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Effects of Cooling and Postharvest Storage Methods on Broccoli Quality

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To the Graduate Council:

I am submitting herewith a thesis written by Sarah Parker entitled "Effects of Cooling and Postharvest Storage Methods on Broccoli Quality." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Carl Sams, Major Professor

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Effects of Cooling and Postharvest Storage Methods on Broccoli Quality

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ABSTRACT

Broccoli (*Brassica oleracea* L. var. *italica*) is a cool-weather vegetable that is grown for its edible flowering heads and stalks. Broccoli inflorescences are immature plant organs with high respiration rates, resulting in a rapid loss of quality after harvest. The effects of cooling and storage methods on postharvest broccoli quality were evaluated based on metabolite contents of broccoli samples stored for 0, 7, 14, 21, 28, and 35 days. Sugar and organic acid contents were measured for broccoli harvested Fall 2018. Contents were compared for two cultivars ('Diplomat' and 'Arcadia') and two temperature treatments (not precooled and stored at 6 [superscript zero] ºC, and precooled with an ice slurry and stored at 0 ºC in ice). Glucosinolate, volatile, carotenoid, and chlorophyll contents were measured for broccoli harvested Summer 2019. Contents were compared for two cultivars ('BH053' and 'Emerald Crown') and two temperature treatments (precooled with top icing and stored at 7 ºC, and precooled with an ice slurry and stored at 0 ºC in ice). Cultivar, storage temperature, and storage time significantly affected metabolite contents in broccoli. Sucrose content was significantly greater for 'Diplomat,' while organic acid content was greater for 'Arcadia.' Carotenoid, and chlorophyll contents were significantly greater for 'BH053,' while glucosinolate and dimethyl disulfide content was significantly greater for 'Emerald Crown.' Broccoli stored at 7 ºC had significantly greater dimethyl disulfide contents while broccoli stored at 0 ºC had significantly greater sucrose and glucosinolate contents. Sugars, organic acids, carotenoids, and chlorophyll significantly decreased within 21 days during storage, while glucosinolates were unaffected by storage time. However, the sulfur-containing volatiles increased from 21 to 35 days. These results indicate that the postharvest quality of broccoli was significantly greater for 'Diplomat' than for 'Arcadia,' and greater for 'BH053' than for 'Emerald Crown.' In addition, these results suggest that storage

at lower temperatures helps to maintain postharvest quality of broccoli by decreasing the loss of nutritionally important glucosinolates and sugars, while preventing the production of volatiles responsible for off-odors.

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INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *Italica*) is a cool-season cabbage species belonging to the mustard family (*Brassicaceae*), characterized by its green inflorescences and stalks (King and Morris, 1994). Cultivation of *Brassica* plants is thought to have originated near the eastern Mediterranean area, and broccoli was domesticated in southern Italy (Buck, 1956). Today, broccoli is a popular vegetable consumed worldwide due to its vitamin, mineral and phytonutrient contents.

Temperature and duration of storage are key factors in determining the storage life of broccoli (Hackert, 1987; Pramanik et al., 2006). The loss of cellular energy reserves through respiration causes a loss in the nutritional quality, flavor, and salable weight of produce (Hansen et al., 1992; King et al., 1994). Lowering the temperature during storage increases the postharvest life of fruits and vegetables by lowering respiration rates (Batal et al., 1982; Kader et al., 2003). Because broccoli has a higher rate of respiration than most vegetables, it is crucial to maintain cool temperatures throughout the entire postharvest handling and shipping process (Forney et al., 199).

California is responsible for over 90% of the total broccoli production in the nation, followed by Arizona (5%) (USDA Economic Research Service, 2011). Consequently, most of the fresh broccoli sold in the Eastern U.S. has been processed and shipped thousands of miles across the country before reaching supermarkets. Due to its high respiration rate, broccoli has a shelf life of 2-3 weeks, leaving little time between arrival at the supermarket and consumption before losing consumer acceptability. Establishing a locally sourced broccoli industry on the East Coast of the United States will reduce the time between harvesting and consumer availability (Atallah et al.,

2014; Fan et al., 2014). Therefore, localization of the broccoli industry along the East Coast would help reduce the time between harvest and consumption for East Coast consumers, resulting in fresher, higher-quality broccoli (Wheeler et al., 2018). In addition, localization of the broccoli market will reduce the cost of shipping, as well as, reduce the carbon footprint. It's estimated that increasing the Eastern market acreage by 30%, would decrease the usage of diesel fuel by 63,000 gallons per year, reducing CO_2 emissions by 1.4 million pounds per year (Atallah et al., 2014; Fan et al., 2014).

During postharvest senescence, produce quality decreases over time due to the breakdown of cellular components, resulting in the loss of nutritionally important compounds (King et al., 1994). The quality of fruits and vegetables can be determined analytically by measuring the concentration of sugar, organic acid, glucosinolate, volatile, carotenoid, and chlorophyll contents. Total and individual concentrations of these compounds differ among cultivars, as well as change over time due to postharvest senescence, and the sensory perception of quality is affected as a result (Bruckner et al., 2005; Farnham and Kopsell, 2009; Pellegrino et al., 2019). Therefore, measuring the effect of different cooling/storage methods on these biochemical compounds for different cultivars will help to determine the proper shipping and storage conditions for maintaining the postharvest quality of broccoli that will be grown and distributed along the East Coast.

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CHAPTER 1: EFFECTS OF COOLING AND STORAGE METHOD ON SUGARS, ORGANIC ACIDS, AND THE SUGAR/ACID RATIO IN BROCCOLI

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Abstract

Broccoli (*Brassica oleracea* L. var. *italica*) is a cool-weather vegetable that is grown for its edible flowering heads and stalks. Broccoli inflorescences are immature plant organs with high respiration rates, resulting in a rapid loss of quality after harvest. The effects of cooling and storage methods on the postharvest quality of broccoli were evaluated based on primary metabolite contents. Changes in sugar and organic acid content were investigated for two cultivars (‘Diplomat’ and ‘Arcadia’), two temperature treatments (not precooled and stored at 6 ºC, and precooled with an ice slurry and stored at 0 ºC in ice), and six different days in storage (0, 7, 14, 21, 28, and 35 days). Cultivar significantly affected sucrose content, malic and citric acid content, and the sugar/acid ratio. Sucrose content was significantly greater for ‘Diplomat’ than for ‘Arcadia.’ Citric acid, malic acid, total organic acid contents, and the sugar/acid ratio were significantly greater for ‘Arcadia’ than for ‘Diplomat.’ Storage time significantly affected all sugar contents (sucrose, glucose, and fructose), all organic acid contents (malic and citric), and the sugar/acid ratio. Average total sugar contents significantly decreased at 7 and 28 d in

storage but did not significantly change from 7 to 21 d or from 28 to 35 d. Organic acid contents significantly decreased at 7 and 14 d, then remained stable from 14 to 35 d. The sugar/organic acid ratio significantly decreased at 7 d, significantly increased at 21 d, and significantly decreased at 28 d but did not significantly change from 7 to 14 d or from 28 to 35 d. Interactions of cultivar and storage time significantly affected fructose, citric acid, and total organic acid contents. Fructose content in 'Diplomat' significantly decreased at 7 d and significantly increased at 21 d. After that, fructose content did not significantly change from 21 to 35 d. For 'Arcadia,' fructose content decreased at 7 d and significantly increased at 35 d but did not significantly change from 7 to 28 d. Citric acid and total organic acid contents in 'Diplomat' did not significantly change during storage, while citric and total organic acid contents for 'Arcadia' significantly decreased at 7 and 14 d but did not significantly change from 14 to 35 d. Interactions of cultivar and storage temperature had a significant effect on the sugar/acid ratio. For 'Diplomat,' broccoli stored at 6 ºC had a significantly greater sugar/acid ratio than broccoli stored at 0 ºC in ice, while storage temperature did not significantly affect the sugar/acid ratio for 'Arcadia.' The sugar/acid ratio in broccoli stored at either temperature was significantly greater for 'Diplomat' than for 'Arcadia.' Results from this study show that cultivar and storage time were the main determinants of sugar content, organic acid content, and the sugar/acid ratio. Significantly greater sucrose levels indicate reduced metabolic activity for 'Diplomat' compared to 'Arcadia,' and a significantly higher sugar/acid ratio indicates potentially increased sweetness and consumer acceptance for 'Diplomat' broccoli compared to 'Arcadia.' Greater sucrose content accompanied by a higher sugar/acid ratio suggests broccoli quality is greater for 'Diplomat' than for 'Arcadia.' Results from this study also confirm that lower storage

temperatures help maintain postharvest quality of broccoli by reducing the rapid depletion of sucrose through metabolic activities, resulting in decreased deterioration rates.

Introduction

The United States (U.S.) broccoli industry is currently centered on West Coast production. California is responsible for over 90% over the total broccoli production in the nation, followed by Arizona (5%) (USDA Economic Research Service, 2011). Consequently, most of the fresh broccoli sold in the Eastern U.S. has been processed and shipped thousands of miles across the country before reaching supermarkets. Establishing a locally sourced broccoli industry on the East Coast will reduce the time between harvest and consumer availability (Atallah et al., 2014; Wheeler et. al., 2018). Broccoli is known to have a high respiration rate and these changes in the time between harvesting and consumer availability have potential consequences on postharvest physiology. Postharvest senescence of broccoli is accompanied by the degradation of metabolites through respiration (Hasperué et al., 2015; King and Morris., 1994), resulting in a loss of nutritional and sensory quality (Bruckner et al., 2005; Hansen et al., 1997; Pellegrino et al., 2019).

Sugars are primary metabolites that serve as the main energy source for respiration, and act to serve as precursors for lipid, protein, and polysaccharide biosynthesis (Duffus and Duffus, 1984). Because broccoli inflorescences are immature organs with high rates of respiration, they require large amounts of sugars to maintain postharvest quality (Lemoine et al., 2008). Cultivar has an impact on the expression of genes that regulate sugar synthesis and consumption as metabolic substrates (Rosa et al., 2001). These metabolic pathways can be altered by storage conditions, subsequently affecting the rate of postharvest senescence (Pramanik et al., 2006). As

sugars serve as the primary energy source for metabolism, sugar levels are closely associated with the physiological and biochemical properties of vegetables (Tian et al., 2016).

Organic acids are important metabolites required for the maintenance of postharvest vegetable quality (Ferreres et al., 2007; Vaughan and Gessler, 1997). Organic acids are thought to have beneficial health effects due to their antioxidant activity (Ayaz et al., 2006), and are often used as antioxidants or acidulants in food industries (Cunha et al., 2002; Shui and Leong, 2002). Organic acids are also known to affect the organoleptic properties in many fruits and vegetables (Vale et al., 2015; Vaughan and Geissler, 1997). Species, cultivar, tissue type, and storage conditions have all been shown to influence organic acid contents (King and Morris, 1994; Lopez-Bucio et al., 2000; Souse et al., 2009; Vale et al., 2015) The main organic acids in broccoli are citric and malic acid, which are mainly produced by the tricarboxylic acid (TCA) cycle in mitochondria of plant cells (Murcia et al., 2000). Storage temperature and time influence the metabolic rate of plants, consequently affecting the rate of organic acid formation and consumption (Carrari and Fernie, 2006). As primary metabolites involved in the synthesis of cellular tissues, postharvest quality is closely associated with organic acid content in horticultural crops (Schouten et al., 2016; Zapata et al., 2013).

Flavor is one of the main factors affecting quality and consumer acceptability of fruits and vegetables. Sweetness and bitterness are key attributes of broccoli flavor (Pellegrino et al., 2019). Although bitterness is often associated with glucosinolate contents in broccoli (Bhandari et al., 2014), the details of this association are still unclear (Bell et al., 2017). The sugar/acid ratio is commonly used as an index of quality and acceptability of produce (Paull, 1999), and an increased sugar/acid ratio is known to contribute to the bitterness in other *Brassica* species

(Fukuda et al., 2016). Therefore, the sugar/acid ratio in broccoli may also be a contributing factor in determining flavor quality and acceptability.

For this study, changes in sugar contents, organic acid contents, and the sugar/acid ratio in broccoli was investigated for two cultivars (‘Diplomat’ and ‘Arcadia’), two temperature treatments (not precooled and stored at 6 ºC, and ice slurry cooled and stored at 0 ºC in ice), and six different days in storage (0, 7, 14, 21, 28, and 35 days). This will help determine the proper shipping and storage conditions for maintaining the postharvest quality of broccoli that will be distributed along the east coast.

Materials and Methods

Plant materials and storage.

Broccoli was supplied by a small-scale producer located in East Tennessee. Broccoli was grown according to recommended management practices for the southeastern U.S. (Kemble et al., 2017). Broccoli was harvested when the majority of the heads had reached commercial maturity. The average head diameter was 9.2 cm ± 1.2. Two cultivars of broccoli, ‘Diplomat’ and ‘Arcadia’ were harvested on 13 Nov. 2018. The average head diameter was 9.3 cm ± 1.2 for ‘Diplomat’ and 9.1 cm ± 1.3 for ‘Arcadia.’ Each cultivar was separated into two treatment groups immediately after harvest. One treatment group was placed into waxed corrugated boxes and not precooled, while the other treatment group was cooled by submerging in an ice slurry. Broccoli was then transported to The University of Tennessee Institute of Agriculture for cold room storage. The internal temperature for non-iced broccoli was 6 ºC ± 2 when it reached the storage cooler (Fig. 1). Broccoli from this treatment group was then placed in cold storage and kept in waxed corrugated boxes without ice. The cold room temperature was maintained at 6 ºC ± 0.4 and the internal broccoli temperature was maintained at 6 ºC ± 0.5. For the other treatment

group, broccoli that was placed in an ice slurry was cooled to 0 ºC ± 0.4 at 1 h after the slurry was applied. Broccoli from this treatment group was then placed in cold storage and kept in coolers filled with ice. The cold room was temperature was maintained at 4ºC ± 0.1 and the internal broccoli temperature was maintained at 0 ºC ± 0.4 (Fig. 1). Internal broccoli temperatures were recorded every 30 min with Watch Dog® data loggers (Spectrum® Technologies, Inc., Aurora, IL, USA).

Postharvest analysis.

Broccoli was removed from storage at 0, 7, 14, 21, 28, and 35 d. Three replications, consisting of two broccoli heads per replication, were subsampled for each cultivar and treatment combination. For each replication, 30 ± 1 g fresh tissue was placed into plastic bags and stored in a -80 ºC freezer overnight, and then freeze-dried the following day. Freeze-dried tissue was ground to a fine powder, using a mortar and pestle in liquid nitrogen, for extraction and analysis.

Sugars, extractions and analysis.

Sugars were extracted from broccoli tissues using a modified version of the method by Kerepesi et al. (1996). A 0.1 g ± 0.01 subsample of homogenized tissue was weighed into a 16 x 100 mL glass centrifuge tube and 2.5 mL of water purified by reverse osmosis (RO water) heated to 80 ºC was added to each sample. Centrifuge tubes were vortexed, shaken at 60 rpm for 15 min, and centrifuged at 4400 g_n for 20 min. The supernatant was then transferred into a new centrifuge tube. This process was repeated one more time for the remaining precipitate. The liquid supernatant was then filtered twice, once through a 0.45 µm Nylon filter and once through a 0.20 µm Nylon filter, into a 12 x 13 mm clear crimp top high performance liquid chromatography (HPLC) vial.

Sugars were analyzed using an Agilent 1200 series HPLC unit with an evaporative light scattering detector (Agilent Technologies, Santa Clara, CA). The column temperature was set at 80ºCfor a 300 x 7.7 mm i.d. 8 µm analytical scale Hi-Plex Ca column equipped with a PL Hi-Plex Ca 7.7 x 50 mm i.d. guard cartridge (Agilent Technologies, Santa Clara, CA). The flow rate was set at 0.600 mL min^{-1}, and 5.0 µL of each sample were injected for a total run time of 25 min per sample. Separations were achieved isocratically using a mobile phase of 100% water. Sucrose, glucose, and fructose peaks were assigned based on external standards. Sugars were expressed on a dry mass basis in $mg \cdot g^{-1}$. Data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies, Palo Alto, CA).

Sugars, extractions and analysis.

Organic acids were extracted from broccoli tissues using a modified version of the method by Barickman et al. (2016). A 0.1 g ± 0.01 subsample of homogenized tissue was weighed into a 15 mL plastic centrifuge tube and 2.5 mL of 80% ethanol/20% RO water was added. Samples were then placed in an ultrasonic bath for 5 min, then centrifuged at 1090 g_n for 5 min. The supernatant was then transferred to a 16 x 100 mL glass centrifuge tube. This process was repeated one more time for the remaining precipitate. The supernatant was then evaporated to dryness using a nitrogen stream. Dried samples were dissolved in 5.0 mL of RO water. The liquid was then filtered twice, once through a 0.45 µm Nylon filter and once through a 0.20 µm Nylon filter, into a 12 x 13 mm clear crimp top HPLC vial.

Organic acids were analyzed using a 1200 series HPLC unit with a refractive index detector (Agilent Technologies, Santa Clara, CA). The column temperature was set at 50 ºC for a 300 x 7.7 mm i.d., 8 µm analytical scale Hi-Plex Ca column, equipped with a Zorbax NH_2 4.6 x 12.5 mm i.d. guard cartridge (Agilent Technologies, Santa Clara, CA). The flow rate was set at 0.600

$mL \cdot min^{-1}$, and 10 µL of each sample were injected for a total run time of 20 min per sample. Separations were achieved isocratically using a mobile phase of 100% 0.05 M H_2SO_4. Malic acid and citric acid peaks were assigned based on external standards. Organic acids were expressed on a dry mass basis in $mg \cdot g^{-1}$. Data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies, Palo Alto, CA).

Statistical Analysis.

SAS statistical software (9.4 for Windows; SAS Institute, Cary, NC) was used for data analysis. Cultivar, storage temperature, storage time, and their interactions were treated as fixed factors, while replication was considered the random factor. Analysis of variance (ANOVA) tests were performed using the GLIMMIX procedure, and means were compared by the least significant difference (LSD) test ($\alpha = 0.05$). ANOVA results are presented for sugars (Table 1), organic acids (Table 2), and the Sugar/acid Ratio (Table 3).

Results

Sugars, effects of cultivar.

Cultivar had a significant impact on sucrose ($F = 7.84$, df = 1, 46, and $p \leq 0.01$) content alone (Fig. 2). 'Diplomat' had significantly higher average total sucrose content compared to 'Arcadia.'

Sugars, effects of cooling/storage method.

Storage temperature significantly affected sucrose ($F = 6.79$, df = 1, 46, and $p \leq 0.05$) content alone (Fig. 3). Broccoli that was precooled with an ice slurry and stored at 0 ºC in ice had significantly higher sucrose content than broccoli that was not precooled and stored at 6 ºC.

Storage time significantly affected all sugars measured (Fig. 4). Sucrose ($F = 43.67$, df = 5, 46, and $p \leq 0.0001$), glucose ($F = 2.22$, df = 5, 46, and $p \leq 0.0001$), fructose ($F = 16.07$, df = 5,

46, and $p \leq 0.0001$), and total sugar (F = 23.26, df = 5, 46, $p \leq 0.0001$) contents significantly decreased during storage for 35 d. Sucrose content significantly decreased at 7 and 14 d in storage, then increased at 21 d, but not significantly. After that, sucrose content significantly decreased at 28 d, then increased at 35 d, but not significantly. Glucose content significantly decreased at 7 d in storage, then decreased at 14 d and increased at 21 d, but not significantly. After that, glucose content significantly decreased at 28 d, then significantly increased at 35 d. Fructose content significantly decreased at 7 d in storage, then decreased at 14 d, but not significantly. Fructose content significantly increased at 21 d, then decreased at 28 d, but not significantly, then remained stable from 28 to 35 d in storage. Total sugar contents significantly decreased at 7 d in storage and remained stable from 7 to 14 d, then increased at 21 d, but not significantly. After that, total sugar contents significantly decreased at 28 d, then increased at 35 d, but not significantly.

Sugars, interaction of cultivar and storage method.

Sugar contents were not significantly affected by the interaction of cultivar and storage temperature (Table 4). The interaction between cultivar and storage time significantly affected fructose (F = 2.43, df = 5, 46, and $p \leq 0.05$) content alone (Table 5). For ‘Diplomat,’ fructose content significantly decreased at 7 d in storage and remained stable from 7 to 14 d, while fructose content for ‘Arcadia’ significantly decreased at 7 d, then decreased at 14 d, but not significantly. After that, fructose content for ‘Diplomat’ significantly increased at 21 d, then decreased at 28 and 35 d, but not significantly. In contrast, fructose content for ‘Arcadia’ remained stable from 14 to 21 d, then decreased at 28 d, but not significantly. After that, fructose content for ‘Arcadia’ significantly increased at 35 d in storage. Sugar contents were not

significantly affected by the interaction of storage temperature and storage time (Table 6) or by the interaction of cultivar, storage temperature, and storage time (Table 7).

Organic acids, effects of cultivar.

Cultivar had a significant impact on all organic acid contents measured (Fig. 5). Citric acid ($F = 33.46$, $df = 1, 46$, and $p \leq 0.0001$), malic acid ($F = 34.09$, $df = 1, 46$, and $p \leq 0.0001$), and total organic acid ($F = 34.85$, $df = 1, 46$, and $p \leq 0.0001$) contents were significantly greater for 'Arcadia' than for 'Diplomat.'

Organic acids, effects of cooling/storage method.

Storage temperature did not significantly affect any of the organic acids measured (Fig. 6). There were no significant differences in organic acid contents for broccoli that was precooled with an ice slurry and stored at 0 ºC in ice and broccoli that was not precooled and stored at 6 ºC.

Storage time had a significant impact on all organic acids measured (Fig. 7). Citric acid ($F = 17.67$, $df = 5, 46$, and $p \leq 0.0001$), malic acid ($F = 11.19$, $df = 5, 46$, and $p \leq 0.0001$), and total organic acid ($F = 14.62$, $df = 5, 46$, and $p \leq 0.0001$) contents significantly decreased during storage for 35 d. Citric, malic, and total organic acid contents significantly decreased at 7 and 14 d in storage, then remained stable from 14 – 35 d.

Organic acids, interaction of cultivar and storage method.

Organic acid contents were not significantly affected by the interaction of cultivar and storage temperature (Table 8). The interaction between cultivar and storage time significantly affected both citric acid ($F = 3.70$, $df = 5, 46$, and $p \leq 0.01$) and total organic acid ($F = 2.90$, $df = 5, 46$, and $p \leq 0.05$) contents in broccoli (Table 9). For 'Diplomat,' both citric and total organic acid contents significantly decreased during storage, but not significantly. In contrast, both citric and total organic acid contents for 'Arcadia' significantly decreased at 7 and 14 d in storage, then

continued to decrease from 21 to 35 d in storage, but not significantly. Citric and total organic acid contents were significantly greater for ‘Arcadia’ than for ‘Diplomat’ throughout the first 21 d in storage. After that, there was no significant difference between organic acid contents for ‘Diplomat’ and ‘Arcadia.’ Organic acid contents were not significantly affected by the interaction of storage temperature and storage time (Table 10) or by the interaction of cultivar, storage temperature, and storage time (Table 11).

Sugar/acid ratio, effects of cultivar.

Cultivar had a significant impact on the sugar/acid ratio ($F = 30.77$, $df = 1, 46$, and $p \leq 0.0001$) in broccoli (Fig. 8). The average total sugar/organic ratio was significantly greater for ‘Diplomat’ than for ‘Arcadia.’

Sugar/acid ratio, effects of cooling/storage method.

Storage temperature did not significantly affect the sugar/acid ratio (Fig. 9). There was no significant difference in the sugar/acid ratio for broccoli that was precooled with an ice slurry and stored at 0 ºC in ice and broccoli that was not precooled and stored at 6 ºC.

Storage time significantly affected the sugar/ acid ratio ($F = 4.54$, $df = 5, 46$, and $p \leq 0.01$) in broccoli (Fig. 10). The sugar/ acid ratio significantly decreased at 7 d in storage, then increased at 14 d, but not significantly. After that, the sugar/organic acid ratio significantly increased at 21 d, then significantly decreased at 28 d, then increased at 35 d, but not significantly.

Sugar/organic acid ratio, interaction of cultivar and storage method.

The interaction between cultivar and storage temperature had a significant impact on the sugar/acid ratio ($F = 4.20$, $df = 1, 46$, and $p \leq 0.05$) in broccoli (Fig. 11). For ‘Diplomat,’ the sugar/organic acid ratio was significantly greater for broccoli that was not precooled and stored at 6 ºC than for broccoli that was precooled with an ice slurry and stored at 0 ºC in ice. In

contrast, for ‘Arcadia,’ storage temperature had no effect on the sugar/organic acid ratio. The sugar/organic acid ratio was significantly greater for ‘Diplomat’ stored at and 0 ºC than for ‘Arcadia’ stored at either temperature. The sugar/acid ratio was not significantly affected by the interaction of cultivar and storage time (Table 12), the interaction of storage temperature and storage time (Table 13), or the interaction of cultivar, temperature, and storage time (Table 14).

Discussion

Sugars, effects of cultivar.

Sugars are primary compounds involved in plant metabolism, serving as an energy source and building blocks for compound synthesis (Rosa et al., 2001). They serve as precursors for the biosynthesis of lipids, proteins, polysaccharides, and other compounds (Duffus and Duffus, 1984), and can regulate plant growth by acting as a signal molecule, which alters enzyme activity and gene expression (Bhandari and Kwak, 2015a; Cheng et al., 2002). Sugar profiles are dependent on many factors, including genotype, cultivar, tissue type, developmental stage, and growing season (Bhandari and Kwak, 2015a; Hodges et al., 2006; Hounsome et al., 2008; Nunes, 2008; VandenLangenberg et al., 2012).

Previous studies have found glucose and fructose to be more abundant than sucrose (Hasperué et al., 2014; Hasperué et al., 2015; Nishikawa et al., 2005). In this study, glucose was found to be the most abundant sugar, followed by fructose and sucrose. Similarly, previous studies have found glucose to be the main sugar in ‘Green Belt’ (King and Morris, 1994), ‘Amagi,’ Baeridom,’ Cheongjae,’ ‘Grace,’ ‘Grandeur,’ ‘JikNok No. 28,’ NokJae,’ ‘NokYeom No. 1,’ TS-2319,’ and ‘YuDoRi No. 1’ (Bhandari and Kwak, 2015a) broccoli cultivars. Rosa et al. (2001) found that glucose was also the most abundant sugar found in secondary inflorescences of ‘Bejo,’ ‘Durango,’ ‘Green Valiant,’ ‘Legend,’ ‘Marathon,’ ‘Shogun,’ ‘SK3,’

and ‘SK4’ cultivars. In contrast, fructose was the most abundant sugar in primary inflorescences of ‘Bejo,’ ‘Claudia,’ ‘Durango,’ ‘Green Valiant,’ ‘Legend,’ ‘Marathon,’ ‘Senshi,’ ‘Shogun,’ SK3,’ ‘SK4,’ and ‘Tokyodome’ broccoli cultivars (Jahangir et al., 2008; Rosa et al., 2001), white cabbage (Rosa et al., 2001), and kale (Ayaz et al., 2006). Genetic influences may shift the metabolic pathway in favor of glucose accumulation (Rosa et al., 2001). Thus, the greater glucose content compared to fructose may be due to genetic factors controlling the metabolism of glucose in broccoli, which is determined by cultivar.

Results from this study show that sucrose content was significantly greater for ‘Diplomat’ than for ‘Arcadia.’ Similarly, previous studies have found differences in sucrose contents among ‘Amagi,’ Baeridom,’ Cheongjae,’ ‘Grace,’ ‘Grandeur,’ ‘JikNok No. 28,’ NokJae,’ ‘NokYeom No. 1,’ TS-2319,’ and ‘YuDoRi No. 1’ (Bhandari and Kwak, 2015a), ‘Emperor,’ ‘Marathon,’ Shogun’ (Bruckner et al., 2005), and various other broccoli cultivars (Rosa et al., 2001). In contrast, Siomos et al. (2004) found that sugar contents were not significantly different among ‘Marathon’ and ‘Samurai’ broccoli cultivars. Bhandari and Kwak (2015a) also found significant differences in glucose, fructose, and total sugar contents among cultivars, and Rosa et al. (2001) found a significant difference among broccoli cultivars for fructose and total sugar contents as well. In contrast, glucose, fructose, and total sugar contents in this study were not significantly influenced by cultivar alone. Sucrose is the main translocated sugar and is either stored or metabolized in sinks, and it can be cleaved by either sucrose synthase or invertase activities. Sucrose synthase, in the presence of uridine 5’-diphosphate (UDP), converts sucrose to UDP-glucose and fructose, while sucrose invertase hydrolyzes sucrose, cleaving it into glucose and fructose (Rosa et al., 2001). Results from this study support the premise that variation in sucrose content is more dependent on cultivar than either glucose or fructose contents in broccoli

(Bhandari and Kwak, 2015b). Greater sucrose content in ‘Diplomat’ may be due to the genetic control of enzyme activity, leading to increased invertase and/or synthase activities that resulted in decreased sucrose content for ‘Arcadia.’ However, Sucrose synthase may also lead to sucrose synthesis in some fruits and vegetables (Pramanik et al., 2004). Thus, greater sucrose content in ‘Diplomat’ may actually be due to greater sucrose synthesis as a result of increased synthase activity in broccoli.

