AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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Blackberry (*Rubus sp.*) fruit are a good source of phenolic compounds and anthocyanins, and are consumed in fresh and processed forms. Though organic products become more popular, limited information is available about how different organic production methods affect the post-harvest quality of blackberry fruit. The objective of this project was to investigate the effects of different organic production systems and harvest times on the physicochemical and nutraceutical properties of four cultivars of blackberry fruit during refrigerated and frozen storage. Trailing blackberry 'Obsidian' and semi-erect blackberry 'Triple Crown' were studied under refrigerated

storage while two trailing blackberry ‘Marion’ and ‘Black Diamond’ were studied under frozen storage.

‘Obsidian’ and ‘Triple Crown’ were grown organically and treated with three different organic fertilizers: processed poultry litter, soy meal, and a blend of fish emulsion and hydrolysate. Each fertilizer was applied at the same rate of 56 kg nitrogen/ha in 2012 and 2013. Samples were hand-picked three times per season at 1 wk intervals, packed immediately into clamshell containers, e and stored at 4.0 ± 0.2 ºC and 90 ± 5% relative humidity for up to 12 d. Physicochemical properties, including decay, leakage, pH, titratable acidity (TA), weight loss, firmness, and moisture content, and antioxidant content and capacities, including total phenolic content (TPC), total monomeric anthocyanins (TMA), radical scavenging activity (RSA), oxygen radical absorbance capacity (ORAC), and ferric reducing ability of plasma (FRAP), were measured prior to and during refrigerated storage. Harvest date and storage time showed more effect on the physicochemical properties than that of fertilizer type. During storage, late-harvest fruit of both cultivars had the least decay in 2012 while early-harvest fruit exhibited the least decay in 2013Fruit leakage in both cultivars increased during storage, reaching 54.3% and 62.5% in ‘Obsidian’ and 62.3% and 73.0% in ‘Triple Crown’ in 2012 and 2013, respectively. Fruit pH increased while titratable acidity decreased during storage in both cultivars. Firmness of ‘Obsidian’ fruit was significantly higher in 2012 than in 2013. Overall, fruit firmness decreased during storage. ‘Obsidian’ fruit had a 2.52% weight loss while that of ‘Triple Crown’ fruit had a 3.15% weight loss after 10 d of storage. The type of fertilizer only affected

fruit weight loss in ‘Obsidian’. ‘Obsidian’ also had as much as 37% higher ORAC values than ‘Triple Crown’ at harvest. Late-harvest fruit from plants fertilized with fish emulsion showed 29% higher ORAC values than fruit harvested from plants in the other fertilizer treatments. Generally, ‘Obsidian’ blackberry showed greater variability in antioxidant properties than ‘Triple Crown’.

‘Marion’ and ‘Black Diamond’ were also grown organically but, in this case with three different weed management strategies: non-weeding, hand weeding, and weed mat. Fruit were machine-harvested three times at 1 wk intervals in 2012, sorted by hand to exclude molded and damaged samples, frozen in a forced-air freezer at -25 ºC, and stored at the freezer for up to 9 mo. Physicochemical properties, including pH, TA, and total soluble solids (TSS), and antioxidant content, including TPC and TMA were measured prior to and during frozen storage. Antioxidant capacities, including RSA, ORAC, and FRAP were also measured prior to frozen storage. Although weed management had no significant effect on the physicochemical properties of the fruit, it had numerous effects on TPC, TMA, RSA, ORAC and FRAP. Late-harvest ‘Marion’ fruit had the highest ORAC and FRAP values compared to ‘Black Diamond’ fruit and ‘Marion’ fruit from the earlier two harvests. Fruit from the hand-weeded treatments had up to 30% higher antioxidant content and capacity during the first second harvests than fruit from the non-weeded and weed mat treatments.

This study provided important information about the effects of organic production systems on post-harvest quality of blackberry fruit during refrigerated and frozen storage. Such information will be helpful for providing guidelines to the organic berry

industry (growers, packers and processors) for making decisions on the selection of organic fertilizers and weed management practices, the timing of fruit harvest, and the maximum amount of time in which the fruit should be refrigerated or frozen without a significant loss in quality.

Effect of Organic Production System and Harvest Date on the Quality of Blackberry Fruit for Fresh and Processed Markets

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Mingyang Liu, Author

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CHAPTER 1

Introduction

Blackberries (*Rubus spp.*) are valuable fruit with unique color, flavor and taste. In the past decades, blackberry fruit have been widely studied for its potential health benefit to human body owning to their high phenolic compounds, vitamins, minerals and fibers (Wang and Lin 2000; Cho et al. 2004; Siriwoharn et al. 2004; Eyduran 2006; Kaume et al. 2011; Granelli et al. 2012). Epidemiological studies have shown that the consumption of blackberry fruit help reduce chronic diseases since the phytochemicals in blackberries participate biological metabolism in human body (Beattie et al. 2005; Seeram 2013). Blackberries were also found to reduce brain aging in rats (Shukitt-Hale et al. 2009).

Fresh blackberry fruit are highly perishable and easily to be damaged during harvest due to their soft tissues and fragile skins. The postharvest storage life of fresh blackberries is also very short because of their high respiration rate, rapid mold growth, and quick loss of water (Joo et al. 2011). During storage, fruit quality can be significantly deteriorated, including mold growth, leakage, loss of physicochemical and nutraceutical properties (Perkins-Veazie et al. 1999; Perkins Veazie and Collins 2002; Joo et al. 2011). Refrigerated storage may help slow down quality deterioration and mold growth of blackberry fruit (Antunes et al. 2003). Under storage condition of 2±0.5 ºC and 90-95 % relative humidity (RH), conventional grown blackberry fruit may keep marketable quality for up to 7 d (Perkins-Veazie et al. 1996).

Due to their short production season and limited shelf-life, fresh blackberries are commonly processed by freezing, canning, drying, and making into jams and jellies (Hager et al. 2008; Veberic et al. 2014). Among those processing methods, freezing process is considered as the least destructive processing method for phenolic compounds of blackberry fruit (Wu et al. 2010). Many studies have investigated the effect of freezing process on the quality of conventional blackberry fruit (Hager et al. 2008; Türkben et al. 2010; Kopjar et al. 2012; Veberic et al. 2014). Although thawed products may show structural collapse, freezing process can significantly extend the shelf life of blackberry fruit (Petzold and Aguilera 2009).

Organic foods have become more popular because of their environmental friendly production and free in pesticides, fungicides, antibiotics and genetically modified organisms (GMOs) (Aertsens et al. 2009). The market for organic blackberries has expanded significantly with the production of organic blackberry in the United States increased 173% from 2005 to 2008 (Strik et al. 2007; USDA 2010). Fertilizer and weed management method are both very important factors affecting organic production. Nitrogen fertilizer provides the necessary N nutrient for the growth of blackberry plant and the yield of blackberry fruit (Martin et al. 2006). Weed control also help maintain soil properties and plant growth (Buhler 2002). However, few studies have investigated the impact of organic fertilizer and weed management strategies on the physicochemical and antioxidant properties of fresh market and processed blackberry fruit.

Therefore, the current study was aimed to investigate the effect of organic production system on the quality of blackberry fruit and quality change during post-harvest storage. The first part of the research was to study the impact of different organic fertilizers and harvest date on the physicochemical and nutraceutical qualities of two hand-picked organically grown blackberries ('Obsidian' and 'Triple Crown') during refrigerated storage, and the second part was to evaluate the effect of weed management strategies and harvest date on physicochemical and nutraceutical qualities of two machine harvested organically grown blackberries ('Marion' and 'Black Diamond') during frozen storage.

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CHAPTER 2

Literature Review

2.1 Blackberry fruit

2.1.1 Introduction

Blackberries (*Rubus sp.*) were first found growing wildly by the early settlers of Europe and North America (Darrow 1937). Due to their thorniness and vigorous growth nature, most blackberries were considered as worthless and harmful to soil cultivation, and only a small part was harvested for food applications (Darrow 1928). Production of blackberries were started after several cultivars from the wild were selected and propagated (Swartz et al. 1981). However, picking and handling of the fruits were very difficult because of their thorny property, which delayed the development of fruit production.

In 1930, a thornless plant of the cut-leaf European blackberry was discovered. This variety was named as 'Thornless Evergreen' and has been planted extensively in the northwest part of the United States (Darrow 1931).

