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Extending Raspberry Condition and Sensory Quality with CO2 Atmospheres

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Extending Raspberry Condition and Sensory Quality with CO_2 Atmospheres

By

MOHD REZAUL ISLAM
THESIS

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Abstract

Raspberry (*Rubus ideaeus L.*) fruit are high value but have a short shelf life due to fungal decay, leakiness, off-flavor, and loss of firmness. High CO_2 in combination with low O_2 can increase raspberry storage time and quality. In fall 2020 and 2021, we examined raspberry shelf life and sensory quality periodically during storage at 5℃ for two weeks in four different CO_2 atmospheres; 15 kPa CO_2, 6 kPa O_2 (15 kPa); 8 kPa CO_2, 13 kPa O_2 (8 kPa); 5 kPa CO_2, 16 kPa O_2 (5 kPa); or 0.03 kPa CO_2, 21 kPa O_2 (0.03 kPa) to identify an ideal postharvest atmosphere that will increase raspberry shelf life without degrading the sensory quality. Raspberry visual attributes, color, glossiness, decay incidence and leakiness deteriorated over time in all atmospheres, but high CO_2 atmospheres reduced fruit discoloration and decay, and slowed the rate of deterioration and leakiness. After 5 days, the quality of air stored raspberries was significantly degraded, while raspberries stored in elevated CO_2 maintained firmness with bright red color up to 10 days. In 2021, a trained sensory panel conducted a descriptive sensory evaluation of the raspberry fruit periodically during storage and found that raspberries stored in 8 or 15 kPa atmospheres had significantly lower off-flavor and higher tartness scores. Raspberries stored in 8 kPa atmosphere scored highest in raspberry flavor with substantial juiciness and sweetness scores. The fermentative volatiles; acetaldehyde and ethanol were higher in raspberries stored in 15 kPa atmosphere, while most other raspberry volatiles decreased with increasing CO_2 concentration, including flavor-related volatiles α-ionone, α-terpineol, limonene and linalool. While raspberries stored in 15 kPa atmosphere had longer shelf life, the decrease in flavor-related volatiles was most pronounced in this fruit. However, fruit stored in 8 kPa atmosphere had better sensory quality and 10 days of shelf life and was the optimal atmosphere for raspberry

in our study. Raspberries should not be stored in air beyond 5 days without modified or controlled atmospheres.

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Chapter 1.

Introduction

Raspberry Morphology

Raspberry (*Rubus idaeus L*) fruit are produced on a perennial shrub belonging to the vastly diversified Rubus genera of the Rosaceae family. There are mainly two types of raspberries: red and black. Yellow raspberries are a mutated version of red raspberries that prevents the formation of red color (Chad et.al., 2014). Purple raspberries are a hybrid between black and red raspberries. Raspberries are an aggregate fruit, a combination of individual drupelets that stay together with the help of an invisible hair-like structure. Each small drupelet is developed from a carpel, a significant characteristic of Rubus, where the mesocarps become fleshy and the endocarps become hard and form a tiny pit that encloses a single seed within each drupelet. (Graham et al., 2007). The drupelets separate from the receptacle at harvest, creating a hollow, thimble-shaped fruit.

Raspberry Production

The primary planting material for raspberries is the cane. Cane traditionally refers to aerial raspberry shoots but also often refers to rooted suckers (Hudson, 1959). Raspberry plants generally start fruiting in the second year and can continue up to 15 years if properly managed. However, the canes are biennial. Usually, the cane grows in one year and fruits the next year. Canes sprouting in the first year are called primocanes (summer-bearing), and in the second year are called floricanes (fall-bearing). Both canes are present during the growing season (Chad et.al., 2014). Primocane-fruiting types (also known as ever bearer) can produce two crops per year; one in the summer from the floricanes and the other in the fall from the primocanes. With the onset of cold temperatures, floricanes often go into a period of dormancy. Six weeks or more at 4℃ or lower is required to break dormancy. After fruiting, the entire cane senesces and dies. When second-year canes (floricanes) are flowering, first-year canes (primocanes) are growing from the crown or roots. Like other brambles, raspberry is a self-pollinated species (Bushway et al., 2008).

Fruit development occurs for 30-36 days in most cultivars (Jennings, 1988; Roach, 1985). The best yields take place under sunny, cool summer conditions (Bushway et al., 2008). Recently, the use of the high tunnels has extended the berry cultivation season both at the beginning and the end. This extension enables growers to gain more profit from the market as berry prices are usually higher during early and late seasons (Both et al., 2019). Like all crops, water is very important for raspberry production. According to Prange and DeEll (1997), lack of water can affect the production and quality of berry fruit after harvest. However, excessive water, mostly due to high rainfall during the fruit growing period, made raspberries more susceptible to mechanical damage during transportation and storage (Kader, 2002).

History and production trends

The red raspberry (*Rubus idaeus L*) is believed to have originated in Turkey's Ide mountains. The Romans may have expanded raspberry cultivation throughout Europe. However, the British improved and popularized raspberries throughout the middle ages and had the plant exported to New York by 1771 (Jennings, 1988). In the early 1900s, raspberry cultivation got its momentum, and in 1920, New York State growers harvested more than 10,000 acres of raspberries. In 2018, there were three major raspberry production regions: Russia, Europe (mostly Serbia, Poland, Spain, Ukraine, Portugal, Bosnia and Herzegovina), and the Pacific Coast of North America (United

States and Mexico) (Bojkovska et al., 2020). World raspberry production has grown 80% over the last 10 years. From 2010 to 2019, production increased from 373,000 tons to 684,000 tons. (https://www.internationalraspberry.net/). In 2020, the total area under raspberry cultivation in the United States (US) was 16,900 acres producing 111,000 tons of raspberries valued at US$469 million. In California, there were 8,000 acres, valued at US$395 million for fresh raspberries only. Canada is the biggest importer of US raspberries, and in 2021, Canada imported a total of 24,400 tons of fresh raspberries valued at $154 million from the US (USDA, 2021).

Raspberry quality factors

A fruit's quality is generally assessed based on its visual appearance, texture, flavor, and nutritional compounds (Pelayo et al., 2003). Raspberry quality and shelf life can be adversely affected by a variety of pre-harvest and postharvest factors. Pre-harvest factors include genetics, environment, and cultural practices. Postharvest factors such as handling, transportation, storage temperature, condition and duration, relative humidity also play important role on maintaining raspberry fruit quality.

Raspberry is renowned for its aroma and flavor. Raspberry is a non-climacteric fruit and their taste and flavor mostly develop while they are ripening on the plant. The ratio of sugar and organic acids determines raspberry taste (Ponder et al. 2020). Soluble solids (SS) from 9 to 10% and titratable acidity (TA) from 1.5 to 1.8% constitute good raspberry taste according to De Ancos (2000). Wang et al. (2009) evaluated raspberry fruit harvested at 5%, 20%, 50%, 80% and 100% ripe and they concluded that berries that were 50% to 80% ripe developed the same degree of SS, TA, and sugars as berries that were 100% ripe, whereas berries that were 5 to 20% ripe never attained those properties. There are many volatile compounds, notably, α and β-ionone, linalool, α and β-pinene, caryophyllene and citral, contributing to raspberry flavor (De Ancos,2000).

Berry purchases are linked to several factors, the most important being freshness and origin, while price does not play a significant role (Girgenti et al., 2016). Visual quality is also very important for raspberry and a good indicator of shelf life. Brighter color without any visible decay and leakiness is perceived as fresher. Krüger et al. (2003; 2011) categorized raspberries in three groups based on their ripening stage; semi-ripe, ripe and over-ripe. They concluded that semi-ripe raspberries were potentially more suitable for shipping while maintaining acceptable sensory quality (Kruger et al., 2003).

Health benefits of raspberry consumption

Consumers have always been concerned about food quality and appearance, in general. However, consumer preference has been shifting toward fruit flavor and nutritional qualities, including their composition and level of bioactive compounds, such as vitamins, minerals, fiber, and even phenolic compounds (Paredes-López, 2010). Raspberry fruit are 85-90% water, ~9% SS and the remaining are insoluble solids. Raspberry fruit contain 13.6–31.1 mg/100 g Vitamin C and 0.2–83.6 mg/100 g anthocyanins (Akimov et al., 2021). These compounds vary by cultivar, harvest time, cultural practices, environment and weather conditions (Fu et al., 2015; Duarte et al., 2010). Raspberry fruit also contain a broad range of polyphenolic compounds; phenolic acids, flavanols, anthocyanins, proanthocyanidins, and ellagitannins (Dincheva et al., 2013). These compounds have been extensively studied for their antioxidant capacity and impact on human health (Deighton et al., 2000). High antioxidant capacity is believed to contribute to health benefits by ameliorating the detrimental effects of reactive oxygen species (ROS) generated in the body through oxygen metabolism (Halliwell, 2007). Berry polyphenols also have been shown to protect against ROS-induced neurological diseases such as Alzheimer's (Spencer, 2010), and red raspberries have been reported to have a neuroprotective effect (Burton-Freeman et al., 2016). However, the impact of raspberry fruit's high antioxidant capacity might be limited by very low (µM – nM) uptake into the bloodstream from dietary intake (Koli et al., 2010). Nonetheless, they could have beneficial effects on the gastrointestinal tract (GIT) as they pass along the digestive system, thus preventing oxidation from foods already in the stomach and GIT (Gorelik et al., 2005) or by affecting food digestion, glucose levels, and calorie usage (McDougall et al., 2008). In addition, raspberry contains a significant amount of ellagitannins; a large group of polyphenols that are beneficial to fight cervical cancer, cardiovascular disease, and diabetics (Ross et al., 2007; Scazzocchio et al., 2011)

Postharvest handling of raspberry

Raspberries have a short shelf life of 2 to 5 days because of their natural soft texture and sensitivity to mold and other pathogens. Postharvest handling and storage conditions, including packaging, relative humidity, temperature, and light, can affect the bioactive compounds in brambles (Nunes et al., 2009). Cooling is by far the best technology for increasing the shelf-life of horticultural produce. Low temperatures slow pathogen growth and reduce the rate of deterioration of freshly harvested commodities, thus extending shelf life and the marketing period (Sommers et al., 1973). The recommended temperature for raspberry storage is 0-1℃ (Haffner et al., 2002), but it is challenging to maintain this recommended temperature during transportation and marketing. Although low storage temperatures can slow the development of *Botrytis cinerea* infections, they do not provide adequate control when pathogen inoculum loads are high (Maude, 1980). Acidity and SS as well as pigment compounds such as anthocyanins play an important role in berry marketability after storage (Wang et al., 2009), and the presence of light and temperature during storage might affect anthocyanin compound stability (Kalt et al., 1999).

Atmosphere modification

Modification of storage or transport atmospheres help maintain raspberry shelf life and quality. Controlled atmospheres (CA) or modified atmospheres (MA) are created by reducing O_2 and/or elevating CO_2 concentrations and have the general effect of slowing senescence and extending shelf life (Kader, 1992). The fundamental difference between CA storage and MA packaging (MAP) systems is that in the CA storage system, gas levels are rigidly maintained, whereas in the MAP system, the gas mixture is flushed into the package once, if at all, and concentrations vary over time with product respiration and package permeability (Choubert and Baccaunaudb, 2006). Active MAP is performed by removing some amount of air from the package and replacing it with the desired gas combination (Kader et al., 2000). High CO_2 concentrations have a general inhibitory effect on microorganism growth and development. CA composed of high CO_2 and low O_2 was found to be fungistatic in controlling *Botrytis alli, Rhizopus nigricans*, and *Penicillium expansum* (Littlefield et al., 1966). Nine red raspberry genotypes were tested in CA storage at 1℃ and decay was strongly suppressed across all the genotypes (Forney et al., 2015). Raspberries exposed to CO_2 levels of 20% or higher exhibited delayed gray mold decay and extended shelf life (Goulart et al., 1992). High CO_2 concentration also slows further ripening and softening in berries. Applying CA, even for a short time of 0.5 to 3 days, increased strawberry shelf life by 3 days, as well as reduced the endogenous ethylene production and ultimately maintained lighter and brighter colored and firmer fruit (Alamar et al., 2017). In addition, lowering the O_2 concentration in the storage atmosphere can be beneficial in extending the shelf life of fresh produce (Robinson et. al., 1975). In mangoes, respiration rate decreased about 20-25% in low O_2 atmosphere compared to air (Rattanapanone et. al., 2000).

Storing fruit with higher CO_2 atmospheres; however, can result in off-flavor development, perhaps due to the initiation of anaerobic respiration and production of fermentative volatiles. Also, oxygen levels less than 2 kPa may cause fermentation of raspberry fruit (Joles et.al., 1994; Haffner et al., 2002). MA packaged strawberries developed off-flavor which the authors suggested might be linked to a specific cultivar's susceptibility to accumulate ethyl acetate (Larsen, 1994). The raspberry cultivar, Qualicum, produced more ethyl acetate in modified atmosphere packaging compared to "Meeker" and "Chilliwack" (Toivonen et al., 1999).

Hypothesis

Storing raspberries in high CO_2 atmospheres will extend their shelf life through a fungistatic effect and inhibit fruit softening by slowing their ripening without negatively affecting sensory quality.

We hypothesize that high CO_2 atmospheres will act as a fungistatic agent and slow ripening as well as reduce the respiration rate of the raspberries, which ultimately will increase their shelf life without comprising flavor.

Objectives:

1. Determine the effects of different cold storage atmospheres on the postharvest quality and shelf life of raspberry fruit.

2. Determine the effects of different cold storage atmospheres on the sensory quality of raspberry fruit.

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Chapter 2. Extending raspberry shelf life and maintaining postharvest quality with CO_2 atmospheres

Abstract

Raspberry (*Rubus ideaeus L.*) generally have a very short shelf life. Decay, leakiness, and loss of firmness are the most common limiting factors contributing to short storage life. However, storing in elevated CO_2 and reduced O_2 atmospheres can delay senescence of fruit by reducing softening, respiration and ethylene production rates, and pathogen growth. Our study explored the effects of holding raspberries at 5℃ in four different CO_2 atmospheres; 15 kPa CO_2, 6 kPa O_2 (15 kPa); 8 kPa CO_2, 13 kPa O_2 (8 kPa); 5 kPa CO_2, 16 kPa O_2 (5 kPa); or 0.03 kPa CO2, 21 kPa O_2 (0.03 kPa) atmospheres on fruit quality and shelf life. Berries were evaluated periodically during two weeks of storage in 2020 and 2021. Raspberries stored in 15 kPa atmosphere followed by 8 kPa atmosphere had higher firmness and brighter red color, with the least leakiness and fungal decay. In all atmospheres, total anthocyanin content increased over time, although the rate of increase was slowed by high CO_2. Raspberry visual attributes deteriorated over time in all atmospheres, but high CO_2 atmospheres slowed the rate of deterioration. After five days, the quality of air stored raspberries was significantly degraded, while raspberries stored in elevated CO_2 maintained good quality for up to 10 days.

Introduction

Raspberry (*Rubus idaeus L.*) is a high value fruit but their shelf life is impacted by high perishability. In 2020, the United States produced 111,000 tons of raspberries, at a value of US$469 million and California alone contributed fresh raspberries worth of US$395 million (USDA, 2021). Raspberry's delicate morphology coupled with high respiration and transpiration rates make them vulnerable to rapid deterioration after harvest. The typical shelf life of raspberries ranges from 3 to five days (Adobati et al., 2015). Decay, leakiness, loss of firmness, darkening of the red color, and off-flavors are common limiting factors contributing to short storage life of raspberries (Haffner et al., 2002). It is well established that cooling is by far the best technology for increasing the shelf-life of horticultural produce. Low temperatures slow pathogen growth and reduce the rate of deterioration of freshly harvested commodities, thus extending shelf life and the marketing period (Sommers et al., 1973). The recommended temperature for raspberry storage is 0-1℃ (Haffner et al., 2002), but it is challenging to maintain this recommended temperature during transportation and marketing. Although low storage temperatures can slow the development of *Botrytis cinerea* infections, they don't provide adequate control when pathogen inoculum loads are high (Maude, 1980). Atmospheres enriched with CO_2 can create fungistatic conditions, and therefore, inhibit the growth of fungi. Raspberries exposed to CO_2 levels of 20 kPa or higher had delayed gray mold decay and extended shelf life (Goulart et al., 1992). Nine red raspberry genotypes were stored in controlled atmospheres (CA) with 12.5 kPa CO_2 and 7.5 kPa O_2 for 50 days at 1℃, and decay development was strongly suppressed across all genotypes (Forney et al., 2015). Our objective was to determine the optimum atmosphere to extend raspberry shelf-life and maximize quality during transit or storage by assessing fruit response to a range of CO_2 atmospheres.

Materials and Methods

Freshly harvested raspberries (*Rubus idaeus L.*, cv. Maravilla) were obtained immediately after harvest in fall 2020 and 2021. Berries were field packed into clamshells (170 gm) and precooled at a commercial facility in Watsonville, California. Cooled fruit were transported on the same day in an air-conditioned vehicle to the UC Davis postharvest laboratory within 3 hours. Raspberries were held at 5℃ overnight, and the next day, the baseline quality of a sample of fruit was analyzed before randomly assigning the remaining clamshells to different atmosphere treatments at 5℃. Fruit were removed from the atmosphere treatments after 6, 10 and 14 days in 2020 and 5, 10, and 13 days in 2021 and immediately evaluated to assess changes in the fruit's physical quality over time in storage. The performance of fruit in each treatment atmosphere was evaluated from the perspective of raspberry shelf life and quality.

Treatment atmospheres and experimental setup

In both years, raspberries packed in clamshells were stored at 5℃ for up to 14 or 13 days in 2020 or 2021, respectively, in one of four atmosphere treatments: 15 kPa CO_2, 6 kPa O_2 (15 kPa); 8 kPa CO_2, 13 kPa O_2 (8 kPa); 5 kPa CO_2, 16 kPa O_2 (5 kPa); or 0.03 kPa CO2, 21 kPa O_2 (0.03 kPa). These atmospheric treatments simulate the mixture of O_2 and CO_2 concentrations that would be present in a modified atmosphere package aiming for 15, 8, and 5 kPa CO_2 as well as an unmodified atmospheric package (air). The gas concentrations were measured during set up and periodically with a CO_2/O_2 gas analyzer (Systec Gas Advance Micro-GS3, Boston, MA). The four atmospheres were humidified by bubbling through water prior to fruit exposure in a continuous flow-through system at a rate of 100 mL/minute. Raspberry fruit remained in the clamshells during treatments, and there were nine plastic bags, each holding six clamshells inside for each atmosphere (a separate bag for each evaluation day (3) and replication (3)), which had been modified with inlet and outlet ports and connected to a separate gas flow board. Instrumental fruit quality was evaluated again on each removal date.