Although fructose content was not significantly affected by cultivar alone, the interaction between cultivar and storage time did have a significant impact on fructose content in this study. Fructose content did not significantly change among cultivars during storage from 0 to 14 d. However, fructose content was significantly greater at 21 d for ‘Diplomat’ than for ‘Arcadia.’ Fructose content continued to decrease from 21 to 35 d for ‘Diplomat,’ but not significantly, while fructose levels for ‘Arcadia’ decreased from 21 to 28 d, but not significantly, then significantly increased at 35 d. Fructose content in broccoli may be under genetic and/or environmental control, so the relative content among cultivars may be due to different hydrolysis pathways or changes in the type/activity of hexokinases in broccoli tissues (Rosa et al., 2001). Hexokinases have a preferential substrate affinity to fructose in some plant species, and fructokinases are known to be substrate-inhibited by fructose accumulation (Quick and Schaffer, 1996). Results from this study suggest that the variation in fructose content among cultivars during storage may be due to a shift in the metabolic pathways as a result genetic influence.

Sugars, effects of cooling/storage method.

Broccoli inflorescences are immature organs with high respiration rates, resulting in a high requirement of sugars to maintain postharvest quality (King and Morris, 1994). Sugars play a key role in the quality and shelf life of broccoli, serving as the main source of energy for many

chemical reactions responsible for the synthesis of new compounds and maintaining tissue integrity (De Vries, 1975; Hasperué et al., 2016). Many studies have associated the rate senescence with sugar content in broccoli (Hasperué et al., 2011; Nishikawa et al., 2005; van Doorn, 2004). Sugar losses during storage are due to their consumption as a respiratory substrate, as well as their transformation into cell wall material (Pramanik et al., 2006). Because sugar serves as an important energy source and is a main substrate for respiration, sugar levels are thought to be closely related to the physiological and biochemical properties of vegetables (Tian et al., 2016).

Sucrose content alone was significantly affected by storage temperature. The average total sucrose content was significantly greater for broccoli that was cooled with an ice slurry and stored at 0 ºC in ice than for broccoli that was not precooled and stored at 6 ºC. Similarly, previous studies found that broccoli sucrose content decreases at a slower at lower storage temperatures (Page et al., 2001; Downs and Somerfield, 1997; Pramanik et al., 2004; Xu et al., 2016; Pogson et al., 2004; McKenzie et al., 2004). For this study, broccoli sucrose content at 0 and 7 d in storage was significantly greater for broccoli stored at 0 ºC than at 6 ºC. When harvested, immature broccoli heads suffer from severe stress that leads to the expression of genes controlling the onset of senescence. Expression of broccoli senescence genes are activated within 24 h of harvest (Chen et al., 2008; Coupe et al., 2004; Eason et al., 2005; Hasperué et al., 2016; Page et al., 2001). As broccoli heads are harvested while florets are still immature, they require a continuous supply energy to support high respiration rates (King and Morris, 1994). Because sucrose is one of the main compounds consumed in metabolic reactions, sucrose content in broccoli stored at air temperature drops by 50% within 6 hours after harvest (Downs et al., 1997). Postharvest sugar losses are determined by respiration rate, and increased storage

temperature leads to greater respiration rates in broccoli (Tian et al., 2016). Thus, greater sucrose content at 0 and 7 d for broccoli stored at 0 ºC was most likely a result of decreased metabolic activity in response to lower storage temperature compared to 6 ºC. It may have also been due to rapid cooling to 0 ºC within 1 h after harvest, leading to less severe effects from harvesting. Sucrose contents for broccoli stored at 0 ºC significantly decreased at 7 and 14 d, while sucrose content in broccoli stored at 6 ºC only significantly decreased at 7 d. Sucrose contents did not significantly change after that for either storage temperature. Because sucrose content was significantly greater and continued to decrease for a longer period of time for broccoli stored at 0 ºC than at 6 ºC, this suggests that sucrose content may have been depleted to a non-functional level for metabolism (King and Morris,1994) during the first 7 d of storage for broccoli stored at 6 ºC, while sucrose contents were not completely depleted until after 14 d for broccoli stored at 0 ºC. Sucrose levels at 0 d were significantly greater for broccoli cooled with an ice slurry and stored at 0 ºC in ice, indicating that both precooling and storage temperature have a significant effect on sucrose content. Thus, precooling with an ice slurry and maintaining a storage temperature of 0 ºC leads to higher quality broccoli by reducing the metabolic activity, and preserving sucrose content.

In this study, total sugar contents significantly decreased during storage for 35 d. Similarly, previous studies found that total sugar contents decreased in broccoli stored for 3 d at 20 ºC (King and Morris, 1994), 5 d at 22 ºC under continuous low intensity white light (Büchert et al., 2011), 4 d at 15 ºC in control and treated with 1-methylcyclopropene (Xu et al., 2016), 0, 14, or 21 d at 4 ºC (Hasperué et al., 2015), 12 and 16 d at 5 ºC (Cefola et al., 2015), 30 d at 5 ºC (Tian et al., 2016), and 35 or 70 d at 1 ºC (Pogson et al., 1997). Previous studies found that sucrose content decreased in broccoli stored for 3 d at 20 ºC (King and Morris, 1994; McKenzie et al.,

2004), 5 d at 20 ºC after low intensity light treatments (Lemoine et al., 2008), 4 d at 15 ºC with or without 1-methylcyclopropene treatment (Xu et al., 2016), 7 and 14 d at 1 ºC (Pramanik et al., 2006), 21 d at 4 ºC (Hasperué et al., 2015), and 35 or 70 d at 1 ºC (Pogson et al., 1997). In this study, sucrose content significantly decreased in broccoli stored for 35 d. In contrast, Hasparue et al. (2016) observed an increase in sucrose content in broccoli stored for 35 and 42 d at 5 ºC. In this study both glucose and fructose contents decreased in broccoli stored for 35 d. Similarly, glucose and fructose contents decreased in broccoli when stored for 4 d at 20 ºC after low intensity light treatments (Favre et al., 2018; Lemoine et al., 2008), 5 d at 25 ºC under hydrogen sulfide fumigation (Li et al., 2014), 5 d at 22 ºC under continuous low intensity white light (Büchert et al., 2011), 4 d at 15 ºC with or without 1-methylcyclopropene treatments (Xu et al., 2017), 4 d at 20 ºC with or without sucrose feeding (Xu et al., 2016), 35 or 42 d at 5 ºC (Hasperué et al., 2016), 30 d at 5 ºC (Tian et al., 2016), 0, 14, or 21 d at 4 ºC (Hasperué et al., 2015), and 35 or 70 d at 1 ºC (Pogson et al., 1997). In contrast, some studies reported stable glucose and fructose levels in broccoli stored for, 3 d at 20 ºC in air or controlled atmosphere conditions (McKenzie et al., 2004) and 7 or 14 d at 1 ºC (Pramanik et al., 2006). Sucrose contents significantly decreased at 7 and 14 d, while glucose and fructose contents only significantly decreased at 7 d in storage. This may have been due to increased activity of sucrose degrading enzymes, such as invertase or sucrose synthase, acting on sucrose to produce hexose (glucose and fructose) (Schouten et al., 2016). Hexose sugars can then be oxidized as a source of energy for biosynthetic processes (Li et al., 2017). Results from this study suggest that sucrose was consumed in the formation of glucose and fructose during the first 14 d of storage, which led to greater sucrose losses than either glucose or fructose during this time. Both sucrose and glucose increased at 21 d, but not significantly, while fructose content significantly increased at

21 d. Similarly, both sucrose and glucose contents significantly decreased at 28 d, while fructose content remained stable from 21 to 28 d. After that, sucrose and fructose contents remained stable from 28 to 35 d, while glucose contents significantly increased. Metabolic activity in broccoli decreases as the duration of storage at lower temperatures increases (Coupe et al., 2003). The variation in sugar contents from 21 to 35 d may have been due to the activation or inhibition of different genes controlling metabolic activity in broccoli, leading to changes in individual sugar compounds at different lengths of time during storage. An increase in fructose content at 21 d and increased glucose at 35 d may have a result of cell wall material breakdown, increasing the amount of soluble sugars (Li et al., 2017).

Organic acids, effects of cultivar.

Organic acids play an important role in maintaining the postharvest quality of fruits and vegetables (Ferreres et al., 2007; Vaughan and Geissler, 1997) and contribute to a healthy diet (Ayaz et al., 2006). They are also used as antioxidants or acidulants in many food industries (Cunha et al., 2002; Shui and Leong, 2002) and are important factors for the organoleptic properties in produce (Vale et al., 2015; Vaughan and Geissler, 1997). It is known that organic acid content contributes to flavor in horticultural crops (Davies et. al., 1981). Increased sourness is commonly attributed higher organic acid contents, which often has a negative effect on consumer preference (Casals et al., 2011). Organic acid profiles are dependent on many factors, including species, cultivar, developmental stage, tissue type, growing conditions, and harvesting conditions (Lopez-Bucio et al., 2000; Sousa et al., 2009; Vale et al., 2015).

Citric and malic acids are the main organic acids commonly found in many *Brassica* species (Ayaz et al., 2006; Fernandes et al., 2007; Ferreres et al., 2007; Ferreres et al., 2006; Sousa et al., 2005; Sousa et al., 2009). Broccoli used in this study contained both citric and malic organic

acids. Malic acid was the most abundant organic acid in both ‘Diplomat’ and ‘Arcadia’ cultivars. Similarly, King and Morris (1994) found that malic acid was the most abundant organic acid in ‘Green belt’ broccoli. In contrast, previous studies found that citric acid was the most abundant in ‘Parthenon’ (Zapata et al., 2013) and ‘Calabrese’ (Vale et al., 2015) broccoli cultivars, as well as cabbage (Ferreres et al, 2006) and kale (Ayaz et al., 2006). Whether citric or malic acid is the most abundant organic acid in broccoli may depend on the TCA cycle (Fernie et al., 2004). After glycolysis, hexoses combine with an intermediate to form citrate, which is turned into malate. After that, malate is either consumed to restart the TCA cycle or used to begin hexose synthesis during gluconeogenesis. Schouten et al. (2016) demonstrated that greater hexose breakdown, followed by increased hexose synthesis from the breakdown of malate in gluconeogenesis, results may higher levels of citrate. Thus, higher levels of citric acid may be due to increased hexose turnover.

In this study, cultivar had a significant impact on average total organic acid contents in broccoli. Citric, malic, and total organic acid contents were significantly greater for ‘Arcadia’ than for ‘Diplomat.’ Similarly, previous studies found significantly higher organic acid contents for ‘Marathon’ compared to ‘Samurai’ (Siomos et al., 2004), and ‘Hartland’ compared to ‘Sairin’ (Pramanik et al., 2004). Cultivar effects on organic acids have also been observed in other *Brassica* species, such as pak choi (Kim et al., 2017). Interaction between cultivar and storage time also significantly affected both citric and total organic acid contents in broccoli. Citric and total organic acid contents were significantly greater for ‘Arcadia’ than for ‘Diplomat’ from 0 to 21 d in storage but did not significantly change after that. Both citric and total organic acid contents significantly decreased at 7 and 14 d for ‘Arcadia,’ then continued to decrease, but not significantly. In, contrast, citric and total organic acid contents decreased throughout storage but

did not significantly change for ‘Diplomat.’ The expression of genes controlling organic acid synthesis can change among different plant cultivars (Ayaz et al., 2006). Substrate availability is also a determining factor in the formation of organic acids (Rontein et al., 2003). Sucrose acts as a substrate, and is cleaved into glucose or fructose, which serve as the immediate precursors in organic acid formation (Schouten et al., 2016). Significantly greater organic acid contents in ‘Arcadia’ compared to ‘Diplomat’ broccoli may have been due to increased metabolic activities controlling the conversion of sucrose into hexoses that were then used for the formation of organic acids. This is supported by significantly lower sucrose contents in ‘Arcadia’ compared to ‘Diplomat,’ indicating that greater amounts of sucrose in ‘Arcadia’ may have been consumed for the formation of organic acids. The increased rate of decline in organic acid content during storage for ‘Arcadia’ compared to ‘Diplomat’ may have been due to increased metabolic activity in ‘Arcadia,’ which resulted in a faster rate of organic acid depletion. The less severe decrease in organic acid contents for ‘Diplomat,’ accompanied by significantly greater sucrose contents, compared to the rapid decline in organic acid contents and significantly lower sucrose levels for ‘Arcadia,’ indicates that ‘Diplomat’ broccoli was less metabolically active during storage. As decreased metabolic activity during storage is associated with increased postharvest broccoli quality (King and Morris, 1994), results from this study suggest that ‘Diplomat’ broccoli quality is superior to that of ‘Arcadia.’

Organic acid, effects of cooling/storage method.

Storage temperature is the main factor influencing the rate of deterioration and potential market life of broccoli (Mitchell, 2002; Pramanik et al., 2004). Organic acids are important attributes of quality (King and Morris, 1994), which have an impact on the organoleptic properties as well (Siomos et al., 2004). Storage temperature and length of time have an effect on

the conversion of sugars and acids in plants (Carrari and Fernie, 2006). In the TCA cycle, sucrose is converted into hexoses by hydrolyzing enzymes (Koch, 2004). Hexoses then combine with an intermediate to form citrate. Citrate is converted to malate, and malate is then either consumed to restart the cycle or used for hexose synthesis during gluconeogenesis (Schouten et al., 2016). The rate of metabolism is influenced by storage temperature, which affects the rate of biosynthesis and breakdown of organic acids (Pramanik et al., 2004).

King and Morris (1994) found that organic acid contents decreased and were totally depleted in broccoli stored for 1 d at 20 ºC, while Zapata et al. (2013) found that citric acid content decreased in broccoli stored for 2 and 5 d at 20 ºC, but remained stable from 5 to 8 d. They also found that malic acid content decreased at 2, 5, and 8 d. For this study, broccoli was either not precooled and stored at 6 ºC or cooled with an ice slurry and stored at 0 ºC in ice. Results from this study indicated that storage temperature did not have a significant impact on organic acid contents. However, storage time significantly affected both citric and malic acid contents in broccoli. Citric, malic, and total organic acid contents significantly decreased at 7 and 14 d in storage. In contrast, Pramanik et al. (2006) found that malic and citric acid contents remained stable in broccoli stored for 7 and 14 d at 1 ºC, but decreased when transferred to storage at 20 ºC.

Sucrose is a substrate, while fructose and glucose are intermediates consumed in the formation of organic acids (Kays, 1991; Pramanik et al., 2004). A rapid decline was observed for broccoli sugar contents, as well as organic acid contents, during the first 14 d in storage. As sugars and organic acids are both consumed in the formation of cellular materials, the rapid decline in both sugars and organic acids is most likely due to increased metabolic activity as a result of harvesting stress (Tietz and Wild, 1991). Although organic acid formation may have

been occurring during this time, both organic acids and sugars were also probably being oxidized (King and Morris et al., 1994). Thus, the rate of organic acid oxidation may have been too great for the rate of organic acid synthesis to compensate for this loss, resulting in a net loss of both sugar and organic acid contents. As a result of substrate depletion, long term storage of fruits and vegetables leads to a reduction in metabolism (Toivonen et al., 1997). Therefore, the rapid decrease and subsequent stabilization of organic acid contents in broccoli during storage may have been caused by metabolic changes due to substrate, i.e. sugar, depletion (Pramanik et al., 2006).

Sugar/organic acid ratio, effects of cultivar.

Flavor is of one of the main factors affecting quality and consumer acceptability of fruits and vegetables (Montero et al., 1996). Often, the balance between sweetness, due to sugar contents, and sourness, due to organic acid contents, is an important determining factor of quality in many horticultural crops (Shamaila et al., 1992; Shaw et al., 1990). Sweetness and bitterness are key attributes influencing flavor in broccoli (Pellegrino et al., 2019). Bitterness is often attributed to glucosinolate content in broccoli, while sweetness is attributed to sugar content, and it is thought that an increase in sugar levels may help to mask the bitterness associated with glucosinolate contents (Bhandari and Kwak 2015b; Schohnof et al., 2004). However, there is no direct relationship between bitterness with glucosinolate, nor sweetness with sugar, content alone (Baik et al., 2006; Bell et al., 2017). The perception of bitterness is commonly confused with sourness among untrained panelists (Gregson and Baker, 1973), and organic acids contribute to the bitterness in other *Brassica* species (Fukuda et al., 2016). Thus, it may be that increased sourness, as a result of increased organic acid contents, plays a role in the perception of the bitter taste associated with broccoli.

Cultivar is one of the determining factors of produce quality (Lee and Kader, 2000), and It has been demonstrated that cultivar has a significant impact on the sugar/acid ratio in many fruits, including apple (Hecke et al., 2006), raspberry (Shamaila et al., 1993), strawberry (Pineli et al., 2011), cherry (Dever et al., 1996), pomegranate (Al-Said et al., 2009), pear (Chen et al., 2007), pineapple (Bartolome et al., 1995), and tomato (Mohammed et al., 1999) fruits. However, Siomos et al. (2004) found no significant difference in the sugar/organic acid ratio among 'Marathon' and 'Samurai' broccoli cultivars. In contrast, results from this study indicate that sugar/acid ratios in broccoli are significantly affected by cultivar. The sugar/acid rate was significantly greater for 'Diplomat' than for 'Arcadia.' To our knowledge, results from this study are the first to confirm that cultivar does have a significant impact on the sugar/acid ratio in broccoli. The expression of genes involved in the biosynthesis and consumption of sugars and organic acids during postharvest storage is often determined by cultivar (Bhandari and Kwak, 2015a; Rosa et al., 2001; Quick and Schaffer, 1996). Thus, differences in the sugar/organic acid ratio among cultivars may be due to differences in the expression of genes involved in sugar and organic acid metabolism (Zhang et al., 2020). Because increasing the sugar/organic acid ratio increases the sweetness and reduces the sourness, a higher sugar/organic acid ratio leads to improved flavor and consumer acceptability in fruits, such as tomatoes (Malundo et al., 1995). Results from this study suggest that a higher sugar/acid ratio may result in higher quality, in terms of sensory perception, for 'Diplomat' than for 'Arcadia' broccoli.

Sugar/organic acid ratio, effects of cooling/storage method.

The sugar/acid ratio is often used as in index of quality and consumer acceptability of fruits (Paull, 1999; Siomos et al., 2004), and the relationship between the sugar/acid ratio and produce quality has been extensively studied for many horticultural crops (Beckles 2012; Casals et al.,

2011; Charles et al., 2016). Previous studies have investigated the impact of storage conditions on sugars and organic acids in broccoli (King and Morris, 1994; Pramanik et al., 2006; Zapata et al., 2013), and the effect of growing conditions on the sugar/acid ratio has been studied for broccoli (Siomos et al., 2004) and other *Brassica* plants (Qui et at., 2013). However, to our knowledge, the effect of cooling/storage method on the ratio of sugars to organic acids has not been previously studied for broccoli.

The sugar/acid ratio during storage significantly decreased at 7d, then significantly increased at 21 d. After that, the sugar/acid ratio significantly decreased at 28 d and slightly increased at 35 d, but not significantly. Organic acid contents significantly decreased at 7 and 14 d, then remained stable. An increase in the sugar/acid ratio at 21 d can be attributed to increased sugar contents, mainly fructose, at 21 d in storage. This increase in sugar/acid ratio may have been a result of a decline in metabolic activity, or the breakdown of cellular wall components as broccoli tissues were deteriorated, resulting in increased sugar contents at 21 d but no change in organic acid contents (Lemoine et al., 2007).

Although cooling/storage temperature alone did not have a significant impact on sugar/acid ratios in broccoli, the interaction between cultivar and storage temperature on the sugar/acid ratio was significant. For ‘Diplomat,’ storage at 6 ºC resulted in a significantly higher sugar/acid ratio compared to storage at 0 ºC, while storage temperature had no effect on ‘Arcadia.’ This may be due to a difference in the expression of genes controlling the metabolic activity in response to different temperatures (Escalona et al., 2006). The enhanced expression of genes controlling sucrose hydrolyzing enzyme activity occurs during postharvest senescence of broccoli (Coupe et al., 2003). The higher sugar/acid ratio for ‘Diplomat’ stored at 6 ºC compared to 0 ºC is due to lower fructose and glucose contents for ‘Diplomat’ stored at 0 ºC, which may have been caused

by a higher conversion rate of sucrose into glucose and fructose as a result of increased invertase or synthase activities (Toivonen et al., 1997). This may have occurred because of increased stress during precooling and/or storage. The increased storage temperature for 'Diplomat' broccoli may have led to increased expression of genes controlling sucrose hydrolysis, as a response to greater stress experienced after harvest compared to broccoli cooled with an ice slurry (Coupe et al., 2003). Interestingly, this effect was not observed in 'Arcadia,' which had roughly equal sugar/acid ratios for broccoli stored at 0 ºC compared to 6 ºC. This may be due to genetic differences among cultivars, resulting in different patterns of gene expression in response to harvesting stress (Page et al., 2001). For 'Arcadia,' the sugar/acid ratio was significantly lower than it was for 'Diplomat,' due to significantly greater organic acid levels. Sucrose levels were also significantly lower for 'Arcadia' than for 'Diplomat.' This may have been a result of increased metabolic activity for 'Arcadia,' which converted sucrose into fructose and glucose, then glucose/fructose into organic acids at a faster rate than 'Diplomat' (Schouten et al., 2016). Although the sugar/acid ratio was roughly equal for 'Arcadia' stored at either temperature, the sucrose content was significantly lower for 'Arcadia stored at 6 ºC compared to 0 ºC, while sucrose content was not significantly different for 'Diplomat' stored at either temperature. These results indicate that 'Diplomat' broccoli may have a greater ability to regulate metabolic activity in response to different cooling/storage conditions, suggesting that 'Diplomat' is superior to 'Arcadia' in terms of maintaining postharvest quality.

Although the compositional quality of 'Diplomat' broccoli may have been improved by storage at lower temperatures, the significantly lower sugar/acid ratio for broccoli stored at 0 ºC may have led to a decrease in the sensory perception of quality. Thus, investigating the impact of sugar/acid ratios on the sensory perception of quality in broccoli would help to determine the

appropriate storage conditions for a balance between the maintenance of compositional and sensory quality of broccoli during postharvest storage.

Conclusion

Results from this study show that cultivar, storage temperature, and storage time significantly influence postharvest broccoli quality. Cultivar and storage time were the main determinants of sugar content, organic acid content, and the sugar/acid ratio. However, sucrose content and the sugar/acid ratio in broccoli was dependent on storage temperature as well.

For sugars, only sucrose was significantly affected by cultivar alone, while interactions of cultivar and storage time significantly affected fructose alone. The variation in fructose content among cultivars may have been due to the expression of different genes controlling the balance between fructose synthesis and degradation during postharvest storage of broccoli (Rosa et al., 2001). Because sucrose is the main energy source consumed during respiration of plants, sucrose losses are thought to mirror the rate of senescence (King and Morris, 1994). As a signaling molecule, the rapid loss of sucrose after harvest is thought control gene expression during senescence. Sucrose decline is correlated with increased invertase activity, thereby increasing the rate of irreversible sucrose hydrolysis into glucose and fructose (Eason et al., 2007). Thus, significantly greater sucrose content suggests superior postharvest quality for ‘Diplomat’ compared to ‘Arcadia’ broccoli cultivars.

The sugar/acid ratio significantly decreased from 0 to 7 d, began to increase from 7 to 14 d, then significantly increased from 14 to 21 d. After that, the sugar/acid ratio significantly decreased from 21 to 28 d and slightly increased from 28 to 35 d, but not significantly. Variation in the sugar/acid ratio from 14 to 35 d can be attributed to variations in sugar contents at this time. Both sugar and organic acid contents significantly decreased during storage. All sugars

(sucrose, glucose, and fructose) significantly decreased at 7 d but only sucrose content significantly decreased from 7 to 14 d. Fructose content alone significantly increased from 14 to 21 d, while sucrose and glucose increased slightly, but not significantly. Fructose content remained stable from 21 to 35 d, while both sucrose and glucose significantly decreased from 21 to 28 d. Glucose content significantly increased from 28 to 35 d, while sucrose increased, but not significantly. Variation in individual sugar contents, particularly after 21 d in storage, may have been due to the breakdown of cell wall materials, resulting in higher sugar contents for different compounds at different lengths of storage. However, as respiration is known to decline during cold storage (Toivonen et al., 1997), this variation may have been due to alterations in metabolism as storage time increased. Organic acid contents significantly decreased from 0 to 7 and 7 to 14 d, then remained stable from 14 to 35 d. Rapidly declining organic acid contents during the first 14 d of storage suggest that they were being consumed as metabolism substrates during this time. The subsequent stabilization after 14 d suggests that metabolic activity had been reduced as a result of substrate depletion.

There was no significant difference in glucose or fructose contents, which are the main contributors to total sugar content, among cultivars. The higher sugar/acid ratio for ‘Diplomat’ is due to lower organic acid contents compared to ‘Arcadia.’ Arcadia had significantly greater organic acid levels from 0 to 28 d in storage. This may have been a result of different gene expression rates during development, indicated by initially higher organic acid contents after harvest, or it may be due to a difference in metabolic pathways during storage. Although storage temperature did not affect the sugar/acid ratio for ‘Arcadia,’ significantly lower sucrose content was observed for ‘Arcadia’ broccoli stored at 6 ºC compared to 0 ºC. However, sucrose content for ‘Diplomat’ was unaffected by temperature. This suggests that metabolic activity in ‘Arcadia’

was significantly greater when stored at 6 ºC. However, equal sucrose levels for ‘Diplomat’ stored at either temperature indicates that the difference in storage temperature on sucrose consumption was less severe for ‘Diplomat’ compared to ‘Arcadia.’ This may have been due to decreased invertase or synthase activities as a result of lower storage temperature, indicating decreased metabolic activity. As sucrose is hydrolyzed into hexoses by invertase and synthase enzymes (Schouten et al., 2016), a lack of invertase activity may have been responsible for the lower glucose and fructose levels in ‘Diplomat’ stored at 0 ºC compared to 6 ºC. These results suggest that ‘Diplomat’ broccoli may have a greater ability to regulate and optimize sugar metabolism in response to different storage temperatures during postharvest storage.

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Appendix

Table 1. Analysis of variance results for sugar contents in broccoli for 'Diplomat' and 'Arcadia,' not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

Source of Variance	Sucrose	Glucose	Fructose	Total Sugars
Cultivar (C)	* [z]	NS	NS	NS
Treatment (T)	*	NS	NS	NS
Storage Time (S)	***	***	***	***
C x T	NS	NS	NS	NS
T x S	NS	NS	NS	NS
C x S	NS	NS	*	NS
C x T x S	NS	NS	NS	NS

[z] Significance is denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 2. Analysis of variance results for organic acid contents in broccoli for 'Diplomat' and 'Arcadia,' not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

Source of Variance	Malic Acid	Citric Acid	Total Organic Acids
Cultivar (C)	*** [z]	***	***
Treatment (T)	NS	NS	NS
Storage Time (S)	***	***	***
C x T	NS	NS	NS
T x S	NS	NS	NS
C x S	NS	**	*
C x T x S	NS	NS	NS

[z] Significance is denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 3. Analysis of variance results for the sugar/acid ratio in broccoli for 'Diplomat' compared with 'Arcadia,' not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

Source of Variance	Sugar/Acid Ratio
Cultivar (C)	*** [z]
Treatment (T)	NS
Storage Time (S)	**
C x T	*
T x S	NS
C x S	NS
C x T x S	NS

[z] Significance is denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 4. Average sugar contents in broccoli ($mg \cdot g^{-1}$ dry mass), across time, for 'Diplomat' and 'Arcadia,' and broccoli that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice.

Cultivar (C)	Storage Temperature (T)	Sucrose	Glucose	Fructose	Total
Diplomat	6 ºC	52.06 a [zy]	178.19 a	127.95 a	358.20 a
	0 ºC	58.91 a	145.31 b	107.84 ab	312.06 ab
Arcadia	6 ºC	32.65 b	140.15 b	105.36 ab	278.16 b
	0 ºC	51.12 a	149.11 ab	104.04 b	304.27 ab
Interaction Effects		NS [x]	NS	NS	NS

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual sugar, letters beside means for one cultivar and storage temperature that are not different from letters beside means for other cultivars and storage temperatures, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between cultivar and storage temperature on sugar contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 5. Average sugar contents in broccoli ($mg \cdot g^{-1}$ dry mass), stored at both temperatures, for 'Diplomat' compared with 'Arcadia,' and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Cultivar	Storage Time (d)	Sucrose	Glucose	Fructose	Total
Diplomat	0	134.95 a [zy]	280.83 a	203.25 a	619.03 a
	7	60.20 b	144.38 bc	79.47 de	284.06 bc
	14	39.41 bcd	132.34 bcd	90.12 de	261.87 bcd
	21	47.29 bc	180.02 b	136.01 bc	363.32 b
	28	22.40 d	115.63 cd	110.76 cde	248.78 cd
	35	28.66 cd	117.28 cd	87.77 de	233.71 cd
Arcadia	0	113.18 a	251.95 a	173.38 ab	538.52 a
	7	54.06 b	138.52 bc	97.94 cde	290.52 bc
	14	23.20 d	110.48 cd	76.60 de	210.29 cd
	21	28.73 cd	131.97 bcd	88.91 de	249.62 cd
	28	16.37 d	77.65 d	75.54 e	169.55 d
	35	15.75 d	157.21 bc	115.83 cd	288.80 bc
Interaction Effects		NS [x]	NS	*	NS

Table 5. Continued.