2.1.2 Cultivars of blackberries

There are many different cultivars of blackberry fruits with three predominant types: trailing (thorny and thornless), thorny erect, and thornless semi-erect (Poling 1997). Thorny erect blackberries were developed from blackberries in the eastern U.S. They are more vigorous than the others. The University of Arkansas developed a new primocane fruiting erect blackberry in 2004 which flower and fruit quite late in the

ripening season (Clark et al. 2005). The cultivars of erect blackberries include but no limit to ‘Cheyenne’, ‘Cherokee’, ‘Shawnee’, ‘Navaho’, ‘Kiowa’, ‘Apache’, ‘Chickasaw’ and ‘Ouachita’ and were released between 1970s and 2000s (Table 2.1). Trailing blackberries do not grow well in the cold climate regions with significant low yields. The plants produce primocanes grow along the ground. It’s very helpful to have primocanes physically tied to have a good production. Trailing blackberries have more aromatic flavor as well as less seeds than other two types (Finn 2001). The cultivars of trailing blackberries include but no limit to ‘Marion’, ‘Olallie’, ‘Pacific’, ‘Waldo’, ‘Black Diamond’, ‘Evergreen’ and ‘Obsidian’, and were mostly released in the 20th century (Table 2.1). ‘Shawnee’ cultivar is characterized as productive plant and the fruits are extremely large and sweet during the harvest season. Since ‘Shawnee’ cultivar is cold sensitive, it has not been widely planted in the Midwest or Northeast part of U.S. Semi-erect blackberries tend to grow towards the ground with vigorous and large canes, and the canes tend to be more naturally branched after several years planting. The plants are usually productive but the fruits are cold sensitive, thus extra precautions should be taken when growing in the cold regions (Kafkas et al. 2006). The cultivars of this type of blackberries are primarily ‘Smoothstem’, ‘Thornfree’, ‘Black Satin’, ‘Dirksen Thornless’, ‘Chester Thornless’, ‘Himalaya’, ‘Hull Thornless’, ‘Triple Crown’, ‘Loch Ness’ and ‘Loch Tay’ (Table 2.1). Among these cultivars, ‘Kiowa’ fruits are large and flavorful, and the plants have a long ripening season and relatively productive. This cultivar is mostly grown in south central U.S. ‘Olallie’ fruits are medium size with bright black color and firm texture and the plants are

vigorous and only grown in Pacific Northwest area. ‘Navaho’ usually fruits late during fruiting season with medium size and flavorful fruit. Moreover, this cultivar is resistant to anthracnose and root rot. ‘Hull Thornless’ fruits are sweeter than other trailing cultivars and can retain its color even in high temperature environment. ‘Dirksen Thornless’ is quite firmer than other trailing cultivars, and compared with other early cultivars, ‘Dirksen Thornless’ fruits have excellent quality and flavor. ‘Chester Thornless’ fruits ripen later than ‘Hull Thornless’ and ‘Dirksen Thornless’ cultivars while better flavor and more cold resistance. ‘Evergreen’ fruits are medium size and firm with dark black color. This productive cultivar is mostly grown in Pacific Northwest region in U.S. (Hall and Jo Stephens 1998; Guzman-Baeny 2004; Coyner et al. 2005; Strik et al. 2007)

In this project, ‘Obsidian’, ‘Triple Crown’, ‘Marion’ and ‘Black Diamond’ were studied. ‘Obsidian’ is a trailing blackberry with high yield and excellent quality which was released by U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS/Oregon State University Cooperative) breeding program in Corvallis, Oregon. ‘Obsidian’ fruits are large and conical with good firmness, though they are not as uniform as ‘Siskiyou’ and ‘Black Diamond’. Excellent color makes fruit remain black in refrigeration and flavor is full and rich and much more pleasant than ‘Chester Thornless’ (Finn et al. 2005b). ‘Triple Crown’ is semi-erect and named for its flavor, productivity and vigor. It’s first released by USDA-Beltsville and Pacific West Agricultural Research Service. Fruits are quite large, flavorful and ripening earlier than ‘Chester’, and grown uniformly for easy picking. ‘Marion’ cultivar is also

developed by USDA–ARS/Oregon State University Cooperative breeding program in Corvallis, Oregon. It's a trailing type and the fruits show black color and turn dark purple after frozen and thawed. 'Marion' has somewhat tart flavor and larger size than 'Evergreen'. 'Black Diamond' is also a trailing type blackberry and firstly selected from USDA–ARS/Oregon State University Cooperative breeding program in Corvallis, Oregon. The high-yielding, vigorous fruits are more suitable for machine harvest. Moreover, the fruits are firm enough to be used for fresh market. The medium size fruit has excellent blackberry flavor but not as intense as 'Marion' (Finn et al. 2005a). These four cultivars as well as 'Evergreen', 'Boysen', 'Ouachita', 'Chester Thornless', 'Loch Ness', 'Navaho', 'Arapaho', 'Natchez' and 'Hull Thornless' are the most commonly grown in United States (Strik 1992; Strik and Finn 2011).

2.1.3 Chemical composition of blackberries

Blackberry fruits are soft fruits containing plenty of vitamins and minerals as well as various phytochemicals. The exact chemical composition is depending on the cultivar, growing location, harvest time, maturity stage, and storage conditions (Zhao 2007a). According to the National Nutrient Database for Standard Reference (USDA 2011), the nutrition content of blackberry fruits are summarized in Table 2.2.

Carbohydrates are the essential compounds in blackberry fruit and directly impact the ripeness and sensory quality of the fruits. Among them, glucose, fructose and sucrose are the predominant sugars (Fan-Chiang and Wrolstad 2010). During fruit ripeness, the amount of glucose, fructose, sucrose and other soluble solids

significantly accumulated along with the color change from light red to dark purple, even black color (Acosta-Montoya et al. 2010). Therefore, fruits should be harvested until enough amounts of sugars are accumulated. The sugar amount varies depending on not only ripeness, but also the growing environment and cultivars. The total soluble solid content of different cultivars of blackberry fruits is partially summarized in Table 2.3.

Besides sugar, blackberries also contain some organic acids which are important for the fruit sensory quality. The major organic acids in blackberry fruits are citric, malic and tartaric acid, in which citric and malic acids are predominant acids (Kafkas et al. 2006; Veberic et al. 2014). Moreover, high concentration of organic acid in blackberry fruits is important for preservation and pH maintenance in processed products (Kafkas et al. 2006; Famiani and Walker 2009). There is also a group of phenolic acids including hydroxybenzoic acids like p-hydroxybenzoic, protocatechuic, gallic, gentisic and hydroxycinnamic acids like caffeic, m-coumaric, p-coumaric and ferulic. These two predominant phenolic acids are present in free, ester and glycoside forms (Talcott 2007). Combination of organic acids and phenolic acids is responsible for total titratable acidity of the fruits, which is an important parameter for fruit processing, storage and sensory evaluation. Like sugar content, different cultivars contain different types and amounts of organic and phenolic acids. Total titratable acidity of some blackberry fruit cultivars are listed in Table 2.4.

Blackberries contain various essential vitamins and minerals as shown in Table 2.2 and are good source of vitamin A, vitamin B, vitamin C and folate, as well as

potassium, phosphorus, magnesium, and iron (Seeram et al. 2006a). Ascorbic acid is important for human health since it acts as a reducing agent for preventing oxidation by promoting the function of oxidase enzymes. Other than that, ascorbic acid may also promote hormone synthesis and immunity enhancement (Pantelidis et al. 2007; Patras et al. 2009).

Other than the chemical substances indicated above, blackberries contain many bioactive compounds including various enzymes. The hydrolase and oxidase in blackberries play an important role in quality deterioration of the fruits, such as changes in color, texture and flavor. Among those enzymes in the fruits, oxidizing enzymes are especially important. Polyphenol oxidase (PPO) and peroxidase (POD) attribute to the enzyme browning reaction and quality deterioration of fruit during ripening and post-harvest storage. PPO catalyzes the oxidation of o-diphenolic compounds to quinone compounds, and then further transformed to polymerized brown pigment (González et al. 2000; Patras et al. 2009). Since blackberry fruits contain large amount of phenolic compounds which are the substrates of PPO and POD, the activity of PPO and POD could be much high during fruit ripening. Fruit softening is another important quality indicator. Cellulase is related to texture quality during ripening and storage by changing the structure of cell wall. Polygalactouroase (PG) and pectin methyl esterase (PME) take part in pectin modification of cell wall in the fruit, further affecting fruit softening (Barnes and Patchett 1976; Nunan et al. 2001; Martínez and Civello 2008; Famiani and Walker 2009).

2.1.4 Phytochemicals and natural pigments in blackberries

Blackberry fruits are rich source of polyphenols, especially anthocyanins, flavonols, flavan-3-ols, proanthocyanidins and ellagitannins as well as some phenolic acids (Bushman et al. 2004; Cho et al. 2005). Phenolic compounds in fruit are generated during plant development and fruit ripening with phenylalanine ammonialyse, cinnamate 4-hydroxylase and other enzymes involved (Macheix and Fleuriet 1990). Phenolic compounds are important factors influencing the color and antioxidant properties of blackberry fruits depending on the cultivar, ripeness, process methods and storage conditions. In fresh fruit, the color can be changed due to oxidation of phenols and enzyme related reactions (Kähkönen et al. 1999). Range of total phenolic content reported in the literatures is partially listed in Table 2.5, and the structures of major phenolic compounds in blackberry fruits are illustrated in Fig. 2.1.