Quality evaluations

Respiration rate and ethylene production were measured at 5°C on each evaluation date. Fruit for the 0-day evaluation were cooled overnight before measurement. After removing stored raspberries from the atmospheres, fruit were held at 5°C in air for 18-20 hours to off-gas before being sealed inside a 10-liter container for 1 hour at 5°C prior to headspace gas sample collection. Headspace gas samples were analyzed for CO_2 (Horiba infrared gas analyzer, Irvine, CA) and ethylene (Carle gas chromatograph, Tulsa, OK) concentrations. Respiration and ethylene production rates were calculated and expressed as ml CO_2/kg/hr and µl ethylene /kg/hr, respectively.

One clamshell per treatment and replication was weighed before sealing in the plastic bags. Percent weight loss was calculated by deducting the measured final weight from the initial weight, dividing by the initial weight, and multiplying by 100. Leakiness was assessed subjectively on one clamshell per treatment and replication. In 2020, a single layer of paper towel was laid on a tray. The whole clamshell of raspberries was gently poured onto the tray, then the tray was shaken five times, back and forth; gently, but enough to move the berries. The tissue paper was evaluated for the juice marks resulting from berry leaking and ranked based on their intensity;

where 1 = none, 2 = very slight, 3 = slight, 4 = moderate, and 5 = severe. In 2021, an improved method was used. The raspberries from one clamshell were arranged on a white paper divided into 40 square blocks; an individual raspberry was placed horizontally on each block for leakiness evaluation. A similar paper (with printed square blocks) was used to cover the raspberries and pressed very gently onto the fruit for 1 second. The top paper and the fruit were removed, and the papers' printed square blocks (bottom and top) were evaluated and scored for liquid stains resulting from berry leaking. The scores for each fruit (block) were assigned based on the intensity, where 1 = none, 2 = very slight, 3 = slight, 4 = moderate and 5 = severe (Supplemental Fig. 2.1). The number of fruit with a score of 2 or higher were divided by the total number of fruit to determine the percentage of affected fruit. Leakiness severity was calculated by summing up the severity scores of fruit with a score of 2 or higher (leaky fruit) and dividing by the total number of leaky fruit.

Decay evaluation was done visually on the fruit from the same clamshell as leakiness. The severity of infection on each fruit was scored using a scale of 1 to 5, where 1 = none; 2 = very slight, 1-3 decayed drupelets; 3 = slight, 4-6 decayed drupelets; 4 = moderate, 7-9 decayed drupelets; and 5 = severe, >9 decayed drupelets (Supplemental Fig. 2.2). The number of fruit with a score of 2 or higher was divided by the total number of fruit and multiplied by 100 to determine the percentage of decayed fruit. Decay severity was calculated by summing up the severity scores of fruit with a score of 2 or higher (decayed fruit) and dividing by the total number of decayed fruit.

Ten raspberries were randomly selected from one clamshell per treatment and replication to evaluate color using a chromameter (Konica Minolta Sensing Americas, Inc, Ramsey, NJ, USA) with the CIELAB color space. Coloration of the external surface of the raspberries was measured and expressed as L*, C*, and h color coordinates, indicating lightness, chroma and hue angle, respectively. Only one side, close to the apex of the fruit was measured.

The same fruit were evaluated for glossiness. Glossiness refers to the light reflection intensity of the fruit. The fruit were visually inspected and subjectively scored from 1 to 3, where 1 = dull, 2 = moderately glossy, and 3 = glossy (Supplemental Fig. 2.3). The same ten berries were used to evaluate the degree of discoloration based on the number of discolored (whitish/pale) drupelets, and scored on a 1 to 5 scale where 1 = none, 2 = very slight, 1-3 discolored drupelets; 3 = slight, 4-7 discolored drupelets; 4 = moderate, 8-11 discolored drupelets; and 5 = severe, >11 discolored drupelets (Supplemental Fig. 2.4).

Fruit firmness was also assessed subjectively using the same raspberries that were used to evaluate glossiness and discoloration. Each raspberry was pressed slightly with the thumb and middle fingers. Based on the palpability, the berries were scored from 1 to 5, where, 1 = very firm, rebounds from compression, high resistance; 2 = firm, partial rebound; 3 = soft, partial rebound; 4 = very soft, partial rebound and 5 = no resistance.

Ten randomly selected raspberries (the same raspberries used for glossiness) were juiced together using a hand juicer and cheesecloth, yielding 10-15 ml of juice. The juice was used for measuring total soluble solids (TSS) content with a tabletop automatic refractometer (Atago RX 5000i, Bellevue, WA), and results were expressed as the percentage of TSS. Four grams of juice were diluted with 20 ml of dH_20 and then titrated with an automatic titrator (TIM850 Titration Manager, Radiometer Analytical, France). Titratable acidity (TA) was expressed as percentage of citric acid (g/100 g juice), the dominant organic acid in raspberries.

A second clamshell of raspberries from each treatment and replication was frozen with liquid N_2, and immediately broken into drupelets with a mortar and pestle. Drupelets were mixed among fruit from each clamshell and stored in a –80 ℃ freezer until analyzed. These frozen raspberries were used for measuring total anthocyanin content (TAC). The TAC was measured using a microvolume UV-Vis spectrophotometer (Nanodrop, Thermo Fisher Scientific, Waltham, MA) by adapting a method from Abdel-Aal & Hucl (1999). Liquid N_2 was added to the frozen raspberry drupelets and then immediately ground with a blender (Osterizer 12 speed blender, Mexico) for 1 min and turned into a fine powder. An aliquot (400 mg) of raspberry powder was added to 10 mL of acidified ethanol solution (96% ethanol and 1 N HCL 85:15 v/v) and vortexed for 1 min. The solution was incubated for 30 min at 50℃ and then filtered through a 0.45-micron polytetrafluorethylene filter (Agilent Technologies, Santa Clara, CA). The supernatant was collected and held in a –20℃ freezer until evaluated by spectrophotometry. Absorbance (A) was measured at 530 and 700 nm on cyanidin 3-glucoside equivalence. The acidified ethanol solution was used as a blank. Total anthocyanin content per sample (mg/kg) was calculated as cyanidin 3-glucoside equivalent, the most dominant anthocyanin in raspberry:

$$C = (A/\varepsilon) \times (\text{vol}/1{,}000) \times MW \times (1/\text{sample wt.}) \times 10^6$$

where C is the concentration of total anthocyanin (mg/kg), A is the difference (530 nm-700 nm) between the absorbance readings, ε is the molar absorptivity (cyanidin 3-glucoside = 25,965 cm^{-1} M^{-1}), vol is the total volume of anthocyanin extract, and MW is molecular weight of cyanidin 3-glucoside = 449. (Abdel-Aal & Hucl, 1999).

Statistical analysis

Data were statistically analyzed using R statistical program (Core Team, 2013). In addition to base statistical analysis, ggplot2 and dplyr packages were used. A total of 4 atmospheres (treatments) and 3 replications across the 4 evaluation dates were analyzed for quality characteristics of raspberry fruit. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$) among treatments and evaluation days.

Results

Firmness

Holding raspberry fruit after harvest in high CO_2 atmospheres reduced softening in a concentration dependent manner (Fig. 2.1, Table 2.1). Raspberry firmness showed a similar trend for both years of the experiment (Fig. 2.1). Raspberries stored in 15 kPa atmosphere were significantly higher in firmness than raspberries stored in lower CO_2 atmospheres and in air in both years, and had the highest firmness among all the atmospheres throughout storage in both years, followed by raspberries stored at 8 kPa and 5 kPa atmosphere, respectively. Air (0.03 kPa atmosphere) stored raspberries lost firmness most quickly during storage, and had the lowest firmness among all treatments on each evaluation day (Fig. 2.1).

Decay

Storage of raspberry fruit under high CO_2 atmospheres reduced decay development (Table 2.1, Fig. 2.2). However, decay increased over time during storage in all atmospheres, and there was more decay in 2021 than 2020. In 2021, decay control was similar in fruit stored in 15 kPa and 8 kPa atmosphere (Fig. 2.2 Table 2.1), while fruit stored in 5 kPa atmosphere was intermediate between fruit stored in 0.03 kPa and 8 kPa or 15 kPa atmosphere (Table 2.1). In both years, raspberries stored in 0.03 kPa atmosphere had significantly higher decay incidence compared to raspberries stored in other atmospheres. Raspberries stored in air (0.03 kPa atmosphere) also had the highest decay severity, along with raspberries stored in 5 kPa CO_2 in 2021 (Table 2.1).

Weight loss

Overall weight loss was low (<1.5%) during raspberry storage in 2021; however, all of the stored raspberries lost weight over time (Fig. 3). After five days, weight loss slowed in raspberries held in 15 kPa atmosphere. Weight loss was lower in raspberries stored in 8 kPa or 15 kPa atmosphere compared to raspberries stored in 0.03 kPa atmosphere (Fig. 2.3, Table 2.1).

Discoloration

There was an increase in raspberry discoloration during storage, particularly after five days of storage; however, storage in elevated CO_2 atmospheres slowed the increase in discoloration, especially at 8 and 15 kPa atmosphere (Fig. 2.4, Table 2.1). All raspberries showed an increase in discoloration between 10 and 13 days except raspberries stored in 15 kPa atmosphere (Fig. 2.4). Raspberries stored in 0.03 kPa and 5 kPa atmosphere had the highest discoloration score (Table 2.1).

Glossiness

Raspberries stored in 15 kPa atmosphere maintained similar glossiness scores to the glossiness values at harvest across all evaluation dates, while the glossiness score of other raspberries decreased during storage (Fig. 2.5). The glossiness score of raspberries stored in 15 kPa atmosphere was highest, while the glossiness scores of raspberries stored in 8 kPa or 5 kPa atmosphere were similar and lower than at 15 kPa atmosphere (Table 2.1). Raspberries stored in air (0.03 kPa atmosphere) had the lowest glossiness scores throughout storage (Table.2.1, Fig. 2.5), and raspberries stored in 0.03 kPa or 5 kPa atmosphere exhibited a rapid decrease in glossiness (Fig. 2.5).

Leakiness

There was a steady increase in the percentage of raspberry fruit showing leakiness throughout the storage period for all atmospheres (Fig. 2.6). The increase was significantly slower for raspberries stored in 15 kPa atmosphere and was higher for raspberries stored in air and 5 kPa atmosphere; raspberries stored in 8 kPa atmosphere were intermediate between 15 and 8 kPa atmosphere. Leakiness severity scores were also reduced by storage in CO_2; fruit in the 15 kPa atmosphere had the lowest leakiness scores and fruit stored in 0.03 kPa atmosphere had the highest scores (Table. 2.1).

Color

Raspberry color tone (hue angle) decreased (indicating darker red color) rapidly and then stabilized during 13 days of storage (Fig. 2.7). The hue angle stabilized after ten days in storage at values that were concentration dependent, with higher hue angle values in raspberries stored in higher CO_2 concentrations (Fig. 2.7, Table 2.1). The exception was for raspberries stored in 15 kPa atmosphere where the hue angle stabilized around five days of storage (Fig. 2.7). L value and chroma decreased with time in storage, but while there was no impact of CO_2 concentration on L value, chroma was maintained at higher levels in raspberries stored in 15 kPa atmosphere (Table 2.1).

Total anthocyanins content

Total anthocyanin content increased during air storage, particularly up to 10 days (Fig. 2.8); however, raspberries stored in elevated CO_2 atmospheres experienced a slower rate of increase and a lower total anthocyanin content on day 13 compared with air stored raspberries. The higher the CO_2 concentration, the stronger the reduction in anthocyanin accumulation (Fig. 2.8, Table 2.1).

Total soluble solid and titratable acidity

Raspberry TSS showed a declining trend over the storage period, regardless of the CO_2 concentration (data not shown). Raspberry TA did not change during storage, but raspberries stored in 5 kPa had significantly higher TA than the ones stored in 0.03 kPa atmosphere (data not shown).

Respiration and ethylene

Respiration rate and ethylene production of stored raspberries increased over time (Table 2.1). However, high CO_2 atmospheres reduced both respiration rate and ethylene production. Raspberries held in 15 kPa atmosphere had the lowest respiration rates, which were similar to raspberries stored in 8 kPa atmosphere, but significantly different from raspberries stored in 5 or 0.03 kPa atmosphere (Table 2.1). Ethylene production rate of raspberries stored in 15 kPa atmosphere was lowest, while raspberries stored in 5 and 8 kPa atmosphere were intermediate and air stored raspberries had the highest ethylene production (Table 2.1).

Discussion

Firmness is an important indicator of quality in raspberry fruit, as well as many other fruit. The decrease in raspberry firmness after harvest was inhibited or slowed by storage under increasing CO_2 concentrations, and high CO_2 stored fruit had significantly higher firmness than air stored raspberries. CO_2 has other effects on fruit physiology, it influences ethylene biosynthesis by regulating 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis and oxidization. ACC synthase is inhibited by high (5-20 kPa) CO_2. ACC oxidase activity is stimulated by low levels (< 5 kPa) of CO_2 and inhibited by higher CO_2 (Mathooko, 1996). The association of high CO_2 atmospheres with the maintenance of raspberry fruit firmness was further supported by González et al. (2020) who found that raspberries stored in a continuous flow of CO_2 (15 or 10 kPa CO_2) for 14 days had higher firmness than berries exposed to CO_2 for 3 days or an intermittent CO_2 treatment. In strawberries, elevated CO_2 has also been shown to enhance firmness (Smith, 1992). Strawberry fruit exposed to high CO_2 atmospheres exhibited changes in apoplastic pH levels and in turn may have increased cell to cell adhesion by precipitation of soluble pectin (Harker et al., 2000). Solubilization of CO_2 produces H^+ and $HCO3^-$ that could influence pH (Bown, 1985). The increase in firmness following exposure to high CO_2 atmospheres, as related to pectin polymerization, is mediated by calcium. In strawberry, modification of pectic polymers decreased the amount of water soluble pectins (WSP) and increased the chelator soluble pectins (CSP), which is the major factor in firmness increase (Hwang et al., 2012). However, in our study, we did not find any increases in raspberry firmness as a result of exposure to up to 15 kPa atmosphere for 14 days, although the rate of softening was reduced. Forney et al. (2015) found that CA (12.5 KPa CO_2 with 7.5 KPa O_2) did not maintain raspberry firmness during 2-3 days storage at 1°C, and resulted in fruit softening compared to air stored raspberries.

The effect of the modified atmospheres in delaying further ripening, as evidenced by differences in other raspberry quality parameters such as color, may be one reason why the firmness was maintained. Bing cherries stored in low O_2 (0.5-2%) maintained a higher percentage of green stems, brighter color and higher TA, indicating delayed ripening as compared to air stored cherries (Chen et al., 1981). However, O_2 may not have had much effect in our experiment because the lowest O_2 concentration we utilized was 6 kPa and the other O_2 concentrations were $\geq$ 13 kPa. The 15 kPa atmosphere could be the one exception. Given the relatively low O_2 content and the high CO_2 content, the combination of 15 kPa CO_2 and 6 kPa O_2 may have had additional effects on fruit metabolism beyond the effects of the high CO_2 alone, strengthening the effect of the 15 kPa atmosphere on fruit quality. However, elevated CO_2 atmospheres can delay ripening without the added effect of low O_2.

In our study we observed an increase in leakiness and a decrease in glossiness during storage. Leakiness is initiated in raspberries by physiological breakdown (PB) of the cells, a typical symptom of a plant tissues' senescence (Bhattacharjee, 2005). Physiological breakdown is evidenced by juice leakage and softness, and contributes to the fast deterioration of raspberry fruit quality (Perkins-Veazie, 2004). We observed a significant increase in leakiness over time after harvest; however, the rate of increase was slower with less leaky raspberries when stored in 15 kPa atmosphere. The effect of high CO_2 in slowing further ripening and overripening likely contributed to the slower rate of leakiness development. When evaluating different raspberry cultivars, Harshman et al. (2014) did not detect a clear association between fruit firmness and PB resistance, indicating that initial fruit firmness is not related to PB incidence. Forney et al. (2015) reported that storage in 12.5 KPa CO_2 and 7.5 KPa O_2 was less effective in delaying PB than delaying decay. Perhaps, their fruit had already begun senescence prior to CA exposure.

Visible decay on the fruit surface significantly reduces raspberry fruit quality. Decay incidence in our studies was reduced by storage under high CO_2 concentrations, with the maximum effect achieved at 8 and 15 kPa atmosphere. In agreement with our study, Haffner et al. (2002) found significant inhibition of raspberry decay by using high CO_2 atmospheres (10-30 kPa CO_2 in combination with 10 kPa O_2) as compared to air stored fruit. High CO_2 concentrations create a fungistatic effect that slows microbial activity of fungi as well as the metabolic activity of fruit. High CO_2's fungistatic effect is due to its solubility in the aqueous phase of the produce and fungi. CO_2 in the intercellular environment lowers the pH, inhibiting enzyme-catalyzed processes and enzyme production, interacting with cell membranes, and affecting the physicochemical characteristics of proteins (Farber, 1991). Altered expression of proteins in both fungi and fruit tissues can therefore alter decay development (Chan, 2013). In addition, maintaining cellular integrity as a result of CO_2’s firming effect may have also inhibited fungal activity. Petrasch et al. (2022) also, reported mycelium developed faster on softer strawberry fruit than on firmer fruit. In apple and pear CA storage, Von Schelhorn et al. (1951) determined that control of fungal development was a secondary impact, and the major prolongation of shelf life was due to delayed ripening of the fruit. While the atmospheres and time-frame of apple and pear storage are very different from those for raspberry, we also found some strong effects of atmosphere on raspberry senescence, apart from decay, which may have contributed to the fruit's ability to resist decay. CA impacts on fruit physiology may promote decay resistance in addition to direct effects on fungal development. Modified atmospheres reduce respiration rates and delay fruit ripening (Watkins and Zhang, 1998), which is also in agreement with our findings. In addition, higher firmness can reduce fruit damage and stronger cell walls resist cell wall degrading enzymes produced by pathogens, hindering a microbe's capacity to infect the fruit (Lagaert et. al, 2009).