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual sugar, letters beside means for one cultivar and storage time that are not different from means for letters beside other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between cultivar and storage time on sugar contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 6. Average sugar contents in broccoli ($mg \cdot g^{-1}$ dry mass), for both cultivars, that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Storage Temperature	Storage Time (d)	Sucrose	Glucose	Fructose	Total
6 ºC	0	109.66 b [zy]	262.03 a	186.83 a	558.53 a
	7	41.75 de	134.45 bcde	73.86 cd	250.06 bc
	14	31.68 de	151.25 bc	103.92 bc	286.86 bc
	21	28.30 de	146.68 bcd	113.37 b	288.35 bc
	28	18.55 e	107.57 cde	105.70 bc	231.80 bc
	35	24.20 de	153.02 bc	116.27 b	293.49 bc
0 ºC	0	138.47 a	270.75 a	189.80 a	599.02 a
	7	72.51 c	148.46 bc	103.55 bc	324.52 b
	14	30.93 de	91.56 de	62.80 d	185.30 c
	21	47.72 d	165.31 b	111.55 bc	324.58 b
	28	20.22 e	85.70 e	80.62 bcd	186.54 c
	35	20.21 e	121.48 bcde	87.30 bcd	229.02 bc
Interaction Effects		NS [x]	NS	NS	NS

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 6. Continued.

[y]For each individual sugar, letters beside means for one storage temperature and storage time that are not different from means for letters beside other storage temperatures and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between storage temperature and storage time on sugar contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 7. Average sugar contents in broccoli ($mg \cdot g^{-1}$ dry mass), for 'Diplomat' compared with 'Arcadia,' broccoli that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Cultivar	Storage Temperature	Storage Time (d)	Sucrose	Glucose	Fructose	Total
Diplomat	6 ºC	0	118.33 ab [zy]	286.17 a	204.82 a	609.32 a
		7	49.97 def	141.44 defg	56.08 h	247.49 cdef
		14	45.36 defg	178.00 cde	120.20 cdef	343.57 cd
		21	43.17 defg	195.81 bcd	160.63 abcd	399.61 bc
		28	20.47 efg	126.47 defg	120.74 cdef	267.68 cdef
		35	35.07 efg	141.22 defg	105.22 defgh	281.52 cdef
	0 ºC	0	151.58 a	275.50 ab	201.67 a	628.74 a
		7	70.43 cd	147.32 def	102.87 efgh	320.62 cde
		14	33.46 efg	86.67 fg	60.03 gh	180.16 ef
		21	51.41 de	164.22 cdef	111.40 defgh	327.03 cde
		28	24.32 efg	104.79 efg	100.78 efgh	229.88 def

Table 7. Continued.

Diplomat	0 ºC	35	22.25 efg	93.34 fg	70.32 fgh	185.91 def
Arcadia	6 ºC	0	100.99 bc	237.90 abc	168.85 abc	507.74 ab
		7	33.53 efg	127.45 defg	91.65 efgh	252.62 cdef
		14	18.00 efg	124.50 defg	87.64 efgh	230.15 def
		21	13.43 g	97.54 fg	66.12 fgh	177.09 ef
		28	16.62 fg	88.67 fg	90.62 efgh	195.91 def
		35	13.33 g	164.82 cdef	127.31 bcde	305.46 cde
	0 ºC	0	125.37 ab	266.00 ab	177.92 ab	569.30 a
		7	74.60 cd	149.59 def	104.22 efgh	328.41 cde
		14	28.41 efg	96.46 fg	65.57 fgh	190.43 def
		21	44.04 defg	166.41 cdef	111.70 defg	322.14 cde
		28	16.12 fg	66.62 g	60.46 gh	143.19 f
		35	18.18 efg	149.61 def	104.36 efgh	272.14 cdef
Interaction Effects			NS [x]	NS	NS	NS

Table 7. Continued.

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual sugar, letters beside means for one cultivar, storage temperature, and storage time that are not different from letters beside means for other cultivars, storage temperatures, and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects among cultivar, storage temperature, and storage time on sugar contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 8. Average organic acid contents in broccoli ($mg \cdot g^{-1}$ dry mass), across time, for 'Diplomat' compared with 'Arcadia,' and broccoli that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice.

Cultivar	Storage Temperature	Malic	Citric	Total
Diplomat	6 ºC	4.69 b [zy]	3.80 b	8.49 b
	0 ºC	5.30 b	4.42 b	9.71 b
Arcadia	6 ºC	9.07 a	7.66 a	16.73 a
	0 ºC	7.56 a	6.95 a	14.51 a
Interaction Effects		NS [x]	NS	NS

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual organic acid, letters beside means for one cultivar and storage temperature that are not different from letters beside means for other cultivars and storage temperatures, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between cultivar and storage temperature on organic acid contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 9. Average organic acid contents in broccoli (mg·g^{-1} dry mass), stored at both temperatures, for 'Diplomat' compared with 'Arcadia,' and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Cultivar	Storage Time (d)	Malic	Citric	Total
Diplomat	0	7.20 cd [zy]	6.48 c	13.69 c
	7	6.51 cde	5.94 cd	12.45 cd
	14	4.45 def	3.67 def	8.12 de
	21	3.01 f	2.31 f	5.33 e
	28	4.26 ef	3.04 ef	7.30 de
	35	4.52 def	3.20 ef	7.72 de
Arcadia	0	14.07 a	14.39 a	28.46 a
	7	10.03 b	9.80 b	19.83 b
	14	7.54 bc	6.42 c	13.97 c
	21	6.58 cde	5.25 cde	11.83 cd
	28	6.24 cde	4.18 cdef	10.43 cde
	35	5.43 cdef	3.78 cdef	9.21 cde
Interaction Effects		NS [x]	**	*

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 9. Continued.

[y]For each individual organic acid, letters beside means for one cultivar and storage time that are not different from letters beside means for other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between cultivar and storage time on organic acid contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 10. Average organic acid contents in broccoli ($mg \cdot g^{-1}$ dry mass), for both cultivars, that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Storage Temperature	Storage Time (d)	Malic	Citric	Total
6 ºC	0	10.74 a [zy]	10.50 a	21.24 a
	7	8.62 abc	8.16 ab	16.78 ab
	14	6.20 cde	4.97 cd	11.17 cd
	21	5.04 e	3.66 d	8.70 d
	28	5.39 de	3.47 d	8.86 d
	35	5.29 de	3.64 d	8.92 d
0 ºC	0	10.54 ab	10.37 a	20.91 ab
	7	7.92 bcd	7.59 bc	15.51 bc
	14	5.80 de	5.12 cd	10.92 cd
	21	4.55 e	3.91 d	8.46 d
	28	5.11 e	3.76 d	8.87 d
	35	4.66 e	3.34 d	8.01 d
Interaction Effects		NS [x]	NS	NS

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 10. Continued.

[y]For each individual organic acid, letters beside means for one storage temperature and storage time that are not different from letters beside means for other storage temperatures and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between storage temperature and storage time on organic acid contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 11. Average organic acid contents in broccoli (mg·g^{-1} dry mass), for 'Diplomat' compared with 'Arcadia,' broccoli that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Cultivar	Storage Temperature	Storage Time (d)	Malic	Citric	Total
Diplomat	6 ºC	0	5.79 defghi[zy]	5.45 efg	11.25 defg
		7	6.59 defghi	6.13 defg	12.72 defg
		14	4.99 efghi	3.91 efgh	8.89 efgh
		21	2.09 i	1.19 h	3.28 h
		28	4.88 efghi	3.29 fgh	8.17 fgh
		35	3.81 ghi	2.83 gh	6.65 gh
	0 ºC	0	8.62 bcde	7.52 cde	16.14 cde
		7	6.43 defgh	5.76 defg	12.19 defg
		14	3.92 ghi	3.43 fgh	7.35 fgh
		21	3.94 ghi	3.44 fgh	7.38 fgh
		28	3.64 hi	2.78 gh	6.42 gh
		35	5.23 efghi	3.57 fgh	8.80 efgh
Arcadia	6 ºC	0	15.69 a	15.55 a	31.24 a
		7	10.65 bc	10.19 bc	20.83 bc
		14	7.41 cdefgh	6.04 defg	13.45 cdefg
		21	7.99 cdef	6.12 defg	14.11 cdefg
		28	5.91 defghi	3.65 fgh	9.55 efgh

Table 11. Continued.

		35	6.76 cdefgh	4.44 efgh	11.20 defg
Arcadia	0 ºC	0	12.45 ab	13.23 ab	25.68 ab
		7	9.41 bcd	9.42 bcd	18.84 bcd
		14	7.68 cdefg	6.81 cdef	14.49 cdef
		21	5.16 efghi	4.39 efgh	9.55 efgh
		28	6.58 defgh	4.73 efgh	11.31 defg
		35	4.10 fghi	3.12 fgh	7.21 fgh
Interaction Effects			NS [x]	NS	NS

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual organic acid, letters beside means for one cultivar, storage temperature, and storage time that are not different from letters beside means for other cultivars, storage temperatures, and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects among cultivar, storage temperature, and storage time organic acid contents are denoted by NS, *, **, ***: Non-significant or significant at $p \leq$ 0.05, 0.01, and 0.001, respectively

Table 12. Average sugar/acid ratio in broccoli ($mg \cdot g^{-1}$ dry mass), stored at both temperatures, for ‘Diplomat’ compared with ‘Arcadia,’ and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Cultivar	Storage Time (d)	Sugar/Acid Ratio
Diplomat	0	52.13 a [zy]
	7	22.72 bc
	14	35.22 b
	21	58.63 a
	28	35.74 b
	35	27.48 bc
Arcadia	0	21.07 bc
	7	16.51 c
	14	16.30 c
	21	27.40 bc
	28	18.06 c
	35	25.39 bc
Interaction Effects		NS [x]

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 12. Continued.

[y]Letters beside sugar/acid means for one cultivar and storage time that are not different from letters beside means for other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between cultivar and storage time are denoted by NS, *, **, ***: Non-significant or significant at $p \leq v0.05$, 0.01, and 0.001, respectively.

Table 13. Average organic acid contents in broccoli ($mg \cdot g^{-1}$ dry mass), for both cultivars, that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Storage Temperature	Storage Time (d)	Sugar/Acid Ratio
6 ºC	0	41.72 ab [zy]
	7	15.79 d
	14	28.54 abcd
	21	42.81 ab
	28	26.57 bcd
	35	30.65 abcd
0 ºC	0	31.48 abc
	7	23.44 cd
	14	22.97 cd
	21	43.21 a
	28	27.20 bcd
	35	22.22 cd
Interaction Effects		NS [x]

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 13. Continued.

[y]Letters beside sugar/acid means for one storage temperature and storage time that are not different from letters beside means for other storage temperatures and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between storage temperature and storage time on the sugar/acid ratio are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 14. Average sugar/acid ratio in broccoli (mg·g^{-1} dry mass), for 'Diplomat' compared with 'Arcadia,' broccoli that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Cultivar	Storage Temperature	Storage Time (d)	Sugar/Acid Ratio
Diplomat	6 ºC	0	63.38 ab [zy]
		7	19.28 efg
		14	38.56 cdef
		21	72.09 a
		28	32.51 cdefg
		35	33.98 cdefg
	0 ºC	0	40.88 cde
		7	26.26 cdefg
		14	31.88 cdefg
		21	45.17 bc
		28	38.97 cdef
		35	20.99 defg
Arcadia	6 ºC	0	20.06 defg
		7	12.40 g
		14	18.53 fg
		21	13.53 g
		28	20.63 defg
		35	27.32 cdefg

Table 14. Continued.

0 ºC	0	22.08 defg
	7	20.62 defg
	14	14.07 g
	21	41.26 cd
	28	15.44 g
	35	23.46 cdefg
Interaction Effects		NS [x]

[z]Means are the average of three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]Letters beside sugar/acid means for one cultivar, storage temperature, and storage time that are not different from letters beside means for other cultivars, storage temperatures, storage times are not significantly different by the LSD test ($\alpha = 0.05$).

[x]Significance of interaction effects between cultivar, storage temperature, and storage time are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

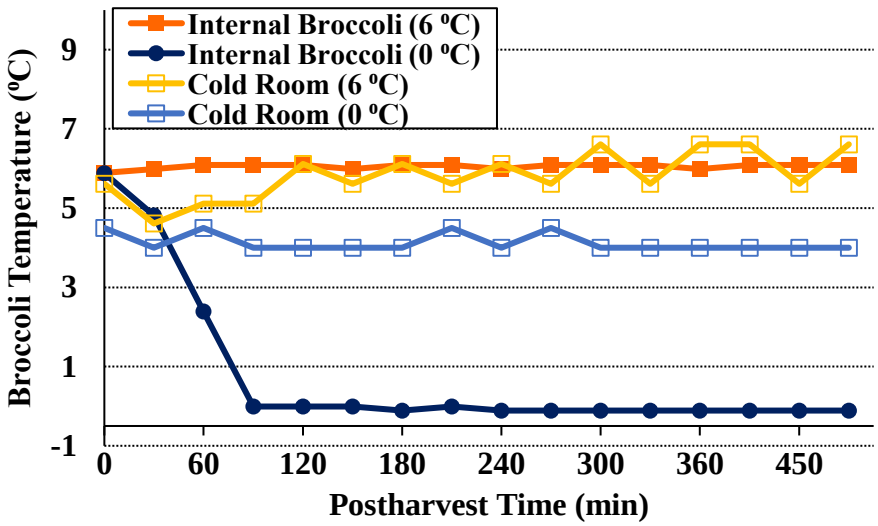

Fig. 1. Mean internal broccoli temperature for broccoli cooled with top icing and stored 6 ºC, and broccoli cooled with an ice slurry and stored at 0 ºC. Postharvest broccoli temperatures were recorded every 30 minutes during field cooling and transportation to cold room storage. Means are the average of three replications per cultivar and storage temperature combination.

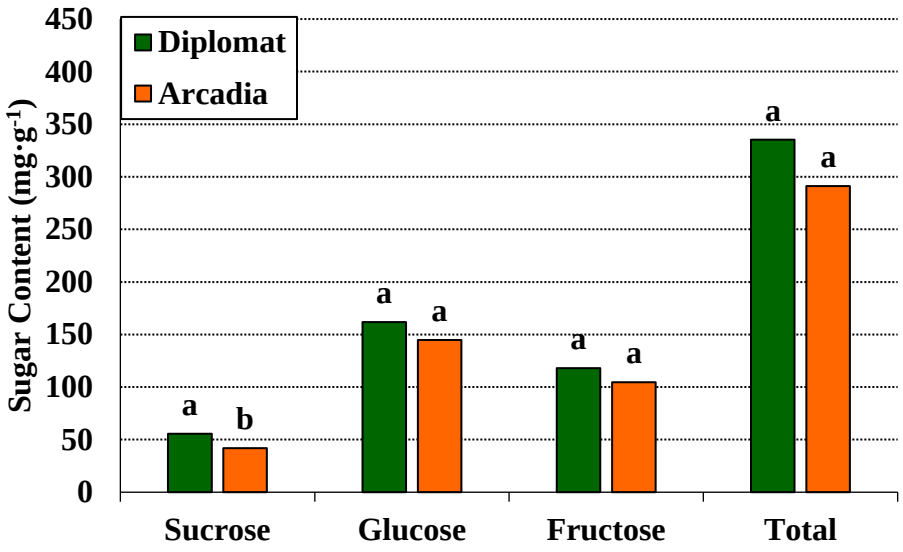

Fig. 2. Average sugar contents in broccoli, across time for both storage temperatures, for ‘Diplomat’ compared with ‘Arcadia’ broccoli. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above an individual sugar for one cultivar that are not different from letters above that sugar the other cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

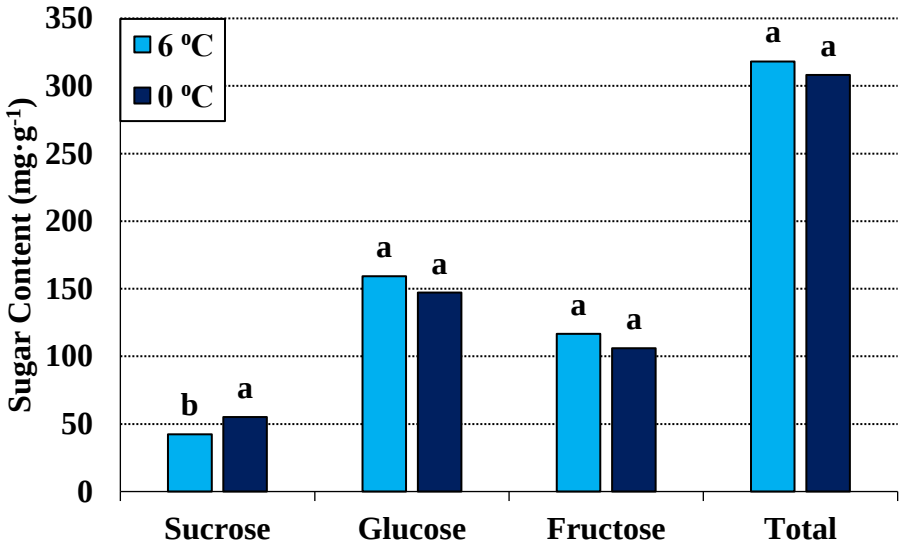

Fig. 3. Average sugar contents in broccoli, across time for both cultivars, that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above an individual sugar for one storage temperature that are not different from letters above that sugar for the other storage temperature, are not significantly different by the LSD test ($\alpha = 0.05$).

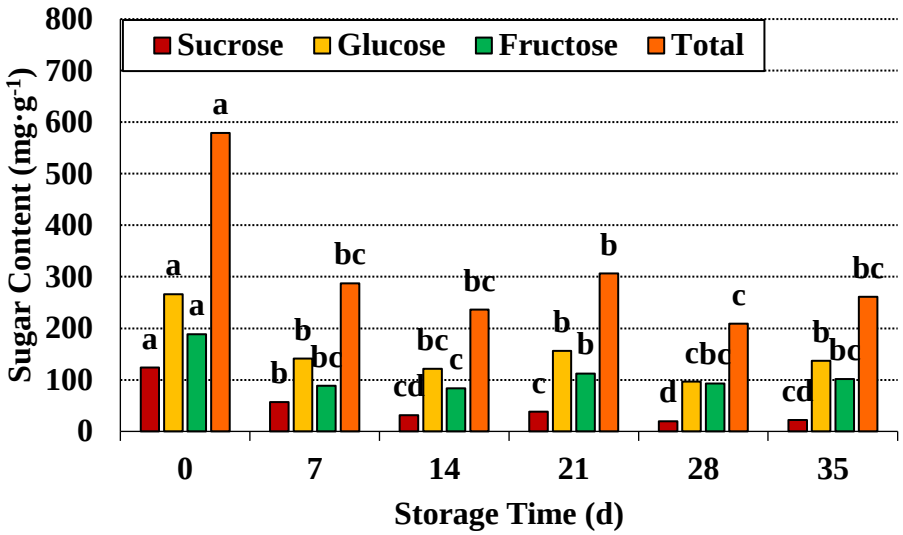

Fig. 4. Sugar contents in broccoli, for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination. Letters above an individual sugar for one storage time that are not different from letters above that sugar for other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

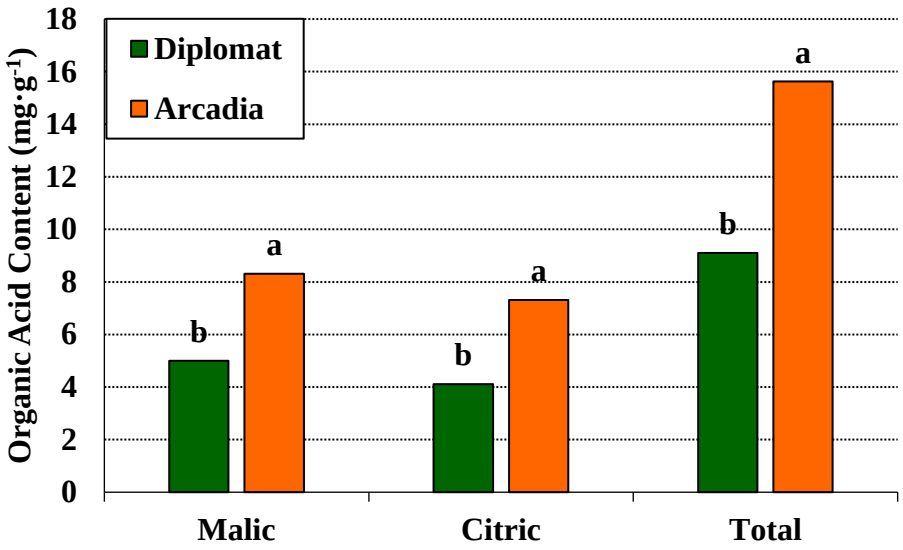

Fig. 5. Average organic acid contents in broccoli, across time for both storage temperatures, for 'Diplomat' compared with 'Arcadia.' Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above an individual organic acid for one cultivar that are not different from letters above that organic acid for the other cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

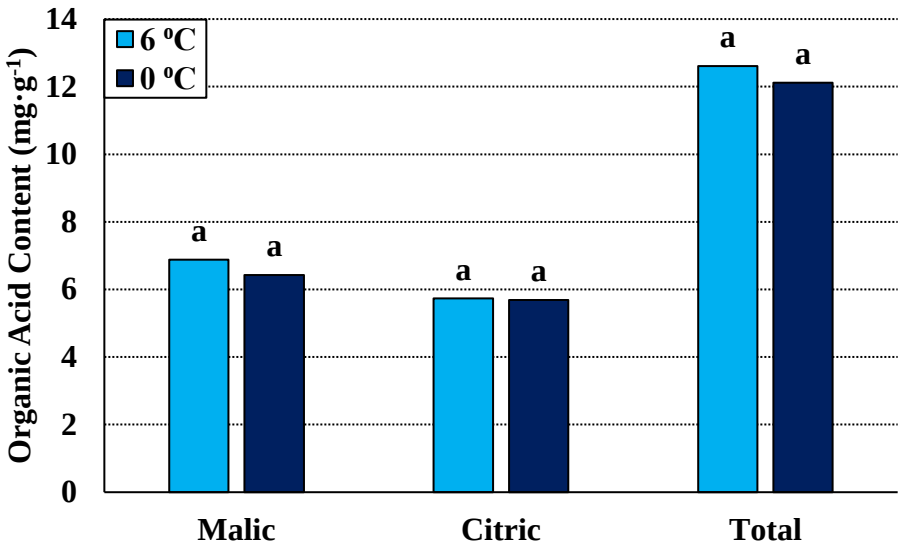

Fig. 6. Average organic acid contents in broccoli, across time for both cultivars, that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above an individual organic acid for one storage temperature that are not different from letters above that organic acid for the other storage temperature, are not significantly different by the LSD test ($\alpha = 0.05$).

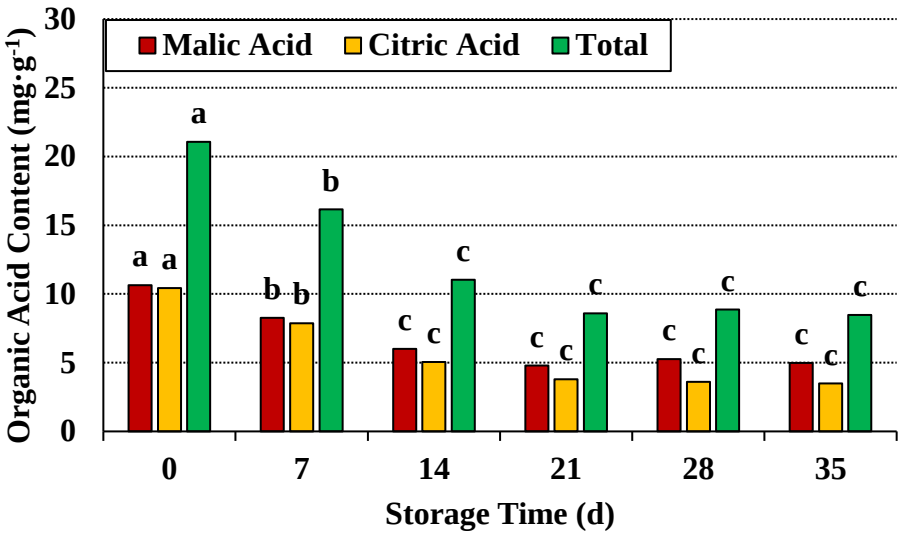

Fig. 7. Average organic acid contents in broccoli, for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination. Letters above an individual organic acid for one storage time that are not different from letters above that organic acid for other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

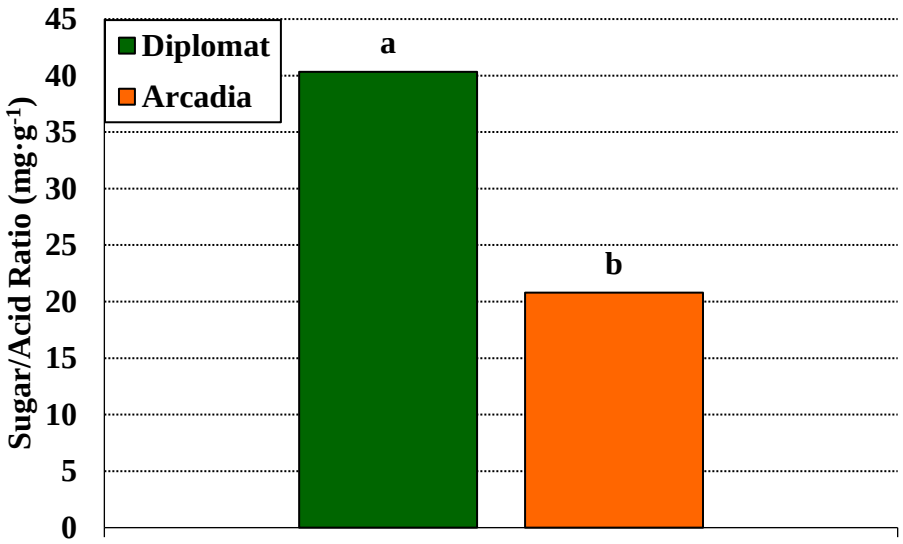

Fig. 8. Average sugar/acid ratio in broccoli, across time for both storage temperatures, for ‘Diplomat’ compared with ‘Arcadia.’ Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one cultivar that are not different from letters above another cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

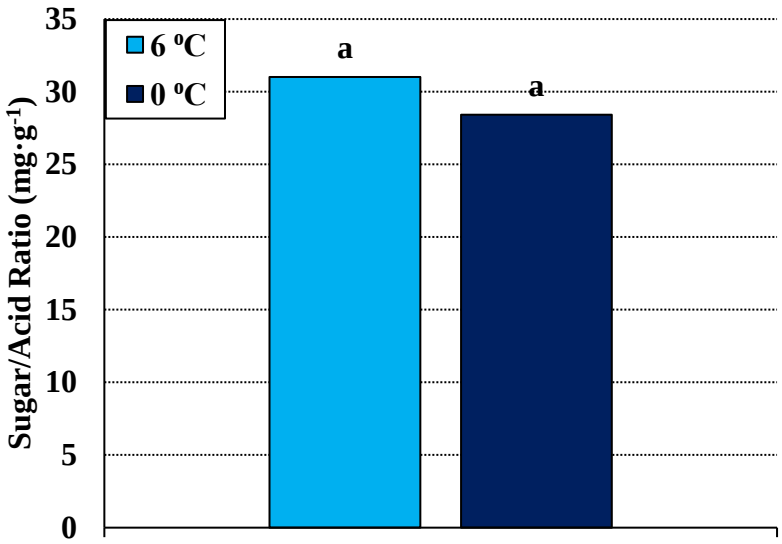

Fig. 9. Average sugar/acid ratio in broccoli, across time for both cultivars, that was not precooled and stored at 5 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one storage temperature that are not different from letters above the other storage temperature, are not significantly different by the LSD test ($\alpha = 0.05$).