Wang and Lin (2000) also reported that maturity of fruit affect the total polyphenol values in blackberry fruits. They found that total polyphenol content of 'Triple Crown' and 'Hull Thornless' decreases significantly when fruits became ripe ($P<0.05$).

Phenolic acids are important phytochemicals, and the primary ones in blackberry fruits are hydroxybenzoic acids and hydroxycinnamic acids (Schuster and Herrmann 1985; Zadernowski et al. 2005). These two phenolic acids usually form as esters and glycosides instead of free acids (Dai et al. 2007). The common hydroxybenzoic acids in blackberries are p-hydroxybenzoic, protocatechuic, gentisic, vanillic, salicylic and gallic acid, of which salicylic acid in ester and glycoside forms are predominating (Zadernowski et al. 2005). The hydroxycinnamic acids in blackberries include m-

coumaric, p-coumaric, caffeic, and ferulic, of which m-coumaric, 3,4-dimethoxycinnamic acid in ester form and hydroxycaffeic acid are mostly common (Zadernowski et al. 2005). Among all kinds of phenolic acids, ones with ester, glycoside and free form account for 53.1%, 43.6% and 3.3%, respectively (Zadernowski et al. 2005). Ellagic acid is a specific hydroxybenzoic acid which can be quantified and reported as "ellagic acid equivalent" following acid hydrolysis. Ellagic acid has shown excellent antimutagenic and anticarcinogenic activity against specific carcinogens (Maas et al. 1991). Siriwoharn et al. (2005) reported that the ellagic acid content in 'Marion' and 'Evergreen' blackberry cultivars was about 18 and 12 mg/100 g fresh weight of berry, respectively, while González et al. (2003) found that Spanish wild blackberry fruits contain 25.93 mg ellagic acid/100 g fresh weight of berry.

Anthocyanins are a group of phenolic compounds and are responsible for the dark red color of blackberry fruits (Fan-Chiang and Wrolstad 2005). Due to their health benefits, anthocyanins are recognized a good dietary supplement recommended by many scientists (Zafra-Stone et al. 2007; He and Giusti 2010). Anthocyanins are primarily existed in berry fruits and red grapes having a structure of anthocyanidin glycosylated with sugars attached at the C3 position of the flavan structure (Cho et al. 2005; Zhao 2007a). About 93% of anthocyanins in blackberry fruits are in the form of monoglycosides and 7% are in the form of diglycosides (Wu et al. 2006). Generally, there are six individual anthocyanins primarily existed in berry fruits, including Pelargonidin, Cyanidin, Delphinidin, Peonidin, Petunidin, and Malvidin (Howard and Hager 2007). The primary anthocyanins in blackberry fruits are listed in Table. 2.6,

and among them cyaniding 3-glucoside was reported as the predominating one (Cho et al. 2005).

Total anthocyanins content of blackberry fruits depends on cultivars, grown location, and harvest and storage conditions (Beattie et al. 2005). Sellappan et al. (2002) reported that total anthocyanin of 'Kiowa' and 'Choctaw' cultivars grown in Georgia are 122.66 and 110.52 mg cyanidin-3-glucoside equivalent/100 g FW respectively based on the pH differentiation method, similar value was reported by Cho et al. (2005) that anthocyanin content range of 6 cultivars is from 114.4 to 241.5 mg cyanidin-3-glucoside equivalent/100 g FW. However, lower content of anthocyanin (average of 88.7 mg cyanidin-3-glucoside equivalent/100 g FW) was found in 'Black Diamond', 'Smoothstem', 'Darrow', 'Chester', 'Hull Thornless' and 'Black Satin' blackberries grown in Italy (Benvenuti et al. 2004). Moreover, identification of different forms of anthocyanins in blackberry fruits has been studied using HPLC-MS (High Performance Liquid Chromatography- Mass Spectrometry). Fan-Chiang and Wrolstad (2005) found that in 51 testing samples, cyanidin-3-glucoside takes the highest portion, 44-95%, follows with cyanidin 3-rutinoside (0.01%-53%), cyanidin 3-xyloside (0.01%-11%), and cyanidin 3-(malonyl)glucoside (0.01%-5%).

Blackberry fruits are also a good source of flavonols, which is a group of flavonoids found in the drupelet (Iriwoharn and Wrolstad 2004). The most common flavonols in berry fruit are quercetin, myricetin, and karmpferol (Seeram et al. 2006b). Cho et al. (2005) reported that quercetin 3-galactoside and quercetin 3-glucoside are

the main flavonols in ‘Apache’, ‘Arapaho’, ‘Kiowa’, ‘Navaho’ and ‘Chickasaw’ blackberry cultivars.

Tannins in blackberries are a group of polyphenol compounds with oligomeric and polymeric constituents (Shahidi and Naczk 2003). Based on the structure, tannins are divided into two groups: condensed tannins and hydrolysable tannins. Blackberry fruits are a good source of hydrolysable tannins, such as ellagitannins that is mostly existed in the seeds (Daniel et al. 1989). Siriwoharn et al. (2005) found that ‘Evergreen’ blackberry cultivar contain 33.4% higher amount of ellagitannin than ‘Marion’ cultivar.

Other than the phytochemicals stated above, blackberry fruits also contain secoisolariciresinol which is a kind of lignans and might be used for preventing cancer and heart disease (Mazur et al. 2000).

2.1.5 Antioxidant activity and health benefits of blackberry fruits

Many studies have found that the high content of phytochemicals such as anthocyanins, phenols, flavonoids in berry fruits can help reduce the risk of heart disease, chronic disease, stroke, and cancers (Van Duyn and Pivonka 2000; Vinson et al. 2001; Beattie et al. 2005; Duthie et al. 2006). Flavonoids are most important phenolic compounds in blackberries which primarily consist of flavonols, anthocyanidins, proanthocyanidins, catechins, and flavons. Flavonoids have been shown having antiviral and anti-inflammatory functions (Li et al. 2000; Guardia et al. 2001; González-Gallego et al. 2007; Rathee et al. 2009). Moreover, flavonoids,

together with other phenolic compounds, have the ability to inhibit low-density lipoprotein (LDL) oxidation by decreasing the amount of oxidized LDL in atherosclerotic lesions (Aviram and Fuhrman 2002; Chung et al. 2004). High level LDL is highly risk to cardiovascular disease (CD), and the phenolic compounds have been shown with high inhibition activity on human LDL and lecithin liposomes (Heinonen et al. 1998). Moreover, flavonoids also help protect platelet by decreasing superoxide anions and increasing nitric oxide produced by platelet.

Phenolic acid such as caffeic acid, vanillic acid and chlorogenic acid are well recognized as antioxidants, and their antioxidant activities are associated with the hydroxyl groups in the molecules (Rice-Evans et al. 1996). Chlorogenic acid was found to be the most active antioxidant (Chu et al. 2000). Other phenolic derivatives are also high in antioxidant and antimicrobial activity (Sofos et al. 1998).

Anthocyanins are found with anti-inflammatory, antiviral and anticarcinogenic properties (Skrede and Wrolstad 2002), and help prevent obesity (Prior et al. 2008; Prior et al. 2010). Similar functions were also found for ellagic acid and ellagitannins. Polyphenols were reported to help slow down brain aging by relieving the stress (Shukitt-Hale et al. 2008). Anthocyanins also help protect human endothelial cell by inhibiting monocyte chemotactic protein-1 (MCP-1), which is a protein involved in atherogenesis in infection and inflammation (Garcia-Alonso et al. 2009). Anthocyanins may also be involved in cancer prevention by inducing phase II enzymes which further inactivate carcinogens that cause DNA damage of human cells (Giusti and Jing 2007).

Serraino et al. (2003) reported that blackberry extract shows prevention against peroxynitrite-induced DNA strand damage in vascular endothelial cells. Moreover, *in vitro* studies showed that blackberry extract can inhibit proliferation of human lung cancer cells and reduces neoplastic transformation in mouse cells (Duthie 2007). The authors reported that blackberry anthocyanins can inhibit cancer cell by modifying cell signaling pathways as well. Blackberry extract also exhibited inhibition properties on human cancer cells from oral, breast, prostate and colon based on *in vitro* study (Seeram et al. 2006a).

Other than cancer inhibition, blackberry fruits are found to prevent neurodegenerative diseases like Alzheimer's disease (Shukitt-Hale et al. 2009). Anthocyanins in bilberry was reported to significantly reduce polymeric collagen and structural glycoprotein production which may result in capillary thickness in diabetes (Boniface et al. 1985). Bone mineral density of ovariectomized rats fed by 5% blackberry supplementation was increased significantly (Kaume et al. 2010).