Maintaining a bright red color is an important postharvest quality attribute for raspberries, as dark red color is associated with overripe fruit (Madrid and Beaudry, 2020). High values of hue angle indicate more orange-red color and low values more blue-red color. Our results showed that raspberry fruit stored in 15 kPa atmosphere maintained a stable hue angle after five days, but the hue angle declined in raspberries stored in air (0.03 kPa) or lower CO_2 concentrations. In strawberries, holding fruit in 15 kPa CO_2 and 5 kPa O_2 decreased endogenous ethylene biosynthesis and resulted in a lighter, brighter hue (Alamar et al., 2017) and this finding is also aligning with our finding where high CO_2 held raspberries had significantly lower ethylene production rate than air held raspberries.

pH also plays a crucial role in raspberry fruit color. CO_2 in the intercellular environment lowers the pH (Farber, 1991). Hydration of CO_2 and the production of HCO_3^- and H^+ may reduce intracellular pH (Bown, 1985). In strawberry, reducing pH from 3.81 to 3.21 resulted in a 37 to 13 percent shift in flavylium form, and also increased the stability of fruit color more than any other factors (Wrolstad et al., 1970). The red flavylium cation (AH^+) remains stable only in acidic conditions (Brouillard et. al., 1982). In addition, elevated CO_2 atmospheres during storage and/or transportation were found to maintain a lighter, brighter color in strawberry (Alamar et al., 2017).

Anthocyanins play a vital role in raspberry color expression. The visual appeal of raspberry fruit decreases with time after harvest, along with increased levels of certain anthocyanins (Stavang et al., 2015). In our study, total anthocyanins increased over time, except in raspberries stored in 15 kPa atmosphere; storing raspberries in 15 kPa atmosphere maintained the anthocyanin content (as well as hue angle) close to the levels at harvest. In agreement with our finding, Gil et al. (1997) found that high CO_2 concentrations inhibited the increase in anthocyanin content after harvest by affecting its biosynthesis, degradation or both. These results indicate the ability of high CO_2 atmospheres to maintain raspberry fruit color tone, even after two weeks of storage. Anthocyanin content is also related to raspberry skin color. Palonene et al. (2019) found a significant correlation between anthocyanin concentration and color values, as the darkest raspberries had higher anthocyanin content. In our research, we also found higher anthocyanin content and low hue angle in raspberries stored in air or low CO_2 atmospheres. Moore (1997) also stated that the hue angle or a*/b* could predict raspberry anthocyanin content.

We observed an increase in raspberry discoloration after harvest, which has not been reported previously to our knowledge. Discoloration occurred when the raspberry drupelets changed color from red to light pink. In blackberries, a similar phenomenon, red drupelet reversion (RDR), occurs, a type of physiological disorder (Morris et al., 1980). Edgley et al. (2020) reported RDR was associated with a decrease in anthocyanin content and was primarily caused by mechanical damage during harvest which causes lost membrane integrity and a decrease in cellular structural integrity. There may also be some change in pH from membrane leakiness leading to color changes in the anthocyanins. Slight changes in pH significantly impact anthocyanins, as acidity of the solution impacts the ratio between different forms (colors) of the pigments (Holcroft et al., 1999). In our study, discoloration increased with time in storage, but was inhibited by high CO_2; anthocyanin content was also maintained close to harvest levels with high CO_2. Also, high CO_2 atmospheres maintained fruit firmness and the integrity of the cell wall, and reduced senescence. It seems that these effects may be related to the decrease in discoloration development with high CO_2 atmospheres.

Overall, high CO_2 atmospheres were effective in increasing raspberry shelf life and maintaining postharvest quality. Raspberries held in 15 kPa atmosphere maintained the highest firmness and glossiness, and the brightest red color, with the least leakiness and decay, followed by raspberries held in 8 kPa atmosphere. Total anthocyanin content increased over time after harvest in all raspberries, regardless of storage atmosphere, but the increase was greatly inhibited by high CO_2 in a concentration dependent manner. Raspberry visual attributes deteriorated over time after harvest, but the atmosphere influenced the rate of deterioration. High CO_2 slowed ripening and created fungistatic conditions. Air (0.03 kPa) stored raspberries rapidly lost shelf life and quality after five days at 5℃ and should not be stored longer without modified or controlled atmospheres. As little as 5 kPa atmosphere can contribute to maintaining raspberry quality for very short periods (<five days) and 8 kPa atmosphere can maintain quality for up to 10 days be potential an alternative to 15 kPa atmosphere for storing below 10 days. It would be beneficial to investigate the effects of these atmospheres on the sensory quality of raspberry, to ensure that flavor quality is maintained.

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table 2.1: Quality attribute means and mean separation by treatment and evaluation day for raspberry fruit stored under different atmospheres in 2021.

Treatment (kPa)	Firmness score	Decay (%)	Decay severity score	Weight loss (%)	Dis coloration score	Glossiness score	Leakiness (%)	Leakiness score	Color (hue°)	L value	Chroma	TAC (mg Cy3Glu ·kg)	Respiration (ml·kg^{-1}· h^{-1})	Ethylene (µl·kg^{-1}· h^{-1})
0.03	2.98 a	45.16 a	2.372 a	0.68 a	1.87 a	1.98 c	94.08 a	3.31 a	19.6 d	30.39	31.70 b	485.41 a	18.89 a	7.79 a
5	2.39 b	31.12 b	2.14 ab	0.59 ab	1.70 ab	2.25 b	84.83 a	3.01 ab	21.18 c	30.55	32.14 b	409.47 ab	18.24 a	5.29 b
8	2.20 b	15.42 c	1.97 b	0.41 b	1.49 bc	2.33 b	57.58 b	2.66 bc	22.58 b	31.05	32.96 ab	377.28 bc	16.81 ab	4.43 b
15	1.67 c	13.13 c	1.89 b	0.42 b	1.43 c	2.57 a	30.45 c	2.40 c	25.52 a	31.14	33.73 a	303.02 c	13.18 b	3.1 c
	***	***	**	**	***	***	***	***	***	NS	**	**	**	***
Days														
0	1.56 d	1.9 c	1.33 c	0.00 d	1.23 c	2.46 a	2.00 d	1.33 d	27.02 a	34.34 a	35.38 a	35.38 a	16.09 ab	1.72 c
5	2.10 c	11.90 c	2.09 b	0.37 c	1.35 c	2.39 a	47.16 c	2.82 c	21.09 b	30.80 b	33.10 b	33.10 b	14.82 b	4.39 b
10	2.46 b	35.71 b	2.25 b	0.77 b	1.76 b	2.19 b	98.75 b	3.44 b	20.03 c	29.50 c	31.52 c	31.52 c	17.38 ab	5.28 b
13	3.23 a	59.09 a	2.77 a	1.01 a	2.21 a	2.04 b	127.09 a	3.91 a	20.40 bc	28.25 d	30.24 c	30.24 c	19.35 a	9.78 a
	***	***	***	***	***	***	***	***	***	***	***	***	*	***
T X Day	***	***	*	*	***	***	***	***	***	NS	NS	*	*	***

Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Means followed by different letters were significantly different. Significance level of each attribute by treatment or day; *** = 0.001, ** =0.01, and * = 0.05 based on their p values. NS = not-significant, TAC = total anthocyanin content.

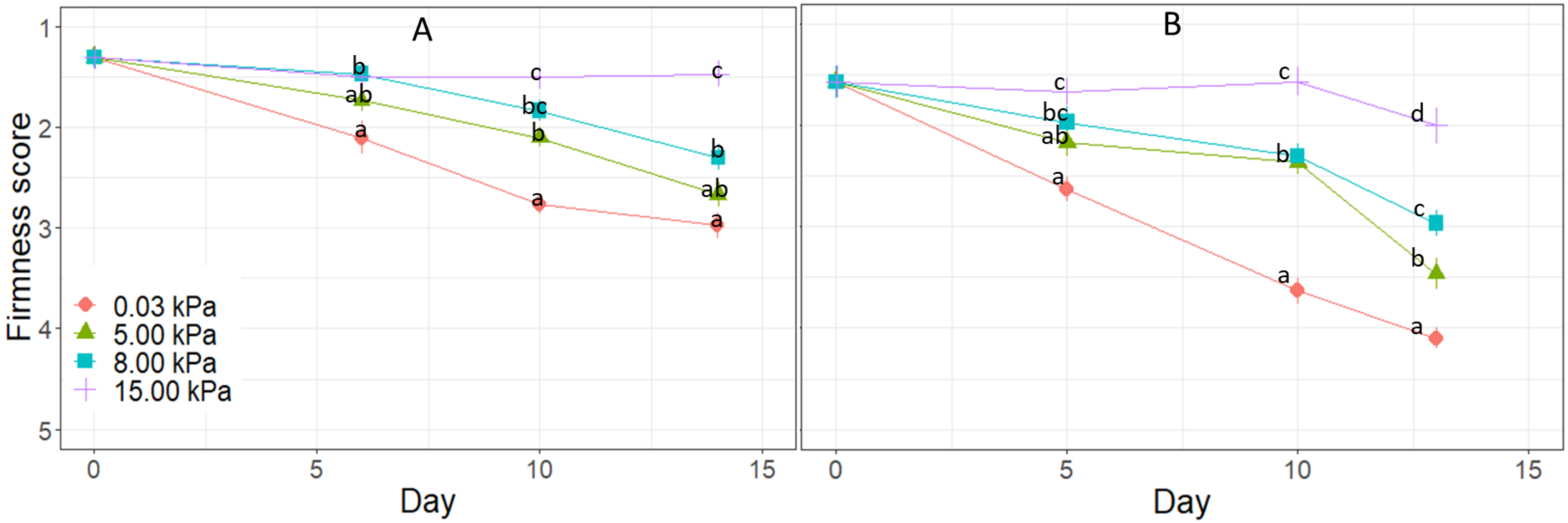


Fig. 2.1: Changes in raspberry firmness score following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5°C for 14 (2020, A) or 13 (2021, B) days. Firmness scale; 1 = very firm, 2 = firm, 3 = soft, 4 = very soft and 5 = no resistance. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences (p<0.05). Different letters indicate significant differences while same letters represent no significant differences.

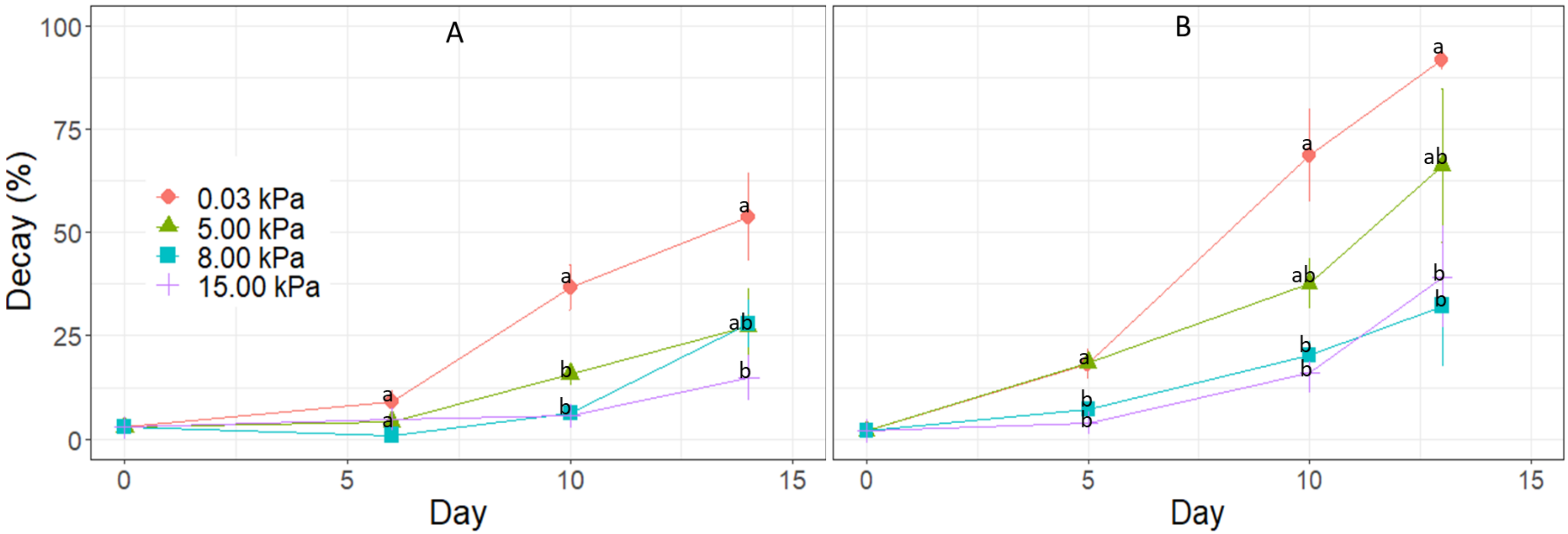


Fig. 2.2: Changes in raspberry fungal decay (%) following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5°C for 14 (2020, A) or 13 (2021, B) days. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

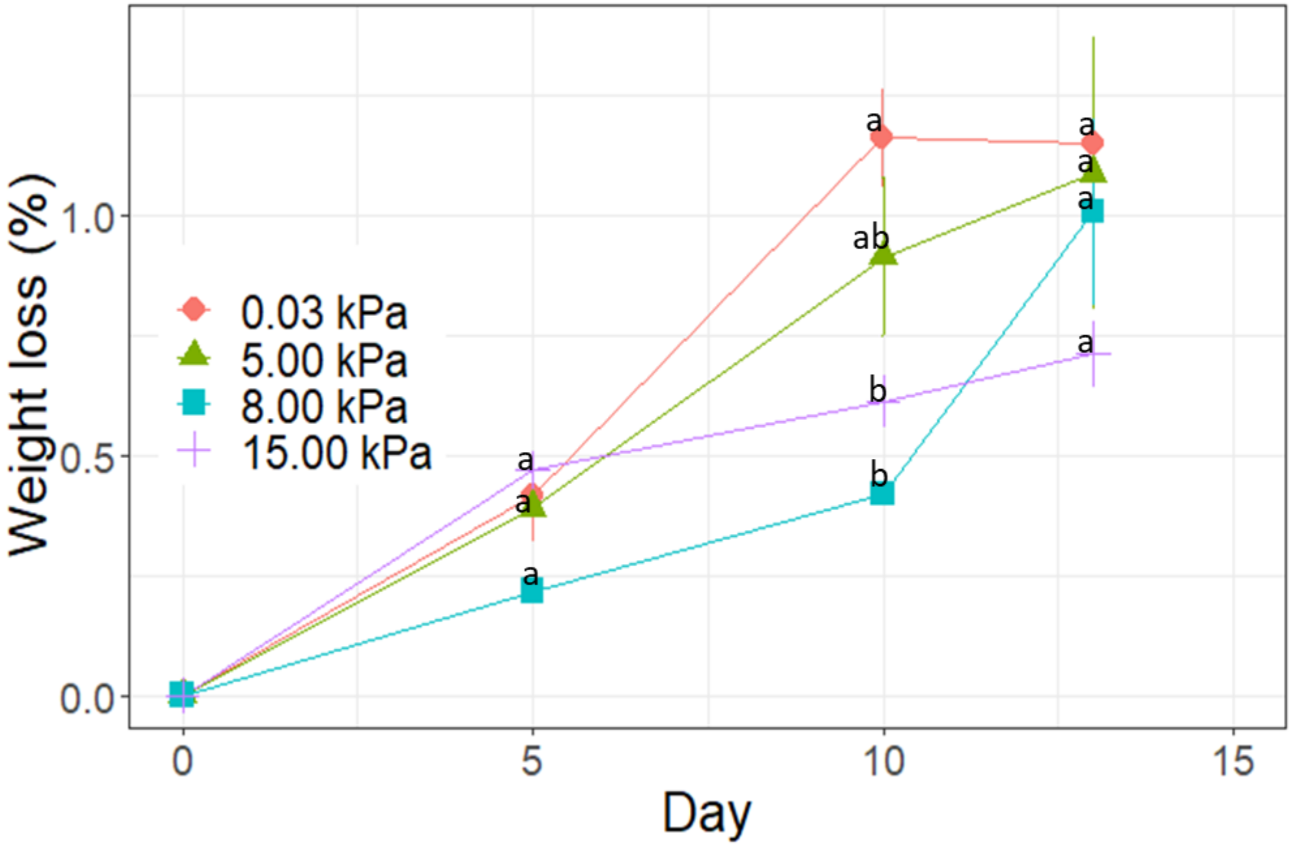


Fig. 2.3: Changes in raspberry weight loss (%) following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days in 2021. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

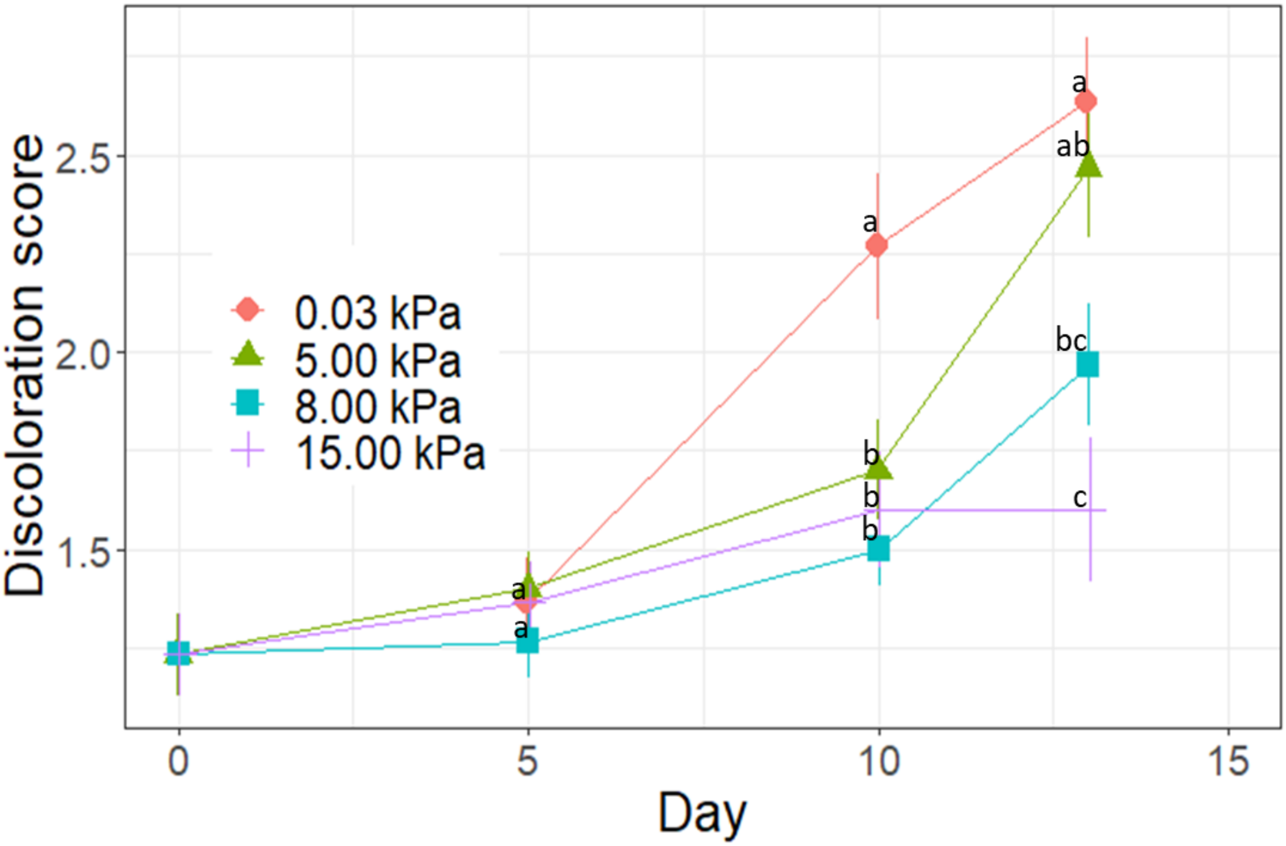


Fig. 2.4: Changes in raspberry discoloration score following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days in 2021. Discoloration score scale; 1 = none, 2 = very slight, 3 = slight, 4 = moderate, and 5 = severe. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