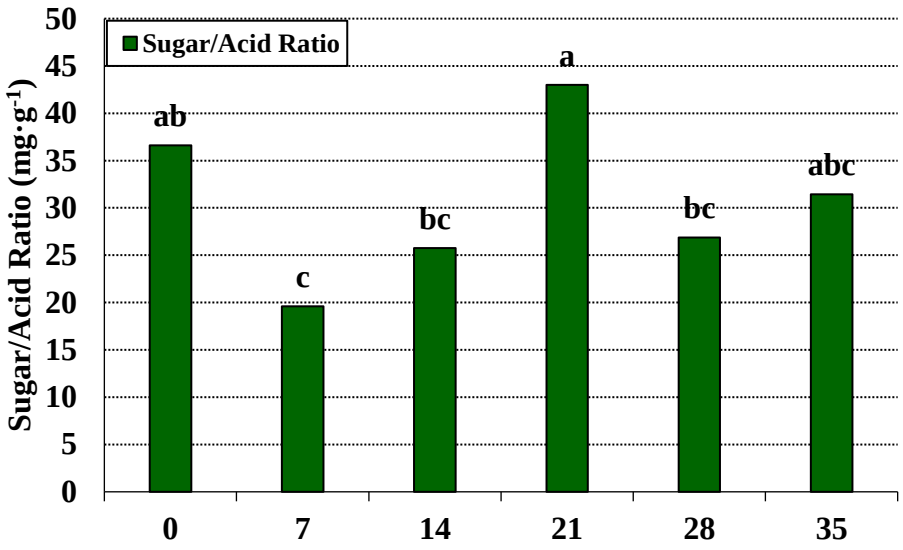

Fig. 10. Average sugar/acid ratio in broccoli, for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one storage time that are not different from letters above other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

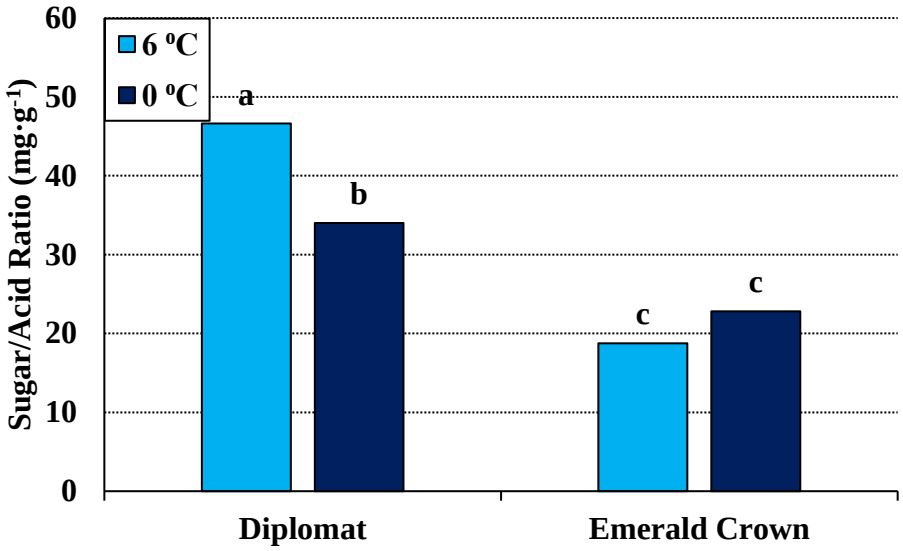

Figure 11. Average sugar/acid ratio in broccoli, across time, for 'Diplomat' compared with 'Arcadia,' and broccoli that was not precooled and stored at 6 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice. Means are the average three replications, two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Interaction effects between cultivar and storage temperature were significant ($P \leq 0.05$). Letters above one cultivar and storage temperature that are not different from letters above other cultivars and storage temperatures, are not significantly different by the LSD test ($\alpha = 0.05$).

CHAPTER 2: EFFECTS OF COOLING AND POSTHARVEST STORAGE METHODS ON GLUCOSINOLATES AND VOLATILES IN BROCCOLI

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Additional index words. Brassica oleracea Italica group, pre-cooling, temperature, chemical properties

Abstract

Broccoli (*Brassica oleracea* L. var. *italica*) is a cool-weather vegetable that is grown for its edible flowering heads and stalks. Glucosinolates and volatiles affect the nutritional, flavor, and aroma characteristics associated with broccoli quality. Cooling and postharvest storage conditions affect the quality of broccoli by altering the levels of glucosinolate and volatile contents. Changes in glucosinolate and volatile contents were investigated for two cultivars ('BH053' and 'Emerald Crown'), two temperature treatments (precooled with top icing and stored at 7 ºC, and precooled with an ice slurry and stored at 0 ºC in ice), and six different days in storage (0, 7, 14, 21, 28, and 35 days). Cultivar and storage temperature significantly affected the total glucosinolate content in broccoli. 'Emerald Crown' had significantly higher levels of indole and total glucosinolates than 'BH053.' Broccoli stored at 0 ºC had significantly higher levels of aliphatic, aromatic, and total glucosinolates compared to broccoli stored at 7 ºC. 'Emerald Crown' had significantly higher levels of glucoprogoitrin, glucoraphanin, gluconapin,

glucoerucin, 4-methoxyglucobrassicin, gluconasturtiin, and neoglucobrassicin. ‘BH053’ had significantly higher levels of epiprogoitrin and glucosinalbin. Broccoli stored at 0 ºC had significantly higher levels of epiprogoitrin, glucosinalbin, glucobarbarin, glucoerucin, gluconasturtiin, glucobrassicin, 4-methoxyglucobrassicin, and glucoraphanin than broccoli stored at 7 ºC. Average total glucosinolate content remained stable throughout the duration of storage. The sulfur-containing volatile dimethyl disulfide was significantly greater for ‘Emerald Crown’ than for ‘BH053.’ Dimethyl disulfide content was significantly greater for broccoli stored at 7 ºC than for broccoli stored at 0 ºC. Both sulfur-containing volatiles, dimethyl disulfide and dimethyl sulfide, began to increase at 21 d in storage, and reached their highest level at 35 d in storage. The cooling/storage method had no effect on (E)-2-pentenal, propanal, or 2-ethylfuran contents. ‘Emerald Crown’ had significantly higher levels of (E)-2-Hexenal than ‘BH053.’ (E)-2-Hexenal content significantly decreased throughout the entire 5-week duration of storage. This study suggests that storage at lower temperatures helps to maintain postharvest quality of broccoli by decreasing the loss of nutritionally important glucosinolates, while preventing the production of volatiles responsible for off-odors.

Introduction

The United States (U.S.) broccoli industry is currently centered on West Coast production. California is responsible for over 90% of the total broccoli production in the nation, followed by Arizona (5%) (USDA Economic Research Service, 2011). Consequently, most of the fresh broccoli sold in the Eastern U.S. has been processed and shipped thousands of miles across the country before reaching supermarkets. Establishing a locally sourced broccoli industry on the U.S. East Coast will reduce the time between harvesting and consumer availability (Atallah et al., 2014; Wheeler et al., 2018). Broccoli is known to have a high respiration rate, and these

changes in the time between harvesting and consumer availability have potential consequences in postharvest physiology. Postharvest senescence of broccoli is accompanied by the degradation of metabolites through respiration (Hasparue et al., 2015; King and Morris., 1994). The loss of metabolites varies among cultivars and affects nutritional quality, as well as perceived sensory quality, of broccoli (Bruckner et al., 2005; Hansen et al., 1997; Pellegrino et al., 2019).

Glucosinolates are sulfur-containing compounds that are found in *Brassica* crops. The glucosinolate molecule is comprised of a b-thioglucoside N-hydroxysulphate, which has a side chain and b-D-glucopyranose moiety (Hansen et al., 1995). Glucosinolates have three classes: aliphatic, indole, and aromatic. Classes depend on their amino acid precursor. For aliphatic glucosinolates, amino acid precursors include alanine, leucine, isoleucine, valine, and methionine. Precursors for aromatic and indole glucosinolates are phenylalanine or tyrosine and tryptophan, respectively (Ishida et al., 2014). Glucosinolates are biologically inactive until they have been hydrolyzed enzymatically to their breakdown products by the enzyme myrosinase (Kushad et al., 1999). Nutritionally important glucosinolates include sinigrin, glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin, glucoraphanin, gluconasturtiin, and glucoerucin. Sinigrin and gluconasturtiin interrupt the cell cycle and cause apoptosis in cancer cells (Bell et al., 2018; Engel et al., 2002; Fenwick et al., 1983; Jakubikova et al., 2005; Sultana et al., 2003; Van Doorn et al. 1999). The indolyl glucosinolates (glucobrassicin, neoglucobrassicin, and 4-methoxyglucobrassicin) may inhibit prostate cancer (Sarkar et al., 2004; Fenwick et al., 1983). Glucoraphanin also suppresses the formation of prostate cancer (Beaver et al., 2017; Bell et al., 2017; Jones et al., 2006), and glucoerucin inhibits the proliferation of prostate and adenocarcinoma cells (Bennet et al., 2002; Jirovetz et al., 2002; Melchini et al., 2013). Glucoraphanin (aliphatic) comprises over half of the total glucosinolates found in broccoli. Other

common glucosinolates in broccoli belonging to the aliphatic class include sinigrin, progoitrin, gluconapin, as well as the indole glucosinolate, glucobrassicin (Badelek et al., 2012). Glucosinolate levels at harvest are mainly determined by genetic factors but can also be influenced by growing environment (Hanschen et al., 2014). Postharvest storage conditions, such as temperature and length of storage period, can also affect glucosinolate content (Jones et al., 2006; Paulsen et al., 2018; Verkerk et al., 2009).

In humans, glucosinolates act as chemoprotective agents, which have an influence on carcinogenesis during cancer development. Isothiocyanates and indoles, which are both autolytic breakdown products of glucosinolates, protect against many types of cancer at various stages of development, including the induction of phase II enzymes (detoxifying) and inhibition of phase I enzymes (activation) (Hanschen et al., 2012). The most effective glucosinolates for inducing the phase II enzymes that neutralize potential carcinogens in mammalian cells are sulforaphane, iberin, and erucin (Fahey et al., 2002; Hecht 2000; Zhang et al., 1994), which are the hydrolysis breakdown products of glucoraphanin, glucoiberin, and glucoerucin isothiocyanates, respectively (Velasco et al., 2008). Phase I enzymes metabolically activate most carcinogens in human cells. Inhibition of these phase I enzymes is required for the breakdown of some glucosinolates. Another potential chemo preventative mechanism of glucosinolates is their ability to regulate cancer cell development by interrupting the cell cycle and inducing apoptosis, and reducing metastasis and tumor growth. Also, these glucosinolate breakdown products have been known to block estrogen receptor function, preventing some cervical and breast cancers (Nilsson et al., 2006).

Volatiles impact the quality of broccoli by affecting flavor and aroma, which is known to change during postharvest storage (Zapata et al., 2013). Sulfur compounds are responsible for the

characteristic aroma associated with broccoli, and account for over 50% of the total volatile content in broccoli. Sulfuric volatiles are also the main chemicals responsible for off-odors produced by broccoli during senescence (Vidal-Aragon et al., 2009). These volatiles are formed due to cellular deterioration of lipid membranes and loss of intracellular compartmentalization, allowing enzymatic reactions to occur (Caleb et al., 2016; Forney et al., 1998). Previous studies have shown that increased membrane deterioration occurs at higher temperatures, which enhances aroma development (Chin et al., 1993; Dan et al., 1997). Because membrane deterioration is temperature dependent, different storage temperatures can result in the production of different volatiles (Jacobsson et al., 2004). Ketones and aldehydes make up less than 1% of the total volatile compounds found in broccoli. However, as aldehydes have a low olfactory detection threshold (i.e. highly sensitive) and characteristic aromas, this group of volatiles might play a part in broccoli aroma. (E)-2-hexenal is formed by the oxidative degradation of unsaturated fatty acids (Yu et al., 2009). It is described as having aromatic notes similar to freshly cut grass and bitter almonds (Vidal-Aragon et al., 2009). Aldehyde and ketone volatile contents tend to decrease during storage (Luo, 2018).

This study aims to measure the effects of cooling and postharvest storage method on the glucosinolate and volatile contents for two cultivars of broccoli. This will help to determine the proper storage conditions for maintaining postharvest quality of broccoli grown and distributed along the U.S. East Coast.

Materials and Methods

Plant materials and storage.

Broccoli was supplied by the Upper Mountain Research Station in Laurel Springs, North Carolina. Broccoli was grown according to recommended management practices for the southeastern U.S. (Kemble et al., 2018). Broccoli was harvested when the majority of the heads had reached commercial maturity. The average head diameter was 8.4 cm ± 1.5. Two cultivars, 'BH053' and 'Emerald Crown,' were harvested on 31 July 2019 and 5 Aug. 2019, respectively. The average head diameter of 'BH053' was 8.2 cm ± 1.4, while the average head diameter of 'Emerald Crown' was 8.7 cm ± 1.5. Each cultivar was separated into two treatment groups immediately after harvest. One treatment group was cooled by top icing to remove field heat, while the other treatment group was cooled by submerging in an ice slurry. Broccoli was then transported to The University of Tennessee Institute of Agriculture for cold room storage. Top iced broccoli was cooled to 16 ºC ± 4 when it reached the storage cooler (Fig. 12) and the top icing had melted during transportation. Broccoli from this treatment group was then placed in cold storage and kept in waxed corrugated boxes without ice. The cold room temperature was maintained at 6 ºC ± 0.4 and the internal broccoli temperature was maintained at 7 ºC ± 1. For the other treatment group, broccoli that was placed in an ice slurry was cooled to 1 ºC ± 1 at 2 h after the slurry was applied. Broccoli from this treatment group was then placed in cold storage and kept in coolers filled with ice. The cold room temperature was maintained at 4ºC ± 0.2 and the internal broccoli temperature was maintained at 0 ºC ± 0.3 (Fig. 12). Internal broccoli temperatures were recorded every 30 min with Watch Dog® data loggers (Spectrum® Technologies, Inc., Aurora, IL, USA).

Postharvest analysis

Broccoli was removed from storage at 0, 7, 14, 21, 28, and 35 days. Four replications of 'BH053' and three replications of 'Emerald Crown,' consisting of two broccoli heads per replication, were subsampled for each cultivar and treatment combination. For each replication, 3.0 g ± 0.1 fresh tissue was placed into clear glass headspace vials for immediate volatile analysis, and 30 g ± 1 fresh tissue was placed into plastic bags and stored in a -80 ºC freezer overnight, and frozen tissue was freeze-dried the following day. Freeze-dried tissue was ground to a fine powder, using a mortar and pestle in liquid nitrogen, for extraction and analysis.

Glucosinolate extraction and analysis

Glucosinolates were extracted from broccoli tissue using a modified version of the method by Charron et al. (2005), and desulphated based on the procedure by Raney and MacGregor (1990). A 0.2 g ± 0.01 subsample of finely ground broccoli tissue was placed into a 16 x 100 mm glass centrifuge tube. 2.0 mL of methanol, 0.1 mL of 0.6 M barium-lead acetate, and 1.0 mL of benzyl glucosinolate standard solution were added to the centrifuge tube. Centrifuge tubes were shaken at 60 rpm for 60 min, then centrifuged at 2000 g_n for 20 min. Next, 0.5 mL of supernatant from each sample was added to a 1.0 mL column containing 0.3 mL DEAE Sephadex A-25 (Sigma-Aldrich). Columns were washed with 900 mL of 67% methanol, 900 mL of pyridine acetate, and 3.60 mL of water purified by reverse osmosis (RO water). A 0.5 mL solution of sulfatase was then added to each column. The following day, 900 mL of RO water was eluted through the columns and collected in 12 x 13 mm clear standard crimp top vials.

Desulphonated glucosinolates were separated using an Agilent 1100 series high-performance liquid chromatography unit with a photodiode array detector (Agilent Technologies, Santa Clara, California). The column temperature was set at 40 ºC for a reverse-phase 250 x 4.6 mm i.d., 5

mm Luna C18 column (Phenomenex, Torrance, California) at a wavelength of 230 nm. The flow rate was set at 1.0 $mL \cdot min^{-1}$. The gradient elution was set to 100% pure water for 1 min, followed by a linear gradient to 75% water and 25% acetonitrile over 15 min. This was held constant for 5 min before returning to 100% pure water for 5 min. A comparison of retention times of authentic standards was used to identify glucosinolates (Hansen et al., 1995; Kushad et al., 1999). Glucosinolates were expressed on a dry mass basis in $mg \cdot g^{-1}$. Data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies, Palo Alto, California).

Volatile extraction and analysis

Whole floret tissue samples were collected immediately after removal from storage, and 3.0 g ± 0.1 of broccoli was weighed into a headspace vial for immediate analysis.

Volatiles were analyzed using an Agilent series 6890 Network Gas Chromatography System with an Agilent series 5973 Mass Selective Detector and a G1888 Agilent series and a Headspace Sampler (Agilent Technologies, Santa Clara, California). Volatiles were identified based on previously calibrated standard curves. Data were collected, recorded, and integrated using ChemStation Software (Agilent Technologies, Palo Alto, California).

Statistical analysis

SAS statistical software (9.4 for Windows; SAS Institute, Cary, North Carolina) was used for the analyses of data. Cultivar, storage temperature, storage time, and their interactions were treated as fixed factors, while replication was considered the random factor. Analysis of variance (ANOVA) tests were performed using the GLIMMIX procedure, and means were compared by the least significant difference (LSD) test ($\alpha = 0.05$). ANOVA results are presented for glucosinolates (Table 15) and volatiles (Table 16).

Results

Glucosinolates, effects of cultivar.

Cultivar had a significant impact on 4-methoxyglucobrassicin ($F = 23.34$, $df = 1, 57$, and $p \leq 0.0001$), neoglucobrassicin ($F = 32.32$, $df = 1, 57$, and $p \leq 0.0001$), gluconasturtiin ($F = 7.96$, $df = 1, 57$, $p \leq 0.01$), glucosinalbin ($F = 11.62$, $df = 1, 57$, and $p \leq 0.01$), glucoraphanin ($F = 13.24$, $df = 1$, $p \leq 0.001$), epiprogoitrin ($F = 142.46$, $df = 1, 57$, and $p \leq 0.0001$), progoitrin ($F = 43.06$, $df = 1, 57$, and $p \leq 0.0001$), glucoerucin ($F = 46.59$, $df = 1, 57$, and $p \leq 0.0001$), gluconapin ($F = 11.10$, $df = 1, 57$, and $p \leq 0.01$), total indole ($F = 32.81$, $df = 1, 57$, and $p \leq 0.0001$), and total glucosinolate ($F = 15.89$, $df = 1, 57$, and $p \leq 0.001$) contents (Table 17). 'Emerald Crown' had significantly higher levels of 4-methoxyglucobrassicin, neoglucobrassicin, gluconasturtiin, glucoraphanin, progoitrin, glucoerucin, gluconapin, total indole, and total glucosinolate contents, while 'BH053' had significantly greater glucosinalbin and epiprogoitrin contents. For 'BH053,' glucobrassicin was the most dominant glucosinolate, followed by glucoraphanin and neoglucobrassicin. Neoglucobrassicin, glucobrassicin, and glucoraphanin were the dominant glucosinolates for 'Emerald Crown.'

Glucosinolates, effects of cooling/storage method.

Storage temperature significantly affected glucobrassicin ($F = 7.95$, $df = 1, 57$, and $p \leq 0.01$), gluconasturtiin ($F = 9.31$, $df = 1, 57$, and $p \leq 0.01$), glucoraphanin ($F = 7.85$, $df = 1, 57$, and $p \leq 0.01$), epiprogoitrin ($F = 34.00$, $df = 1, 57$, and $p \leq 0.0001$), glucobarbarin ($F = 5.24$, $df = 1, 57$, and $p \leq 0.05$), total aliphatic ($F = 8.70$, $df = 1, 57$, and $p \leq 0.01$), total aromatic ($F = 8.77$, $df = 1, 57$, and $p \leq 0.01$), and total glucosinolate ($F = 5.80$, $df = 1, 57$, and $p \leq 0.05$) contents (Table 18). Broccoli that was ice slurry cooled and stored at 0 ºC in ice had significantly higher glucobrassicin, gluconasturtiin, glucoraphanin, and glucobarbarin contents than broccoli that was

top icing cooled and stored at 7 ºC without ice. Broccoli stored at 7 ºC had significantly higher epiprogoitrin content than broccoli stored at 0 ºC in ice. Storage time alone did not significantly impact glucosinolate content (Table 19).

Glucosinolates, interaction of cultivar and storage method.

Interaction effects between cultivar and storage temperature were significant for epiprogoitrin ($F = 142.46$, df = 1, 57, and $p \leq 0.001$) content alone (Table 20). For 'BH053,' epiprogoitrin content was significantly greater for ice slurry cooled broccoli stored at 0 ºC in ice than for top icing cooled broccoli stored at 7 ºC. For 'Emerald Crown,' epiprogoitrin levels were unaffected by storage temperature and were significantly lower than epiprogoitrin levels for 'BH053' stored at either temperature.

Interaction effects between cultivar and storage time were significant for glucobrassicin ($F = 2.64$, df = 5, 57, and $p \leq 0.05$), 4-methoxyglucobrassicin ($F = 4.08$, df = 5, 57, and $p \leq 0.01$), gluconasturtiin ($F = 2.44$, df = 5, 57, and $p \leq 0.05$), glucosinalbin ($F = 4.28$, df = 5, 57, and $p \leq 0.01$), epiprogoitrin ($F = 4.38$, df = 5, 57, and $p \leq 0.01$), total aliphatic ($F = 2.59$, df = 5, 57, and $p \leq 0.05$), and total aromatic ($F = 2.94$, df = 5, 57, and $p \leq 0.05$) contents (Table 21 and 22). Glucobrassicin, 4-methoxyglucobrassicin, and gluconasturtiin contents were significantly greater for 'Emerald Crown' than for 'BH053' at 0 d in storage. After that, glucobrassicin levels did not vary significantly for either cultivar. 4-methoxyglucobrassicin content at 14 and 21 d was significantly greater for 'Emerald Crown' than for 'BH053' but then decreased for the remaining days in storage. For 'BH053,' 4-methoxyglucobrassicin remained stable. Gluconasturtiin content did not significantly vary for either cultivar. However, gluconasturtiin levels decreased during storage for 'Emerald Crown' but increased for 'BH053.' Glucosinalbin levels decreased throughout the first 28 d in storage for 'Emerald Crown,' then increased at 35 d, but not

significantly. For ‘BH053,’ glucosinalbin content significantly increased at 14 d, then significantly decreased at 21 d but did not significantly vary after that. Glucosinalbin levels were significantly greater for ‘BH053’ than for ‘Emerald Crown’ at 14 and 28 d in storage. Epiprogoitrin levels decreased for ‘Emerald crown’ at 7 d, but not significantly, then remained stable. In contrast, epiprogoitrin content significantly increased at 7 d, then remained stable. Total aliphatic and aromatic glucosinolate contents were significantly greater for ‘Emerald Crown’ than for ‘BH053’ at 0 d in storage. For ‘BH053,’ total aliphatic contents remained stable throughout the entire duration of storage, while total aliphatic contents for ‘Emerald Crown’ significantly decreased from their initial levels at 14 d and remained stable after that. Total aromatic contents were significantly greater than their initial levels at 14 and 21 d in storage, but did not significantly vary other than that. In contrast, total aromatic contents decreased throughout storage for ‘Emerald Crown,’ but were only significantly lower than their initial levels at 28 d in storage.

Interaction effects between storage temperature and storage time were significant for 4-methoxyglucobrassicin ($F = 3.00$, $df = 5, 57$, and $p \leq 0.05$), glucosinalbin ($F = 2.80$, $df = 5, 57$, and $p \leq 0.05$), glucoerucin ($F = 2.72$, $df = 5, 57$, and $p \leq 0.05$), and epiprogoitrin ($F = 3.64$, $df = 5, 57$, and $p \leq 0.01$) contents (Table 23). 4-methoxyglucobrassicin content was significantly greater for broccoli stored at 0 ºC on the day of harvest but significantly decreased at 7 d, then continued to decrease for the remaining duration of storage, but not significantly. In contrast, for broccoli stored at 7 ºC without ice, 4-methoxyglucobrassicin content significantly increased at 14 d, then began to decrease, but not significantly. Glucosinalbin content remained stable throughout the entire 5-week duration of storage for broccoli stored at 0 ºC in ice, and was significantly greater than glucosinalbin content for broccoli stored at 7 ºC at 35 d. Glucosinalbin

content significantly increased at 14 d for broccoli stored at 7 ºC, then began to decrease. Epiprogoitrin levels decreased throughout the duration of storage for broccoli stored at 7 ºC, but not significantly. For broccoli stored at 7 ºC, epiprogoitrin levels significantly increased at 28 d, then significantly decreased at 35 d in storage. Compared to storage at 7 ºC, broccoli stored at 0 ºC had significantly higher epiprogoitrin content when in storage for 14, 28, or 35 d.

The interaction between cultivar, cooling temperature, and storage time significantly affected 4-methoxyglucobrassicin ($F = 2.39$, $df = 5, 57$, and $p \leq 0.05$) and epiprogoitrin ($F = 4.38$, $df = 5, 57$, and $p \leq 0.001$) contents (Table 24). For 'BH053,' 4-methoxyglucobrassicin content did not significantly vary during storage for broccoli stored at either temperature. However, 4-methoxyglucobrassicin content for 'Emerald Crown' significantly increased at 14 d for broccoli stored at 7 ºC, then significantly decreased at 28 d and remained stable from 28 to 35 d. In contrast, 4-methoxyglucobrassicin content significantly decreased at 7 d for broccoli stored at 0 ºC, then continued to decrease and was no longer detected when stored for 35 d. Epiprogoitrin content did not significantly vary for broccoli receiving either treatment for 'Emerald Crown.' For 'BH053,' epiprogoitrin content significantly decreased at 28 d for broccoli stored in 7 ºC. In contrast, epiprogoitrin levels significantly increased at 7, 14, and 28 d for broccoli stored at 0 ºC in ice, then significantly decreased at 35 d in storage.

Volatiles, effects of cultivar.

Cultivar had a significant effect on the dimethyl disulfide ($F = 36.81$, $df = 1, 57$, and $p \leq 0.0001$) and (E)-2-hexenal ($F = 5.28$, $df = 1, 57$, and $p \leq 0.05$) contents in broccoli (Fig. 13 and 14). 'Emerald Crown' had significantly higher dimethyl disulfide and (E)-2-hexenal contents than 'BH053.'

Volatiles, effects of cooling/storage method.

Storage temperature had a significant effect on the dimethyl disulfide ($F = 24.65$, $df = 1, 57$, and $p \leq 0.0001$) content alone (Fig. 15). Dimethyl disulfide content was significantly greater for top icing cooled broccoli stored at 7 ºC than for ice slurry cooled broccoli stored at 0 ºC in ice. Storage time had a significant effect on dimethyl disulfide ($F = 9.04$, $df = 5, 57$, and $p \leq 0.0001$), dimethyl sulfide ($F = 23.50$, $df = 5, 57$, and $p \leq 0.0001$), (E)-2-hexenal ($F = 7.91$, $df = 5, 57$, and $p \leq 0.0001$), (E)-2-pentenal ($F = 9.91$, $df = 5, 57$, and $p \leq 0.0001$), 2-ethylfuran ($F = 10.86$, $df = 5, 57$, and $p \leq 0.0001$), and propanal ($F = 5.65$, $df = 5, 57$, and $p \leq 0.001$) contents (Figs.16 – 19). Dimethyl disulfide concentration significantly decreased at 7 d in storage, then significantly increased at 21 d in storage. Dimethyl disulfide content continued to increase at 28 and 35 d in storage and reached a final content level that was significantly greater than the initial value at harvest. Dimethyl sulfide significantly decreased from its initial value at 7 d in storage, then significantly increased at 28 and 35 d and reached a final content level that was significantly greater than its initial value at harvest. Propanal content significantly increased at 7 d in storage, then decreased at 14 and 21 d in storage, but not significantly. Propanal levels significantly decreased at 28 d, then remained stable from 28 to 35 d in storage. (E)-2-hexanal content remained stable throughout the first 14 d in storage, then significantly decreased at 21 d. After that, (E)-2-hexenal content decreased at 28 d, but not significantly, and was no longer detected when stored for 35 d. Both (E)-2-pentenal and 2-ethylfuran contents significantly increased at 7 d, then significantly decreased at 21 d, and were no longer detected when stored for 35 d.

Volatiles, interaction of cultivar and storage method.

Dimethyl sulfide ($F = 4.43$, $df = 5, 57$, and $p \leq 0.01$) content alone was significantly affected by the interaction between cooling/storage temperature treatment and storage time (Fig. 20). For

broccoli stored at 7 ºC without ice, dimethyl sulfide contents were only significantly greater than their initial levels at 35 d in storage. For broccoli stored at 0 ºC in ice, dimethyl sulfide contents significantly decreased at 7 d in storage. After that, dimethyl sulfide levels began to increase for the remaining weeks in storage, but were only significantly greater than their initial levels when stored for 35 d.