2.2 Production of blackberries

2.2.1 Conventional production

Blackberries have long been consuming by humans. For the past decades, production of commercially cultivated blackberries increased significantly worldwide from 13,958 ha in 1995 to 20,035 ha in 2005 which is about 45% increase, and among 20,035 ha production in 2005, 2,528 ha was organically produced (Strik et al. 2007). Europe and North America are the primary production regions of blackberries in the

world. There were 7,692 ha of blackberries grown in Europe and 7,159 ha grown in North America in 2005. From year 2009 to 2011, 7,100, 7,500 and 7,300 acres of blackberry were harvested, respectively within the United States (USDA 2014). United States took 67% of planted areas in North America region and the primary blackberry production location within the United States was Oregon, in which planted area increased 25% from 1995 to 2005 (Strik and Finn 2011). Most blackberries grown in Oregon were trailing types, including 'Marion', 'Boysen' and 'Evergreen'. Only 1% of blackberries grown in Oregon were erect types such as 'Cherokee' and 'Navaho'. Other than Europe and North America regions, Central America, South America, Asia, Oceania and Africa all make contribution to the worldwide blackberry production in the percent range of 0.50-8.19%. Blackberries have various production system based on the classifications (trailing, semi-erect, and erect) (Morris et al. 1970). In general, erect and semi-erect types were harvested for fresh market because the fruits have longer shelf life and more firm while trailing type are harvested for processed market since fruits are not firm enough to ensure good quality during transportation. However, several new trailing types like 'Obsidian' are used for fresh market (Strik et al. 2007).

2.2.2 Organic production of blackberries

There were about 2,527 ha of organic blackberry production worldwide in 2005, of which 72.8 ha were in the United States (Strik et al. 2007). In the year of 2008, 199 ha of organic blackberries were harvested in the United States (USDA 2010). Growers

expect significant increase of organic blackberry production in the next decades due to the demand of organic products by consumers.

2.2.2.1 Consumer awareness and knowledge about organic produce

Nowadays, protection to the environment has attracted more attention by the growers and consumers. Among all three agricultural production protocols (conventional, organic, and integrated production) studied by many researchers, organic production is regarded as the most environment friendly one (Granatstein and Kupferman 2006; Kaltsas et al. 2007; La Rosa et al. 2008; de Barros et al. 2009). As there is not much difference about exterior appearance between organic and conventional products, consumers may not recognize whether a product is organic or conventional, but consumer knowledge and awareness about organic production affects their willingness to pay higher price and make purchase decision for organic products (Giannakas 2002). According to the USDA National Organic Program Standard (USDA 2000), "organic" refers to

"*The food or other agricultural product has been produced through approved methods that integrate cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity. Synthetic fertilizers, sewage sludge, irradiation, and genetic engineering may not be used*".

However, some studies reported that consumers have different perception about what organic food is. Jolly et al. (1989) reported that consumers in California believe that organic products are products without any pesticides and artificial fertilizer. Hill and Lynchehaun (2002) conducted a survey in Britain and indicated that organic food is more natural and healthy without further detailed information. Moreover, Wolf

(2002) found that the term "environmental friendliness" associated with organic lettuce is more appreciated by consumers than the term "certified organic" on the label. Another survey conducted in UK found that less than 50% respondents notice the organic label and some of them don't recognize the organic logo or the symbol (Hutchins and Greenhalgh 1997). In contrast, researchers did find organic logos and labels help consumer identify organic products (Chang and Kinnucan 1991; Mathios 1998). Even if consumers noticed the products were grown organically, they may not make purchase decision because they are skeptical about the organic quality of products, especially in the regions where the certification and standardization are not well developed.

In general, the facts that consumers are not informed about organic products as well as lack of enough information about the difference between organic and conventional products are the primary reason holding consumers back from organic products purchase (Kaume et al. 2011).

2.2.2.2 Consumer attitudes and perceptions towards organic produce

Though consumers recognize the produce in front of them are organic, the quality and attributes of organic produce may also affect consumer's preference. Consumers may compare organic and conventional products by the methods of production and specific characteristics of products they are interested in when they were asked about the preference between those two types of products (Yiridoe et al. 2005). However, complexity of human behaviors affects their purchase decision based on not just consumer's knowledge and perception, but also nutrition consideration and social

psychological status leading to various attitudes about organic products purchase (Hay 1989).

Due to the concerns on the potential risk of some artificial chemicals used in conventional production of produce as well as environmental issues, more consumers tend to buy organic products (Yiridoe et al. 2005). Many studies found that consumers tend to purchase organic products due to the primary perception that such products are safer, healthier, and environmental friendly (Schifferstein and Oude Ophuis 1998; Gregory 2000). People from different regions showed various preferences about organic products. Werner and Alvensleben (1984) reported that consumers in Germany tend to buy organic fresh fruits and vegetables, which is similar to the findings that organic fruits are most frequently purchased by consumers in California (Jolly et al. 1989) and Canada (Hay 1989). Salleh et al. (2010) concluded that in Malaysia the decision to buy organic products was complicated to make by consumers, not only because of environmental friendly of organic products. Similar conclusion was made by Ahmad and Juhdi (2008), where the organic food in Malaysia was still at the introductory stage, consumers' consumption pattern may not change in short time. Sangkumchaliang and Huang (2012) reported that consumers in Thailand purchase organic products with consideration of potential environmental and health benefits, but due to labeling issue, consumers may not notice those organic products in the market.

2.2.2.3 Comparison of organically and conventionally grown produce

Though organic products are difficult to distinguish by appearance alone, consumer believed that organic products are different from conventional products in

the aspects such as nutrition content and safety (Yiridoe et al. 2005). Several studies have assessed the differences between organic and conventional products. Langenkämper et al. (2012) reported that there is no significant difference in most nutrients content between organic and conventional wheat except total protein content was higher in organic wheat. Pieper and Barrett (2009) found higher total soluble solids content, titratable acidity and firmness in organic tomatoes compared with conventional one. However, no significant differences in titratable acidity (TA) and total soluble solids (TSS) between organic and conventional apples were reported (Roussos and Gasparatos 2009). Similar results were found by Juroszek et al. (2009) that no TA or TSS difference between conventional and organic tomatoes. On the other hands, it was reported that ascorbic acid content was significantly higher in organic tomatoes (Borguini 2006; Chassy et al. 2006). In respect to antioxidant activity, Wang et al. (2008b) reported higher phytochemical content and antioxidant activity in organic blueberry that that of conventional one, while You et al. (2011) found that antioxidant activity difference between organic and conventional blueberry depends on cultivars. Moreover, Reganold et al. (2010) found higher phenolic compounds content and antioxidant activity in organic strawberry, and Asami et al. (2003) reported similar results that organic strawberry contained higher phenolics. Higher phenolics content was also found in organic peaches and pears (Carbonaro and Mattera 2001).

Though comparisons between organic and conventional production systems have been conducted by several researchers, direct comparison was sometimes impossible.

According to the U.S. Department of Agriculture National Organic Program (AMS 2000), the farms for producing organic produce must not use synthetic pesticides and herbicides for at least 3 years prior to harvest. It is almost impossible to keep the organic farmland away from the synthetic pesticides and chemical fertilizers due to the interference between the organic and conventional farmland. Therefore, it is impossible to grow organic fruit right next to the conventionally grown farmland to make uniform production conditions for directly comparison between organic and conventional production.

2.2.3 Nitrogen fertilization for the production of organic blackberries

Based on the organic production standard, commercial fertilizers can only be used on the basis of soil and plants analysis and needs of the crops. Well balanced fertilizer application enables the soil to make nutrients available to the plant in adequate amounts at the right time and helps keep the plants in physiological balance.

The nutrients that plants take from the soil to grow and produce fruit are provided by the application of fertilizers (Havlin et al. 2005). However, the nutrients supplied by fertilizers are not fully available for use by the fruit plants, as nutrients may be lost as a result of leaching and runoff (Zhu et al. 2005). On the other hand, nutrients are constantly being made available to the plants through mineralization and the action of weather on the soil (Silgram and Shepherd 1999). The annual nitrogen requirement of fruit plant is relatively low, about 30 kg N/ha (Dasberg 1987). Adequate supply of nitrogen (N) in the critical phase after flowering will help ensure lignification, flower

bud production and quality of fruit (Lind 2003). However, excess of N may lead to excessive shoot growth and risk of physiological disorders, associated with keeping quality. Excessive application of N would increase the incidence of pests and diseases as well (Fageria et al. 2011).

Generally, fertilizers that can be utilized for organic production are classified as composts and manures, plant and root residues, and other fertilizers (Trenkel and Association 1997). Applying organic manures is a traditional method. Other than supplying nutrients, manure is also known to improve soil physical properties (Low 1954). Sauerbeck (1982) found that after organic farmyard manure was added to soil, organic carbon content increased significantly, and Johnston (1975) reported that composted matter showed higher effects on soil organic carbon content than fresh matter. In general, fresh manures may not be used immediately until proper curing which is to humificate the component of the manure. Roots and plants residues help maintain fertility, digestion of nutrients and improve soil property. They have better distribution in soil than organic manures. In some countries, manures are not sufficient for the demand of fertilization so that plant residues can help make supplement. Moreover, turf and sludge are also suitable as organic fertilizer, and the great hygroscopic ability and high water content make them good organic fertilizers (Li et al. 2005).