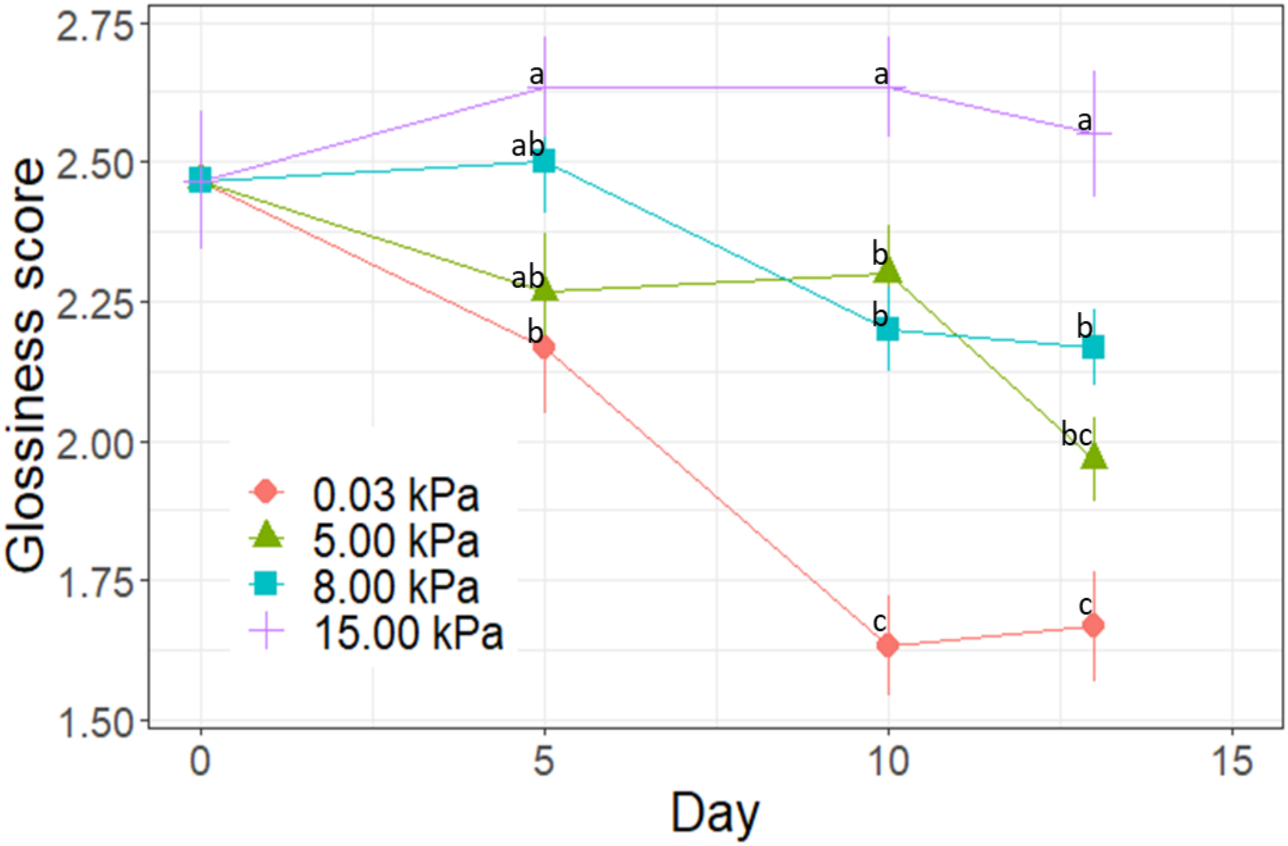


Fig. 2.5: Changes in raspberry glossiness score following exposure to 0.03 kPa (0.03 kPa CO$_2$, 21 kPa O$_2$); 5 kPa (5 kPa CO$_2$, 16 kPa O$_2$); 8 kPa (8 kPa CO$_2$, 13 kPa CO$_2$) or 15 kPa (15 kPa CO$_2$, 6 kPa O$_2$) atmospheres at 5℃ for 13 days in 2021. Glossiness score scale; 1 = dull, 2= moderate, and 3= glossy. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

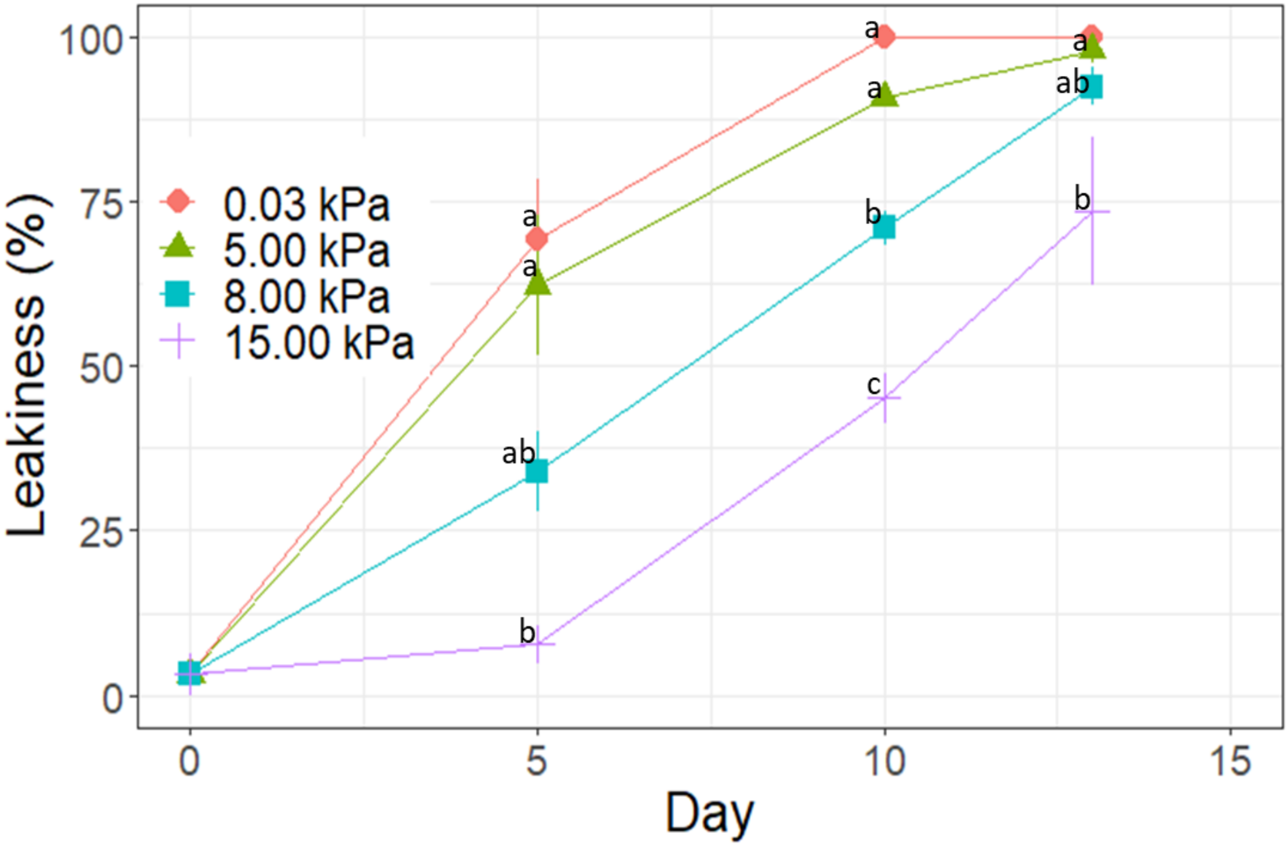


Fig. 2.6: Changes in raspberry leakiness (%) following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5°C for 13 days in 2021. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

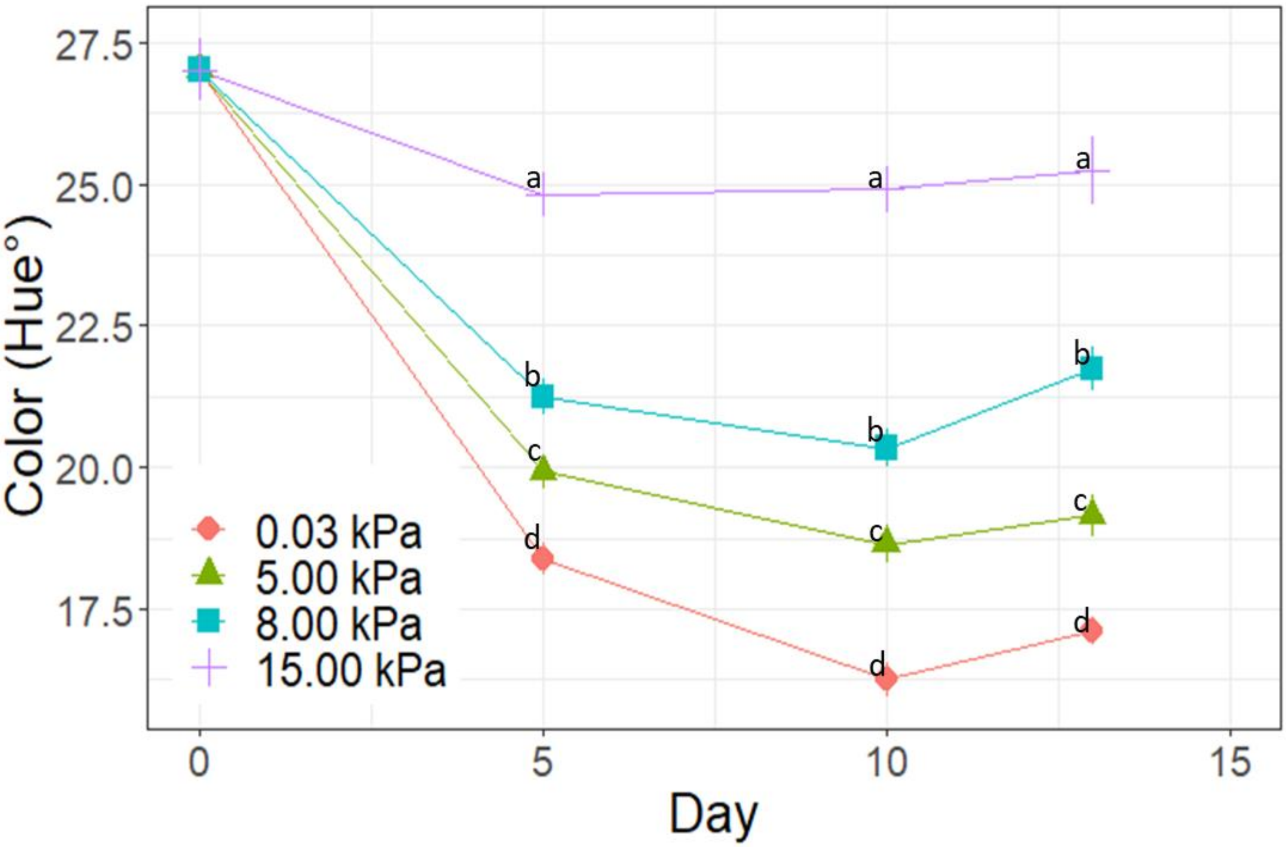


Fig. 2.7: Changes in raspberry color (hue °) following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days in 2021. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

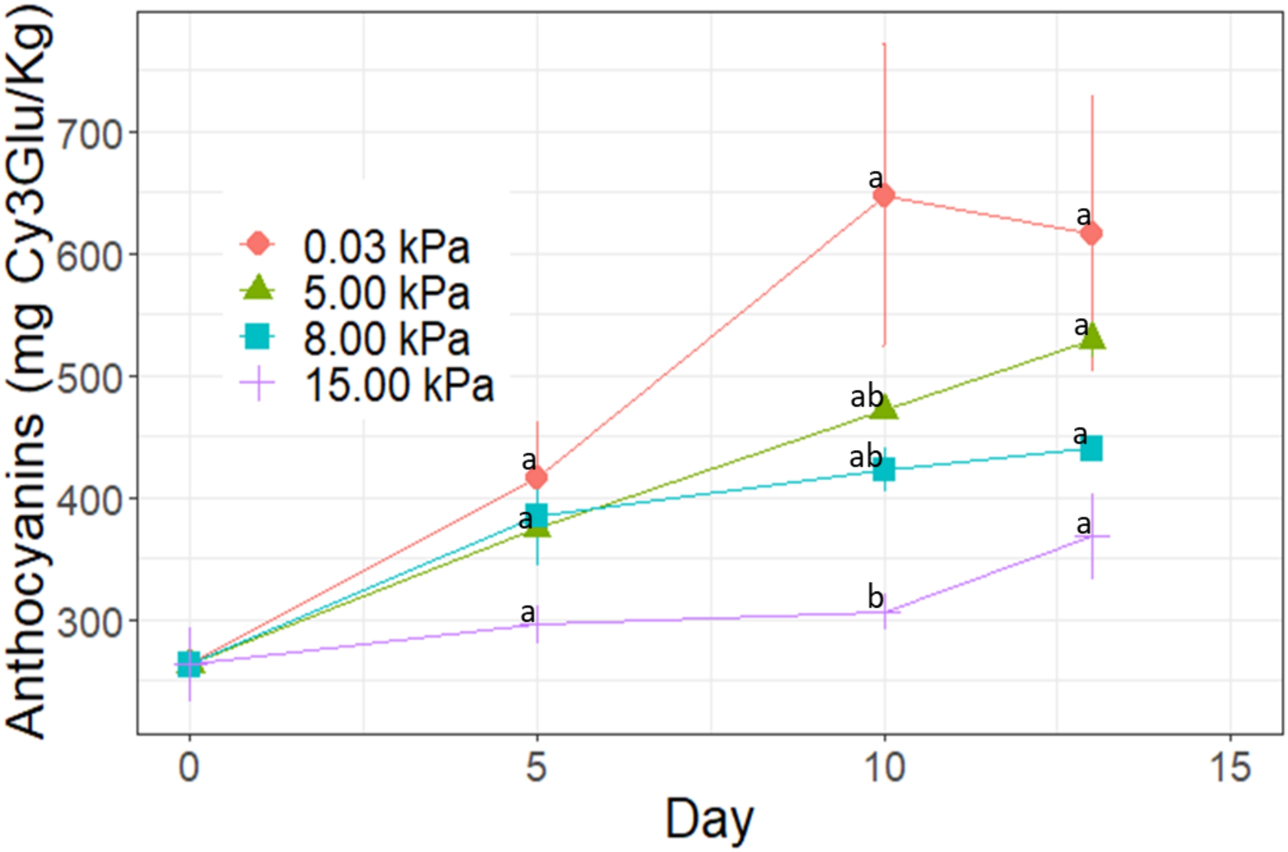
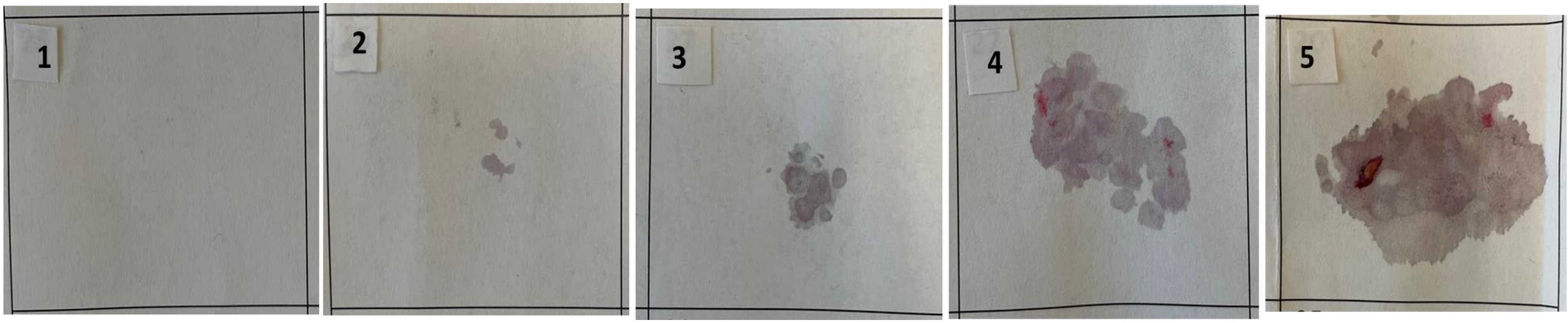


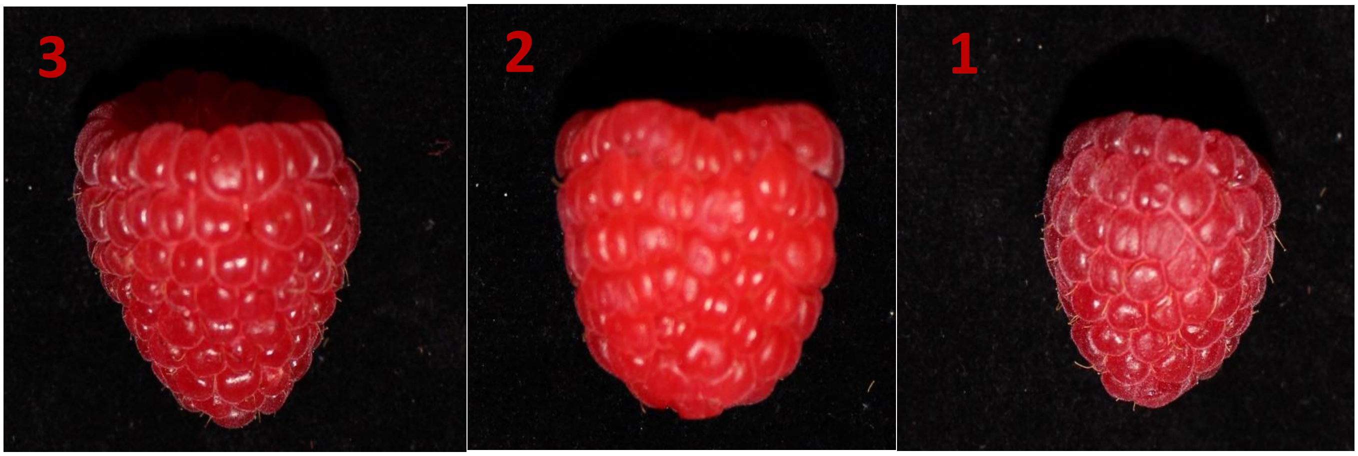
Fig. 2.8: Changes in raspberry total anthocyanins content (mg cyanidin-3-glucoside/kg) following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days in 2021. Data were assessed through ANOVA followed by Honest Significance Difference (HSD) Tukey test to reveal significant differences (p<0.05). Different letters indicate significant differences while same letters represent no significant differences.