Interaction effects between cultivar and storage time were significant for dimethyl disulfide ($F = 10.98$, $df = 5, 57$, and $p \leq 0.0001$), dimethyl sulfide ($F = 7.37$, $df = 5, 57$, and $p \leq 0.0001$), (E)-2-hexenal ($F = 4.91$, $df = 5, 57$, and $p \leq 0.001$), (E)-2-pentenal ($F = 6.68$, $df = 5, 57$, and $p \leq 0.0001$), 2-ethylfuran ($F = 6.20$, $df = 5, 57$, and $p = 0.0001$), and propanal ($F = 2.88$, $df = 5, 57$, and $p \leq 0.05$) contents (Table 25). For 'BH053,' dimethyl disulfide contents significantly decreased from their initial contents at 7 d in storage. At 21 d, dimethyl disulfide contents began to increase, but not significantly. For 'Emerald Crown,' dimethyl disulfide contents increased throughout the duration of storage but were only significantly greater from their initial levels at 28 and 35 d in storage. For 'BH053,' dimethyl sulfide contents significantly decreased from their initial levels at 7 d in storage. Dimethyl sulfide levels remained stable until they significantly increased at 35 d in storage. For 'Emerald Crown,' dimethyl sulfide contents were only significantly greater from their initial levels when stored for 28 and 35 d. For 'BH053,' (E)-2-hexenal, (E)-2-pentenal, propanal, and 2-ethylfuran contents were only detected during 7 – 28 d in storage. For 'Emerald Crown,' propanal was detected throughout the entire duration of storage, (E)-2-hexenal and 2-ethylfuran were only detected during 0 – 21 d in storage, and (E)-2-pentenal contents were only detected during 0 – 14 d in storage. When present, (E)-2-hexenal levels remained stable for 'BH053' but significantly decreased at 21 d in storage for 'Emerald Crown.' (E)-2-pentenal levels significantly increased at 14 d, then significantly decreased at 28 d

for ‘BH053,’ while (E)-2-pentenal contents significantly increased at 7 d and remained stable from 7 to 14 d in storage for ‘Emerald Crown.’ Propanal contents only significantly decreased at 28 d in storage for ‘BH053,’ while propanal contents decreased throughout the entire duration of storage for ‘Emerald Crown,’ but not significantly. For ‘BH053,’ 2-ethylfuran contents significantly decreased at 21 d, then significantly increased at 28 d in storage. For ‘Emerald Crown,’ 2-ethylfuran contents increased at 7 d in storage, then decreased at 14 and 21 d, but not significantly.

Discussion

Glucosinolates, effects of cultivar.

Glucosinolates contribute to the taste and flavor of *Brassica* crops (Schonhof et al., 2004), and are thought to be responsible for the bitterness associated with these plants (Bell et al., 2017; Drewndowski et al., 2000). Sinigrin, glucoiberin, and glucoraphanin are glucosinolates that have been associated with typical *Brassicaceae* flavors. Higher glucosinolate levels in broccoli are linked with stronger flavor, but levels too high can negatively impact consumer acceptance (Bell et al., 2017; Hansen et al., 1997). Sinigrin, gluconapin, progoitrin and epiprogoitrin, glucobrassicin, neoglucobrassicin, and 4-methoxyglucobrassicin have been identified as the main glucosinolate compounds responsible for bitterness (Engel et al., 2002; Jones et al., 2006; Frandsen et al., 2014). Isothiocyanates (ITC) of sinigrin (allyl ITC) and gluconapin (3-Butenyl ITC) are also responsible for pungent or acrid flavors and aromas (Bell et al., 2018; Depree et al., 1999), and the aromatic hydrolysis product of glucosinalbin (4-Hydroxybenzyl ITC) can cause an intense burning sensation when consumed (Ghawi et al., 2014). Glucosinolates, such as glucoraphanin, glucoerucin and glucoiberin, are not associated with bitterness in *Brassica* vegetables. Breakdown products of glucoraphanin and glucoiberin are semi-volatile and are

unlikely to contribute greatly to flavor (Traka et al, 2009). Glucoerucin has a “radish” aroma, but a low intensity (Raffo et al., 2018). In arugala, glucoraphanin and glucoerucin were not significantly correlated with bitterness or consumer rejection (Bell et al., 2017). Off-odors are also often attributed to glucosinolate and isothiocyanate contents in broccoli. These odors are often described as “sulfurous,” “earthy,” or “musty” (Chen et al., 2017). Although previous studies (Baik et al., 2003; Cartea et al., 2008) reported that sulfides and VOCs, rather than glucosinolates, significantly affected taste and flavor in *Brassica* plants, recent studies have found that glucosinolates and ITCs may have a very low detection threshold and could contribute to flavor and aroma more than previously realized (Bell et al., 2017). Glucosinolates and their degradation products also receive much attention due to their beneficial health effects (Bell et al., 2017). Sinigrin, glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin, glucoraphanin, gluconasturtiin, and glucoerucin are nutritionally important compounds known for their role in the prevention of cancer development and cardiovascular and neurodegenerative disorders (Bell et al., 2017; Dinkova-Kostova et al, 2012; Giaccopo et al., 2017; Halkier et al., 2006; Hayes et al., 2008), while epiprogoitrin and progoitrin are potentially harmful glucosinolates. Their breakdown product, goitrin, competes for iodine in humans. For thyroid impaired or iodine deficient individuals, this can cause the condition known as goiter (Steinmetz et al., 1991).

Glucobrassicin is the most abundant glucosinolate reported for ‘Parthenon,’ ‘Monaco’ (Fernández-Leon et al., 2012), ‘Marathon’ (Rosa and Rodrigues, 2001; Rybarczyk-Plonska et al., 2016; Vallejo et al., 2003), ‘Shogun’ (Kushad et al., 1999), and ‘Legacy’ (Paulsen et al., 2018) broccoli. However, other studies reported glucoraphanin as the dominant glucosinolate in ‘Sebastian’ (Cieślik et al., 2007), ‘Youxiu’ (Jia et al., 2009), ‘Lvxiong’ (Yuan et al., 2010), ‘Beneforte’ (Bell et al., 2018), and others (Jones et al., 2006; Oliviero et al., 2012; Song and

Thornalley, 2007). In this study, glucobrassicin was the most abundant glucosinolate for 'BH053,' while neoglucobrassicin was dominant for 'Emerald Crown.' Although glucoraphanin and glucobrassicin appear to be the major glucosinolates present in broccoli, other glucosinolates have shown cultivar-dependent distribution patterns. Significant differences among multiple cultivars have been reported for progoitrin, glucoraphanin, sinigrin, gluconapin, glucoerucin, and glucobrassicin (Bhandari et al., 2014). The interaction between cultivar and storage time significantly affected epiprogoitrin, glucobrassicin, 4-methoxyglucobrassicin, gluconasturtiin, glucosinalbin, total aliphatic, and total aromatic contents in broccoli. These compounds, except for glucosinalbin, either increased or remained stable during storage for 'BH053,' but decreased during storage for 'Emerald Crown.' Glucosinalbin content for 'BH053' increased throughout the first 28 d in storage, then decreased at 35 d. For 'Emerald Crown,' glucosinalbin content decreased throughout the first 28 d, then increased at 35 d. Aliphatic glucosinolates remained stable throughout storage for 'BH053,' while aliphatic contents for 'Emerald Crown' significantly decreased at 14 d in storage, then remained stable from 14 to 35 d. Aromatic glucosinolates increased at 7, 14, and 28 d in storage for 'BH053,' but not significantly. Although not significant, aromatic glucosinolate contents decreased at 7 and 28 d in storage, then increased at 35 d for 'Emerald Crown.' Aliphatic glucosinolates, such as epiprogoitrin, are predominately under genetic control (Magrath et al., 1994), while indole glucosinolates, such as glucobrassicin and 4-methoxyglucobrassicin, are thought to be determined by environmental and physiological factors (Kushad et al., 1999; Mithen et al., 1995). Differences in cultivar for both aliphatic and indole glucosinolates suggest that both genetic and environmental/physical factors may have played a role in regulating glucosinolate concentrations in broccoli. Results from this study show that 'Emerald Crown' had significantly higher initial levels of nutritionally important

glucosinolates, glucobrassicin, 4-methoxyglucobrassicin, and gluconasturtiin. However, 'BH053' was able to prevent the loss of these glucosinolates in storage. Thus, the quality of broccoli after long term storage may be higher for 'BH053' than for 'Emerald Crown.' Although the presence of glucoraphanin and glucoerucin, non-taste affecting compounds, was significantly higher for 'Emerald Crown,' it's sensory quality may be adversely affected due to significantly higher levels of glucosinolates that may contribute to bitterness, such as progoitrin, gluconapin, 4-methoxyglucobrassicin, and neoglucobrassicin (Bell et al., 2017).

Glucosinolates, effects of cooling/storage method.

Glucosinolate levels at harvest are mainly determined by genetic factors but can also be influenced by growing environment (Schreiner, 2005; Charron and Sams, 2004) and postharvest storage conditions (Jones et al., 2006; Rybarczyk-Plonska et al., 2015; Verkerk et al., 2009). The rate of cellular damage increases during storage at higher temperatures, affecting glucosinolate content (Latté et al., 2011). In this study, epiprogoitrin, glucoraphanin, glucobarbarin, glucobrassicin, gluconasturtiin, average aliphatic, average aromatic, and average total glucosinolate contents were significantly greater for broccoli precooled with ice and stored at 0 ºC in ice than for broccoli precooled with top icing and stored at 7 ºC. The interaction between storage temperature and storage time significantly affected epiprogoitrin, glucoerucin, glucobrassicin, 4-methylglucobrassicin, and glucosinalbin contents. In this study, glucobrassicin significantly decreased at 35 d in storage for broccoli precooled with an ice slurry and stored at 0 ºC in ice, but remained stable throughout the entire duration of storage for broccoli precooled with top icing and stored at 7 ºC. However, previous studies found that glucobrassicin remained stable for broccoli stored for 28 d at 1 to 2 ºC (Winkler et al., 2007) and for broccoli stored 21 d at 4 ºC (Paulsen et al., 2018). In this study, 4-methoxyglucobrassicin content significantly

increased at 14 d in storage at 7 ºC, then began to decrease for the following weeks in storage. Similarly, previous studies reported that 4-methyoxyglucobrassicin contents increased for broccoli stored for 21 d at 4 ºC (Paulsen et al., 2018), 5 d at 4 ºC (Rodrigues and Rosa et al., 1999), and 7 d at 4 ºC (Rybarczyk-Plonska et al., 2015). In contrast, 4-methoxyglucobrassicin content decreased throughout the entire duration of storage for broccoli stored at 0 ºC in ice. Glucosinalbin content significantly increased at 14 d in storage at 7 ºC, then began to decrease. For broccoli stored at 0 ºC, glucosinalbin content decreased at 14 d in storage, then increased at 28 and 35 d, but not significantly. In this study glucoerucin content increased during storage at 7 ºC but decreased for broccoli stored at 0 ºC in ice. In contrast, Pardo et al. (2014) reported stable levels of glucoerucin for broccoli stored for 3 d at 15 ºC. In this study, epiprogoitrin increased at 7 and 14 d, then decreased during in storage at 7 ºC. For broccoli stored at 0 ºC, epiprogoitrin increased at 14 and 28 d in storage, then decreased at 35 d. Rodrigues and Rosa (1999) found that progoitrin remained stable for broccoli stored for 5 d at 4 ºC in cling wrap, while others reported a significant decrease in progoitrin content for broccoli stored for 5 d at 4 ºC (Jia et al., 2009). For broccoli precooled with top icing and stored at 7 ºC without ice, total glucosinolate content remained stable throughout the entire duration of storage. Similarly, previous studies found that total glucosinolate content remained stable for broccoli stored for 5 d at 4 ºC (Rodrigues and Rosa, 1999), 4 or 7 d at 4 ºC (Rybarczyk-Plonska et al., 2015), 7 d at 4 to 8 ºC (Song and Thornally, 2007), 7 d at 4 ºC in modified atmosphere packaging (MAP) (Rangkadilok et al., 2002), 21 d at 4 and 8 ºC in MAP (Paulsen et al., 2018), 21 d at 1 to 2 ºC (Fernández-Leon et al., 2013), and 28 d at 1 ºC (Winkler et al., 2007). In contrast, total glucosinolate content significantly decreased for broccoli precooled with an ice slurry and stored at 0 ºC in ice. Similarly, Vallejo et al. (2003) found a decrease in total glucosinolate content in packaged

broccoli stored for 7 d at 1 ºC. Stable glucoraphanin levels were reported for broccoli stored for 28 d at 1 to 2 ºC (Winkler et al., 2007), and 10 d at 4 ºC (Rangkadilok et al., 2002). Although glucoiberin, progoitrin, gluconasturtiin, and neoglucobrassicin contents remained stable, glucobrassicin and glucoraphanin contents significantly decreased in primary florets stored for 5 d at 4 ºC (Rodrigues and Rosa, 1999). Paulsen et al. (2018) found that glucobrassicin and neoglucobrassicin contents remained stable for broccoli stored for 21 d at 4 ºC. They also reported stable glucoraphanin levels during storage for 14 d at 4 ºC but decreased glucoraphanin content at 21 d. In this study, glucoraphanin and neoglucobrassicin contents significantly decreased at 7 d, then remained stable. However, these compounds remained stable throughout the entire 5-week duration of storage for broccoli precooled with top icing and stored at 7 ºC .

Storage conditions, such as temperature and time, affect both total and individual glucosinolate contents in broccoli (Paulsen 2018). Glucosinolate content in storage is dependent on two mechanisms, hydrolysis and biosynthesis. A balance between these two mechanisms may help prevent the loss of glucosinolates during storage, but these mechanisms are still unclear (Fernández-Leon et al., 2013; Rybarczyk-Plonska et al., 2016). Results from this study suggest that the expression of genes controlling the biosynthesis of nutritionally important glucosinolates, 4-methoxyglucobrassicin and glucoerucin, may have been activated at 14 d of storage for broccoli precooled with top icing and stored at 7 ºC, but not for broccoli precooled with an ice slurry and stored at 0 ºC in ice. Studies report that stresses, such as high temperatures, cause activation of primary and secondary metabolism, which increases glucosinolates during postharvest storage (Villarreal-Garcia et al., 2016). Thus, increased levels of 4-methoxyglucobrassicin and glucoerucin during storage at 7 ºC may have been a result of greater tissue damage that occurred during the longer precooling phase, which caused the expression of

genes controlling glucosinolate biosynthesis (Torres-Contreras et al., 2017). The significant loss and subsequent stabilization of 4-methoxyglucobrassicin and glucoerucin at 7 d in storage for broccoli precooled with an ice-slurry and stored at 0 ºC in ice supports this idea. Levels of these compounds may have been significantly higher on the day of harvest because precooling with an ice slurry helped to slow down the rate of respiration faster than top icing, decreasing the rate of deterioration (Deschene et al., 1991; Jacobsson et al., 2014; Nilsson, 2000). This would have prevented myrosinase from coming into contact with glucosinolates (Latté et al., 2011), preventing the immediate loss of 4-methoxyglucobrassicin and glucoerucin. Because these compounds significantly decreased at 7 d in storage at 0 ºC, the rate of deterioration may not have been great enough to initiate biosynthesis but did allow for hydrolysis to occur (Grubb and Abel, 2006). However, this may not be true for other health promoting glucosinolates, including glucobrassicin, neoglucobrassicin, glucoraphanin, and gluconasturtiin. In contrast to 4-methoxyglucobrassicin and glucoerucin, both glucobrassicin and gluconasturtiin slightly increased at 28 d for broccoli stored at 0 ºC in ice but increased at 7 d for broccoli stored at 7 ºC. Thus, biosynthesis of new glucobrassicin and gluconasturtiin compounds seems to have been activated for broccoli stored at either temperature but occurred earlier for broccoli stored at 7 ºC. Similar to 4-methoxyglucobrassicin and glucoerucin, neoglucobrassicin content significantly decreased, then remained stable throughout storage at 0 ºC in ice. However, neoglucobrassicin content remained stable throughout storage at 7 ºC. This may have been due to a balance between mechanisms controlling biosynthesis and hydrolysis (Paulsen et al., 2018), which allowed neoglucobrassicin levels to remain stable during storage. These results suggest that maintenance of healthful glucosinolates is influenced by precooling and subsequent storage

conditions, and that the degree to which glucosinolates are affected by these conditions varies for individual compounds.

Volatiles, effects of Cultivar.

Flavor and aroma are important indicators of quality in produce (Ye et al., 2017). The 6-C aldehyde, (E)-2-Hexenal, has a characteristic green odor associated with the sensory perception of freshness (Hatanaka et al., 1996), and plays an important role in the flavor of *Brassica* vegetables (Banerjee et al., 2013). (E)-2-pentenal is monosaturated fatty aldehyde, described as having a fresh fruit odor and flavor (Maga 1981; Ullrich and Grosch, 1988), while propanal is a saturated 2-C aldehyde, described as having a solvent, pungent odor. The furan compound, 2-ethylfuran, has a sweet, roasted, coffee odor (Birch et al., 2012). These compounds are formed by enzymatic oxidation of fatty acids. Sulfurous compounds are problematic in *Brassica*s because of their low detection threshold in humans (0.04 ppb) (Bell et al., 2017; Lindsay et al., 1986). Sulfides, including dimethyl disulfide and dimethyl sulfide, are responsible for off-odors released by broccoli during senescence, and are often associated with undesirable aroma and flavor attributes (Bell et al., 2017; Chin et al., 1993; Engel et al., 2002; Forney et al., 1991; Hansen et al., 1992; Jacobsson et al., 2004).

Volatile contents vary according to species, cultivar, and developmental stage of the plant (Schaich et al., 2012). Distribution of these compounds have a significant impact on the aroma and quality of vegetables (Banerjee et al., 2013). Previous studies have used sulfurous compound contents to compare cultivars (Di Cesare et al., 2001). Vidal-Aragon et al. (2009) found significantly higher levels of dimethyl disulfide for ‘Merit’ than for ‘Marathon,’ ‘Nubia,’ ‘Parthenon,’ Samson,’ or ‘Shena.’ However, they did not observe a significant difference in dimethyl sulfide content among cultivars. Although there was no significant difference in the

average total dimethyl sulfide content, average total dimethyl disulfide was significantly greater for ‘Emerald Crown’ than for ‘BH053.’ Dimethyl disulfide and dimethyl sulfide contents decreased at 7 d in storage, then began to increase at 21 d in storage and continued to increase for ‘BH053.’ These results are similar to those reported for ‘Marathon,’ in which dimethyl disulfide also significantly decreased at 7 d in storage (Caleb et al., 2016). In contrast, dimethyl disulfide and dimethyl sulfide contents of ‘Emerald Crown’ only began to increase at 28 d in storage. Sulfide contents vary widely among cultivars and maturity stage in *Brassica* species (Raseetha et al., 2013). Availability of precursors for hydrolysis, presence of enzymes, and accessibility between substrate and enzymes are key factors involved in volatile formation (Hansen et al., 1996). Genetic variation among cultivars may affect the presence of volatile precursors, such as glucosinolates (Lewis and Fenwick 1987). Cultivar may also have an effect on S-methylcysteine sulfoxide (SMCO) content as well (Bradshaw and Borzucki, 1983). Dimethyl disulfide originates from either the rupture of S-methyl-Lcysteine sulphoxide (Di Pentima et al., 1995) or from methanethiol (Chin and Lindsay, 1994), while dimethyl sulfide can be derived from S-methyl methionine. The rate of deterioration may also differ among cultivars, which would affect presence and accessibility between substrate and enzymes (Hansen et al., 1996). Thus, the significant difference in sulfurous volatile production for these two cultivars may be due to the genetic control of key factors relating to volatile formation during postharvest storage of broccoli.

Volatiles detected in this study were consistent with those responsible for the green leaf aroma in *Brassica* plants (Jirovetz et al., 2002). Aragon et al. (2009) found significantly greater levels of (E)-2-hexenal content for ‘Merit’ and ‘Nubia’ than for ‘Marathon,’ ‘Parthenon,’ ‘Samson,’ Serydan,’ or ‘Shena.’ In this study, (E)-2-hexenal content was significantly greater for

‘Emerald Crown’ than for ‘BH053.’ For ‘Emerald Crown,’ propanal content decreased throughout the duration of storage, and (E)-2-hexenal, (E)-2-pentenal, and 2-ethylfuran were only detected during the first 14 or 21 d in storage. In contrast, for ‘BH053,’ these compounds were not detected on the day of harvest or at 35 d in storage. Lipase enzymes hydrolyze fatty acids, releasing them from membrane lipids. Fatty acids are then acted upon by lipoxygenase and hydroperoxide lyase, leading to formation of aldehydes and furan (Banerjee et al., 2013; Hatanaka et al., 1996). Genetic differences among cultivars may play a role in regulating lipoxygenase activity in broccoli (Raseetha et al., 2014). This would explain the difference between volatile contents for ‘Emerald Crown’ and ‘BH053.’ However, because these volatiles are associated with wounded tissues in plants, significant variation among cultivars may be due to the rate of tissue deterioration (Banerjee et al., 2013).

Volatiles, effects of storage/cooling method.

Tissue disruption and cellular deterioration are thought to enhance aroma production (Jacobsson et al., 2004; Tulio et al., 2013; Chin and Lindsay et al., 1993). Sulfur compounds are derived from sulfur precursors or from degradation of volatiles derived from glucosinolate breakdown (Banerjee et al., 2013). Temperature influences changes in aroma compounds during storage (Raseetha et al., 2014). Higher storage temperatures lead to increased cellular damage, which allows enzymes to mix uncontrollably with potential substrates, producing sulfurous volatiles (Travers-Martin et al., 2008). During storage, S-methylmethionine is degraded to S-methylmethionine sulfonium salt to dimethyl sulfide (Scherb et al., 2009), while dimethyl disulfide is derived from S-methylcysteine sulfoxide that is acted upon by cysteine sulfoxide lyase or from oxidation of methanethiol (Chin and Lindsay, 1994). Caleb et al. (2016) found that dimethyl disulfide content significantly increased at 4 d in storage, then significantly decreased

at 7 d and remained stable from 7 to 11 d in storage, while dimethyl sulfide content significantly increased at 7 d, then remained stable from 7 to 11 d. In this study, dimethyl disulfide content alone was significantly affected by treatment, while dimethyl sulfide was the only volatile affected by the interaction between treatments and storage time. Dimethyl disulfide content was significantly greater for broccoli precooled with top icing and stored at 7 ºC than for broccoli precooled with an ice slurry and stored at 0 ºC in ice. During storage, dimethyl disulfide content significantly decreased at 7 d, then significantly increased at 21 d and continued to increase. Similarly, Jacobsson et al. (2004) found that production of dimethyl disulfide and dimethyl sulfide was greater for broccoli stored at 10 ºC compared to 4 ºC, and Caleb et al. (2016) found that dimethyl disulfide accumulated in the headspace of packaged broccoli stored for 11 d at 10 ºC. Chen et al. (2019) found that sulfur compounds significantly increased in broccoli during storage for 0, 6, and 12 d at 4 ºC, and dimethyl sulfide was reported to increase continually during storage for *Brassica* species (Hacer et al., 2015; Lv et al., 2017). In this study, dimethyl sulfide did not significantly increase until 35 d in storage for broccoli stored at 7 ºC. For broccoli stored at 0 ºC, dimethyl sulfide significantly decreased at 7 d in storage, then significantly increased at 28 d. However, dimethyl disulfide content was significantly lower at 35 d for broccoli stored at 0 ºC than for broccoli stored at 7 ºC. Sulfide formation is due to several factors, including bacterial metabolism, senescence, and tissue damage. Results from this study show that increased duration of storage results in production of sulfurous volatiles, which have a negative impact on aroma and quality of broccoli. These results indicate that the degree to which dimethyl sulfide is affected during storage is dependent on precooling and subsequent storage methods. Lower sulfide concentrations for ice slurry cooled broccoli stored at 0 ºC in ice suggest

that the degree of deterioration was lower than for broccoli cooled with top icing and stored at 7 ºC .

Previous studies have shown that aldehydes and esters are key aroma compounds involved in the lipoxygenase pathway (Deza-Durand and Petersen, 2011). Banerjee et al. (2013) found that the decrease in linolenic acid was linearly correlated with increased (E)-2-Hexenal content in irradiated cabbage. Caleb et al. (2016) found that (E)-2-hexenal was not detected throughout the duration of storage for 11 d at 10 ºC. They also found that propanal was only detected on the day of harvest, and that 2-ethylfuran significantly increased at 4 and 11 d in storage. In this study, (E)-2-hexenal, (E)-2-pentenal, and 3-ethylfuran contents increased at 14 d in storage, then decreased over 21 and 28 d, and were not detected at 35 d. Propanal decreased throughout the entire duration of storage. These compounds, (E)-2-hexenal (Hatanaka et al., 1996), (E)-2-pentenal (Lund et al., 1996), propanal (Ruiz del Castillo et al., 2010), and 2-ethylfuran (Medina et al., 1999; Vichi et al., 2003; Wang et al., 2001) are derived from lipid oxidation of fatty acids in plant tissues and are thought to be wound-induced volatiles (Banerjee et al., 2013; Cozzolino et al., 2016; Deza-Durand et al., 2014). Lipase enzymes hydrolyze fatty acids, releasing them from membrane lipids. Fatty acids are then acted upon by lipoxygenase and hydroperoxide lyase, forming aldehydes and alcohols (Hatanaka et al., 1996). The presence of these compounds during storage may be due to breakdown of lipid membrane tissues due to senescence. The decrease of these compounds in late storage may be due to depletion of fatty acid precursors during later stages of senescence.

Conclusion

In this study, ‘Emerald Crown’ had significantly higher levels of glucosinolates and sulfurous volatiles than ‘BH053.’ As glucosinolate contents began to decrease for ‘Emerald Crown,’

sulfurous volatile contents began to increase. However, for ‘BH053,’ glucosinolate contents did not significantly change throughout the entire duration of storage, while sulfurous volatiles start out significantly higher, then decrease before increasing over the last two weeks of storage. These results indicate that the effect of storage duration on both glucosinolates and volatiles is determined not only by precooling and storage conditions, but also by genetic factors. Other than their initial values, there was no significant difference between total glucosinolate contents for broccoli stored for 35 d at 0 ºC in ice and broccoli stored at 7 ºC. However, initial contents were significantly greater for broccoli precooled with an ice slurry than broccoli precooled with only top icing. Ice slurry cooled broccoli was cooled to 1 ºC at 2 h after the slurry was applied, while top icing cooled broccoli was cooled to 7 ºC at 6 h after top icing was applied. The increased cooling time for top iced broccoli may have led to the immediate loss of glucosinolate contents, while broccoli that was cooled faster was able to maintain higher levels by preventing the hydrolysis of glucosinolates. This is supported by significantly higher initial levels of dimethyl disulfide and dimethyl sulfide contents, which may have formed due to the breakdown of glucosinolates after harvest.

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Appendix

Table 15. Analysis of variance results for glucosinolate contents in broccoli for 'BH053' and 'Emerald Crown,' cooled with top ice and stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

	Indole Glucosinolates		
Source of Variance	Glucobrassicin	4-Methoxyglucobrassicin	Neoglucobrassicin
Cultivar (C)	NS [z]	***	***
Treatment (T)	**	NS	NS
Storage Time (D)	NS	*	NS
C x T	NS	NS	NS
T x D	NS	*	NS
C x D	*	**	NS
C x T x D	NS	*	NS

	Aliphatic Glucosinolates			
Source of Variance	Glucoraphanin	Epiprogoitrin	Progoitrin	Sinigrin
Cultivar (C)	***	***	NS	NS
Treatment (T)	**	***	***	NS
Storage Time (D)	NS	NS	NS	NS
C x T	NS	***	*	NS
T x D	NS	**	NS	NS
C x D	NS	**	NS	NS
C x T x D	NS	***	NS	NS

Table 15. Continued.

	Aliphatic Glucosinolates			
Source of Variance	Glucoerucin	Gluconapin	Glucoiberin	Glucobarbarin
Cultivar (C)	***	**	NS	NS
Treatment (T)	NS	NS	NS	*
Storage Time (D)	NS	NS	NS	NS
C x T	NS	NS	NS	NS
T x D	*	NS	NS	NS
C x D	NS	NS	NS	NS
C x T x D	*	NS	NS	NS

	Aromatic Glucosinolates	
Source of Variance	Gluconasturtiin	Glucosinalbin
Cultivar (C)	**	**
Treatment (T)	**	NS
Storage Time (D)	NS	NS
C x T	NS	NS
T x D	NS	*
C x D	*	**
C x T x D	NS	NS

[z]Significance is denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 16. Analysis of variance results for volatile contents in broccoli for 'BH053' and 'Emerald Crown,' cooled with top ice and stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

	Sulfurous Volatiles	
Source of Variance	Dimethyl Disulfide	Dimethyl Sulfide
Cultivar (C)	*** [z]	NS
Treatment (T)	***	NS
Storage Time (D)	***	***
C x T	NS	NS
T x D	NS	**
C x D	***	***
C x T x D	NS	NS

	Aldehyde Volatiles			*Furan Volatiles*
Source of Variance	(E)-2-Hexenal	(E)-2-Pentenal	Propanal	2-ethylfuran
Cultivar (C)	*	NS	NS	NS
Treatment (T)	NS	NS	NS	NS
Storage Time (D)	***	***	***	***
C x T	NS	NS	NS	NS
T x D	NS	NS	NS	NS
C x D	***	***	*	***
C x T x D	NS	NS	NS	NS

[z]Significance is denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 17. Average glucosinolate contents in broccoli (mg·g^{-1} dry mass), across time for both storage temperatures, for ‘BH053’compared with ‘Emerald Crown.’

