ± 2.38^{a}	13.64 ± 8.67^{a}	16.96 ± 4.44^{a}	17.74 ± 1.14^{a}	208.14 ± 37.49^{b}	203.37 ± 1.89^{c}	100.12 ± 36.00^{b}
BOPP MAP	34.22 ± 2.63^{b}	44.17 ± 2.80^{a}	40.62 ± 1.25^{a}	12.46 ± 0.60^{a}	16.80 ± 2.64^{a}	14.38 ± 1.47^{a}	13.42 ± 3.21^{a}	16.74 ± 4.72^{a}	15.90 ± 1.06^{a}	263.91 ± 27.74^{b}	265.98 ± 16.27^{b}	110.90 ± 22.35^{b}
PA MAP	Not trialled		41.23 ± 1.51^{a}	Not trialled		13.77 ± 1.41^{a}	Not trialled		15.11 ± 1.24^{a}	Not trialled		101.22 ± 18.94^{b}

For each storage condition, means with different lowercase letters within one column are significantly different ($p < 0.05$).

n = 4