Supplemental Materials

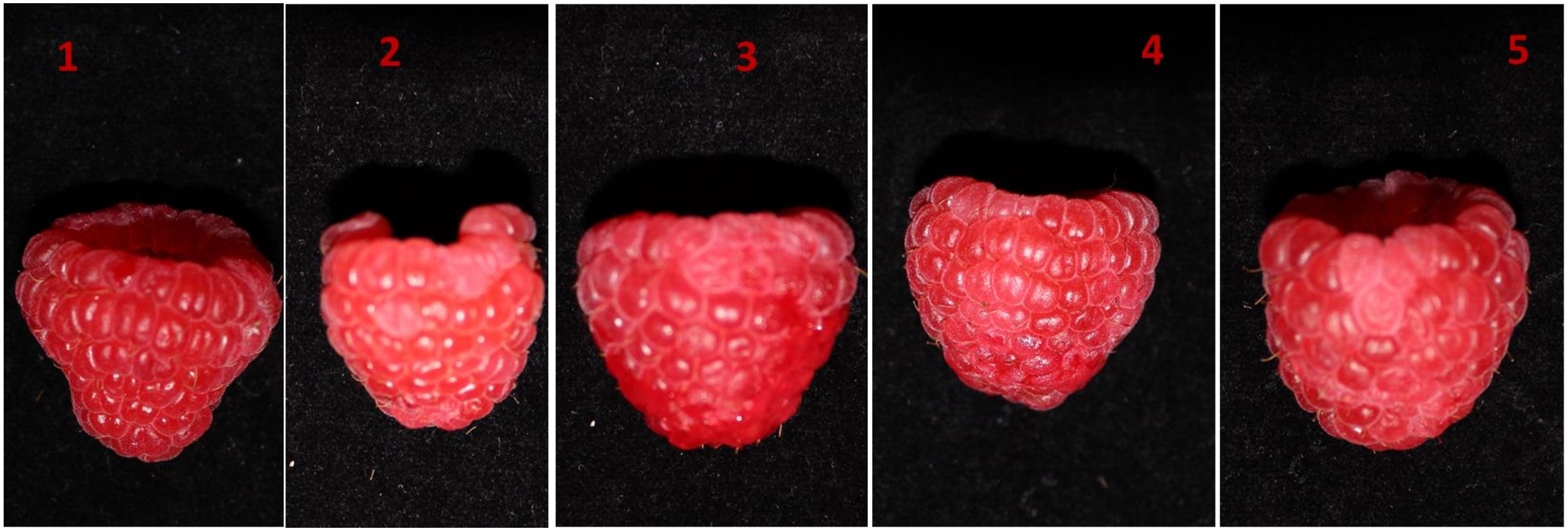


Supplemental Figure 2.1: Pattern of leakiness created by placing a single fruit within a 12.21 cm^2 block and applying slight pressure with a sheet of paper. Leakiness scale (2021): 1 = none, 2 = very slight, 3 = slight; 4 = moderate and 5 = severe.

Supplemental Figure 2.2. Decay severity scale: 1 = none; 2 = very slight, 1-3 decayed drupelets; 3 = slight, 4-8 decayed drupelets; 4 = moderate, 9-13 decayed drupelets; and 5 = severe, >13 decayed drupelets.



Supplemental Figure 2.3: Glossiness scale: 3 = glossy, 2 = moderately glossy, and 1= dull.



Supplementary Fig. 2.4: Discoloration scale: 1=None, 2= Very slight; 1-3 discolored drupelets, 3= Slight; 4-8 discolored, 4= Moderate; 9-13 discolored drupelets and 5= Severe; >13 discolored drupelets.

Chapter 3. Changes in raspberry sensory quality after harvest as affected by CO_2 atmospheres

Abstract

CO_2 atmospheres have the potential to influence raspberry (*Rubus ideaeus L.*) sensory quality after harvest. Our study determined the optimal postharvest atmosphere to extend shelf life without negatively impacting sensory quality. Raspberries were stored at 5℃ in 15, 8, 5 or 0.03 kPa CO_2 in combination with 6, 13, 16 or 21 kPa O_2, respectively. A trained sensory panel conducted a descriptive sensory evaluation of the raspberry fruit after 5, 10, and 13 days of storage. Sensory attributes were influenced by the CO_2 atmospheres. Raspberries stored in 15 kPa CO_2 with 6 kPa O_2 (15 kPa) atmosphere followed by 8 kPa CO_2 with 13 kPa O_2 (8 kPa) atmosphere had higher firmness than 5 kPa CO_2 with 16 kPa O_2 (5 kPa) and 0.03 kPa CO_2 with 21 kPa O_2 (0.03 kPa) atmosphere. Panelists found that raspberries stored in 8 kPa and 15 kPa atmosphere had the least off-flavor and highest tartness. Raspberries stored in 8 kPa scored highest in raspberry flavor with substantial juiciness and sweetness scores. The total content of volatile compounds (both fermentative and aromatic) in the raspberry fruit increased over time during storage. The fermentative volatiles acetaldehyde and ethanol were higher in raspberries stored in 15 kPa despite the low off-flavor scores, while most other raspberry volatiles decreased with increasing CO_2 concentration, including flavor-related volatiles α-ionone, α-terpineol, limonene and linalool. After 10 days, the quality of raspberries stored in air (0.03 kPa) or 5 kPa atmosphere had degraded considerably. While 15 kPa atmosphere prolonged shelf life the longest, 8 kPa atmosphere prolonged shelf life to 10 days while maintaining sensory quality and is the best overall atmosphere.

Introduction

Raspberry fruit are appreciated for their distinctive aroma and flavor. Visual appearance, texture, flavor, and nutritional compounds are generally considered as fruit quality (Pelayo et al., 2003). Visual quality is indicated by color, absence of disease or decay, texture, and aroma, which altogether appeal to consumers as freshness. 'Texture' is a qualitative characteristic of fruit appreciated by the consumer, including firmness, juiciness, and crispness. Multiple irreversible physiological and biochemical changes occur during ripening that impact fruit quality (Klee et al., 2011). According to Ponder et al. (2020) the ratio of sugar and organic acid determines raspberry taste. De Ancos (2000) further reported that total soluble solids (TSS) content ranges between 9 and 10 % and titratable acidity (TA) between 1.5 and 1.8% for good raspberry taste.

Raspberry aroma is composed of volatile chemicals (Aprea et al., 2015). Raspberry volatiles are vital for olfactory sensory quality perception as well as mold resistance (Aprea et al., 2015). It has been reported that raspberry has approximately 200 aromatic volatiles. (Klesk et al., 2004). The main volatile compounds contributing to raspberry flavor are α and β-ionone, linalool, α and β-pinene, caryophyllene and citral (De Ancos, 2000). As a non-climacteric fruit, raspberry taste and flavor mostly develops while they are ripening on the plant. Kader (1997) suggested that berries should be picked when fully ripe to ensure good flavor quality. Some raspberry research has focused on three phases of ripeness: semi-ripe, ripe, and over-ripe (Krüger et al., 2003; Krüger et al., 2011) and suggested that semi-ripe fruit may be more suitable for shipment and good sensory quality (Kruger et al., 2003). Wang et al. (2009) evaluated raspberry fruit harvested at 5%, 20%, 50%, 80% and 100% ripe. They concluded that 50-80% ripe berries developed the same level of TSS, TA and sugars as 100 % ripe berries but, 5-20% ripe berries never attained those qualities.

High CO_2 atmospheres can be beneficial to extend the postharvest life of raspberry fruit, slowing further ripening and reducing decay development (Goulart et al., 1992). However, high CO_2 concentrations have the capacity to disrupt enzyme systems, including the lipoxygenase pathway which is involved in the formation of aromatic volatile compounds (Morales et al., 2014). In addition, use of high CO_2 atmospheres can result in off-flavor development, which might be due to initiation of fermentative respiration. Earlier research by Li and Kader (1989) reported higher accumulation of ethanol in strawberries treated with low (0.5-1%) O_2 and/or high (15-20%) CO_2 than air stored berries. Ke et al. (1994) suggested that low O_2 and high CO_2 concentrations contribute to alcohol production. Oxygen levels less than 2 kPa can result in fermentation of raspberries (Joles et.al, 1994; Haffner et al., 2002). The objective of this research was to investigate the effects of a range of CO_2 atmospheres during cold storage on raspberry fruit sensory quality.

Materials and methods

Freshly harvested raspberries (*Rubus idaeus L.*, cv. Maravilla) were obtained immediately after harvest in Fall 2021. Berries were commercially field packed into clamshells (170 gm) and precooled at a commercial facility in Watsonville, California. Cooled fruit were transported on the same day in an air-conditioned vehicle to the UC Davis postharvest pilot plant within 3 hours. Raspberries were held at 5°C overnight, and the next day, a fruit sample was analyzed for objective and sensory quality to determine the baseline quality. The remaining clamshells of fruit were randomly assigned to different atmosphere treatments at 5°C. Fruit were removed from the atmosphere treatments after 5, 10, and 13 days and immediately evaluated to assess changes in the fruit's objective and sensory quality over time in storage.

Treatment atmospheres and experimental setup

Raspberries in clamshells were stored at 5℃ for up to 13 days in one of four atmosphere treatments: 15 kPa CO_2, 6 kPa O_2 (15 kPa); 8 kPa CO_2, 13 kPa O_2 (8 kPa); 5 kPa CO_2, 16 kPa O_2 (5 kPa); or 0.03 kPa CO2, 21 kPa O_2 (0.03 kPa). These atmosphere treatments represent the mixture of O_2 and CO_2 concentrations that would be found in a modified atmosphere package targeting 15, 8 and 5 kPa CO_2 as well as a package without modified atmosphere (air). The gas concentrations were measured during set up and periodically with a CO_2/O_2 gas analyzer (Systec Gas Advance Micro-GS3, Boston, MA). The four atmospheres were humidified by bubbling through water prior to fruit exposure in a continuous flow-through system at a rate of 100 mL/minute. Raspberry fruit remained in the clamshells during treatment. There were nine plastic bags, each holding six clamshells inside, for each atmosphere (a separate bag for each evaluation day (3) and replication (3)). The bags were modified with an inlet and outlet port and connected to a separate flow board for each atmosphere. Instrumental and sensory fruit quality was evaluated at the start of the experiment and again on each evaluation date.

Quality evaluations

Firmness

Ten raspberries from one clamshell per replication and treatment was used for subjective fruit firmness assessment. Each raspberry was pressed slightly with thumb and middle finger. Based on the palpability, the berries were scored from 1 to 5, where 1 = very firm, rebounds from compression, high resistance; 2 = firm, partial rebound; 3 = soft, partial rebound; 4 = very soft, partial rebound, and 5 = no resistance.

Total soluble solids and titratable acidity

Ten randomly selected raspberries from 1 clamshell per replication were juiced together using a hand juicer and cheesecloth, yielding about 10-15 ml of juice. The juice was used for measuring TSS with a tabletop automatic refractometer (Atago RX 5000i, Bellevue, WA), and results were expressed as the percentage TSS. Four grams of juice were diluted with 20 ml of dH_2O and then titrated with an automatic titrator (TIM850 Titration Manager, Radiometer Analytical, France). Titratable acidity was expressed as percentage citric acid (mg/100 g juice), the dominant organic acid in raspberries (De Ancos et al. 1999).

Volatile compound analysis

A clamshell of raspberries from each treatment and replication was frozen with liquid nitrogen, and immediately broken into drupelets with a mortar and pestle. Drupelets were mixed among the fruit from each clamshell. These raspberry drupelets were held in a –80°C freezer until use. Raspberry volatiles were analyzed using a method modified from Forney et al. (2015). Frozen raspberry drupelets were removed from the -80°C freezer and 5 g were added to 100 g NaCl saturated H_2O, and homogenized in a blender for 1 min. Five mL of the homogenate was transferred to a 20 mL headspace vial (Agilent Technologies, Santa Clara, CA, USA). Headspace volatiles were then analyzed by solid phase micro extraction (SPME) gas chromatography mass spectroscopy (SPME-GC-MS) (Agilent 6890N Network GC System paired with 5975B Inert XL EI/CI MSD, and an FID detector, Santa Clara, CA). Vials were incubated at 50°C for 10 min, and then the headspace was exposed to a gray SPME fiber (50/30 mm DVB/CAR/PDMS [gray] fiber (Supelco Analytical, Bellefonte, PA, USA) for 10 min with agitation. The fiber was desorbed at

250℃ for 15 min onto a BD-WAX UI (30 m X 0.250 mm X0.25 um) column (Agilent Technologies, Santa Clara, CA, USA) held at 35℃ for 5 min, then ramped to 240℃ at 0.167℃/s and held at 240℃ for 4.5 minutes. Helium was the carrier gas at a flow of 16.7 ml/s. Peaks were initially identified through comparison with NIST Mass spectral library (NIST MS, 2005). Retention indices of these compounds were used to further verify identity by comparison against standard compounds and relatively quantified with GC (Agilent 7890B GC, Santa Clara, CA) using a BD-FATWAX UI column. The same method was followed except the sample amount was 1 mL and nitrogen was used as the carrier gas. The concentrations of the volatile compounds were determined by comparison to standard peaks. The samples' peak area was multiplied by the reference standard concentration, and the result was divided by the peak area of the reference standard.

Descriptive sensory analysis

A descriptive sensory analysis was performed with 12 panelists who were trained ahead of the sensory evaluations to align their sensory perception. There were four one-hour training sessions over two weeks. During the training, panelists were provided with references (Table 3.1) for each attribute to compare against the training samples. The sensory evaluations took place in the UC Davis Department of Plant Sciences Sensory Lab, equipped with five separate evaluation booths with individual computers with sensory analysis software (Compusense Inc., Guelph, ON, Canada). Samples were prepared the morning of evaluation and stored at 5℃. The samples were brought to room temperature before being tasted by the panel. One sample included 3-4 raspberries and was provided to the panelists in sealed sensory tasting cups. Each sample was blinded with random 3-digit codes generated by the software (Compusense Inc, Guelph, ON, Canada).

Panelists tasted three replications of raspberries at harvest (baseline), and again for each treatment after 5, 10, and 13-days in atmosphere storage and evaluated their taste, texture, and flavor. The panelists were instructed to cleanse their palates with crackers and water in between samples. On day 10, there was only one replication of the air (0.03 kPa) treatment and two replications each of 15 kPa and 5 kPa atmospheres appropriate for sensory evaluation due to decay growth. On day 13, there were no samples of the air (0.03 kPa) treatment, 5 kPa treatment had two replications, 8 kPa treatment had thee replications and 15 kPa treatment had two replications. The panelists measured the intensity of sensory attributes of the raspberry samples and marked their score for each given attribute on a 10 cm straight line anchored with less and more using sensory evaluation software (Compusense Inc, Guelph, Ontario). This software transmuted the markings for each attribute into a numerical value ranging from 1 to 10 units, where 1 was less and 10 was more intensity. The tasted attributes were sweetness, acidity/tartness, firmness (mouthfeel), juiciness, raspberry flavor, and off-flavor. The tasting lexicons were decided and agreed upon during the training.

Statistical analysis

Data were analyzed using R statistical program (Core Team, 2013). A total of 4 treatments (atmospheres) and 3 replications across the four evaluation dates were analyzed for instrumental and sensory qualities of the raspberries. Data were assessed through ANOVA followed by Fishers Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$) among treatments and evaluation times. A correlation analysis was also conducted to investigate the relationship between volatile compounds and sensory attributes. The sensory data was analyzed using principal component analysis (PCA) using R and R Studio software (Core Team, 2013) and

PCA plots are presented for 5- and 10-day evaluations. The sensory data on day 13 was insufficient for analysis due to decay.

Results

Hand Firmness

Storing raspberries under high CO_2 atmospheres reduced fruit softening in a concentration-dependent manner (Fig. 3.1). Raspberries stored in 15 kPa atmosphere did not soften until day 13, and only slightly. Raspberries stored in 8 and 5 kPa CO_2 had intermediate firmness throughout storage, and softened gradually, while raspberries stored in 0.03 kPa atmosphere lost firmness quickly during storage and had the lowest firmness among all the atmosphere treatments at each evaluation. Across all evaluation's times, raspberries stored in 15 kPa atmosphere were most firm, raspberries stored in air (0.03 kPa) were least firm, and raspberries stored in 5 or 8 kPa atmosphere were intermediate and not different from each other (Table 3.2).

Volatiles

A total of 14 volatile compounds were detected in the raspberry fruit (Table 3.3). There were five terpenes (α-ionone, β-ionone, α-terpineol, limonene and linalool), three alcohols (ethanol, 2-heptanol and 2-methyl-1-butanol), two aldehydes (acetaldehyde and hexanal) and one each of ester (ethyl acetate), ketone (2-heptanone), alkyne (tetradecane) and carboxylic acid (hexanoic acid).

The aromatic volatiles limonene, linalool, hexanoic acid and α-terpineol increased in concentration over time, particularly in 0.03 kPa atmosphere stored raspberries (Figs. 3.4-3.6;

Table 3.3). However, storage in elevated CO_2 atmospheres resulted in significantly lower concentrations of these volatiles as well as α-ionone (Figs. 3.4-3.7; Table 3.3). Raspberries held in 15 kPa atmosphere exhibited a sharp and significant increase in the fermentative volatiles, acetaldehyde and ethanol, on day 5 (Figs. 3.2-3.3) and had higher concentrations than raspberries stored in other atmosphere treatments (Table 3.3). Acetaldehyde, ethyl acetate, and ethanol all increased in concentration overtime, particularly on the last day of storage (Table 3.3).