	Cultivar	
Glucosinolate	BH053	Emerald Crown
Indole Glucosinolates		
Glucobrassicin	18224.57 a [zy]	20742.02 a
4-Methoxyglucobrassicin	188.76 b	750.58 a
Neoglucobrassicin	4352.72 b	52824.24 a
Aliphatic Glucosinolates		
Glucoraphanin	5381.67 b	12363.95 a
Epiprogoitrin	3226.01 a	693.79 b
Progoitrin	63.96 b	2035.87 a
Sinigrin	721.22 a	793.28 a
Glucoerucin	252.90 b	1331.02 a
Gluconapin	214.98 b	430.53 a
Glucoiberin	88.54 a	101.80 a
Glucobarbarin	26.97 a	51.17 a
Aromatic Glucosinolates		
Gluconasturtiin	3740.42 b	5877.34 a
Glucosinalbin	1917.74 a	842.72 b
Group Totals		

Table 17. Continued.

Aliphatic Glucosinolates	27923.77 a	37272.35 a
Aromatic Glucosinolates	5662.42 a	6685.32 a
Indole Glucosinolates	4824.98 b	54955.64 a
Total Glucosinolates	38409.12 b	98777.59 a

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual and grouping of glucosinolates, letters beside means for one cultivar that are not different from letters beside means for the other cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 18. Average glucosinolate contents in broccoli ($mg \cdot g^{-1}$ dry mass), across time for both cultivars, for top icing cooled broccoli stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice.

	Storage Temperature	
Glucosinolate	7 ºC	0 ºC
Indole Glucosinolates		
Glucobrassicin	14615.97 b [zy]	24350.61 a
4-Methoxyglucobrassicin	452.04 a	487.30 a
Neoglucobrassicin	20402.31 a	36774.65 a
Aliphatic Glucosinolates		
Glucoraphanin	6243.18 b	11502.44 a
Epiprogoitrin	1360.27 b	2559.53 a
Progoitrin	779.18 a	1338.65 a
Sinigrin	1098.64 a	1661.81 a
Glucoerucin	830.22 a	753.71 a
Gluconapin	259.17 a	386.34 a
Glucoiberin	116.15 a	74.19 a
Glucobarbarin	14.88 b	63.25 a
Aromatic Glucosinolates		
Gluconasturtiin	3669.98 b	5947.78 a
Glucosinalbin	721.55 a	792.95 a
Group Totals		

Table 18. Continued.

Aliphatic Glucosinolates	24142.29 b	41053.67 a
Aromatic Glucosinolates	4751.25 b	7596.49 a
Indole Glucosinolates	21698.27 a	38081.35 a
Total Glucosinolates	50523.67 b	86662.91 a

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual and grouping of glucosinolates, letters beside means for one storage temperature that are not different from letters beside means for the other storage temperature, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 19. Average glucosinolate contents in broccoli ($mg \cdot g^{-1}$ dry mass), for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, and 35 days.

	Storage Time (d)					
Glucosinolate	0	7	14	21	28	35
Indole Glucosinolates						
Glucobrassicin	25125.49 a [zy]	21151.24 ab	21400.64 ab	17177.62 ab	19459.67 ab	12585.09 b
4-Methoxyglucobrassicin	712.45 a	442.06 ab	752.21 a	516.94 ab	283.53 b	110.82 b
Neoglucobrassicin	52650.74 a	32955.24 ab	26640.18 ab	23949.21 ab	18660.69 b	16674.81 b
Aliphatic Glucosinolates						
Glucoraphanin	13637.62 a	7795.94 ab	9421.72 ab	9714.82 ab	7863.44 ab	4803.31 b
Epiprogoitrin	1645.01 bc	1965.64 abc	2355.46 a	2025.18 abc	2347.40 ab	1420.72 c
Progoitrin	1968.69 a	914.85 b	935.44 b	1175.81 ab	826.66 b	532.05 b
Sinigrin	889.52 a	1210.02 a	614.69 a	532.17 a	963.15 a	333.95 a
Glucoerucin	947.17 a	629.25 a	727.81 a	833.76 a	829.58 a	784.23 a
Gluconapin	419.18 a	318.35 a	353.34 a	298.55 a	284.40 a	262.72 a

Table 19. Continued.

Glucoiberin	39.41 b	183.38 a	58.13 b	91.17 ab	108.84 ab	90.07 ab
Glucobarbarin	21.31 ab	20.67 ab	70.98 ab	85.69 a	6.22 b	29.53 ab
Aromatic Glucosinolates						
Gluconasturtiin	5322.11 a	4648.49 a	4915.54 a	4849.46 a	5245.01 a	3872.68 a
Glucosinalbin	1385.67 ab	1378.35 ab	2182.55 a	963.18 b	1123.48 ab	1248.14 ab
Group Totals						
Aliphatic Glucosinolates	43771.83 a	33585.67 ab	35185.55 ab	31062.19 ab	31900.34 ab	20080.94 b
Aromatic Glucosinolates	6690.40 a	6009.46 a	7080.72 a	5795.27 a	6351.12 a	5116.25 a
Indole Glucosinolates	54330.48 a	34046.03 ab	28190.00 ab	25384.42 ab	19778.84 b	17609.09 b
Total Glucosinolates	104724.76 a	73574.10 ab	70389.52 ab	62173.39 ab	57963.08 ab	42733.82 b

[z]Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual and grouping of glucosinolates, letters beside means for one storage time that are not different from letters beside means for that carotenoid at other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 20. Average epiprogoitrin content in broccoli ($mg \cdot g^{-1}$ dry mass), across time, for 'BH053' compared with 'Emerald Crown,' and top icing cooled broccoli stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice.

		Glucosinolate
Cultivar	Storage Temperature	Epiprogoitrin
BH053	7 ºC	2226.26 b [zyx]
	0 ºC	4225.76 a
Emerald Crown	7 ºC	494.27 c
	0 ºC	893.31 c

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]Interaction effects between cultivar and storage temperature on epiprogoitrin content in broccoli were significant ($p \leq 0.05$).

[x]For epiprogoitrin, letters beside means for one cultivar and storage temperature that are not different from letters beside means for other cultivars and storage temperatures, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 21. Average glucosinolate contents in broccoli (mg·g^{-1} dry mass), stored at both temperatures, for 'BH053' compared with 'Emerald Crown,' and compared during storage at 0, 7, 14, 21, 28, and 35 days.

		Glucosinolate				
		Indole Glucosinolates		*Aliphatic Glucosinolates*	*Aromatic Glucosinolates*	
Cultivar	Storage Time (d)	Glucobrassicin	4-Methoxy-glucobrassicin	Epiprogoitrin	Gluconasturtiin	Glucosinalbin
BH053	0	11880.75 b [zy]	52.16 e	2141.05 bc	1731.66 c	975.20 bcde
	7	18023.10 b	202.35 de	3325.04 a	3264.34 bc	2074.87 bc
	14	24974.56 ab	224.20 de	4070.09 a	4592.16 bc	3787.81 a
	21	15704.32 b	178.58 de	3433.44 a	3699.28 bc	1707.34 bcd
	28	25969.41 ab	311.76 cde	4186.39 a	5852.24 ab	2123.36 b
	35	12795.27 b	163.50 de	2200.07 b	3302.84 bc	837.83 bcde

Table 21. Continued.

	0	38370.23 a	1372.75 a	1796.13 bcd	1148.97 cd	8912.56 a
	7	24279.38 ab	681.76 bcd	681.83 bcde	606.24 d	6032.63 ab
Emerald	14	17826.72 b	1280.21 ab	577.30 cde	640.83 d	5238.91 abc
Crown	21	18650.93 b	855.31 abc	219.02 de	616.92 d	5999.64 ab
	28	12949.93 b	255.30 cde	123.61 e	508.42 d	4637.78 bc
	35	12374.91 b	58.14 e	1658.45 bcde	641.38 d	4442.52 bc

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]Interaction effects between cultivar and storage time on individual glucosinolate contents in broccoli were significant ($p \leq 0.05$).

[x]For each individual glucosinolate, letters beside means for one cultivar and storage time that are not different from letters beside means for other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 22. Average total aliphatic and aromatic glucosinolate contents in broccoli ($mg \cdot g^{-1}$ dry mass), stored at both temperatures, for 'BH053' compared with 'Emerald Crown,' and compared during storage at 0, 7, 14, 21, 28, and 35 days.

		Glucosinolates	
Cultivar	Storage Time (d)	Aliphatic Glucosinolates	Aromatic Glucosinolates
BH053	0	18348.27 b [zyx]	2706.86 c
	7	27694.67 b	5339.21 bc
	14	36360.04 b	8379.97 ab
	21	25917.56 b	5406.62 bc
	28	39306.34 b	7975.60 ab
	35	19914.88 b	4166.28 bc
Emerald Crown	0	69195.40 a	10673.95 a
	7	39478.07 ab	6679.71 abc
	14	34012.26 b	5781.47 abc
	21	36207.02 b	6183.91 abc
	28	24494.16 b	4726.65 bc
	35	20247.99 b	6066.22 abc

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 22. Continued.

[y]Interaction effects between cultivar and storage time on aliphatic and aromatic glucosinolate contents in broccoli were significant ($p \leq 0.05$).

[x]For each glucosinolate group, letters beside means for one cultivar and storage time that are not different from letters beside means for other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 23. Average glucosinolate contents in broccoli ($mg \cdot g^{-1}$ dry mass), for both cultivars, for top icing cooled broccoli stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

Storage Temperature	Storage Time (d)	Glucosinolate			
		Epiprogoitrin	Glucoerucin	4-Methoxy-glucobrassicin	Glucosinalbin
7 ºC	0	1391.88 cde[zyx]	374.72 b	263.62 cd	640.86 cd
	7	1729.08 cd	600.92 b	370.84 cd	1120.27 bcd
	14	1831.83 cd	932.19 ab	1010.10 ab	2945.15 a
	21	1660.57 cde	942.94 ab	684.13 abc	1044.17 bcd
	28	844.04 de	1078.01 ab	253.83 cd	678.67 cd
	35	704.21 e	1052.57 ab	129.72 cd	162.74 d
0 ºC	0	1898.13 bc	1519.62 a	1161.28 a	2130.47 abc
	7	2202.20 bc	657.58 b	513.28 bcd	1636.42 abcd
	14	2879.09 ab	523.44 b	494.32 bcd	1419.95 abcd
	21	2389.78 bc	724.57 b	349.75 cd	882.19 bcd
	28	3850.77 a	581.15 b	313.23 cd	1568.30 abcd
	35	2137.23 bc	515.88 b	91.92 d	2333.54 ab

[z]Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

Table 23. Continued.

[y]Interaction effects between storage temperature and storage time on individual glucosinolate contents in broccoli were significant ($p \leq 0.05$).

[x]For each individual glucosinolate, letters beside means for one storage temperature and storage time that are not different from letters beside means for other storage temperatures and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 24. Average glucosinolate contents in broccoli (mg·g^{-1} dry mass) for 'BH053' compared with 'Emerald Crown,' cooled with top ice and stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days.

			Glucosinolate	
			Indole Glucosinolates	*Aliphatic Glucosinolates*
Cultivar	Storage Temperature	Storage Time (d)	4-Methoxyglucobrassicin	Epiprogoitrin
BH053	7 ºC	0	50.56 e [zyx]	2255.93 efg
		7	132.24 de	3001.61 cdef
		14	342.43 de	3175.21 cdef
		21	218.25 de	2701.64 def
		28	224.09 de	1220.40 ghi
		35	143.16 de	1002.80 ghi
	0 ºC	0	53.75 e	2026.16 efgh
		7	272.46 de	3648.47 cd
		14	105.98 de	4964.97 b
		21	138.91 de	4165.23 bc
		28	399.44 cde	7152.38 a
		35	183.83 de	3397.34 cde

Table 24. Continued.

	7 ºC	0	476.68 cde	527.83 i
		7	609.43 cde	456.54 i
		14	1677.77 ab	488.45 i
		21	1150.02 bc	619.50 hi
		28	283.57 de	467.68 i
Emerald		35	116.27 de	405.63 i
Crown	0 ºC	0	2268.81 a	1770.10 fghi
		7	754.10 cde	755.94 hi
		14	882.65 bcd	793.21 hi
		21	560.60 cde	614.33 hi
		28	227.03 de	549.17 i
		35	0.00 e	877.12 ghi

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]Interaction effects between cultivar, storage temperature, and storage time on individual glucosinolate contents in broccoli were significant ($p \leq 0.05$).

[x]For each individual glucosinolate, letters beside means for one cultivar, storage temperature, and storage time that are not different from letters beside means for other cultivars, storage temperatures, and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 25. Average volatile contents in broccoli (μg·g^{-1} fresh weight), stored at both temperatures, for 'BH053' compared with 'Emerald Crown,' and compared during storage at 0, 7, 14, 21, 28, and 35 days.

		Volatile					
		Sulfurous Volatiles		*Aldehyde Volatiles*			*Furan Volatiles*
Cultivar	Storage Time (d)	Dimethyl Disulfide	Dimethyl Sulfide	(E)-2-Hexenal	(E)-2-Pentenal	Propanal	2-Ethylfuran
BH053	0	123.88 b [zyx]	4.47 b	0.00 d	0.00 d	0.00 d	0.00 d
	7	7.98 c	0.78 c	0.34 bc	14.44 cd	0.91 a	28.83 abc
	14	3.67 c	0.96 c	0.40 abc	32.98 ab	0.65 ab	40.56 a
	21	16.69 c	2.00 c	0.29 bc	26.18 abc	0.62 ab	24.81 bc
	28	39.49 c	1.32 c	0.15 cd	14.27 cd	0.07 cd	34.72 ab
	35	53.27 c	7.20 a	0.00 d	0.00 d	0.00 d	0.00 d

Table 25. Continued.

	0	33.33 c	0.68 c	0.67 a	23.97 bc	0.47 bc	21.95 bc
	7	43.63 c	0.49 c	0.62 a	42.35 a	0.48 bc	37.37 ab
Emerald	14	57.10 c	0.79 c	0.49 ab	34.21 ab	0.29 bcd	26.25 abc
Crown	21	137.08 b	0.65 c	0.18 cd	0.00 d	0.15 cd	17.34 c
	28	170.79 ab	6.57 ab	0.00 d	0.00 d	0.06 cd	0.00 d
	35	205.22 a	8.14 a	0.00 d	0.00 d	0.05 cd	0.00 d

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]Interaction effects between cultivar and storage time on volatile contents in broccoli were significant ($p \leq 0.05$).

[x]For each individual volatile, letters beside means for one cultivar and storage time that are not different from letters beside means for other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

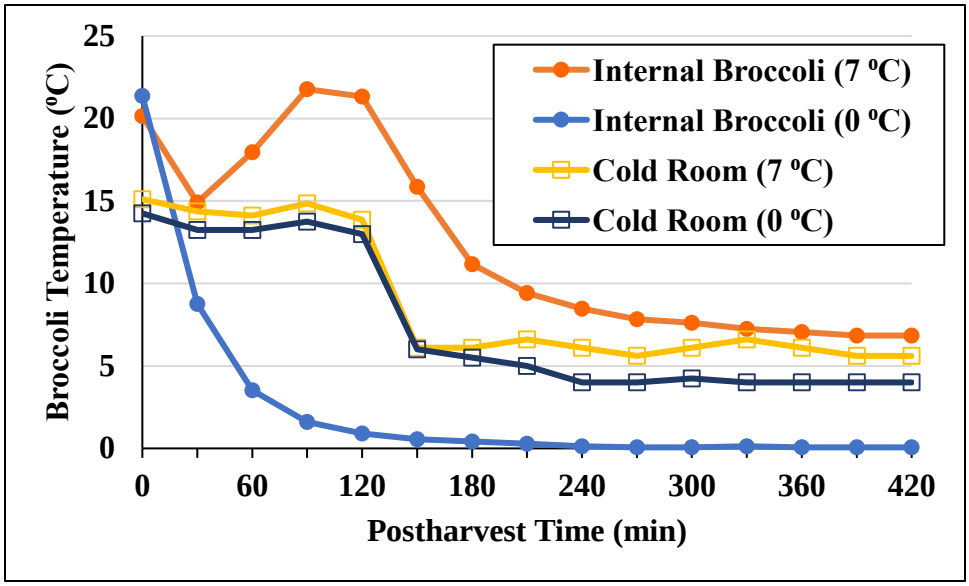

Fig. 12. Mean internal broccoli temperature for broccoli cooled with top icing and stored 5 ºC, and broccoli cooled with an ice slurry and stored at 0 ºC. Postharvest broccoli temperatures were recorded every 30 minutes during field cooling and transportation to cold room storage. Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown' per storage temperature.

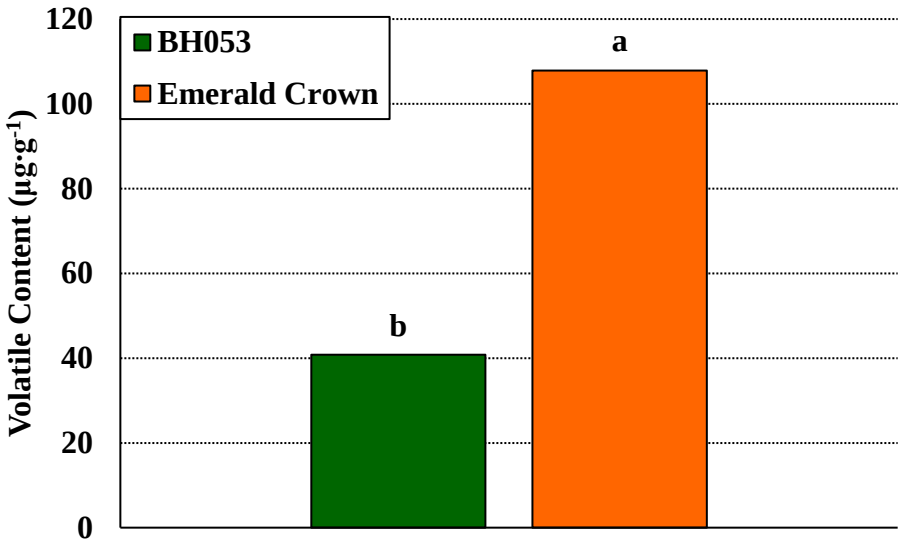

Fig. 13. Average dimethyl disulfide contents in broccoli, across time at both storage temperatures, for ‘BH053’ compared with ‘Emerald Crown.’ Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one cultivar that are not different from letters above the other cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

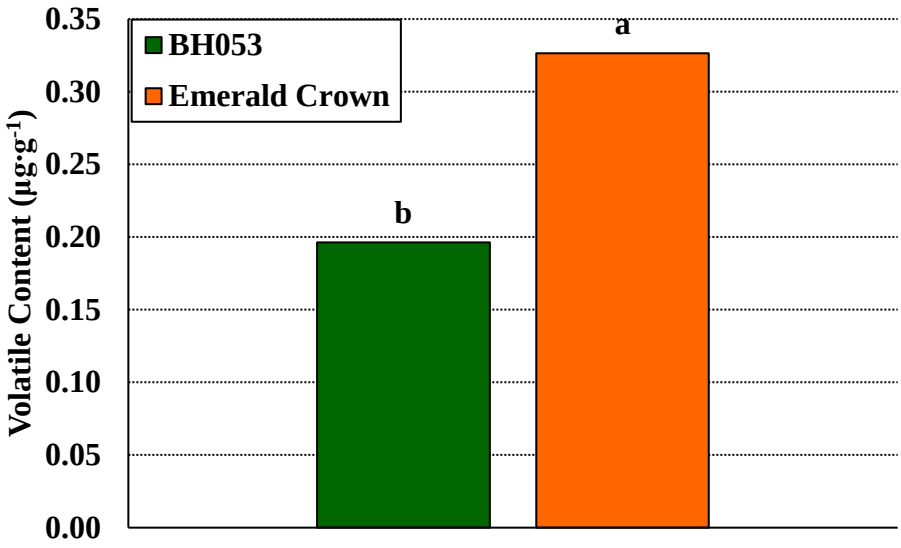

Fig. 14. Average (E)-2-hexenal content in broccoli, across time at both storage temperatures, for ‘BH053’ compared with ‘Emerald Crown.’ Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one cultivar that are not different from letters above the other cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

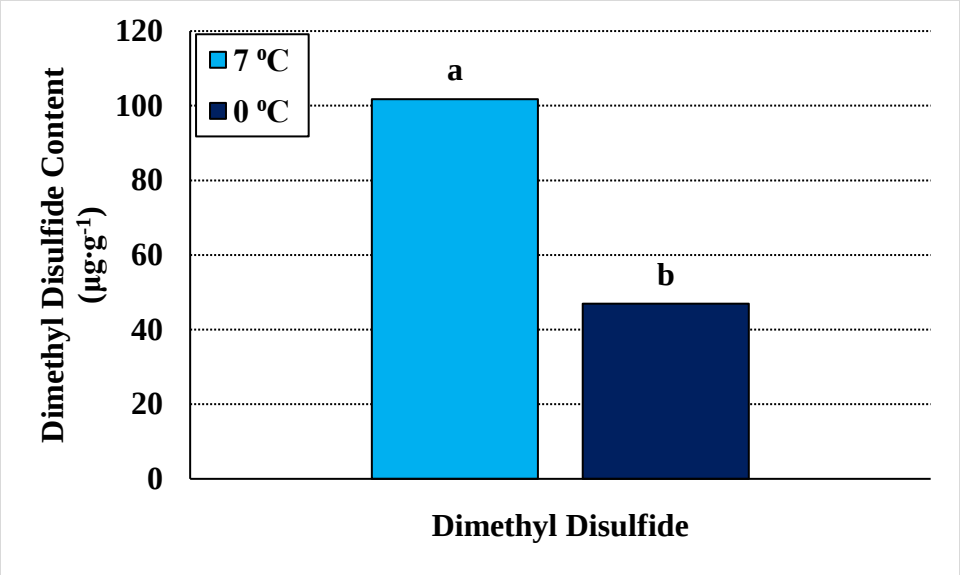

Fig. 15. Average dimethyl disulfide content in broccoli, across time for both cultivars, cooled with top icing and stored at 7 ºC, compared to broccoli cooled with an ice slurry and stored at 0 ºC in ice. Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one storage temperature that are not different from letters above the other storage temperature, are not significantly different by the LSD test ($\alpha = 0.05$).

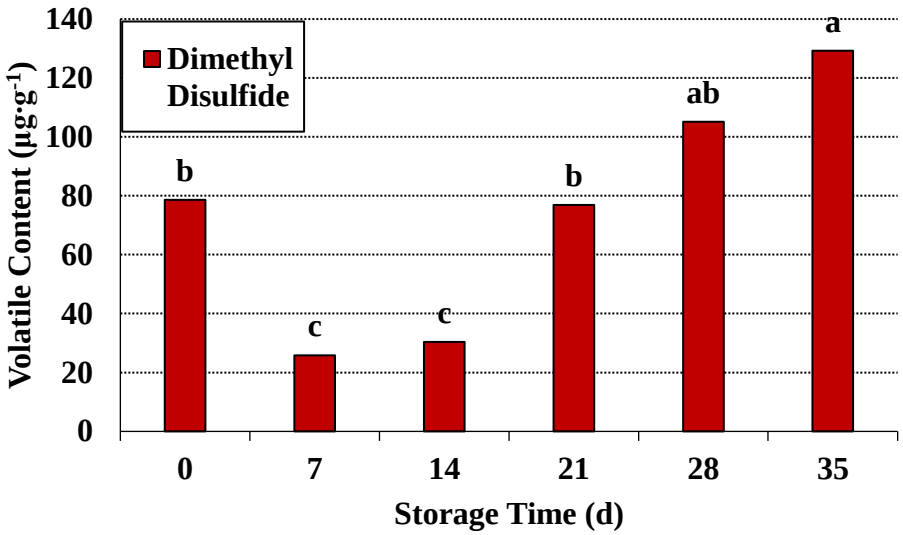

Fig. 16. Average dimethyl disulfide content in broccoli, for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, or 35 days. Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination. Letters above one storage time that are not different from letters above other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

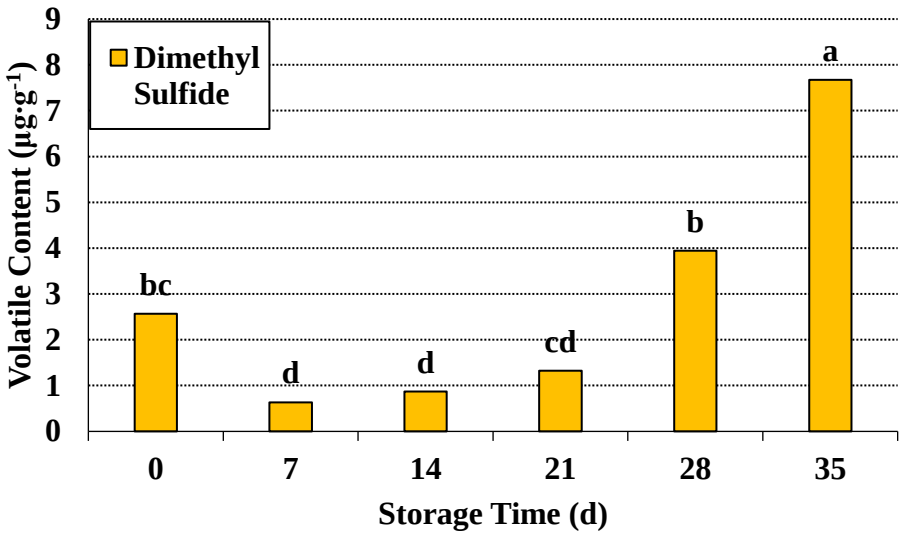

Fig. 17. Average dimethyl disulfide content in broccoli compared during storage at 0, 7, 14, 21, 28, or 35 days. Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination. Letters above one storage time that are not different from letters above other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

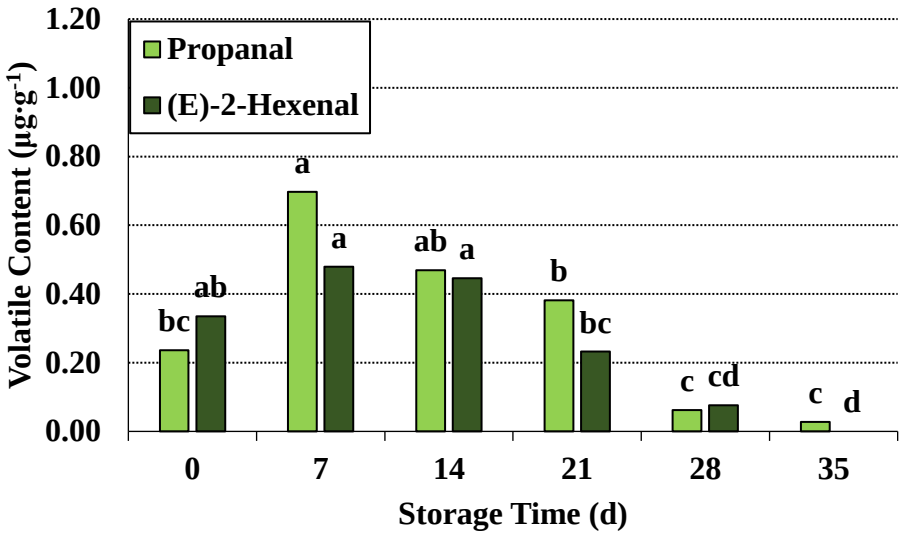

Fig. 18. Average propanal and (E)-2-hexenal in broccoli compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination. For each individual volatile, letters above one storage time that are not different from letters above other storage times for that volatile, are not significantly different by the LSD test ($\alpha = 0.05$).

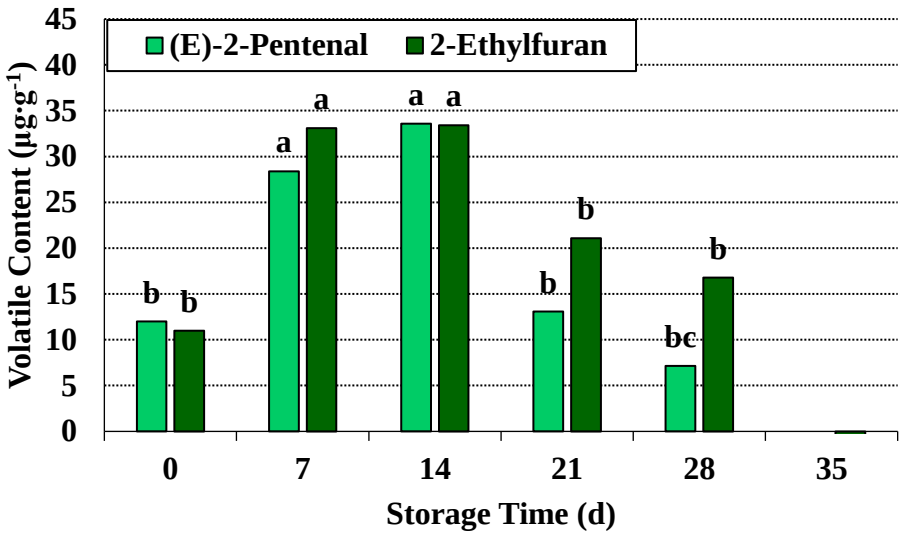

Figure 19. Average (E)-2-pentenal and 2-ethylfuran contents (µg·g^{-1} fresh mass) in broccoli compared during storage at 0, 7, 14, 21, 28, or 35 days Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination. For each individual volatile, letters above one storage time that are not different from letters above other storage times for that volatile, are not significantly different by the LSD test ($\alpha = 0.05$).