2.2.4 Harvest time effect

Harvest time is usually determined based on fruit maturity since it affects fruit quality during post-harvest storage. Immature fruit may not provide good sensory quality for consumers and overripe fruit easily turn to soft and decay with undesired flavor (Kader 1997). Generally, harvest time is a period of dates when fruit are fully ripen with acceptable flavor and appearance as well as at the peak of yield. It is a subjective judgment to distinguish whether a fruit is immature, mature or over-mature. However, a series of objective tests may help identify maturity stage of fruit. These measures are usually easy conducted in the harvest field with some physical and chemical changes including color, texture, soluble solids content and acidity (Thompson 2008). Color is the most common factor used as maturity indices for blackberry. Based on the color, the maturity of blackberry fruit can be classified as green, pink, commercially ripe and over-ripe (Wang and Jiao 2001). The best harvest period for blackberry is about 20-28 d depending on cultivars, climate, and growing location (Strik et al. 2008). Blackberries are usually harvested when dry in early morning. During the peak of harvest season, fruit are harvested 2-3 times a week for fresh market or even more if weather is hot. Hot midday is not proper time, neither as wet humid day since fruits are easily deteriorated.

2.2.5 Harvest methods

Hand harvest and machine harvest are both used for blackberry harvesting. Hand harvest can result in relatively low mechanical damage. However, physical damage of

fruit may occur if workers don't have excellent harvesting skills. The collection of fruit by workers is important. When putting into containers, fruits may be bruised and these bruises may not obvious for the first hours or days right after harvest (Kader 1983). Another problem caused by hand harvest is the un-uniformity of quality of blackberries harvested by different workers since maturity is very subjective judgment depending on workers. Soft fruit like raspberries and blackberries are usually harvested by hands due to their soft texture. Fruit are picked from the plant and put into a suitable container which may be transferred into cooler to remove field-heat and then taken into market or transfer for repacking. While hand picking is a common way for harvesting berries, machine harvest is also used for blackberry harvest.

Fruits harvested by machine are used for the processed market. Soft fruits for processing market like raspberry and blackberry can be harvested by a tractor-mounted machine with combining finger. During harvest, fruits are removed off plant with some leaves and stems left by high frequency vibration and rotation of fingers and then fruits are filtered and processed further. Strik and Buller (2001) reported that fruit yield and percent bud break was not affected by machine harvester and plants were not damaged either. Peterson and Takeda (2003) found that though machine harvest method showed high harvest rate, amount of fruit meeting the requirements for fresh market was much lower due to uniform fruiting canopy and the force applied to pull fruits off the plants. Similar results were reported that total semi-erect blackberries harvested by machine contained 10-70% immature fruit (Takeda et al. 1989).

2.3 Post-harvest handling and storage of blackberry fruit

2.3.1 Quality changes during post-harvest storage

Fresh blackberry fruit have living tissues, and the metabolism continues after harvest. Both physicochemical and nutraceutical properties of the fruit change during post-harvest storage. Sugar and acid contents in the blackberry fruit also change during storage which directly impact the sensory quality of the fruit (Qian and Wang 2005). Perkins Veazie and Collins (2002) reported that titratable acidity of 'Arapaho' and 'Navaho' blackberries decreases during storage at 2 °C due to the possible water loss. Similar results were reported by Wu et al. (2010) that TA of 'Marion' and 'Evergreen' blackberries decrease 36.8% and 46.2%, respectively under 2.0 ± 0.2 ºC and 95 ± 2% relative humidity. Perkins-Veazie et al. (1996) found that total soluble solids of four cultivars of blackberry fruit increase during storage at 2 °C because of the hydrolysis of cell wall materials. They also mentioned that total soluble solids contents of blackberries increase during ripening process since more sugars were accumulated during the ripening process. The firmness of blackberry fruit decreased during ripening and storage depending on the cultivar, storage conditions and time (Joo et al. 2011). Sousa et al. (2007) found an increase of firmness during 2 ºC and 90% RH storage which was probably due to calcium binding of pectins. However, decrease in firmness of blackberry fruit during storage (3~-80 ºC) was also reported by several other researchers (Siriwoharn et al. 2004; Kafkas et al. 2006; Joo et al. 2011). Other than physicochemical properties, nutraceutical properties changed inconsistently as well depending on cultivars and storage conditions. Wu et al. (2010) reported an

increase in anthocyanin content of ‘Marion’ and ‘Evengreen’ fruit during 7 d of storage at 2 °C and 95% RH. The increase in anthocyanin lead to a dark color of the fruit and this change occurred more slowly during cold storage. Besides blackberry, Nunes et al. (2006) mentioned that total anthocyanin content of strawberry is lower during ripening process in cold storage than in the field.

2.3.2 Microbial concerns

Decay and foodbrone pathogen related safety issues are most common microbial concerns for blackberry. Decay caused by fungi and anthracnose, cane and leaf rust and blackberry rosette are the major problems leading to postharvest loss of blackberry fruit (Buckley et al. 1995; Strik et al. 2007; Rueda‐Hernández et al. 2013). During cold storage, high humidity (95-97%) environment is usually applied to ensure the freshness of fruit. However, such high humidity would also promote microbial spoilage unless enough air flow for reducing the amount of moisture on fruit surface. Fungi, such as *Botrytis cinerea* (Ellis et al. 1991), *Rhizopus stolonifer* (Prange and DeEll 1995), and *Mucor* sp. are much more common than bacterial spoilage organisms on blackberry fruit during storage (Washington et al. 1999). Removal of decayed fruit can help control decay of good fruit during storage, but some fungal have spores that can be easily spread by the air even at low temperature, thus making the control on fungal disease very difficult. Moreover, the survival and growth of foodborne pathogens in contaminated fruit can be a potential food safety problem (Buck et al. 2003). *Salmonella, Listeria monocytogenes* and *E. coli* O157:H7 all can survive on the

fruit even no visually evidence was revealed (Shearer et al. 2001). During harvest and transportation, bacteria and viruses could also infect the fruit easily since fruit for fresh market are not washed before sale (Sapers 2001). A case-control study indicated that raw blueberry consumption was involved in 56% of 39 cases of hepatitis A virus (HAV) between January and May in 2002 in New Zealand (Calder et al. 2003). Moreover, frozen blackberry was reported as one of most likely source of an outbreak of acute gastroenteritis occurred in a canteen in Germany (Fell et al. 2007). Ensuring food safety of fresh and processed fruit is extremely important.

2.3.3 Refrigerated storage

Refrigerated storage is an effective method for maintaining the quality of fresh blackberries. Respiration process of blackberry fruits can be slowed down in refrigerated storage (Perkins-Veazie et al. 1999). Generally, recommended cold storage condition for fresh blackberry fruit is about 2 ºC and 90-95% relative humidity and shelf life of blackberry fruit under such conditions is about 2-6 d (Salunkhe and Desai 1984). Moreover, less texture and color changes and loss of flavor and weight were reported (Reyes-Carmona et al. 2005). Due to delayed growth of microorganisms at low temperature, fruit decay was also slowed down in cold storage. However, water loss was quite common at low temperature since cold air couldn't hold as much moisture as air in room temperature, which means specific humidity is needed for maintaining cold storage. Among several refrigeration methods, forced air ventilation is most widely used for blackberry storage. A large refrigerated room with air cooling

system is equipped to ensure constant low temperature. Besides removing field heat, cold air system help evaporate moisture from the surface of blackberry fruit, further lowering the possibility of decay. Another method is refrigeration room without flowing air. This method is not as effective as forced air ventilation because still air could not remove heat as quickly as forced air. Crushed ice may also be used for cold storage when covering the top of fruit, but it is not recommended to cool blackberry fruit because of partial chilling damage of fruit.

2.3.4 Controlled atmosphere storage (CAS) and modified atmosphere packaging (MAP)

Controlled atmosphere storage is a technology to preserve and extend the shelf life of fruits and vegetables under low temperature (0-4 ºC). Respiration and transpiration of fruit are lowered as well as growth of aerobic microorganism and enzymatic activity because of high concentration of carbon dioxide and low concentration of oxygen (Thompson 2010). For blackberry preservation, condition of controlled atmosphere storage with 10-20% carbon dioxide and 5-10% oxygen has been reported by Thompson (2010). However, high concentration of carbon dioxide may result in off-flavor (Pérez and Sanz 2001). Perkins-Veazie and Collins (2002) also reported that controlled atmosphere storage (15 kPa CO_2, 10 kPa O_2) effectively decreased decay of ‘Navaho’ and ‘Arapaho’ blackberries during storage at 2 °C. However, Agar et al. (1997) found that controlled atmosphere storage at 10-30% CO_2 and 2% O_2 is not an optimal method in preserving ascorbic acid in thornless blackberries.