Sensory quality

Raspberry fruit mouthfeel firmness, raspberry flavor and TSS decreased over time and juiciness and off-flavor increased over time (Table 3.2). Firmness scores, both hand and mouthfeel, were significantly higher in raspberries stored in 15 kPa atmosphere followed by fruit held in 8 kPa atmosphere. The trend was opposite for juiciness and sweetness, where raspberries held in 0.03 kPa or 5 kPa atmosphere had the highest juiciness scores, and fruit held in 15 kPa atmosphere had a lower sweetness score than fruit held in air (0.03 kPa) atmosphere. Tartness score was higher in fruit held in 8 kPa or 15 kPa atmosphere than fruit held in 0.03 kPa atmosphere. Raspberry flavor was significantly higher in fruit held in 8 kPa atmosphere than in 5 kPa atmosphere (Table 3.2).

After five days of storage, the PCA biplot showed that raspberry firmness (hand feel and mouthfeel) was strongly associated with the 15 kPa atmosphere treatment, and less so with the 8 kPa atmosphere treatment (Fig. 3.8). Fermentative volatiles: acetaldehyde, ethyl acetate, and ethanol were also clustered with the 15 kPa atmosphere treatment, and less so with the 8 kPa atmosphere treatment. Raspberry flavor was most closely associated with sweetness and tetradecane at the bottom of the biplot. No treatments were closely associated. Tartness, TA, TSS, heptanol, α-terpineol and limonene were associated with each other and the 5 kPa atmosphere treatment at the top of the bi-plot. Sweetness, off-flavor, and juiciness were clustered with each other and the 0.03 kPa atmosphere treatment. Most of the aromatic volatiles were associated closely with the 0.03 kPa and 5 kPa atmosphere treatments, and on the opposite side of the bi-plot from firmness and 8 kPa and 15 kPa atmosphere treatments.

After ten days, firmness (both hand feel and mouthfeel) remained associated with treatments with high CO_2 concentrations (15 kPa and 8 kPa) (Fig. 3.9). Raspberry flavor, TSS, TA, and tartness were associated with each other and the 8 kPa atmosphere treatment, and raspberry flavor shifted to the top of the bi-plot. Juiciness, sweetness, tetradecane and heptanone were clustered on the top left with the 5 kPa atmosphere treatment. At the bottom of the bi-plot, ethanol and ethyl acetate were associated with the 15 kPa and 0.03 kPa atmosphere treatments, respectively, and acetaldehyde was in between 15 kPa and 0.03 kPa atmosphere. Aromatic volatiles maintained their association with lower CO_2 atmosphere treatments, and were also associated with off-flavor as at ten days (Fig. 3.9).

Correlations among quality attributes

Across evaluation days, total soluble solids and juiciness were positively correlated (table 3.4). Sweetness was negatively correlated with hand firmness and tartness, and mouthfeel firmness and juiciness were negatively correlated. Acetaldehyde and ethanol were negatively correlated and tetradecane was positively correlated with juiciness and TSS (Table 3.5). 2-Heptanol was positively correlated with juiciness. Hexanal, hexanoic acid, α-ionone, linalool, and α-terpineol were negatively correlated with hand firmness, and all but hexanal were positively correlated with sweetness. Limonene was the only volatile significantly correlated with off-flavor (0.96), and α-terpineol was the only volatile correlated with tartness (-0.95).

Discussion

Raspberry firmness remained stable or decreased more slowly with increasing CO_2 concentration in storage, with the highest firmness in 15 kPa CO_2. In agreement with our results, Haffner et al. (2002), found that an atmosphere of 15% CO_2, 10% O_2 maintained the firmness of five raspberry cultivars stored for seven days at 1℃. Strawberries exposed to high CO_2 exhibited changes in apoplastic pH, which may have induced cell to cell adhesion by precipitation of soluble pectin (Harker et al., 2000). This may explain why high CO_2 stored raspberries were perceived as firmer by our sensory panelists. However, firmer fruit tasted less sweet to the sensory panelists. Stec et al. (1989) reported that firmer kiwifruit tasted less sweet than softer ones. This finding aligned with the general notion that softer fruit have more ripe fruit characteristics such as sweetness, juiciness and higher aroma intensity (Young & Paterson, 1985). This can explain the negative correlation of juiciness with firmness in our experiment. In addition, storage under high CO_2 atmospheres also might have inhibited further ripening of the fruit which would inhibit fruit softening. High CO_2 atmospheres also reduced development of leakiness and color darkening.

Exposure to CO_2 atmospheres can induce fermentative metabolism (Kennedy et al., 1992), likely due to its capacity to disrupt enzyme systems (Watkins and Zhang, 1998). Elevated CO_2 has been reported to induce development of alcoholic flavors in fruit if the concentration is too high for longer times (Woodward et al., 1972). High CO_2 enhances the activity of pyruvate decarboxylase and alcohol dehydrogenase~~,~~ but reduces activity of alcohol acetyltransferase. As a result, acetaldehyde and ethanol accumulate and this trigger further production of ethyl esters and reduction of other esters, thus, enhancing alcoholic flavor (Ke et al., 1994). Larsen (1994) suggested that accumulation of ethyl acetate was linked to development of off-flavor in raspberries in some cultivars. High CO_2 atmospheres can impact the lipoxygenase pathway which is involved in the formation of aromatic volatile compounds (Morales et al., 2014) through effects on enzymes or by limiting substrates due to production of fermentative volatiles (Watkins and Zhang, 1998). In our study, fermentative volatiles were strongly associated with storage in the higher CO_2 atmospheres early in storage, but after 10 days of storage, raspberries stored in air also accumulated fermentative volatiles, likely due to over-ripening. In addition, very low O_2 atmospheres can contribute to off-flavors. Joles et al. (1994) reported that raspberries stored in 3% O_2 developed off-flavor because fermentative respiration occurs when O_2 levels drop below this critical level (Kader, 1986). However, this might not be the case for our experiment, because our lowest O_2 level was ≥ 6 kPa. The one exception might be the 15 kPa atmosphere. The combination of 15 kPa CO_2 with 6 kPa O_2 could have resulted in additional impacts on fruit metabolism given the relatively low O_2 concentration, resulting in a stronger impact of the 15 kPa atmosphere on fruit quality. The concentration of individual fermentative volatiles was as much as 1000-fold higher than the aromatic volatiles, and the fermentative volatiles were 4 to 300-fold higher in raspberries stored under 15 kPa CO_2 compared to fruit stored in air.

Accumulation of acetaldehyde, ethanol and ethyl acetate can contribute to objectionable changes in taste (Siriphanich, 1980). However, in our experiment, the off flavor sensory score was lower in fruit stored in 15 or 8 kPa atmosphere than in 5 kPa atmosphere, and was more closely clustered on the bi-plot with aromatic volatiles than fermentative volatiles. Limonene was most highly correlated with off-flavor. The concentrations of ethanol detected in our raspberry samples appear to be below the corresponding odor threshold of 990 µl /L, (Supplemental table 3.1; Czerny et al., 2008). This may explain why our sensory panelists did not sense any off-flavor in raspberries stored in 15 kPa atmosphere, even when fermentative volatile concentrations were significantly higher in those fruit than in fruit stored in lower CO_2 atmospheres.

The odor threshold is the lowest concentration of a volatile that can be smelled and can vary as much as 10^6 to 10^8 among volatiles in fruit (Forney, 2001). Therefore, the most abundant volatile is not necessarily always the dominant fruit aroma. Alcohols usually have considerably higher threshold values, near 990 µl /L (Czerny et al., 2008), and therefore contribute less to aroma building than their corresponding aldehydes (Fisher et al., 2007).

The signature raspberry flavor comes from aromatic volatiles, mostly composed of a mixture of ketones and terpenes (Forney, 2001). α-Ionone and β-ionone are carotenoid-derived aromatic volatiles that are mostly responsible for floral notes in fruit (Winterhalter et al., 2001); these compounds usually intensify as raspberries ripen. Also, α-terpineol, which has a sweet, flowery aroma was found to have a positive correlation with sweetness in our study. Aromatic volatiles become prominent during fruit ripening and tend to increase towards senescence, ultimately developing the aroma and flavor for the fruit (Stumpf, 1980). Guichard (1984) reported that in raspberries all the terpenes and sesquiterpenes concentrations significantly increased during ripening with an increase in α -ionone followed by a slight increase in β-ionone. In our research, α-ionone concentration increased over time in air and 5 kPa atmosphere storage, but decreased in raspberries stored in 15 kPa atmosphere, perhaps due to slowing of further ripening. α-

Terpineol, limonene, linalool and hexanoic acid also decreased 3-4-fold with increasing CO_2 concentration in storage. Linalool, α-terpineol, limonene and hexanoic acid showed increases in concentration with time in air storage. In our study, the aromatic volatiles were mostly associated with raspberries stored in lower CO_2 atmospheres (0.03 or 5 kPa). This may be largely due to little or no inhibitory effects on further ripening in these atmospheres. Ripening of fruit is usually accompanied by softening and production of flavor and aroma volatiles (Stec et al., 1989) Some fruity/floral volatiles are known to enhance the perception of sweetness (Baldwin et al., 2004). Volatiles such α-ionone, linalool, and α-terpineol have a sweet floral aroma (Larsen et al., 1992; Czerny et al., 2008; Vilanova et al., 2012). This may explain why we observed a positive correlation of sweetness with these particular volatiles in our study. While raspberries held in higher CO_2 atmospheres had lower concentrations of aromatic volatiles, most, but not all, of the differences can be explained by ripening inhibition. High CO_2/low O_2 atmospheres also restrict enzyme activity, diminishing generation of certain organic volatiles, and reducing the effects of ethylene on CA-stored produce (Thompson, 1998). Off-flavor's association with low CO_2 atmosphere storage may be related to the concentration of limonene which was higher in low CO_2 stored raspberries and positively correlated with off-flavor. Elmaci et al. (2005) also reported an association of off-flavor with increasing percentage of limonene during storage of mandarins. It is possible that off-flavor was also linked to development of decay (even though we removed any visible decay prior to sensory evaluation) or leakiness because the rate of decay and leakiness was higher in fruit stored in low CO_2 atmospheres due to the lack of fungistatic conditions or inhibition of metabolism.

Raspberries stored in 15 kPa atmosphere maintained better firmness over other atmospheres. However, raspberries stored in 8 kPa atmosphere performed better in sensory evaluations in terms of raspberry flavor, juiciness, and sweetness. Raspberries stored in air (0.03 kPa) or 5 kPa atmosphere lost almost all their sensory quality by 10 days. Selection of modified atmospheres for raspberries should be based on the storage time and desired quality. While 15 kPa atmosphere prolonged shelf life the longest, 8 kPa atmosphere prolonged shelf life to 10 days while maintaining sensory quality. Based on these findings, modified atmosphere conditions can be formulated and applied during transportation to further investigate the impacts on quality under commercial conditions. Also, synthesis of volatile compounds and associated gene expression as effected by high CO_2 atmospheres would be an interesting area for further exploration.

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Table 3.1: Raspberry attributes, definitions, and reference standards for sensory descriptive analysis.

Attributes	Higher intensity	Lower intensity
Sweet	Raspberry puree: cane sugar (10: 1)	Raspberry puree: cane sugar (20: 1)
Tart	Raspberry puree: lemon juice (10: 1)	Raspberry puree
Firmness	Freshly harvested raspberry	4-day old raspberry
Juiciness	Fresh cut naval orange	Fresh blueberry
Off-flavor	Off-flavored raspberries (stored under 25% CO_2 at 5 ℃ for 3 day)	Fresh raspberries
Raspberry flavor	Raspberry essence: water (1:10)	Raspberry essence: water (1:100)

Table 3.2: Mean quality attributes across treatments and days of storage for raspberries stored under different atmospheres for 0-10 days.

	Sensory attributes						Quality measurements		
Treatment (kPa)	Firmness score (mouthfeel)	Juiciness score	Raspberry flavor score	Off-flavor score	Sweetness score	Tartness score	Firmness score (hand)	TSS (%)	TA (%)
0.03	4.73 c	4.24 ab	6.65 ab	2.05 ab	5.72 a	3.87 b	3.38 c	10.50	2.51 b
5	4.59 c	4.41 a	6.31 b	2.16 a	5.38 ab	4.31 ab	3.96 b	11.02	2.76 a
8	5.41 b	4.07 b	6.75 a	1.69 b	5.29 ab	4.62 a	4.05 b	10.71	2.73 ab
15	6.27 a	3.41 c	6.56 ab	1.70 b	5.12 b	4.51 a	4.40 a	10.53	2.71ab
	***	***	*	**	*	**	***	NS	*
Days									
0	6.17 a	3.65 C	6.85 a	1.46 b	5.49	4.63 a	4.43 a	11.23 a	2.64
5	4.9 b	4.09 b	6.43 b	2.16 a	5.25	4.30 ab	3.89 b	10.79 a	2.78
10	4.6 b	4.38 a	6.38 b	2.09 a	5.33	4.09 b	3.53 c	10.04 b	2.64
	***	***	**	***	NS	**	***	***	NS

Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Means followed by different letters were significantly different. Significance level of each attribute by treatment or day; *** = 0.001, ** = 0.01, and * = 0.05 based on their p values. NS = not-significant, TSS = Total soluble solids and TA = titratable acidity. 0.03 kPa = 0.03 kPa CO_2, 21 kPa O_2; 5 kPa = 5 kPa CO_2, 16 kPa O_2; 8 kPa = 8 kPa CO_2, 13 kPa O_2 and 15 kPa = 15 kPa CO_2, 6 kPa O_2 atmosphere.

Table 3.3: Mean volatile concentration for raspberries stored under different atmospheres for 0-13 days across treatments and days.

	Fermentative volatiles			Key raspberry volatiles				Other volatiles						
Treatment (kPa)	Acetaldehyde (ul/L)	Ethyl acetate (ul/L)	Ethanol (ul/L)	α -ionone (ul/L)	β-ionone (ul/L)	Linalool (ul/L)	Limonene (ul/L)	2-Heptanone (ul/L)	2-Heptanol (ul/L)	Hexanal (ul/L)	Hexanoic Acid (ul/L)	2-Methyl-1-butanol (ul/L)	α-Terpineol (ul/L)	Tetra decane (ul/L)
0.03	2.92 b	8.16	39.69 b	0.018 a	0.04	1.12 a	0.20 a	0.09	0.11	3.91	2.45 a	5.58	0.93 a	0.01
5	2.84 b	6.72	42.57 b	0.015 ab	0.03	0.35 b	0.18 a	0.07	0.13	2.35	1.38 b	4.46	0.25 b	0.01
8	2.28 b	2.32	15.45 b	0.012 ab	0.03	0.22 b	0.07 ab	0.06	0.12	2.38	1.33 b	4.19	0.15 b	0.01
15	6.34 a	8.15	169.52a	0.006 b	0.03	0.03 b	0.01 b	0.03	0.06	1.86	0.68 b	4.06	0.01 b	0.01
	***	NS	***	**	NS	**	**	NS	NS	NS	***	NS	***	NS
Days														
0	1.88 b	0.40 b	10.90 c	0.011	0.04 a	0.01 b	0.01 b	0.02	0.07	1.50	0.93 bc	4.58	0.01 b	0.01
5	4.13 ab	4.24 b	80.17 ab	0.010	0.03 ab	0.10 b	0.02 b	0.09	0.11	4.16	0.87 c	5.15	0.06 b	0.01
10	3.72 ab	5.40 b	65.04 b	0.017	0.04 a	0.47 b	0.22 a	0.10	0.12	2.71	1.85 ab	4.7	0.28 b	0.01
13	4.50 a	15.95 a	105.82 a	0.013	0.03 b	1.24 a	0.24 a	0.06	0.12	2.16	2.33 a	3.8	1.09 a	0.01
	*	***	***	NS	*	***	***	NS	NS	NS	***	NS	***	NS

Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Means followed by different letters were significantly different. Significance level of each attribute by treatment or day; *** = 0.001, ** = 0.01, and * = 0.05 based on their p values. NS = not-significant. 0.03 kPa = 0.03 kPa CO_2, 21 kPa O_2; 5 kPa = 5 kPa CO_2, 16 kPa O_2; 8 kPa = 8kPa CO_2, 13 kPa O_2 and 15 kPa = 15 kPa CO_2, 6 kPa O_2 atmosphere.

Table 3.4: Correlation among quality attributes for raspberries stored under different atmospheres for 0-10 days.

	Mouthfeel firmness	Juiciness	Raspberry flavor	Off flavor	Sweetness	Tartness	Hand firmness	TSS
Juiciness	**-0.97** *							
Raspberry flavor	0.19 NS	-0.12 NS						
Off-flavor	-0.78 NS	0.77 NS	-0.57 NS					
Sweetness	-0.78 NS	0.67 NS	0.15 NS	0.69 NS				
Tartness	-0.88 NS	-0.48 NS	0.02 NS	-0.71 NS	**-0.94** *			
Hand firmness	0.78 NS	-0.70 NS	-0.25 NS	-0.63 NS	**-0.99** **	0.89 NS		
TSS	-0.88 NS	**0.95** *	0.14 NS	0.56 NS	0.62 NS	-0.36 NS	-0.68 NS	
TA	0.14 NS	-0.03 NS	-0.25 NS	0.06 NS	-0.72 NS	0.74 NS	0.73 NS	-0.09 NS

Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Bold numbers represent significant correlation. Significance level of each attribute by treatment or day; *** = 0.001, ** = 0.01, and * = 0.05 based on their p values. NS = not-significant, TSS = total soluble solids, TA = titratable acidity.

Table 3.5: Correlation among volatile compounds and quality attributes for raspberries stored under different atmospheres for 0 to 10 days.