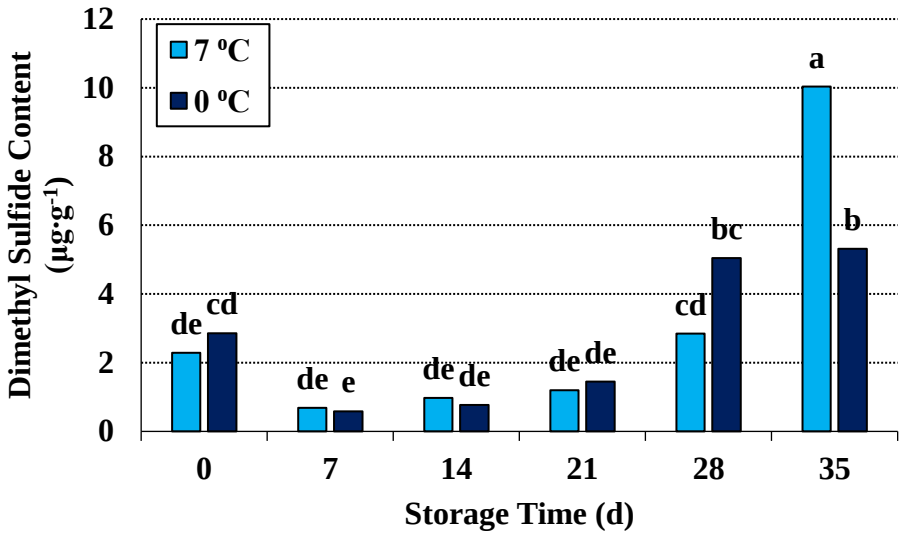

Figure 20. Average dimethyl sulfide content in broccoli, for both cultivars, cooled with top ice and stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, and compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination. Letters above one storage temperature and storage time that are not different from letters above other storage temperatures and times for that volatile, are not significantly different by the LSD test ($\alpha = 0.05$).

CHAPTER 3: EFFECTS OF COOLING AND POSTHARVEST STORAGE METHODS ON CAROTENOIDS AND CHLOROPHYLL IN BROCCOLI

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Additional index words. Brassica oleracea, pre-cooling, temperature, chemical properties

Abstract

Broccoli (*Brassica oleracea* L. var. *italica*) is a cool-weather vegetable that is grown for its edible flowering heads and stalks. Carotenoids and chlorophyll affect the organoleptic and nutritional characteristics that are associated with broccoli quality. Cooling and postharvest storage conditions affect the quality of broccoli by altering the levels of carotenoid chlorophyll contents. Changes in carotenoid and chlorophyll contents were investigated for two cultivars ('BH053' and 'Emerald Crown'), two temperature treatments (cooled with top icing stored at 7 ºC, and cooled with an ice slurry and stored at 0 ºC in ice), and six different days in storage (0, 7, 14, 21, 28, and 35 days). Results from this study indicate that cultivar and storage time significantly influence both carotenoid and chlorophyll contents in broccoli. However, storage temperature did not appear to have a significant impact on carotenoid or chlorophyll contents. Violaxanthin, neoxanthin, antheraxanthin, lutein, β-carotene, α-carotene, chlorophyll *a,* and chlorophyll *b* contents were significantly greater for 'BH053' than for 'Emerald Crown.' Similarly, all carotenoid and chlorophyll contents significantly decreased during storage.

Violaxanthin, neoxanthin, and antheraxanthin contents significantly decreased at 21 d, while lutein and β-carotene contents significantly decreased at 7 d and α-carotene contents significantly decreased at 14 d. Chlorophyll *a* content slightly increased at 7 d, then significantly decreased at 14 d, while chlorophyll *b* content steadily decreased throughout the duration of storage. The interaction of cultivar and storage time significantly affected β-carotene content alone. β-carotene content significantly decreased at 7 d for 'Emerald Crown,' while β-carotene content did not significantly decrease until 21 d for 'BH053.' Results from this study indicate that carotenoid and chlorophyll contents are more dependent on cultivar and storage time than storage temperature. These results show that both carotenoid and chlorophyll contents decrease as the duration of storage increases. Although storage temperature did not appear to have a statistically significant impact, total carotenoid and chlorophyll contents were slightly greater for broccoli cooled with an ice slurry and stored at 0 ºC in ice than for broccoli cooled with top icing and stored at 7 ºC. Due to its significantly greater carotenoid and chlorophyll contents, the postharvest quality appears to be significantly greater for 'BH053' than for 'Emerald Crown.'

Introduction

Broccoli contains high levels of health-promoting antioxidants, including ascorbic acid, phenols, flavonoids, and carotenoids, and chlorophyll (Duarte-Sierra et al., 2017; Fernández-Leon et al., 2013; Li et al., 2014; Raseetha et al., 2013; Vallejo et al., 2003). However, senescence is triggered rapidly after harvest, resulting in a loss of quality and marketability (King and Morris, 1994). Postharvest senescence of broccoli is accompanied by the degradation of metabolites through respiration (Hasparue et al., 2015; King and Morris., 1994), which affects the nutritional, sensory, and physiological quality of broccoli (Bruckner et al., 2005; Hansen et al., 1997; Pellegrino et al., 2019).

The United States (U.S.) broccoli industry is currently centered on West Coast production. California is responsible for over 90% of the total broccoli production in the nation, followed by Arizona (5%) (USDA Economic Research Service, 2011). Consequently, most of the fresh broccoli sold in the Eastern U.S. has been processed and shipped thousands of miles across the country before reaching supermarkets. Broccoli is known to have a high respiration rate, and these changes in the time between harvesting and consumer availability have potential consequences on postharvest physiology. Establishing a locally sourced broccoli industry on the East Coast will reduce the time between harvesting and consumer availability (Atallah et al., 2014; Wheeler et al., 2018).

Sensory quality, including color, aroma, flavor, and tissue morphology, is an important factor for consumer acceptance of broccoli (Luo et al., 2018). Degradation of carotenoids and chlorophyll leads to yellowing in broccoli, which is the main visible symptom of senescence (Eason et al., 2005; King and Morris, 1994). In addition, these phytochemical losses lead to a loss in nutritional value (Caleb et al., 2016). Plant carotenoids act as antioxidants and play an important role in the human diet (Hasperué et al., 2016; Lefsrud et al., 2005). Carotenoids are the main dietary source of provitamin A (Farnham and Kopsell, 2009; Jeffery et al., 2003; Lefsrud et al., 2005). They may also contribute to the prevention of cancer, cardiovascular diseases, and age-related macular degeneration. Chlorophyll also has potential health-promoting benefits, such as cancer prevention, antimutagenic activities, and tumor cell apoptosis (Balder et al., 2006; Egner et al., 2001; Farnham and Kopsell, 2009).

Carotenoids are lipid-soluble phytochemicals that span the chlorophyll thylakoid membranes and play important roles in light harvesting, photoprotection, and structural stabilization in plants (Velasco et al., 2008). The main carotenoids found in broccoli are lutein and β-carotene, an

oxygenated xanthophyll and a hydrocarbon carotene, respectively. Other common carotenoids found in broccoli include α-carotene, and xanthophyll cycle pigments (zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin) (Hasperué et al., 2016; Oelmuller et al., 1985; Niyogi et al., 1997). Chlorophyll *a* and chlorophyll *b* are found in all green plants and edible parts of vegetables (Kidmose et al., 2002). In plants, chlorophyll form photosystem complexes that span the thylakoid membranes of chloroplasts and allow plants to absorb energy from light (Farnham and Kopsell, 2009). Together, these pigments play an essential role in the maintenance of postharvest broccoli quality (Loi et al., 2019).

Storage conditions impact the appearance and quality of broccoli by altering carotenoid and chlorophyll concentrations (Mahn and Reyes, 2012; Serrano et al., 2006). Objectives of this study are to quantify the effects of cooling and postharvest storage method on the carotenoid and chlorophyll contents for two cultivars of broccoli. This will help to determine the proper storage conditions for maintaining postharvest quality of broccoli grown and distributed along the U.S. East Coast.

Materials and Methods

Plant materials and storage.

Broccoli was supplied by the Upper Mountain Research Station in Laurel Springs, North Carolina. Broccoli was grown according to recommended management practices for the southeastern U.S. (Kemble et al., 2018). Broccoli was harvested when the majority of the heads had reached commercial maturity. The average head diameter was 8.4 cm ± 1.5. Two cultivars, ‘BH053’ and ‘Emerald Crown,’ were harvested on 31 July 2019 and 5 Aug. 2019, respectively. The average head diameter of ‘BH053’ was 8.2 cm ± 1.4, while the average head diameter of ‘Emerald Crown’ was 8.7 cm ± 1.5. Each cultivar was separated into two treatment groups

immediately after harvest. One treatment group was cooled by top icing to remove field heat, while the other treatment group was cooled by submerging in an ice slurry. Broccoli was then transported to The University of Tennessee Institute of Agriculture for cold room storage. Top iced broccoli was cooled to 16 ºC ± 4 when it reached the storage cooler (Fig. 12) and the top icing had melted during transportation. Broccoli from this treatment group was then placed in cold storage and kept in waxed corrugated boxes without ice. The cold room temperature was maintained at 6 ºC ± 0.4 and the internal broccoli temperature was maintained at 7 ºC ± 1. For the other treatment group, broccoli that was placed in an ice slurry was cooled to 1 ºC ± 1 at 2 h after the slurry was applied. Broccoli from this treatment group was then placed in cold storage and kept in coolers filled with ice. The cold room temperature was maintained at 4ºC ± 0.2 and the internal broccoli temperature was maintained at 0 ºC ± 0.3 (Fig. 12). Internal broccoli temperatures were recorded every 30 min with Watch Dog® data loggers (Spectrum® Technologies, Inc., Aurora, IL, USA).

Postharvest analysis.

Broccoli was removed from storage at 0, 7, 14, 21, 28, and 35 days. Four replications of 'BH053' and three replications of 'Emerald Crown,' consisting of two broccoli heads per replication, were subsampled for each cultivar and treatment combination. For each replication, 30 g ± 1 fresh tissue was placed into plastic bags and stored in a -80 ºC freezer overnight, and frozen tissue was freeze-dried the following day. Freeze-dried tissue was ground to a fine powder, using a mortar and pestle in liquid nitrogen, for extraction and analysis.

Carotenoid/chlorophyll extraction and analysis.

Carotenoids were extracted from broccoli tissue using the method by Kopsell et al. (2012). A 0.1 g ± 0.01 subsample of finely ground broccoli tissue was weighed into a 16 x 150 mm glass centrifuge tube, then transferred to a tissue grinding tube (Potter-Elvehjem; Kimble Chase-Kontes

Glass, Vineland, NJ). Samples were then hydrated with 800 µL of RO water for 10 min. Then, 800 µL of carotenoid internal standard (Sigma-Aldrich, St. Louis, MO) and 2.5 mL of tetrahydrofuran (THF) were added the grinding tube. Samples were homogenized, with a pestle attached to a drill press, at 540 rpm. Homogenized samples were transferred back into a 16 x 100 mL glass centrifuge tube and centrifuged at 500 g_n for 5 min. The supernatant was collected in a graduated glass centrifuge tube and kept on ice in a light-blocking container. The remaining precipitate was re-suspended with 2.0 mL of THF and homogenized using the same process. This procedure was repeated for a total of 4 extractions. Using a nitrogen airstream, the supernatant was evaporated to 0.5 mL, then brought to 5 mL volume with acetone. The liquid sample was then filtered twice, once through a 0.45 µm PTFE filter and once through a 0.20 µm PTFE filter, into 12x13 mm light-blocking crimp top vials.

Carotenoids and chlorophyll were separated using an Agilent 1100 series high-performance liquid chromatography unit with a photodiode array detector (Agilent Technologies, Santa Clara, CA). The column temperature was set at 30ºCfor a reverse-phase 250 x 4.6 mm i.d., 5 µm analytical scale, 200 Aº polymeric C30 column equipped with a 4.0 x 10 mm guard cartridge and holder (ProntoSIL; MAC-MOD Analytical, Chadds Ford, PA). Separations were achieved isocratically using a binary mobile phase of 11% methyl tert-butyl ether, 88.99% methanol, and 0.01% triethylamine. The flow rate was set at 1.0 $mL \cdot min^{-1}$, and 10.0 µL of each sample were injected for a total run time of 58 min per sample. Carotenoids and chlorophyll were assigned based on external standards and expressed on a fresh mass basis in $\mu g \cdot g^{-1}$. Data was collected, recorded and integrated using ChemStation Software (Agilent Technologies, Palo Alto, CA).

Statistical analysis.

SAS statistical software (9.4 for Windows; SAS Institute, Cary, NC) was used for the analyses of data. Cultivar, storage temperature, storage time, and their interactions were considered fixed factors, while replication was considered the random factor. Analysis of variance (ANOVA) tests were performed using the GLIMMIX procedure, and means were compared by the least significant difference (LSD) test (α = 0.05). ANOVA results are presented for carotenoids (Table 21) and chlorophyll (Table 22).

Results

Carotenoids, effects of cultivar.

Cultivar significantly affected violaxanthin (F = 27.67, df = 1, $p \leq 0.0001$), neoxanthin (F = 12.32, df = 1, $p \leq 0.001$), antheraxanthin (F = 10.43, df = 1, $p \leq 0.01$), lutein (F = 31.82, df = 1, $p \leq 0.0001$), α-carotene (F = 23.75, df = 1, $p \leq 0.0001$), β-carotene (F = 29.73, df = 1, $p \leq 0.0001$), and total carotenoid (F = 31.66, df = 1, $p \leq 0.0001$) contents in broccoli. Violaxanthin, neoxanthin, antheraxanthin, lutein, α-carotene, β-carotene, and total carotenoid contents were significantly greater for 'BH053' than for 'Emerald Crown' (Table 23).

Carotenoids, effects of cooling/storage method.

Storage temperature did not significantly affect carotenoid contents in broccoli. Violaxanthin, neoxanthin, antheraxanthin, lutein, α-carotene, β-carotene, and total carotenoid contents were not significantly different for broccoli cooled with top icing and stored at 7 ºC, and broccoli cooled with an ice slurry and stored at 0 ºC in ice (Table 24).

Storage time significantly affected violaxanthin (F = 10.78, df = 5, $p \leq 0.0001$), neoxanthin (F =24.58, df = 5, $p \leq 0.0001$), antheraxanthin (F = 25.18, df = 5, $p \leq 0.0001$), lutein (F = 24.88, df = 5, $p \leq 0.0001$), α-carotene (F = 11.51, df = 5, $p \leq 0.0001$), β-carotene (F = 24.84, df = 5, p

≤0.0001), and total carotenoid (F = 24.85, df = 5, p ≤0.0001) contents in broccoli. Violaxanthin, neoxanthin, antheraxanthin, lutein, α-carotene, β-carotene, and total carotenoid contents significantly decreased in broccoli stored for 35 d at 5 and 0 ºC (Table 25). Violaxanthin, neoxanthin, and antheraxanthin contents remained stable from 0 to 14 d, then significantly decreased at 21 d. After that, both neoxanthin and antheraxanthin contents significantly decreased at 28 d and remained stable from 28 to 35 d, while violaxanthin content continued to decrease from 21 to 35 d, but not significantly. Both lutein and β-carotene contents significantly decreased at 7 d, remained stable from 7 to 14 d, then significantly decreased at 21 d. Lutein significantly decreased again at 28 d and remained stable from 28 to 35 d, while β-carotene content continued to decrease from 21 to 35 d, but not significantly. α-Carotene significantly decreased from its initial content level (0 d) at 14 d, then remained stable from 14 to 21 d. After that, α-carotene content significantly decreased at 28 d and remained stable from 28 to 35 d. Total carotenoid contents significantly decreased at 7 d, then remained stable from 7 to 14 d. Total carotenoid levels significantly decreased at both 21 and 28 d, then remained stable from 28 to 35 d.

Carotenoids, interactions of cultivar and storage time.

Interactions of cultivar and storage time significantly affected β-carotene (F = 3.78, df = 5, p ≤0.01) contents in broccoli. For both 'BH053' and 'Emerald Crown,' β-carotene contents significantly decreased in broccoli stored for 35 d at 5 and 0 ºC (Fig. 21). For 'BH053,' β-carotene content decreased at 7 d, then increased at 14 d, but not significantly. After that, β-carotene content significantly decreased at 21 d, then continued to decrease from 21 to 35 d but did not significantly change. In contrast, β-carotene content for 'Emerald Crown' significantly decreased at 7 d and remained stable from 7 to 14 d. After that, β-carotene content continued to

decrease from 14 to 35 d but did not significantly change. β-carotene contents were significantly greater for ‘BH053’ than for ‘Emerald Crown’ during the first 14 d in storage. Although slightly greater for ‘BH053,’ there was no significant difference among cultivars from 21 to 35 d.

Chlorophyll, effects of cultivar.

Cultivar significantly affected chlorophyll *a* ($F = 7.95$, $df = 1$, $p \leq 0.01$), chlorophyll *b* ($F = 22.91$, $df = 1$, $p \leq 0.0001$), and total chlorophyll ($F = 13.38$, $df = 1$, $p \leq 0.001$) contents in broccoli. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were significantly greater for ‘BH053’ than for ‘Emerald Crown’ (Fig. 22).

Chlorophyll, effects of cooling/storage method.

Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were not significantly different for broccoli cooled with top icing and stored at 7 ºC, and broccoli cooled with an ice slurry and stored at 0 ºC in ice (Fig. 23).

Storage time significantly affected chlorophyll *a* ($F = 7.52$, $df = 5$, $p \leq 0.0001$), chlorophyll *b* ($F = 22.93$, $df = 5$, $p \leq 0.0001$), and total chlorophyll ($F = 12.22$, $df = 5$, $p \leq 0.0001$) contents in broccoli. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents significantly decreased in broccoli stored for 35 d at 5 and 0 ºC (Fig. 24). Chlorophyll *a* content increased at 7 d, but not significantly, then significantly decreased at 14 d. After that, chlorophyll *a* content slightly decreased at 21 d, but not significantly, and remained stable from 21 to 35 d. Chlorophyll *b* content significantly decreased from its initial content level (0 d) at 14 d, then significantly decreased at both 21 and 28 d, and remained stable from 28 to 35 d. Total chlorophyll contents remained stable from 0 to 7 d, then significantly decreased at 14 d. After that, total chlorophyll contents decreased at 21 and 28 d, but not significantly, then remained stable from 28 to 35 d.

Discussion

Carotenoids, effects of cultivar.

Broccoli is a good source of provitamin A and other carotenoids correlated with the prevention of chronic diseases (Mahn and Reyes, 2012). Increased consumption of vegetables with high carotenoid contents can help decrease the incidence of certain forms of cancer (Mayne, 1996; Podsedek, 2007; Verhoeven et al., 1996), and lutein is known to prevent macular degeneration and cataracts of the eye (Krinsky et al., 2003). In plants, carotenoids are correlated with chlorophyll contents and the intensity of green pigmentation (Khoo et al., 2011). Carotenoids surround chlorophyll complexes along thylakoid membranes, serving as light-harvesting pigments that protect chlorophyll from photodestructive reactions (Braumann et al., 1984). Carotenoid pigments are able to prevent oxidative damage to photosynthetic structures by binding to singlet oxygen and by quenching excited triplet chlorophyll to dissipate excess energy (Farnham and Kopsell, 2009), which helps to maintain broccoli quality during storage. Because the green color of broccoli is associated with the perception of freshness, increased carotenoids are also essential for the maintenance of sensory quality during storage (Paradis et al., 1996).

Carotenoid contents are thought to be primarily dependent on genotype (Ibrahim and Juvik, 2009; Renaud et al., 2014b). Farnham and Kopsell (2009) reported lutein as the most prevalent carotenoid in broccoli, accounting for over 50% of total carotenoids. Similarly, lutein was the most abundant carotenoid in both ‘BH053’ and ‘Emerald Crown’ cultivars in this study. In contrast, Fernández-Leon et al. (2012) found that β-carotene was the main carotenoid in broccoli. However, β-carotene was the second most abundant carotenoid for both cultivars in this study. There is conflicting evidence as to whether genotype has the greatest impact on lutein or β-carotene contents in broccoli. Previous studies found that β-carotene was most dependent on

broccoli genotype (Fernández-Leon et al., 2012; Kurilich et al., 1999). In contrast, others reported that lutein was the carotenoid most strongly influenced by genotype (Farnham and Kopsell, 2009; Ibrahim and Juvik, 2009; Renaud et al., 2014b). In this study, all carotenoids measured (violaxanthin, neoxanthin, antheraxanthin, lutein, α-carotene, β-carotene, and total carotenoid contents) were significantly affected by cultivar, and were significantly greater for 'BH053' than for 'Emerald Crown.' However, many studies only report differences among broccoli cultivars for specific individual carotenoids, mainly β-carotene and lutein. Singh et al. (2007) reported that β-carotene and lutein contents were highest for 'Solar Green,' while β-carotene levels were lowest for 'Fiesta,' and lutein levels were lowest for both 'Fiesta' and 'Lucky' broccoli cultivars. Renaud et al. (2014b) found that 'Oregon' had significantly higher β-carotene and lutein levels compared to 'Maine.' Farnham and Kopsell (2009) found that 'Marathon' had the lowest lutein and violaxanthin contents compared to other broccoli cultivars, and 'Green Valiant' had the highest violaxanthin levels, while lutein was greatest for 'Fiesta.' However, they found that β-carotene was not significantly different among cultivars. In contrast, Kurilich et al. (1999) reported a significant difference among cultivars for both α-carotene and β-carotene. Fernández-Leon et al. (2012) found that β-carotene was significantly greater for 'Parthenon' than for 'Monaco' but reported that lutein was not significantly different among cultivars. Results from this study indicate that all individual carotenoids measured were significantly influenced by genetic factors.

Variation in postharvest carotenoid contents depend on genetic (cultivar), physiological, and abiotic factors (Dominguez-Perles et al., 2011; Fernández-Leon et al., 2012). Significantly greater carotenoid contents may have been due to the earlier maturation date for 'BH053' than for 'Emerald Crown' or other physical characteristics that are influenced by genotype. Renaud et

al. (2014a) found that greater head weight, head diameter, bead size and uniformity, and increasing days to maturity were negatively correlated with carotenoid contents. Negative correlations between physical characteristics and carotenoid contents may be due to increased biomass in certain genotypes that are not accompanied by increased carotenoid synthesis, lowering carotenoid concentration (Farnham and Kopsell, 2009). 'BH053' was harvested 5 days earlier than 'Emerald Crown' and had significantly greater levels of carotenoids than 'Emerald Crown,' indicating that significantly greater contents for 'BH053' may be due to the variation in physical characteristics that are highly influenced by genotype.

Results from this study indicate that postharvest carotenoid contents, including violaxanthin, neoxanthin, antheraxanthin, α-carotene, and β-carotene in broccoli are significantly influenced by cultivar. Carotenoid variations among these cultivars suggest differences in the health promoting properties of broccoli (Kurilich et al., 1999; Singh et al., 2007), as carotenoids are nutritional compounds that are related to the prevention of certain cancers and age-related macular degeneration (Mayne, 1996; Krinsky et al., 2003; Podsedek, 2007; Verhoeven et al., 1996). Because yellowing is the main visible sign of broccoli deterioration, the green color of broccoli is a characteristic of freshness that is preferred by consumers (Kidmose et al., 2002). Recent evidence suggests that increased carotenoid contents result in greener broccoli florets by preventing the loss of chlorophyll (Renaud et al., 2014b). In addition to preventing the loss of color in broccoli, increased carotenoid contents also help to prevent oxidative damage to broccoli tissues during storage, resulting in delayed effects of senescence (Casajús et al., 2019). Thus, significantly greater carotenoid contents suggest that 'BH053' is superior to 'Emerald Crown' in terms of compositional, nutritional, and sensory quality.

Carotenoids, effects of cooling/storage method.

Carotenoids act as lipid-soluble antioxidants (Hasperué et al., 2013; Loi et al., 2019) that help protect cellular membranes by scavenging and quenching free radicals, resulting in delayed effects of postharvest senescence (Casajús et al., 2019; Fernández-Leon et al., 2013; Singh et al., 2007). Storage conditions impact the appearance, nutritional quality, and shelf life of broccoli by altering carotenoid concentrations over time (Mahn and Reyes, 2012; Serrano et al., 2006). Biochemical reaction rates increase up to threefold for every 10 ºC increase in temperature (Kader, 1987). Thus, the postharvest quality of broccoli can be improved by lowering storage temperatures (Price and Flore, 1993), which reduces respiration rates, prevents enzymatic quality losses, and delays senescence (Kidmose et al., 2002).

Nath et al. (2011) found that β-carotene losses in broccoli stored for 6 d were significantly greater for broccoli stored at 15 ºC than at 4 ºC. Cogo et al. (2011) found that carotenoids were significantly greater for broccoli stored at 1 ºC than for broccoli stored at 23 ºC. In contrast, other studies have observed a linear increase in carotenoid contents with increasing storage temperature (Hasparue et al., 2016; Lefsrud et al., 2005). However, carotenoid contents in this study were not significantly different for broccoli cooled with top icing and stored at 7 ºC, and broccoli cooled with an ice slurry and stored at 0 ºC in ice. Although storage temperature did not have a statistically significant impact on broccoli carotenoids, results from this study show that total carotenoid contents were slightly greater in broccoli stored at 0 ºC than at 7 ºC. Carotenoid contents in broccoli may not have been affected by storage temperature because cultivars used in this study were less likely to be influenced by storage temperature (Farnham and Kopsell, 2009). Therefore, lower temperature may still help to preserve the postharvest quality of broccoli in other cultivars that are more influenced by temperature.

Carotenoids are known to decrease during senescence of plants (Biswal, 1995). In this study, storage time significantly affected all carotenoids measured. These carotenoids significantly decreased in broccoli stored for 35 d. The xanthophyll carotenoids, including violaxanthin, neoxanthin, and antheraxanthin, significantly decreased at 21 d. In contrast, β-carotene and lutein contents significantly decreased at 7 d, while α-carotene significantly decreased at 14 d. Previous studies also found that carotenoid contents significantly decreased in broccoli stored for 5 d at 20 ºC (Yuan et al., 2010), 5 d at 25 ºC (Li et al., 2014), 7 d at 5 ºC (Barth et al., 1996), 19 d at 5 ºC (Pintos et al., 2020), and 6, 13, 20, and 27 d at 1 to 2 ºC (Fernández-Leon et al., 2013). In contrast, some studies found that carotenoid contents remained stable in broccoli stored for 7 d at 1 ºC (Cogo et al., 2011), 11 and 19 d at 5 ºC (Pintos et al., 2020), and 35 and 40 d at 5 ºC (Hasperué et al., 2016); while others reported that total carotenoid contents increased in broccoli stored for 5 d at 20 ºC (Casajús et al., 2019), stored from 2 to 5 d at 23 ºC (Cogo et al., 2011), 2, 3, and 4 d at 22 ºC (Hasperué et al., 2016), and 20 d at 4 ºC (Loi et al., 2019). However, results from this study indicate that postharvest carotenoid contents in broccoli decline with increasing storage time.

Radical oxygen species are over-produced, and oxidative damage occurs during storage of vegetable crops (Li et al., 2014). Carotenoids act as important lipid-soluble antioxidants, preventing cellular membrane oxidation by scavenging or quenching free radicals (Apel and Hirt, 2004; Fernández-Leon et al., 2013; Li et al., 2014; Noctor and Foyer, 1998; Page et al., 2001). Increased carotenoid contents in broccoli help maintain postharvest quality by preventing cellular membrane and chlorophyll degradation, resulting in less severe tissue damage and delayed yellowing during senescence (Renaud et al., 2014b). The main causes of carotenoid losses are autooxidation, photooxidation, and coupled lipid oxidation. Autooxidation occurs

spontaneously in the presence of oxygen, forming alkylperoxyl radicals that break down carotenoids to form epoxides (Kidmose et al., 2002). Photooxidation is affected by light intensity in the presence of oxygen. An excited sensitizer produces a singlet oxygen, which is quenched by carotenoids, forming cis isomers that result in color changes (Carnevale et al., 1980; Gross, 1991; Lennersten and Lingnert, 2000; Yanishlieva et al., 1998). Coupled oxidation is associated with the lipoxygenase system in vegetables exposed to stress. Lipid peroxidation forms peroxides, which oxidize carotenoids by a coupled reaction, resulting in carotenoid decoloration. Oxidation rates depend on the availability and presence of specific enzymes, oxygen, and antioxidants (Oszmianski and Lee 1990). Among other factors, such as temperature and light, the rate of oxidation also depends on individual carotenoid structure (Chen et al., 1994). Thus, xanthophyll carotenoids may have decreased slower than carotenes and lutein due to variation in these factors during storage.