Modified atmosphere packaging (MAP) is another packing technology with specialized gas composition for extending shelf life of fruits and vegetables (Kader et al. 1989). Gas composition, storage temperature and package material need to be determined according to the characteristics of food such as respiration rate, mass, temperature requirements, fruit cultivars and maturity for the well-designed modified atmosphere packaging (Fonseca et al. 2002). Many studies have investigated the effectiveness of MAP technology to extend the shelf-life of apples (Rocha et al. 2004), blueberries (Song et al. 2002), and cherries (Petracek et al. 2002), as well as some studies investigating MAP of blackberry fruit. Farber et al. (2003) recommended 15-20% CO_2 and 5-10% O_2 as an optimal MAP condition for blackberry, and Mir and Beaudry (2004) suggested 10-20% CO_2 and 2% O_2 MAP for blackberry.

2.4 Freeze processing and storage of blackberry fruit

2.4.1 Introduction

Due to the short production season and limited shelf-life, fresh blackberries are commonly processed into frozen products. According to the National Agricultural Statistics Service (USDA 2014), 27,183,000 pounds of blackberries were freeze processed between March 31 2013 and February 28 2014. The principle of frozen technology is by reducing temperature and water activity to control the growth of microorganisms, enzyme activity and other reactions, thus resulting in high quality and prolonged shelf-life products. On the other hands, freeze process may advance the structural transformation and diminish biological activities of some nutrients.

2.4.2 Principles of freezing process

Freezing process lowers temperature and reduce water activity of products due to ice formation and concentrated solutes. During the freezing process, some chemical reactions, as well as some effects on the tissue structure and microbial reactions take place and such reactions are associated with various properties of the products.

Freezing rate is the velocity of temperature decrease per minute (ºC/min). It significantly affects quality of frozen fruit since cell wall rupture is affected by freezing rate. When freezing rate is higher than 4 ºC/min, large volume of small ice crystals forms causing less cell wall rupture when comparing with freezing rate lower than 4 ºC/min. Temperature difference between fruit and cooling system, contact area of fruit to cooling agent, initial temperature as well as freezing equipment are all affecting freezing rate (Sousa et al. 2007). Ranđelović et al. (2008) found that both slow and fast freezing may cause color change of blackberry fruit as well as pH and vitamin C change while total soluble solid and total anthocyanins were not changed significantly.

Damage caused by freezing in fruit tissue includes interference of metabolism, changes of enzyme activities, and cell membrance damage (Jul 1984). Fruit will become soft after thawed under situation of ice crystals rupture of cell wall. The bigger the ice crystals are, the more the texture change of fruit. Moreover, the amount of liquid collected, called drip loss, after frozen fruit is thawed has been used as an indicator of quality of frozen fruit (Fuster et al. 1994).

2.4.3 Freezing methods

Berries may be frozen individually, packed in dry sugar, syrup or crushed into purees before freezing depending on the final application of the finished products. Different types of freezers including air-blast, spiral, fluidized bed, liquid immersion, spray, plate, and cryogenic are available for specific applications. The type of freezer selected should be based on freezing rate, cost, function, and feasibility since freezing rate is important factor affecting quality of fruit and economy of equipment is big concern for freezing application (Zhao 2007b).

2.4.4 Quality control during freezing

Liquid nitrogen freezing process effectively retained structure of frozen wild blackberries compared with other plate freezing methods (Marti and Aguilera 1991), and the blackberries frozen at a freezing rate of 2.2 ºC/min showed high firmness after thawed (Sousa et al. 2007). Schmidt et al. (2005) also reported that individual quick freezing (IQF) method may effectively retain antioxidant activity and total phenolics content for both wild and cultivated blueberries in North America. González et al. (2003) reported that after cryogenic freezing in liquid nitrogen at -80 °C, Spanish wild blackberries had 15.48% decrease in total monomeric anthocyanins, 7.51% decrease in total phenolics content and 25.41% decrease in ellagic acid content. They also found total anthocyanins and phenolics remained at the same level for blackberries packed in polyethylene bags during frozen storage at -24°C for 6 months (González et al. 2003). Yorgey and Finn (2005) reported that overall quality of ORUS 1380-1 is significantly

better than ‘Waldo’ blackberry after IQF. Wu et al. (2010) reported that anthocyanins in ‘Evergreen’ blackberry increase up to 5.5% after freezing and phenolic compounds are well maintained. Türkben et al. (2010) found that ferulic acid content decreases significantly after 6 months of frozen storage. Similar results were reported by Dai et al. (2009) that total phenolic content of anthocyanin-containing blackberry extracts decrease 10% after 90 d of frozen storage. Moreover, Ranđelović et al. (2008) found both slow and faster freezing may cause color change of blackberry fruit as well as pH and vitamin C change while total soluble solids and total anthocyanins contents did not change significantly.

2.4.5 Applications of frozen blackberry fruits

Frozen blackberries can be consumed directly (Stanfield et al. 2004), further processed into beverages (Qian et al. 2006), jams, jellies and dried products (Figuerola 2007a), and added into dairy products like yogurts and ice creams (Wade 1991) and bakery goods (Gutierrez 2013).

Dried blackberries can be made into fruit snack for providing phenolic compounds and soluble dietary fiber. Breakfast cereals containing berries are also very popular since the soluble dietary fiber in berries complement the insoluble fiber of cereals (Figuerola 2007b). Blackberries are one of suitable fruits for making jams and jellies because of their color, acidity, flavor, aroma (Guy and Shiun 1986). Blackberry jams and jellies can be used as spread on bread and crackers as well as filling for pastries and cookies because of their thickness, acidity and unique flavors (Figuerola 2007a).

Blackberry juice and wines are also popular because of the unique flavor, color and relatively high content of antioxidant compounds. Juice and wine retain those beneficial chemicals and good texture since seeds are excluded (Cabral et al. 2007). Moreover, Blackberries were added into flavored cottage cheese and the integrity of fruit is important in the final products (Berry 2001). For premium dairy products, IQF blackberries are usually used due to low sugar content and no preservatives. Walker et al. (2006) reported that blackberry yogurt processed by high hydrostatic pressure showed no significant change in color and pH.

2.5 Conclusion

Blackberry fruit have distinguished flavor and color, contain large amount of phenolic compounds with demonstrated antioxidant capacity. Composition varies depending on cultivars, growing location and environment. The market of both fresh and processed blackberries has been expanded significantly in the past decades and further expansion is expected for the next decades. Because of their environmental friendly production and free in pesticide, fungicides, antibiotics and genetically modified organism (GMO), organic produce has become more and more popular.

Nitrogen fertilizers play an important role in blackberry growing as N source provides fruit necessary nutrient. Selection of different nitrogen fertilizers may affect the growth of the plant. Blackberry fruit is generally harvested within one month of time period, and fruit maturity at the harvest is an important factor that could directly impact the postharvest storage life of fruit. However, very limited information is

available about how organic production systems (i.e., fertilizer application, weed management, and harvest date) affect the fruit quality and storage life. And change of the physicochemical and nutraceutical properties of organically grown blackberry fruit during postharvest storage are not well studied. Therefore, it is important to investigate how organic production system and post-harvest refrigerated storage impact the quality of fresh blackberry fruit.

For fresh market, blackberry fruit are hand harvested while they are usually picked by machine harvesters for processed market. Since fresh blackberry fruit have very short shelf life, majority of them are freeze processed to extend the storage time. Again, not much information is available about how freezing process affects the quality of organically grown blackberry fruit. Therefore, another objective of this project was to study freezing effect on the physicochemical and antioxidant properties of organically grown blackberry fruit of the specific cultivars and the quality changes during frozen storage.

Table 2.1 Plant types and cultivars of blackberries

Blackberry Cultivars			Growth region/country
Type	Cultivar	Release Date	
Erect	Cherokee	1970s	Arkansas, USA
	Shawnee	1980s	
	Navaho		Arkansas, USA
	Kiowa	1990s	
	Apache		
	Chickasaw		
	Ouachita	2000s	
Trailing	Evergreen	1850s	Milder parts of the Northwest of USA
	Pacific	1940s	Oregon, USA
	Olallie	1950s	
	Marion		
	Waldo	1970s	
	Black Diamond	1990s	
	Obsidian	2000s	
Semi-erect	Thornfree	1960s	Maryland, USA
	Black Satin		Illinois, USA
	Chester Thornless	1970s	Middle Atlantic and Pacific Northwest, USA
	Himalaya	1930s	
	Hull Thornless	1970s	
	Triple Crown	1980s	
	Loch Ness		U.K.
	Loch Tay	2003	U.K.

Source: Adapted from Clark et al. (2007), Finn (2008), Finn and Strik (2008), and Finn and Clark (2012).