Volatiles	Sensory attributes						Quality measurement		
	Mouthfeel Firmness	Juiciness	Raspberry flavor	Off-flavor	Sweetness	Tartness	TSS	TA	Hand Firmness
Acetaldehyde	0.89 NS	**-0.96** *	-0.06 NS	-0.59 NS	-0.58 NS	0.32 NS	**-0.99** **	0.01 NS	0.64 NS
Ethyl acetate	0.74 NS	-0.86 NS	0.018 NS	-0.42 NS	0.26 NS	-0.01 NS	-0.90 NS	-0.31 NS	0.32 NS
Ethanol	0.85 NS	**-0.94** *	-0.15 NS	-0.51 NS	-0.56 NS	0.28 NS	**-0.99** **	-0.03 NS	0.62 NS
2-Heptanone	-0.89 NS	0.89 NS	0.26 NS	0.60 NS	0.84 NS	-0.63 NS	0.93 NS	-0.42 NS	-0.89 NS
2-Heptanol	-0.90 NS	**0.95** *	-0.34 NS	0.76 NS	0.47 NS	-0.27 NS	0.87 NS	0.26 NS	-0.46 NS
Hexanal	-0.56 NS	0.49 NS	0.5 NS	0.36 NS	0.92 NS	-0.82 NS	0.55 NS	-0.42 NS	**-0.95** *
Hexanoic acid	-0.79 NS	0.75 NS	0.37 NS	0.54 NS	**0.94** *	-0.78 NS	0.79 NS	-0.67 NS	-0.97 *
α-Ionone	-0.63 NS	0.5 NS	0.34 NS	0.51 NS	**0.97** *	-0.91 NS	0.52 NS	-0.86 NS	-0.97 *
B-Ionone	-0.50 NS	0.56 NS	0.74 NS	0.07 NS	0.63 NS	-0.36 NS	0.75 NS	-0.56 NS	-0.72 NS
Limonene	**-0.97** *	0.91 NS	-0.38 NS	**0.96** *	0.76 NS	-0.69 NS	0.76 NS	-0.11 NS	-0.73 NS
Linalool	-0.82 NS	0.73 NS	0.13 NS	0.71 NS	**0.99** **	-0.92 NS	0.68 NS	-0.67 NS	**-0.99** **
2-Methyl-1-	-0.5	0.49	0.63	0.97	0.85	-0.70	0.60	-0.84	-0.90

butanol	NS	NS	NS	NS	NS	NS	NS	NS	NS
α-Terpineol	-0.80	0.69	0.05	0.75	**0.99**	**-0.95**	0.62	-0.67	-0.97
	NS	NS	NS	NS	**	*	NS	NS	*
Tetradecane	-0.88	**0.94**	0.18	0.55	0.65	-0.38	**0.99**	-0.13	-0.71
	NS	*	NS	NS	NS	NS	***	NS	NS

Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Bold numbers represent significant correlation. Significance level of each attribute by treatment or day; *** = 0.001, ** = 0.01, and * = 0.05 based on their p values. NS = not-significant, TSS = total soluble solids, TA = titratable acidity.

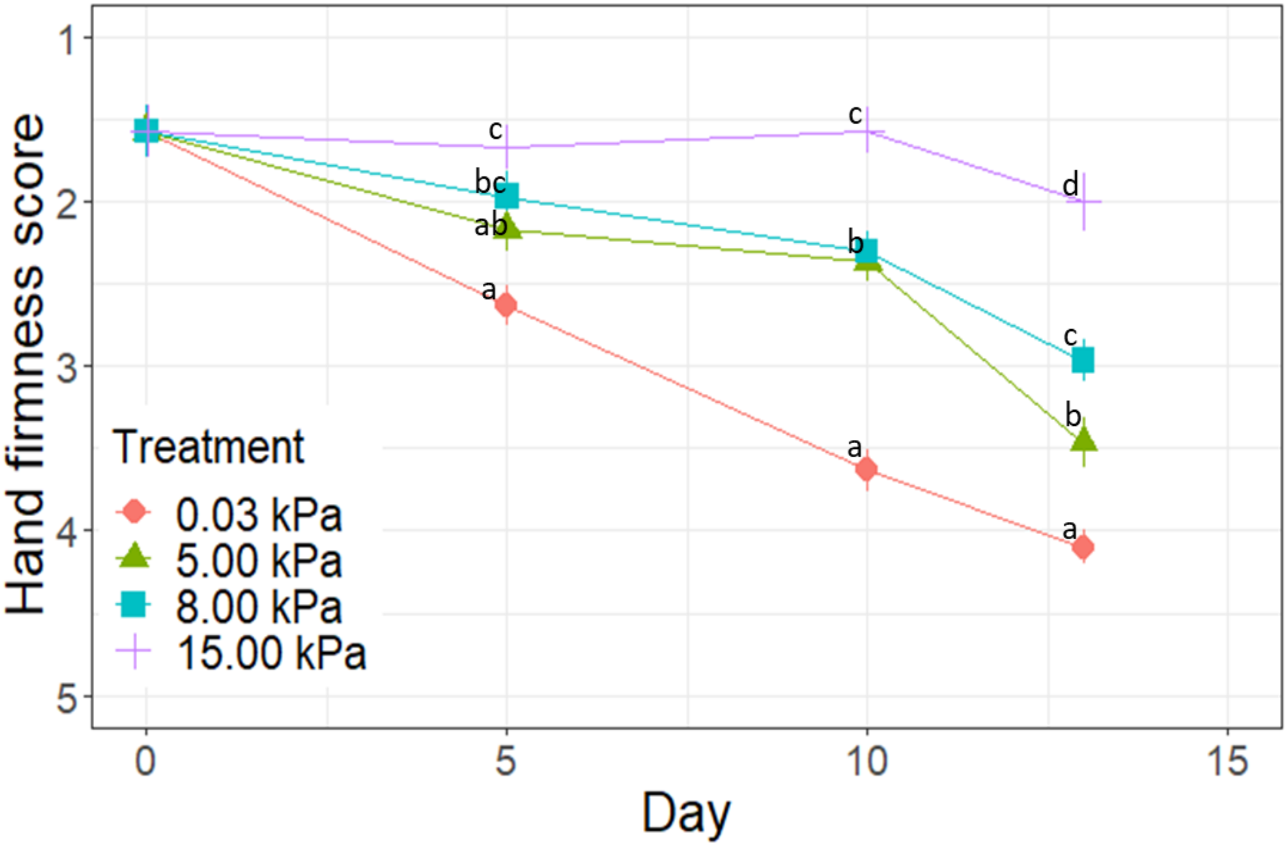

Fig. 3.1: Changes in raspberry hand firmness score following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5°C for 13 days. Firmness scale; 1 = very firm, 2 = firm, 3 = soft, 4 = very soft and 5 = no resistance. Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences (p<0.05). Different letters indicate significant differences while same letters represent no significant differences.

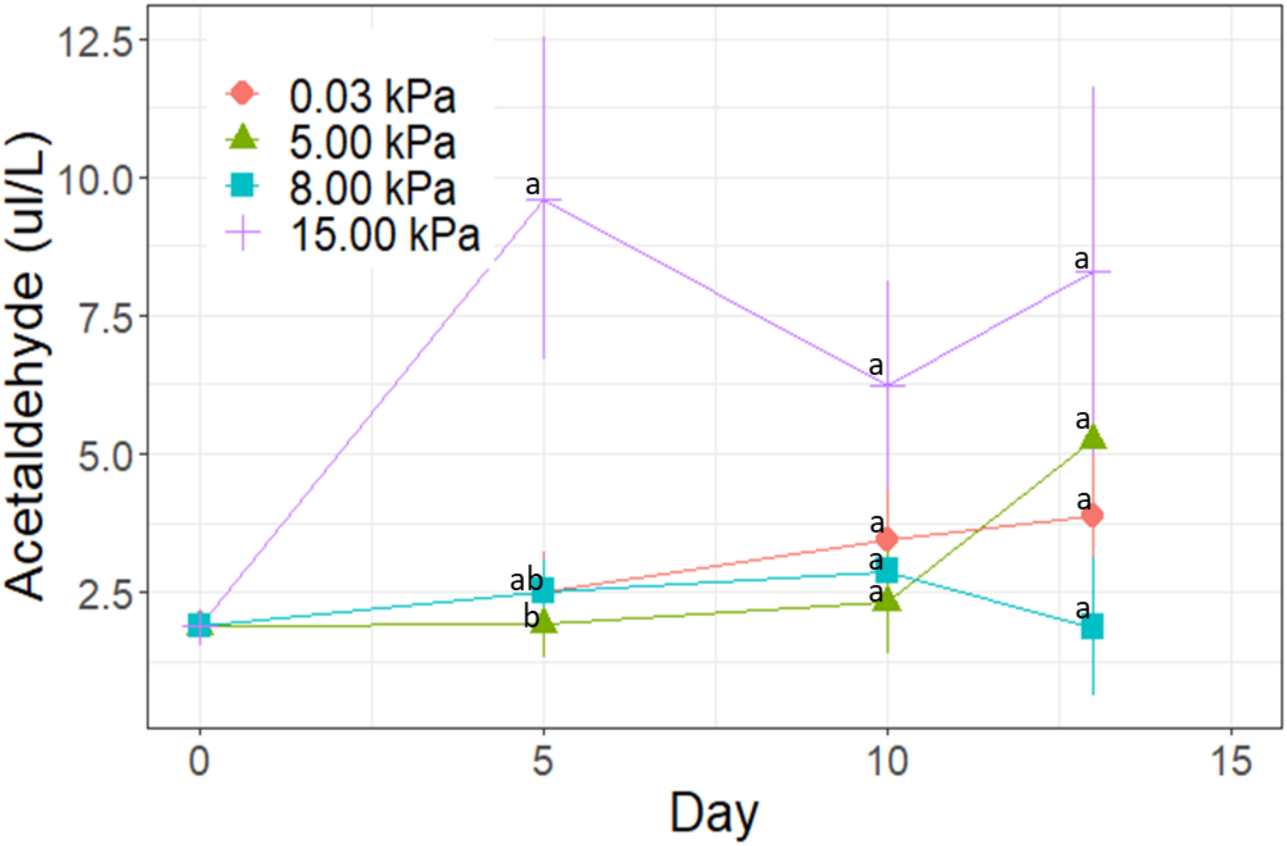

Fig. 3.2: Changes in raspberry acetaldehyde concentration following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days. Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

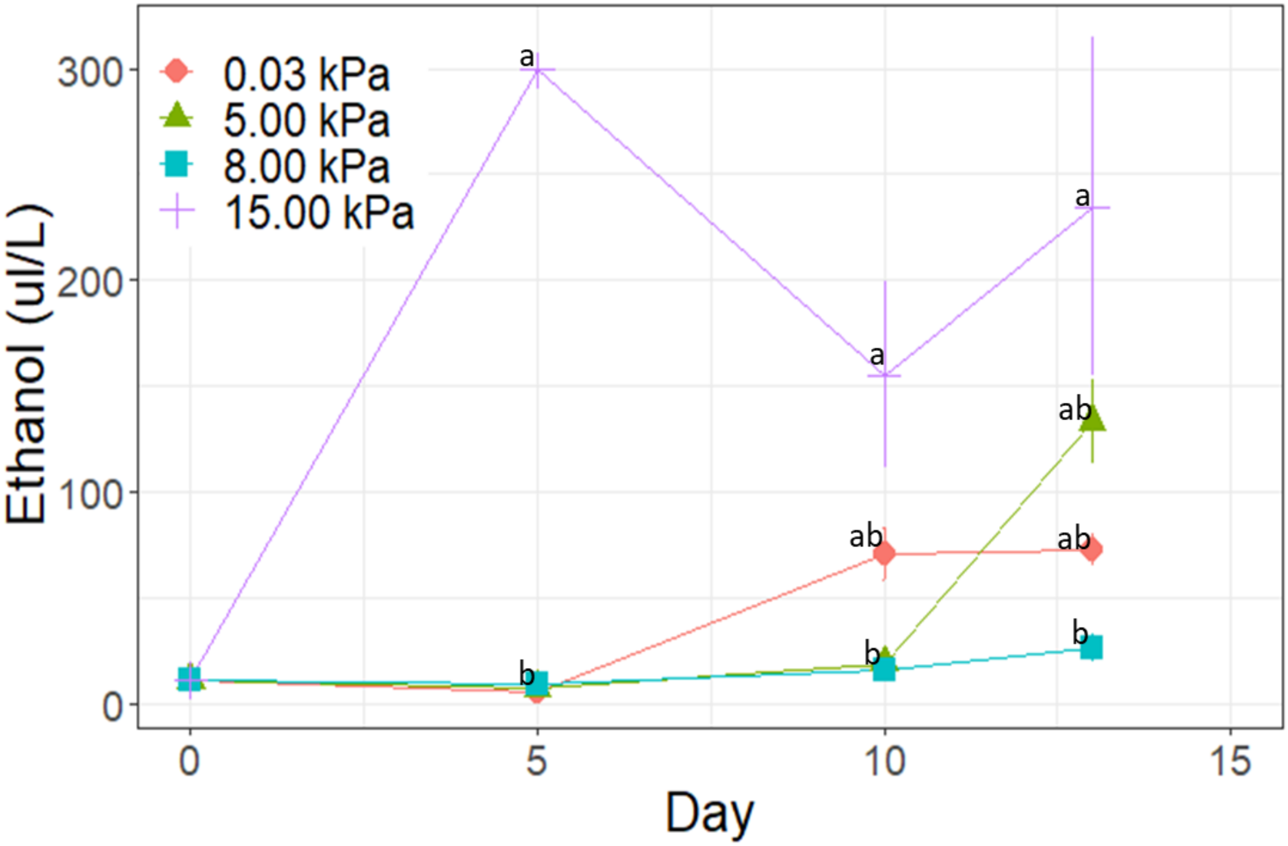

Fig. 3.3: Changes in raspberry ethanol concentration following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days. Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

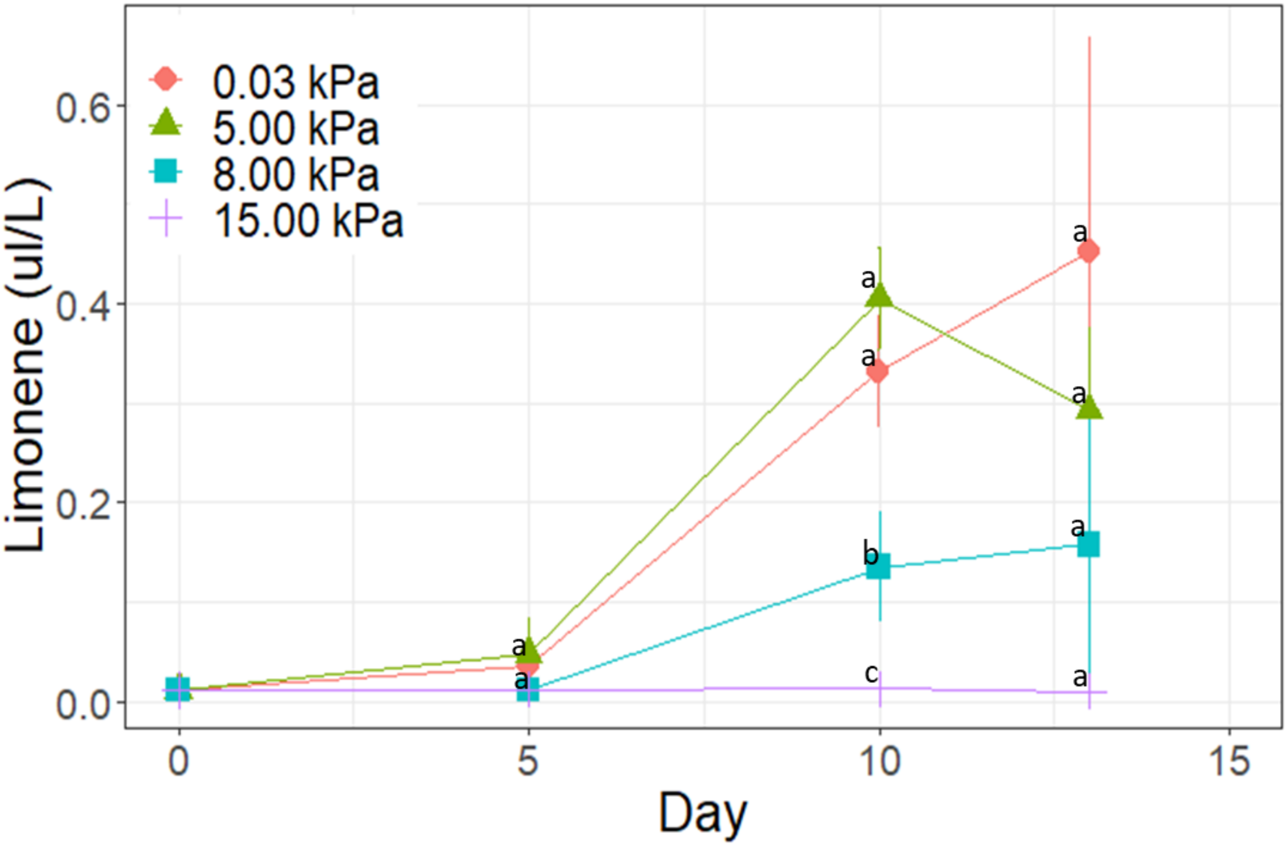

Fig. 3.4: Changes in raspberry limonene concentration following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days. Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

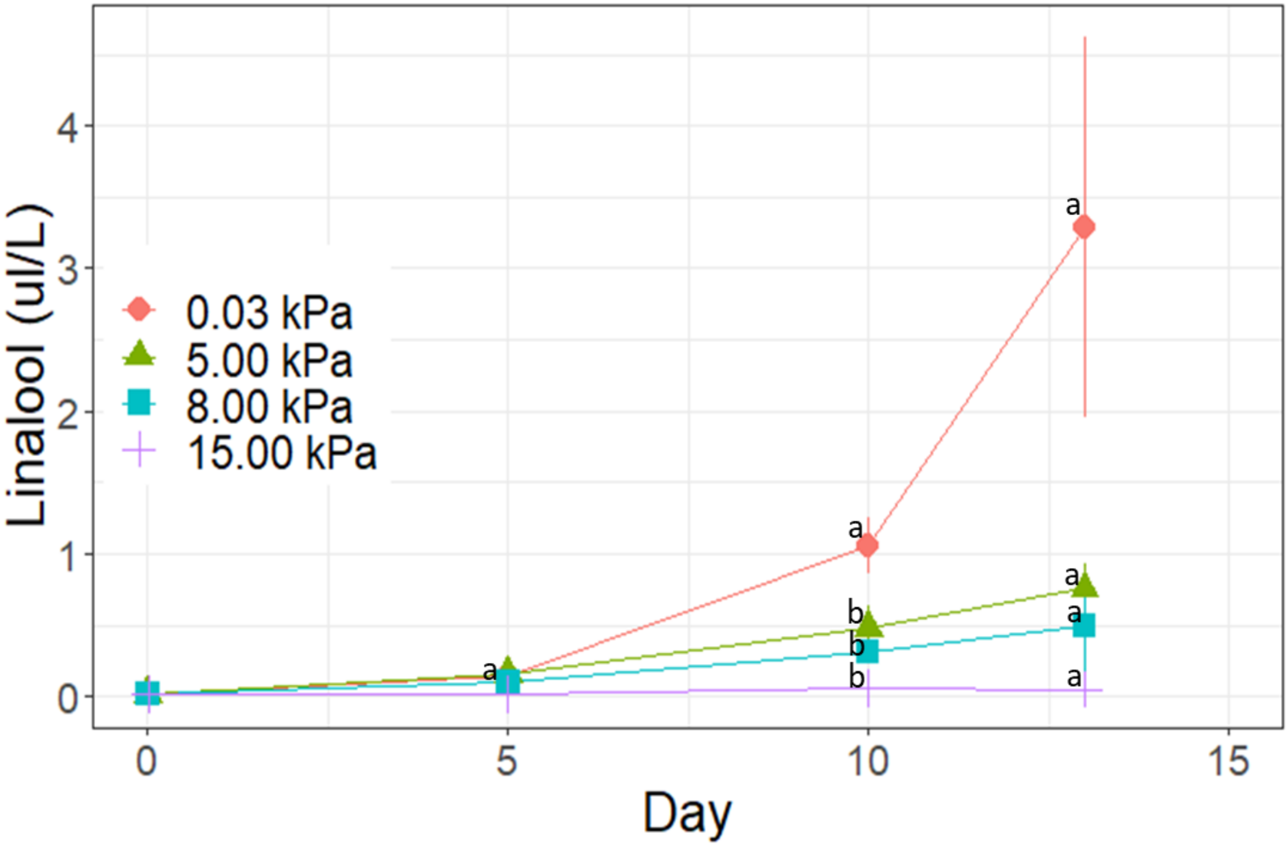

Fig. 3.5: Changes in raspberry linalool concentration following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days. Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