Both β-carotene and lutein contents significantly decreased in broccoli stored for 35 d at 5 and 0 ºC. Similarly, previous studies reported that both lutein and β-carotene contents significantly decreased in broccoli stored for 5 d at 25 ºC (Li et al., 2014), 6, 13, 20, and 27 d at 1 to 2 ºC (Fernández et al., 2013). However, Nath et al. (2011) reported that β-carotene content alone significantly decreased in broccoli stored for 6 d at 15 and 4 ºC. In this study, β-carotene content was also significantly affected by interactions of cultivar and storage time. For 'BH053,' β-carotene content remained relatively stable and was significantly greater than β-carotene content for 'Emerald Crown' throughout the first 21 d in storage. Autooxidation is one of the main causes of carotenoid losses during postharvest storage of vegetables (Kidmose et al., 2002). Apolar carotenoids, such as β-carotene, are more susceptible to autooxidation than xanthophylls (Ramakrishnan and Francis, 1980). Significant interactions of cultivar and storage time for β-

carotene content may be due to the variation in oxidative activity among cultivars during broccoli storage. Greater β-carotene for ‘BH053’ compared to ‘Emerald Crown’ suggests that ‘Emerald Crown’ was experiencing greater autooxidation activity during storage, which led to the significantly greater β-carotene losses. For traits where genotype plays a significant role in contributing to variation (mainly β-carotene and lutein), cultivars with a higher concentration level tend to also be those that are most stable across environments (Renaud et al., 2014b). Thus, greater stability of β-carotene for ‘BH053’ during storage may be due to its increased concentration level compared to ‘Emerald Crown.’

Chlorophyll, effects of cultivar.

Sensory quality, including color, aroma, flavor, and tissue morphology, is an important factor for consumer acceptance of broccoli (Luo et al., 2018). Chlorophyll is important for maintaining the green color of broccoli, which is associated with freshness and preferred by consumers (Nath et al., 2011). Floret yellowing due to chlorophyll catabolism is the main visible sign of postharvest deterioration (Costa et al., 2005; Loi et al., 2019). Because the color of fresh produce is expected to be as close to the original color at harvest, color changes reflect the loss of quality (Gnanasekharan et al., 1992). Chlorophyll may also provide a chemoprotective effect when consumed (Feruzzi and Blakeslee, 2007). Activities of dietary chlorophyll may be associated with cancer prevention, antimutagenic activities, and induction of tumor cell apoptosis (Balder et al., 2006; Egner et al., 2001; Farnham and Kopsell, 2009). Chlorophyll contents in vegetables are determined by interactions of biochemical, physiological, and genetic characteristics (Goldman et al., 1999; Farnham and Kopsell, 2009; Kopsell et al., 2004; Kopsell et al., 2005; Kurilich et al., 1999).

In this study, chlorophyll *a* and chlorophyll *b* contents were significantly greater for 'BH053' than for 'Emerald Crown.' Similarly, Farnham and Kopsell (2009) found that chlorophyll *a* and chlorophyll *b* levels were significantly affected by genotype. They reported that 'High Sierra' had the lowest chlorophyll *a* and chlorophyll *b* contents, while 'Futura' had the highest chlorophyll *a* content, and 'Green Valiant' had the highest chlorophyll *b* contents. They also found that found that Chlorophyll *b* was significantly lower than chlorophyll *a* in multiple broccoli cultivars. Fernández-Leon et al. (2012) also found that 'Parthenon' had significantly greater chlorophyll *a* content than 'Monaco.' However, they did not observe a significant difference between chlorophyll *b* content among cultivars. In this study, chlorophyll *a* was greater than chlorophyll *b* contents for both 'BH053' and 'Emerald Crown.' Carotenoids help maintain broccoli quality by preventing the loss of chlorophyll contents (Braidot et al., 2014; Noichinda et al., 2007; Tracewell et al., 2001). As expected, 'BH053' also had significantly greater carotenoid contents than 'Emerald Crown.'

Chlorophyll degradation is a direct cause of yellowing in broccoli (Cai et al., 2019; Fukasawa et al., 2010; Hasperué et al., 2015; Hörtensteiner and Kräutler, 2011; Shimoda et al., 2016). Dephytilation by the action of chlorophyllase (CHL) was previously thought to be the first step in chlorophyll degradation (Benedetti and Arruda, 2002; Harpaz-Saad et al., 2007; Matile et al., 1999; Takamiya et al., 2000). However, recent studies may indicate that pheophytinase (PPH) and pheophorbide *a* oxygenase (PAO) may be more directly involved in the early stages of chlorophyll degradation than CHL (Büchert et al., 2011; Cai et al., 2019). PPH may be involved in the dephytilation of pheophytin, generating pheophorbide (Yang et al., 2009). Pheophorbide is then consumed by POA to catalyze opening of the pheophorbide porphyrin ring, generating red chlorophyll catabolytes (RCC) (Gómezlobato et al., 2011). Reduction of RCC by RCC reductase

(RCCR) generates primary fluorescent chlorophyll catabolytes (pFCC) that are translocated to vacuoles. pFCCs then undergo modifications and produce non-fluorescent chlorophyll catabolites (NCCs) that are stored inside the vacuole (Hörtensteiner , 2006; Schelbert et al., 2009). Hasperué et al. (2013) found that the expression of one gene controlling CHL activity decreased, while expression of the other gene increased in broccoli stored for 4 d at 20 ºC. Because chlorophyll contents also decreased during this time, they concluded that chlorophyllase may be involved chlorophyll degradation. In contrast, Cai et al. (2019) found that chlorophyllase activity decreased in broccoli stored for 24 d at 4 ºC, while PaO and PPH activity increased. Increased PAO and PPH activity led to decreased chlorophyll contents. The expression of genes controlling chlorophyllase activity decreased, while the expression of genes controlling PaO and PPH increased during storage, indicating that chlorophyllase activity may not be necessary for chlorophyll degradation. Because expression of genes controlling enzymatic activity in broccoli is influenced by cultivar, the difference in chlorophyll contents among cultivars observed in this study may be due to the variation in PAO, PPH, and/or CHL activities. Results from this study suggest that 'BH053' has the ability to better regulate the expression of genes controlling enzymatic activity related to chlorophyll degradation during storage, indicated by significantly greater chlorophyll contents, which would result in delayed yellowing for 'BH053' compared to 'Emerald Crown.'

Chlorophyll, effects of cooling/storage method.

Broccoli is a perishable vegetable with a high rate of senescence (King and Morris, 1994). During senescence, chlorophyll catabolism leads to a loss of green color, resulting in a loss of organoleptic quality (Casajús et al., 2019; Jones et al., 2006). Broccoli inflorescences contain hundreds of florets with petals and sepals containing chlorophyll. Energy, nutritional, and

hormonal supplies are rapidly depleted after harvest, leading to floret opening and senescence, which is accompanied by yellowing and chlorophyll degradation within floret tissues (Li et al., 2014; Page, et al., 2001).

Previous studies have demonstrated that lower storage temperature helps maintain chlorophyll contents in broccoli. Lebermann et al. (1968) found that total chlorophyll losses in broccoli stored for 16 d were significantly greater for broccoli stored at 7 ºC than at 1 ºC. Takeda et al. (1993) found that chlorophyll losses occurred in broccoli stored at 20 or 23 ºC but remained constant when stored at 2 ºC. Deschene et al. (1991) found that chlorophyll contents significantly declined within 4 d at 23 ºC and 10 d at 10 ºC. Nath et al. (2011) found that chlorophyll losses in broccoli stored for 6 d were significantly greater for broccoli stored at 15 ºC than at 4 ºC. In this study, storage temperature did not have a significant effect on chlorophyll contents in broccoli stored for 35 d at 0 and 7 ºC. Similarly, Boonprasom and Boonyakiat (2010) found that chlorophyll contents were not significantly different for broccoli stored at 0 and 7 ºC. However, chlorophyll contents were significantly greater for broccoli stored at 0 or 7 ºC than for broccoli stored at 10 ºC. Thus, the temperature difference between 0 and 7 ºC may not have been great enough to significantly influence the variation in broccoli chlorophyll contents observed in this study.

Although storage temperature did not significantly affect broccoli chlorophyll contents in this study, storage time did have a significant impact chlorophyll. In this study, total and individual chlorophyll contents significantly decreased in broccoli stored for 35 d at 5 and 0 ºC. Similarly, previous studies found that chlorophyll contents significantly decreased in broccoli stored for 4 d at 15 ºC (Yamauchi et al., 1997), 4 d at 20 ºC (Hasperué et al., 2011; Hasperué et al., 2013), 5 d at 20 ºC (Casajús et al., 2019; Yuan et al., 2010), 5 d at 25 ºC (Li et al., 2014), 10 d at 7 ºC (Zhan

et al., 2012), 11 and 19 d at 5 ºC (Pintos et al., 2020), 20 d at 4 ºC (Esturk et al., 2014), 14 and 21 d at 4 ºC (Hasperué et al., 2015), 20 and 27 d at 1 to 2 ºC (Fernández-Leon et al., 2013), and 35 and 40 d at 5 ºC (Hasperué et al., 2016). In contrast, Loi et al. (2019) found that total chlorophyll contents significantly increased in broccoli stored for 20 d at 4 ºC.

Chlorophyll losses have been positively correlated with broccoli deterioration (Aimla-or et al., 2010). As broccoli undergoes senescence, chlorophyll degradation, lipid peroxidation, and cell death occurs (Gómez-Lobato et al., 2012). Chlorophyll and proteins form light harvesting complexes in plants. Prior to chlorophyll degradation, light harvesting complexes must be dismantled (Hasperué et al., 2013). Harvesting allows compartmentalized enzymes to mix with substrates, which increases the rate of chlorophyll decline (Kidmose et al., 2002). As the permeability of cellular membranes change during storage, chloroplast membrane degradation and thylakoid membrane deformation lead to the release of chlorophyll attached to the thylakoid membrane (Kidmose et al., 2002). Released chlorophyll are then degraded by enzymes, including CHL, PPH, and PAO (Hörtensteiner and Krautler, 2011).

Harvesting allows compartmentalized enzymes to mix with substrates, which increases the rate of chlorophyll decline (Kidmose et al., 2002). Chlorophyll are often oxidized by lipoxygenase (LOX), peroxidase (POX), and oxidase enzymes (Gross, 1991). Previous studies have found that POX activity is associated with membrane and pigment breakdown (Barth et al., 1992; Barth et al., 1996). After exposure to stress, fatty acids accumulate in membranes due to phospholipid degradation by senescence-related enzymes (Barclay and McKersie, 1994). These free fatty acids are then oxidized by LOX, forming hydroperoxides (Whitaker, 1990), which can then stimulate the oxidative degradation of chlorophyll (Kidmose et al., 2002; Yamauchi and Watada, 1991). Thus, increased POX and LOX activities during storage may have played a role

in chlorophyll losses during storage. Results from this study show that chlorophyll degradation increased as storage time increased.

Interestingly, chlorophyll contents initially increased from 0 to 7 d. This may have been a result of metabolic activity and development of the immature floral buds (Carr and Irish, 1997; Loi et al., 2019). Previous studies have shown that postharvest chlorophyll losses can be delayed by chlorophyll synthesis at the beginning of senescence (Zhuang et al., 1994), which would explain the increase in total and chlorophyll *a* content at 7 d. However, previous studies have observed faster turnover rate for chlorophyll *b* compared to chlorophyll *a* (Farnham and Kopsell, 2009; Fernández-Leon et al., 2012; Folley and Engel, 1999). Recent evidence suggests that the reduction of chlorophyll *b* to chlorophyll *a* is a key first step in the degradation of chlorophyll (Hörtensteiner and Krautler, 2011). In leaves undergoing senescence, chlorophyll *a* is then demetallated to pheophytin *a* prior to dephytilation of pheophorbide *a*. In contrast, it was observed in ripening fruit, that the dephytilation of chlorophyll *a* into chlorophyllide *a* (by CHL) occurs first. Results from this study indicate that chlorophyll *b* is degraded during the first 7 d of storage, while chlorophyll *a* is synthesized, suggesting that chlorophyll *b* is first reduced to chlorophyll *a* prior to dephytilation of chlorophyll *a* by chlorophyllase (Schelbert et al., 2009; Scheumann et al., 1999; Tanaka et al., 1995; Zhan et al., 2012). Thus, the increase in chlorophyll *a* after harvest may have been due to the reduction of chlorophyll *b*, continued chlorophyll synthesis after harvest, or an interaction of these two mechanisms.

Conclusion

Cultivar and storage time had a significant impact on carotenoid and chlorophyll contents in broccoli, while storage temperature did not appear to have a significant influence on these phytochemicals. There was no significant difference in carotenoid or chlorophyll contents for

broccoli cooled with top icing and stored at 7 ºC and broccoli cooled with an ice slurry and stored at 0 ºC in ice. However, both carotenoid and chlorophyll contents were slightly greater for broccoli stored 0 ºC. This suggests that storage temperature may still play a small role in preventing the loss of these compounds.

All carotenoids measured, including violaxanthin, neoxanthin, antheraxanthin, lutein, β-carotene, and α-carotene, were significantly greater for 'BH053' than for 'Emerald Crown.' Similarly, both chlorophyll *a* and chlorophyll *b* contents were significantly greater for 'BH053' than for 'Emerald Crown.' Significant differences in the carotenoid and chlorophyll contents among broccoli cultivars suggest that these phytochemicals are dependent on genotype. Increased carotenoid contents help to prevent oxidative damage and chlorophyll degradation, which prevents the loss of nutritional and sensory quality during storage. Results from this study suggest that the postharvest quality is greater 'BH053'than for 'Emerald Crown' due to its significantly greater carotenoid and chlorophyll contents.

Carotenoid and chlorophyll contents in broccoli significantly decreased throughout the duration of storage. β-carotene and lutein significantly decreased from their initial values at 7 d, while violaxanthin, neoxanthin, and antheraxanthin significantly decreased at 21 d and α-carotene significantly decreased at 14 d. Chlorophyll *a* significantly decreased at 14 d after slightly increasing at 7 d, while chlorophyll *b* content steadily decreased throughout storage. As carotenoid and chlorophyll contents decreased with increased storage time, results from this study indicate that the decrease in postharvest quality of broccoli is apparent at 7 d and becomes more severe as time progresses.

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Appendix

Table 26. Analysis of variance results for carotenoid contents in broccoli for 'BH053' and 'Emerald Crown,' cooled with top ice and stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

	Carotenoid			
Source of Variance	Violaxanthin	Neoxanthin	Antheraxanthin	Lutein
Cultivar (C)	*** [z]	***	*	***
Treatment (T)	NS	NS	NS	NS
Storage Time (S)	***	***	**	***
C x T	NS	NS	NS	NS
T x S	NS	NS	NS	NS
C x S	NS	NS	NS	NS
C x T x S	NS	NS	NS	NS

Table 26. Continued.

Source of Variance	Carotenoid		
	α-carotene	β-carotene	Total Carotenoids
Cultivar (C)	***	***	***
Treatment (T)	NS	NS	NS
Storage Time (S)	**	***	***
C x T	NS	NS	NS
T x S	NS	NS	NS
C x S	NS	**	NS
C x T x S	NS	NS	NS

[z] Significance of interaction effects are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 27. Analysis of variance results for chlorophyll contents in broccoli for 'BH053' and 'Emerald Crown,' cooled with top ice and stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice, when stored for 0, 7, 14, 21, 28, and 35 days.

	Chlorophyll		
Source of Variance	Chlorophyll *a*	Chlorophyll *b*	Total Chorophylls
Cultivar (C)	*** [z]	***	***
Treatment (T)	NS	NS	NS
Storage Time (S)	***	***	***
C x T	NS	NS	NS
T x S	NS	NS	NS
C x S	NS	NS	NS
C x T x S	NS	NS	NS

[z] Significance of interaction effects are denoted by NS, *, **, ***: Non-significant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Table 28. Average carotenoid contents in broccoli ($\mu g \cdot g^{-1}$ fresh mass), across time for both storage temperatures, for 'BH053' compared with 'Emerald Crown.'

	Cultivar	
Carotenoid	BH053	Emerald Crown
Violaxanthin	3.78 a [zy]	1.53 b
Neoxanthin	3.91 a	2.79 b
Antheraxanthin	0.94 a	0.68 b
Lutein	7.58 a	3.77 b
α-Carotene	3.93 a	1.62 b
β-Carotene	4.86 a	2.31 b
Total	24.69 a	12.87 b

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual carotenoid, letters beside means for one cultivar that are not different from letters beside means for the other cultivar, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 29. Average carotenoid contents in broccoli ($\mu g \cdot g^{-1}$ fresh mass), across time for both cultivars, for top icing cooled broccoli stored at 7 ºC compared with ice slurry cooled broccoli stored at 0 ºC in ice.

	Storage Temperature	
Carotenoid	7 ºC	0 ºC
Violaxanthin	2.55 a	2.77 a
Neoxanthin	3.15 a	3.54 a
Antheraxanthin	0.78 a	0.83 a
Lutein	5.72 a	5.63 a
α-Carotene	2.73 a	2.82 a
β-Carotene	3.71 a	3.46 a
Total	18.52 a	19.05 a

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual carotenoid, letters beside means for one storage temperature that are not different from letters beside means for the other storage temperature, are not significantly different by the LSD test ($\alpha = 0.05$).

Table 30. Average carotenoid contents in broccoli ($\mu g \cdot g^{-1}$ fresh mass), for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, and 35 days.

	Storage Time (d)					
Carotenoid	0	7	14	21	28	35
Violaxanthin	4.39 a	4.08 a	3.67 a	2.21 b	1.02 bc	0.58 c
Neoxanthin	4.90 a	5.25 a	4.52 a	3.04 b	1.43 c	0.94 c
Antheraxanthin	1.36 a	1.13 a	1.13 a	0.73 b	0.30 c	0.20 c
Lutein	11.04 a	8.20 b	7.51 b	1.50 c	1.85 d	0.95 d
α-Carotene	5.18 a	3.90 ab	3.52 b	2.98 b	0.85 c	0.21 c
β-Carotene	7.38 a	4.74 b	5.74 b	1.97 c	1.32 cd	0.36 d
Total	34.41 a	26.46 b	26.18 b	15.52 c	6.86 d	3.33 d

[z]Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days.

[y]For each individual carotenoid, letters beside means for one storage time that are not different from letters beside means for that carotenoid at other storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

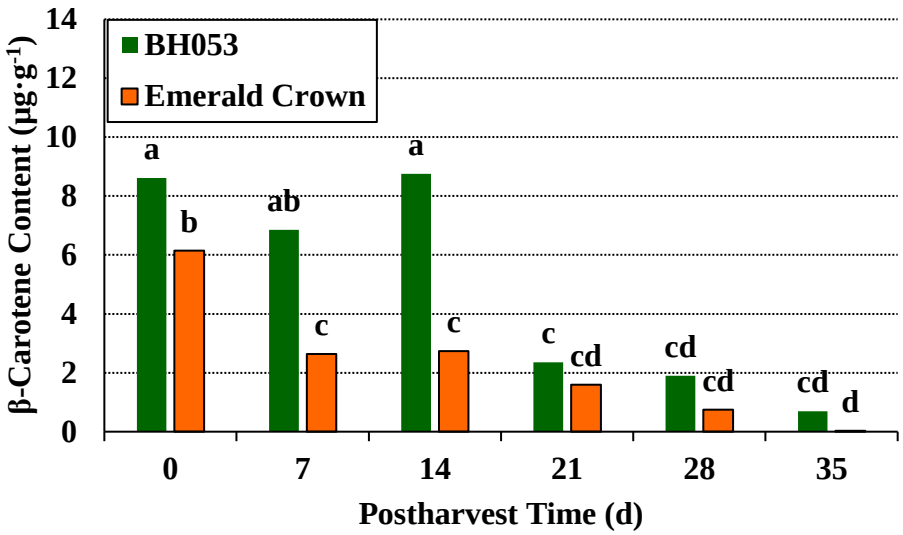

Fig. 21. Average β-Carotene contents in broccoli, stored at both temperatures, for ‘BH053’ compared to ‘Emerald Crown,’ and compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average of four replications for ‘BH053’ and three replications for ‘Emerald Crown,’ two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. Letters above one cultivar and storage time that are not different from letters above other cultivars and storage times, are not significantly different by the LSD test ($\alpha = 0.05$).

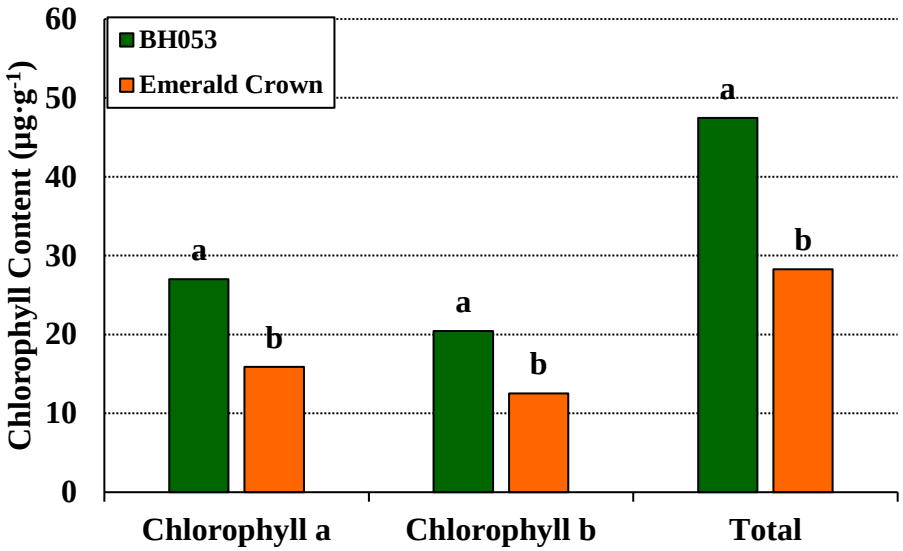

Fig. 22. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in broccoli, across time at both storage temperatures, for 'BH053' compared to 'Emerald Crown.' Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. For each individual chlorophyll, letters above one cultivar that are not different from letters above the other cultivar for that chlorophyll, are not significantly different by the LSD test ($\alpha = 0.05$).

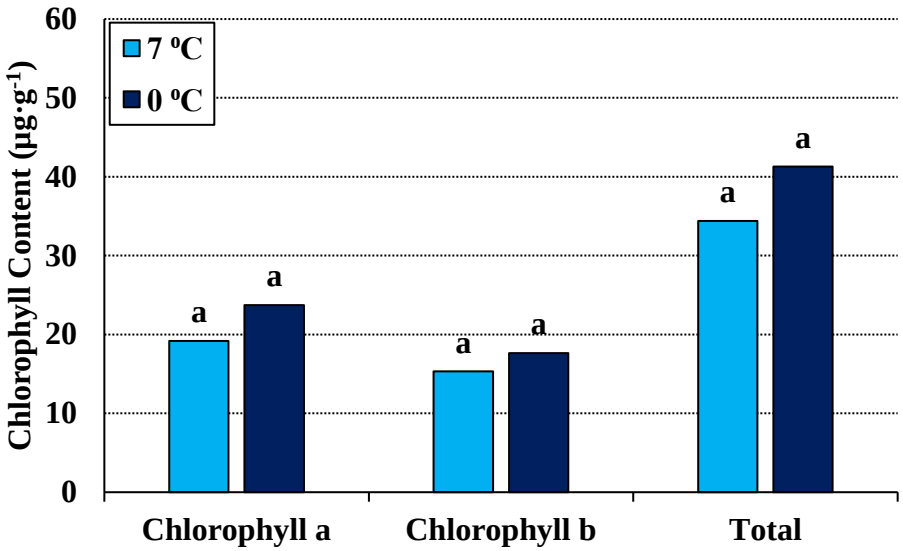

Fig. 23. Average chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in broccoli cooled with top icing and stored at 7 ºC compared with broccoli cooled with an ice slurry and stored at 0 ºC in ice. Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination, and samples stored for 0, 7, 14, 21, 28, and 35 days. For each chlorophyll, letters above one storage temperature that are not different from letters above the other storage temperature for that chlorophyll, are not significantly different by the LSD test ($\alpha = 0.05$).

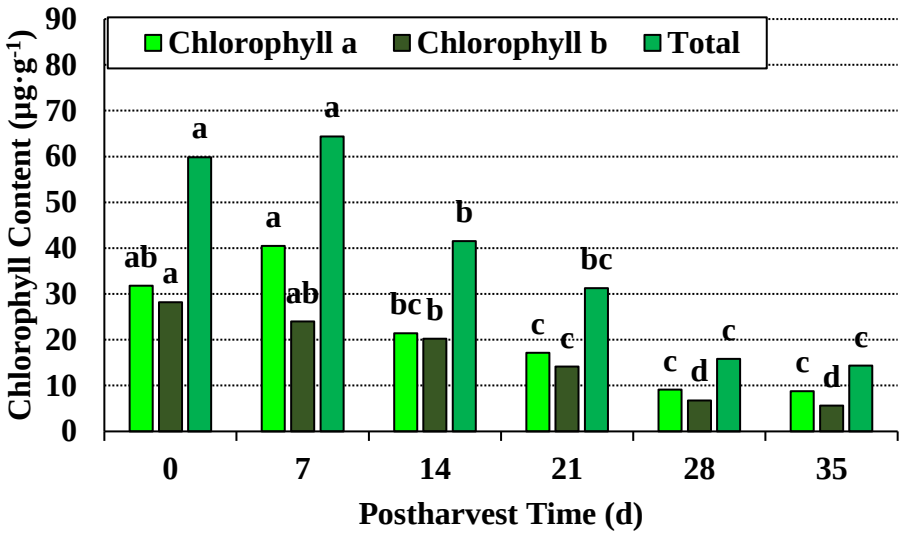

Fig. 24. Average chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents in broccoli, for both cultivars and storage temperatures, compared during storage at 0, 7, 14, 21, 28, and 35 days. Means are the average of four replications for 'BH053' and three replications for 'Emerald Crown,' two broccoli heads for each cultivar and storage temperature combination. For each individual chlorophyll grouping, letters above one storage time that are not different from letters above other storage times for that chlorophyll, are not significantly different by the LSD test ($\alpha = 0.05$).

CONCLUSION

Results from this project indicate that both cultivar and cooling/storage methods have a significant impact on the biochemical factors associated with the maintenance of postharvest broccoli quality. Results from broccoli cultivars harvested in Fall, 2018 reveal that 'Diplomat' had significantly greater sucrose contents and a significantly greater sugar/acid ratio compared to ‘Arcadia.’ As increased sucrose contents are correlated with delaying the effects of senescence, and a higher sugar/acid ratio is often associated with increased consumer acceptance of horticultural crops, these results suggest that the postharvest quality of 'Diplomat' is greater than that of 'Arcadia.’ Results from broccoli harvested in Fall, 2019 show that ‘BH053’ had significantly greater carotenoid and chlorophyll contents than ‘Emerald Crown,’ while glucosinolate, dimethyl disulfide, and (E)-2-hexenal contents were significantly greater for ‘Emerald Crown’ than for ‘BH053.’ Glucosinolates, carotenoids, and chlorophyll offer many potential health benefits when consumed, such as the prevention of certain cancers and cardiovascular diseases. In addition, carotenoids and chlorophyll are essential for maintaining the postharvest sensory quality of broccoli, as increased levels of these metabolites prevent color changes associated with senescence. In contrast, glucosinolates are responsible for the bitterness in *Brassica* plants, and dimethyl disulfide is a sulfur-containing volatile that is associated with the off-odors produced during broccoli senescence. These results suggest that the sensory quality is greater for ‘BH053’ than for ‘Emerald Crown.’

Broccoli has a high rate of respiration due to its developing immature inflorescences. Rapid cooling and lower storage temperatures help to reduce the rate of respiration in vegetables, which prevents the loss of postharvest quality. As expected, the characteristic metabolites related to broccoli quality significantly decreased during storage for 35 d. Broccoli cooled with an ice

slurry and stored at 0 ºC in ice maintained significantly higher levels of sucrose and health-promoting glucosinolates, while storage at 7 ºC resulted in significantly greater levels of the sulfur-containing volatile dimethyl disulfide. Many of the glucosinolates in broccoli have been associated with health benefits, and increased sucrose content helps to delay the effects of senescence, while dimethyl disulfide production indicates cellular deterioration. Thus, these results confirm that storage near 0 ºC, accompanied by immediate precooling in the field, help to prevent the loss of compounds attributed to the maintenance of physiological, nutritional, and sensory quality of broccoli during postharvest storage.

VITA

Sarah Parker was born January 25, 1991 in Athens, TN. She enrolled at the University of Tennessee at Chattanooga in 2009 and earned her Bachelor's degree in Biology in 2014.

In April 2018, she began working as a volunteer for Dr. Carl Sams in the Plant Physiology laboratory at the University of Tennessee and was offered a Graduate Research Assistantship in January 2019. Her responsibilities included harvesting and preparing plant tissue samples for the extraction of metabolites, weighing and recording data, and extracting sugars, carotenoids, flavonoids, organic acids, and minerals. She also had the opportunity to learn various analytical techniques to determine the concentrations of plant compounds. As a Master's student, she was able to use these skills to complete her thesis research.

Her experience in the laboratory and the knowledge she has gained during her studies at the University of Tennessee have undoubtedly prepared her for a future career in plant science research.