Table 2.2 Chemical composition of blackberry fruit from U.S. National Nutrient Database (kg^{-1})

Compounds	Value	Compounds	Value
Water (g)	881.50	Calcium (mg)	290.00
Energy (kcal)	430.00	Iron (mg)	6.20
Protein (g)	13.90	Magnesium (mg)	200.00
Total lipid (g)	4.90	Phosphorus (mg)	220.00
Carbohydrate (g)	96.10	Potassium (mg)	1620.00
Total dietary fiber (g)	53.00	Sodium (mg)	10.00
Total sugar (g)	48.80	Total ascorbic acid (mg)	210.00
Ash (g)	3.70	Total folate (μg)	250.00
Sucrose (g)	0.70	Thiamin (mg)	0.20
Glucose (g)	23.10	Riboflavin (mg)	0.26
Fructose (g)	24.00	Vitamin A (IU)	2140.00
Cyanidin (mg)	999.50	Pelargonidin	4.50
Peonidin	2.10	(+)-Catechin	370.60
Quercetin	35.80	Proanthocyanidin 4-6mers	72.70

Source: Adapted from National Nutrient Database for Standard Reference (USDA 2011).

Table 2.3 Total soluble solids content of different cultivars of fresh blackberry fruit

Cultivar	Total soluble solid	References
Arapaho	9.20 %	Alleyne and Clark (1997)
Arapaho	8.70 %	Perkins Veazie and Collins (2002)
Caingangue	7.60 %	Hassimotto et al. (2008)
Cancaska	9.20 %	Joo et al. (2011)
Chester Thornless	9.63 %	Joo et al. (2011)
Chester Thornless	43.6 $mg/g_{extract}$	Kafkas et al. (2006)
Choctaw	11.50 %	Pantelidis et al. (2007)
Evergreen	16.10 %	Reyes-Carmona et al. (2005)
Evergreen	11.50 %	Pantelidis et al. (2007)
Hull Thornless	9.80 %	Pantelidis et al. (2007)
Loch Ness	52.5 $mg/g_{extract}$	Kafkas et al. (2006)
Marion	10.60 %	Reyes-Carmona et al. (2005)
Navaho	62.5 $mg/g_{extract}$	Kafkas et al. (2006)
Tupy	6.93 %	Hassimotto et al. (2008)

Table 2.4 Total titratable acidity of different cultivars of fresh blackberry fruit

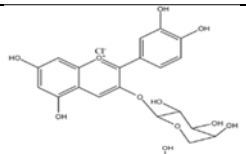
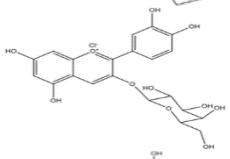
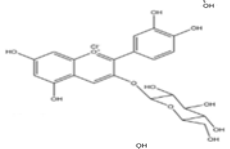
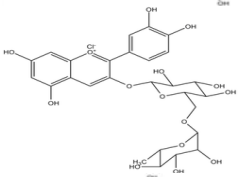
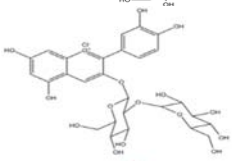
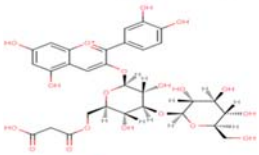
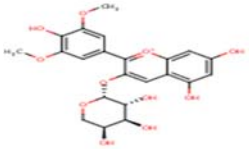
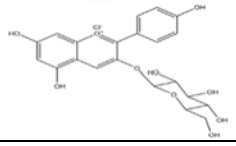
Cultivar	Total titratable acidity	Reference
Apache	1.64 % of oxalic acid	Vrhovsek et al. (2008)
Arapaho	0.82 % of citric acid	Perkins Veazie and Collins (2002)
Chester Thornless	1.88 % of oxalic acid	Vrhovsek et al. (2008)
Chester Thornless	0.80 % of citric acid	Wang et al. (2008a)
Evergreen	1.02 % of citric acid	Reyes-Carmona et al. (2005)
Hull Thornless	0.55 % of citric acid	Wang et al. (2008a)
Hull Thornless	1.38 % oxalic acid	Vrhovsek et al. (2008)
Marion	3.46 % of citric acid	Reyes-Carmona et al. (2005)
Navaho	1.00 % of citric acid	Perkins Veazie and Collins (2002)
Rubus adenotrichus Schltdl.	2.40 % of malic acid	Acosta-Montoya et al. (2010)
Triple Crown	1.72 % oxalic acid	Vrhovsek et al. (2008)
Triple Crown	0.85 % of citric acid	Wang et al. (2008a)

Table 2.5 Total phenolics contents of different cultivars of fresh blackberry fruit

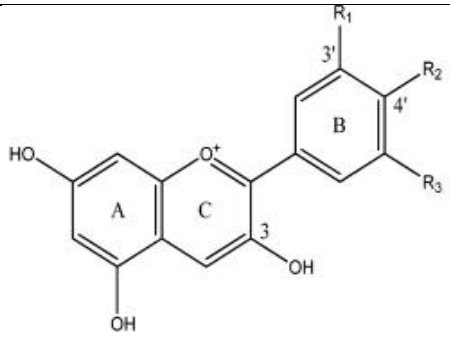
Cultivar	Total phenolics (mg GAE/ 100 g FW)[a]	Reference
Andean	2167 ± 835	Vasco et al. (2008)
Chester Thornless	351.7 ± 7.2	Benvenuti et al. (2004)
Chester Thornless	225	Koca and Karadeniz (2009)
Chester Thornless	2515.7 ± 18.6	Sariburun et al. (2010)
Evergreen	960 ± 101	Siriwoharn et al. (2004)
Hull Thornless	405	Connor et al. (2005)
Hull Thornless	248 ± 5.9	Wang and Lin (2000)
Hull Thornless	236.7 ± 9.5	Benvenuti et al. (2004)
Marion	513	Connor et al. (2005)
Marion	903 ± 145	Siriwoharn et al. (2004)
Navaho	446.4	Cho et al. (2005)
Navaho	210	(Koca and Karadeniz 2009)
Navaho	459	(Connor et al. 2005)
Shawnee	373	(Connor et al. 2005)
Triple Crown	204.0 ± 2.0	(Wang and Lin 2000)

[a] FW, fresh weight; GAE, gallic acid equivalent.

Table 2.6 Anthocyanins in blackberry fruit

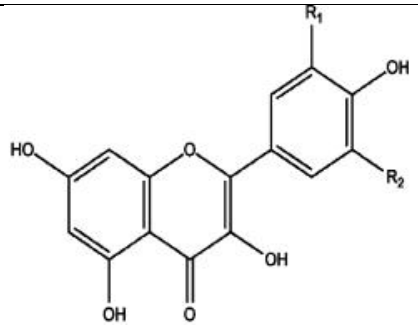
Anthocyanins	**Structure**	**Color**
cyanidin 3-arabinoside		dark red /purple
cyanidin 3-galactoside		dark red /purple
cyanidin 3-glucoside		dark red /purple
cyanidin 3-rutinoside		dark red /purple
cyanidin 3-sophoroside		dark red /purple
cyanidin 3-(6-malonyl)glucoside		dark red /purple
malvidin 3-arabinoside		dark blue/purple
perlargonidin 3-glucoside		dark red /purple

Source: Adapted from Howard and Hager (2007) and Shahidi and Naczk (2003).



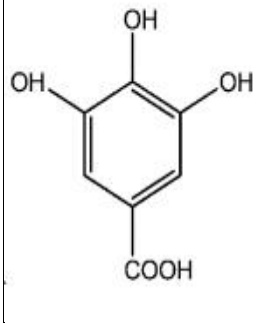
Chemical Structure of Anthocyanin

Anthocyanindin	R_1	R_2	R_3
Cyanidin	-OH	-OH	-H
Delphinidin	-OH	-OH	-OH
Pelargonidin	-H	-OH	-H
Malvidin	$-OCH_3$	-OH	$-OCH_3$
Peonidin	$-OCH_3$	-OH	-H
Petunidin	-OH	-OH	$-OCH_3$

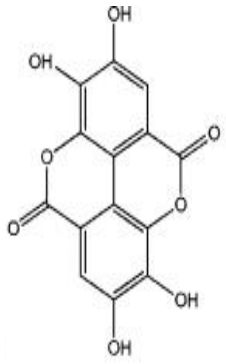


Chemical Structure of Flavonols

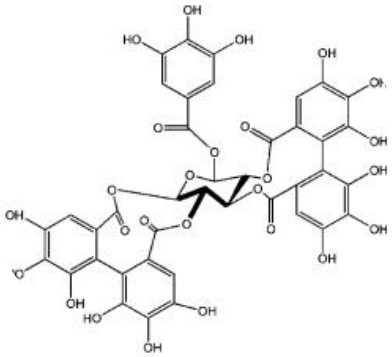
Flavonol	R_1	R_2
Quercetin	-OH	-H
Kaempferol	-H	-H
Myricetin	-OH	-OH



Gallic Acid



Ellagic Acid



Galloyl-bis-HHDP glucose

Figure 2.1 Structure of major phenolic compounds in blackberry fruits.
Source: Adapted from Balasundram et al. (2006), Wu et al. (2006), and Zhao (2007a).