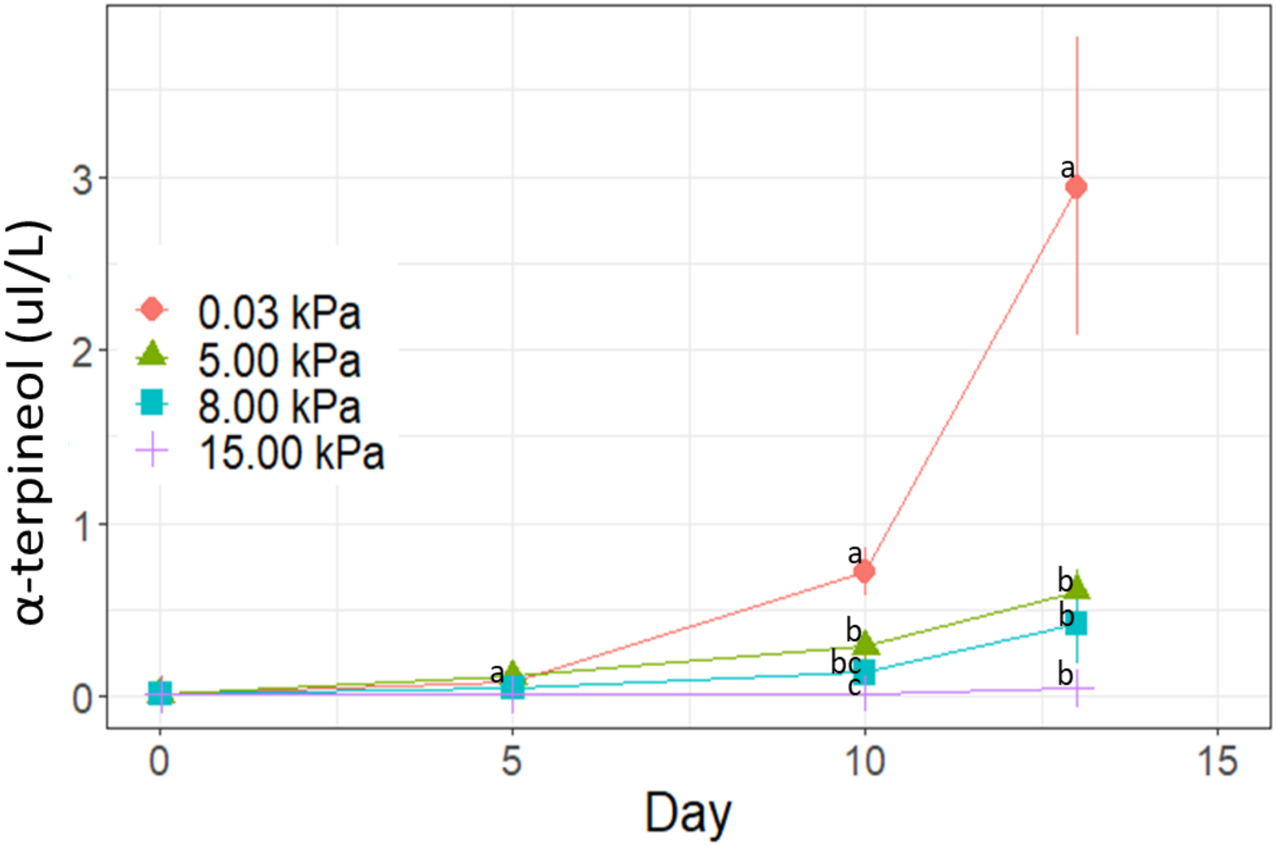

Fig. 3.6: Changes in raspberry α-terpineol concentration following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days. Data were assessed through ANOVA followed by Least Significance Difference (LSD) test to reveal significant differences ($p<0.05$). Different letters indicate significant differences while same letters represent no significant differences.

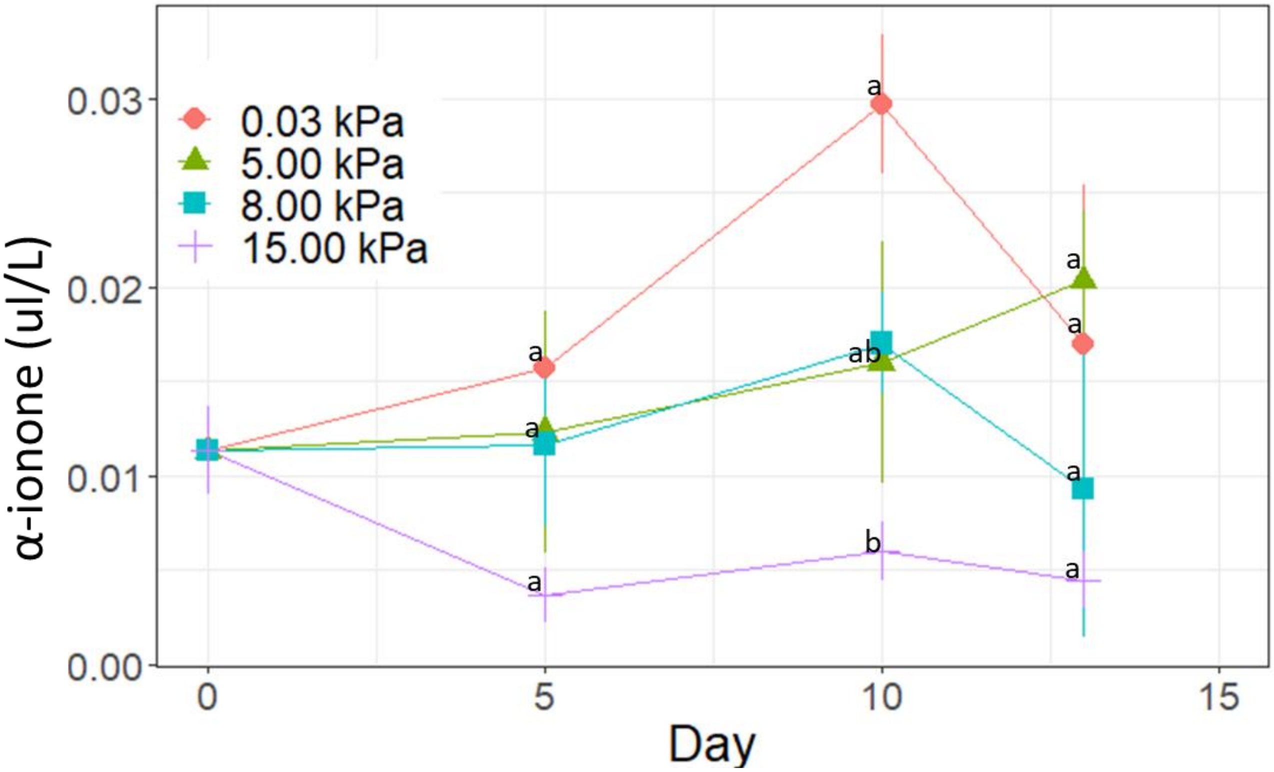

Fig. 3.7: Changes in raspberry α-ionone concentration following exposure to 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) or 15 kPa (15 kPa CO_2, 6 kPa O_2) atmospheres at 5℃ for 13 days. Different letters indicate significant differences while same letters represent no significant differences.

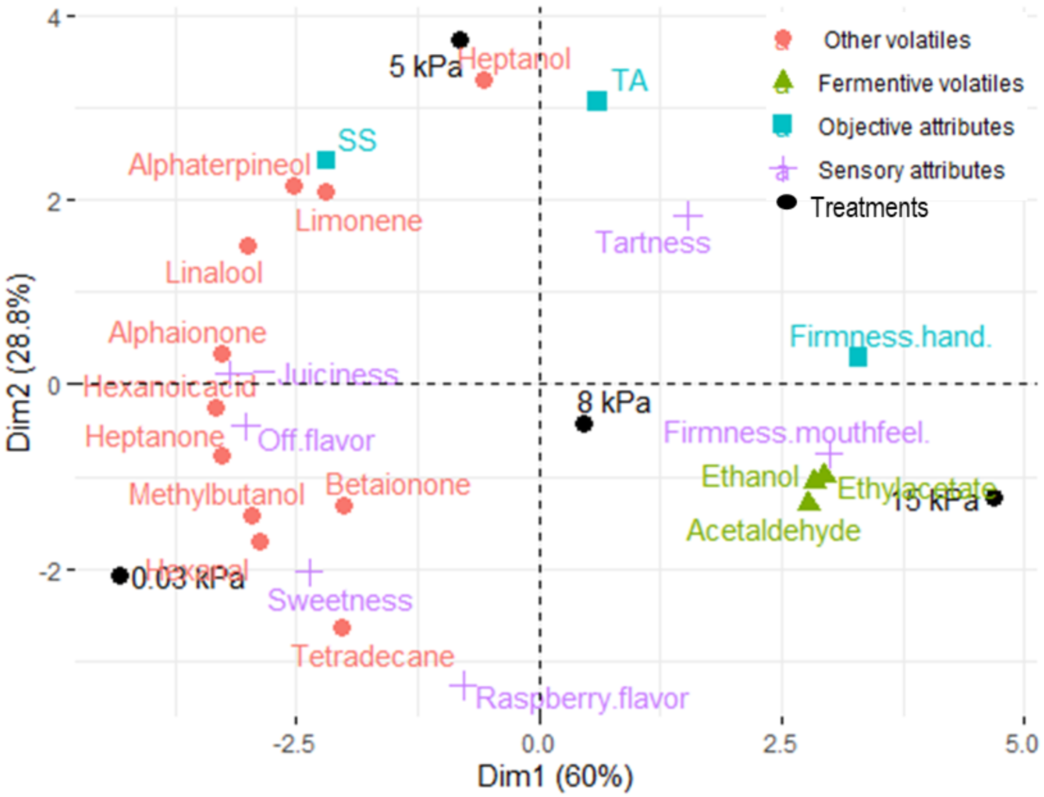

Fig. 3.8: Principal component analysis of the sensory profile of raspberry fruit after 5 days exposure to four modified atmospheres at 5℃: 0.03 kPa (0.03 kPa CO_2, 21 kPa O_2); 5 kPa (5 kPa CO_2, 16 kPa O_2); 8 kPa (8 kPa CO_2, 13 kPa CO_2) and 15 kPa (15 kPa CO_2, 6 kPa O_2).

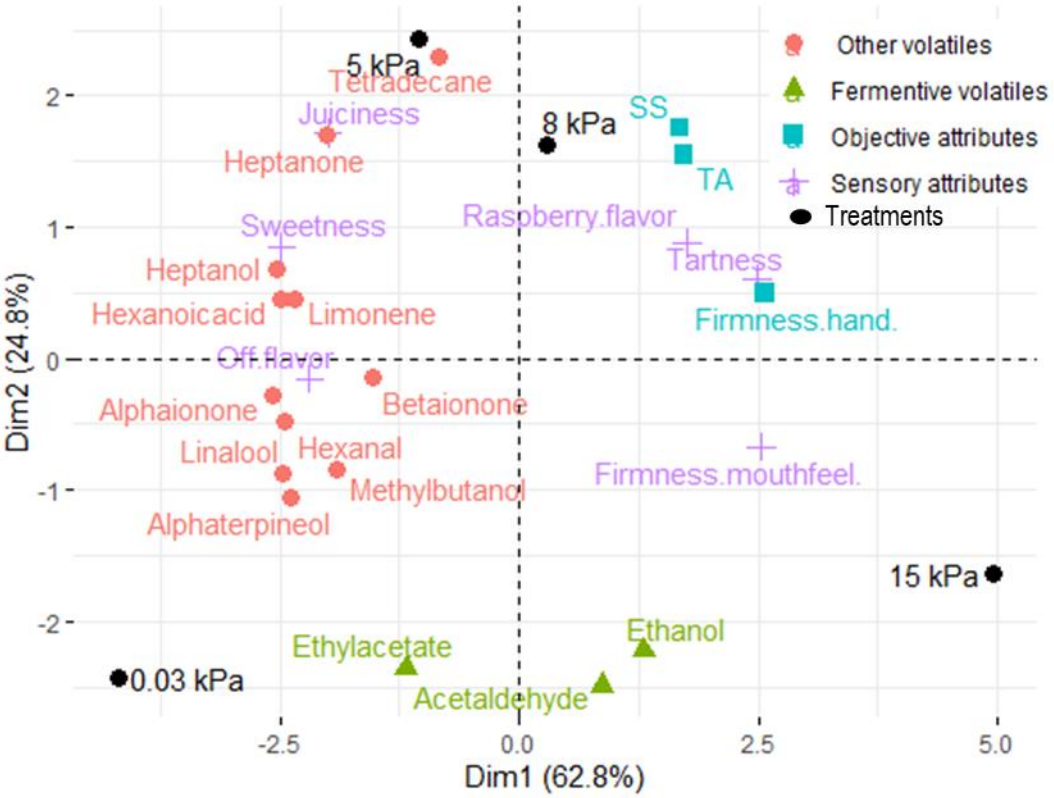

Fig. 3.9: Principal component analysis of the sensory profile of raspberry fruit after 10 days exposure to four modified atmospheres at 5℃: 0.03 kPa (0.03 kPa CO$_2$, 21 kPa O$_2$); 5 kPa (5 kPa CO$_2$, 16 kPa O$_2$); 8 kPa (8 kPa CO$_2$, 13 kPa CO$_2$) and 15 kPa (15 kPa CO$_2$, 6 kPa O$_2$).

Supplemental Materials

Supplemental Table 3.1: Odor threshold of the detected volatiles.

Compounds	Odor description	Odor threshold(ul/L)	references
Acetaldehyde	fresh green	0.025	Czerny et al., 2008
Ethyl acetate	solvent, fruity	7.5	Larsen et al., 1992
Ethanol	alcohol	990	Czerny et al., 2008
2-Heptanone	sulfur, pungent, green	0.14	Steinhaus et., 2005
2-Heptanol	green leaves	0.15	Meilgaard, 1975
Hexanal	grass, tallow	0.03	Kirchoff et al., 2001
Hexanoic acid	goaty, fatty acid, wet dog	3	Guth, 1997
α-Ionone	raspberry, cedarwood	0.0026	Czerny et al., 2008
B-Ionone	flowery, violet like	0.0035	Czerny et al., 2008
Limonene	fruity, lemon	0.015	Noguerol-Pato, et al., 2012
Linalool	sweet, floral scent	0.001	Larsen et al., 1992
2-Methyl-1-butanol	Malty, solvent-like	1.2	Czerny et al., 2008
α-Terpineol	pine, lily of the valley	0.25	Vilanova et. al., 2012; Noguerol-Pato, et al., 2012
Tetradecane	odorless	NA	Tetradecane, 2021

Conclusions

High CO_2 atmospheres were effective in increasing raspberry shelf life and maintaining sensory quality. After harvest, raspberry visual quality degraded over time, but the atmosphere affected how quickly they degraded. The maximum firmness and glossiness, the brightest red color, and the least leakiness were all found in raspberries stored in 15 kPa CO_2 with 6 kPa O_2 atmosphere, followed by raspberries stored in 8 kPa CO_2 with 13 kPa O_2 atmosphere. High CO_2 atmospheres slowed further ripening and created fungistatic conditions, thus reducing decay incidence. Raspberry discoloration (light pink colored druplets) was reported for the first time, and found to increase during storage. This discoloration resembled red drupelet reversion of blackberries. High CO_2 atmospheres inhibited this discoloration in raspberry fruit. Despite the increase in raspberry discoloration, total anthocyanin content increased over time in all raspberries following harvest; however, the increase was significantly hindered by high CO_2 in a concentration-dependent manner.

A trained sensory panel evaluated the effect of CO_2 atmospheres on raspberry sensory quality and found that raspberries stored in 8 kPa CO_2 with 13 kPa O_2 atmosphere performed best, with higher raspberry flavor, juiciness, and sweetness scores than raspberries from other treatments. Off flavor was detected in raspberries stored in air or 5 kPa CO_2 with 16 kPa O_2 atmospheres, but not in raspberries stored in 8 kPa CO_2 with 13 kPa O_2 or 15 kPa CO_2 with 6 kPa O_2 atmospheres. The concentration of fermentative volatiles was higher in raspberries stored in high CO_2 atmospheres, but ethanol concentration remained below the odor threshold and did not impact sensory quality. Olfactory perception depends on a volatile's odor threshold and not necessarily on their abundance. Other aromatic volatiles were mostly associated with low CO_2 or air stored raspberries, but were generally low in concentration in our study.

In order to maintain raspberry visual quality while storing/distributing for less than ten days, 8 kPa CO_2 with 13 kPa O_2 atmosphere was optimal, and CO_2 as low as 5 kPa can contribute to maintaining raspberry quality for relatively short times (<5 days). However, by 10 days, raspberries stored in 0.03 kPa CO_2 with 21 kPa O_2 or 5 kPa CO_2 with 16 kPa O_2 atmospheres lost their sensory qualities. Raspberries stored in air (0.03 kPa CO_2 with 21 kPa O_2) rapidly lost shelf life and quality after 5 days; therefore, raspberries should not be stored longer without modified or controlled atmospheres.

It is best to determine the concentration of CO_2 for atmospheric modification based on the intended storage (or transportation) duration, and this time period should not exceed beyond 10 to 12 days. For future research, it would be interesting to investigate further the quality of raspberries once they are removed from MA, to get an idea about their performance in retail stores. In addition, the effects of high CO_2 atmospheres on the synthesis of volatile compounds and related gene expression could be explored.