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CHAPTER 3

Effect of Different Commercial Fertilizers, Harvest Date and Storage Time on the Physicochemical and Antioxidant Properties of Two Organically Grown Blackberry Cultivars

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ABSTRACT

Despite increased consumer interest in organic produce, little is known about how different organic production methods affect both the traditional measures of quality and the naturally occurring health promoting (bioactive) compounds of food. In this study, 'Obsidian' and 'Triple Crown' blackberries (both hybrids in the genus *Rubus*) were cultivated organically and fertilized at a rate of 56 kg/ha nitrogen with one of three commercially available organic fertilizers: soy meal, fish emulsion/hydrolysate blend, and processed poultry litter. Fruit were hand-harvested three times during their peak production period at approximately 1-wk intervals and stored at 4 °C and 85% RH for up to 12 d. Harvest date and storage time had greater influence on the physicochemical properties than that of fertilizer type. During storage, late-harvest fruit of both cultivars had the least decay in 2012 while early-harvest fruit exhibited the least decay in 2013. Fruit leakage in both cultivars increased during storage, reaching 54.3% and 62.5% in 'Obsidian' and 62.3% and 73.0% in 'Triple Crown' in 2012 and 2013, respectively. Fruit pH increased while titratable acidity decreased during storage in both cultivars. Total soluble solids varied among the fertilizer treatments and between the two cultivars. During 10 d of storage, fruit weight decreased by 2.52% in 'Obsidian' and by 3.15% in 'Triple Crown'. Antioxidant data revealed an interesting pattern: 'Obsidian' had as much as 37% higher ORAC and 40% less sugar than 'Triple Crown' at harvest and also had greater differentiation due to fertilizer treatments. Fertilizer effects differed based on harvest date and cultivar, with late-harvest fruit fertilized with fish emulsion showing as much as 37% higher

TPC and 29% higher ORAC than other fertilizer treatments, while the early and middle-harvest fruit had similar or greater responses to soymeal-based fertilizer. Time of harvest and length of storage also affected the antioxidant properties and sugar profiles in different ways, depending on the cultivar, again with the ‘Obsidian’ fruit showing greater variability in general. This study demonstrated that the two cultivars of organically grown blackberry fruit have different physicochemical and antioxidant properties, thus potentially different shelf-lives in the fresh market.

Keywords: Organic blackberry, antioxidant activity, physicochemical property, sugar profile

Introduction

Consumer interest in organic produce has resulted in the rapid growth of organic agriculture in the United States, with total acreage of organic crops increasing by 76% between 2002 and 2007 (USDA 2010). One of the major reasons for consumer choosing organic produce is the belief that the products are "healthier for me and my children" (OTA 2011). Meanwhile, public health experts are recommending increased consumption of fruit due to high natural antioxidants, which have been linked to reduced risk of various health maladies, including cancer, coronary heart disease, metabolic disorders, and inflammatory responses (Srivastava 2009; Halvorsen et al. 2002; Wang et al. 2009; Obrenovich et al. 2011; Kang et al. 2003; Hagiwara et al. 2001).

Conventional farming has kept pace with increased demand for blackberries through new cultivars and improved agricultural practices. In 2005, 4,818 ha of land was planted with blackberry and produced 31,840 Mg of fruit (Strik et al. 2007). While many of the improved practices for blackberry (e.g., irrigation system and weed management strategies) translate well into organic production systems, there are some limitations. For example, one of the most important factors that can affect plant productivity is the fertilizer regimen used. While several studies have been undertaken to determine the ideal rate and timing of conventional fertilizer applications (Strik 2005), published guidelines and recommendations focus primarily on nitrogen and make little or no distinction among fertilizer sources, aside from the general caveat

that nitrate-nitrogen is better than ammonium-nitrogen in blackberry (Naraguma and Clark 1998; Kuepper et al. 2003; Strik 2005).

Fertilizer source is particularly problematic for organic growers. While conventional/synthetic fertilizers are composed of a handful of industrial chemical compounds, organic fertilizers are derived from plant/animal wastes and, as such, are much more complex. Even if it was possible to extrapolate conventional fertilizer guidelines to organic, it is entirely possible that the plants will respond differently to various organic fertilizers.

Studies that compared conventional and organic production methods in various crops have shown marked differences in performance and fruit/vegetable quality (Reganold et al. 2010; Györe-Kis et al. 2012; Hallmann 2012; Macoris et al. 2012; Griffiths et al. 2012; Strik 2005). Even among different types of organic fertilizer, great variability was found in horticultural measures of performance, such as yield and nitrogen uptake (Gaskell and Smith 2007; Berry et al. 2002). Further, it is unknown how the different organic fertilizer sources can affect the overall fruit quality, thus a question that needs to be answered, particularly given that Bulluck et al. (2002) showed that different organic fertilizer sources not only affect crop yield, but also modify the physical, chemical and microbial properties of the soil itself, and in turn the quality of fruit (Crisosto et al. 1995; Dris et al. 1998; Santos et al. 2003; Harkins et al. 2013).

Fruit quality and health properties may also vary over the course of the growing season. (Basiouny 1995) found that anthocyanin content and shelf life decreased in

later-harvest blackberries, and (Thompson et al. 2009) noted a decline in total soluble solids, total phenolic content, anthocyanin content, and pH as the harvest season progressed in primocane fruiting blackberries. Recent studies have shown that harvest time also has a marked effect on total yield, average berry weight, and total soluble solids in organically produced blackberries (Fernandez-Salvador et al. 2013; Harkins et al. 2013).

Given the focus on perceived health benefits of organically grown produce, it is essential to quantify the bioactive compounds and antioxidant potential of organic blackberry fruit. The former allows the characterization of known compounds that may be linked to particular health benefits, while the latter attempts to give an overall measure of how well all of the compounds scavenge specific types of free radicals. Further measurements of antioxidant potential will vary based on the free radical and comparative standard used in the assay, making comparison between different methods difficult. These benefits and drawbacks lead most researchers to perform one or more assays of each type in an attempt to get a better overall grasp of the antioxidant properties, as well as allow for more avenues of comparison with previous studies. Most antioxidant studies involving blackberries have focused on total phenolic compounds and anthocyanins, as blackberries are well known to be high in both, and also often included one or two measures of antioxidant activity (Srivastava 2009; Fan-Chiang and Wrolstad 2005; Siriwoharn et al. 2004; Halvorsen et al. 2002; Siriwoharn 2001). Despite the potential effect of fertilizer source on the antioxidant properties of blackberries, there have been no published studies on the subject.

The aim of this study is to examine the effects of differing organic fertilizer sources and harvest dates on the physicochemical and antioxidant properties during refrigerated storage in fruit collected from two fresh-market cultivars of organically grown blackberry. Specifically, decay, leakage, fresh weight loss, acidity, moisture content, berry firmness, the sugar profile, the content of the two major antioxidant fractions, and the overall antioxidant potential were measured. It is important to note that this study did not compare organically and conventionally produced fruit. In order to properly compare organic and conventional fruit, the plants must be grown in the same planting area with replicated treatments. This is extremely difficult if not impossible, as the "organic" could never be certified organic due to the close proximity of the conventional plots and the size of the required buffer zones. Such a comparison has not been done to date. Planting at different locations could possibly alleviate the proximity issues, but given the variability normally encountered with minor changes in growing conditions, such studies run the risk of confounding the effects of the production system with geographically-based differences. Previous studies that tried comparing organic and conventional fruit from separate fields (Asami et al. 2003; Mitchell and Barrett 2004) have been criticized for such a comparison (Felsot and Rosen 2004). Hence, it is important not to compare what might not technically be "organic" fruit to conventional ones.

Materials and Methods

Materials

All chemical reagents were analytical grade, except for the ultra-pure (<18.2 MΩ cm) water used as a mobile phase in HPLC analysis of the sugar profile, which was prepared *in situ* using a Millipore filtration system (Millipore Corp., Bedford, MA).

Two blackberry cultivars, ‘Obsidian’ and ‘Triple Crown’, were evaluated in this study. The cultivars were chosen for their potential suitability for organic fresh market production in the Pacific Northwest. ‘Obsidian’ is an early–season cultivar (late-June to mid-July), while ‘Triple Crown’ is a late-season cultivar (early- to late-August). Both cultivars were grown on a certified organic farm (Riverbend Organic Farms, Jefferson, OR) in nine plots per cultivar. Complete details of the growing conditions were described in (Fernandez-Salvador et al. 2013). Briefly, all plots were grown using the same management system (e.g., irrigation, pest control scheme, and weed management technique) but fertilized using one of three different commercial organic fertilizers: processed poultry litter (PPL- “Nutri Rich 4-3-3 Ca 7%”, Stutzman Farms, Canby, OR), soy meal (SM- “Phyta-grow leafy green special", California Organic Fertilizers Inc., Fresno, CA), and a blend of fish emulsion and fish hydrolyzate (FE- "True 402", True Organic Products Inc., Helm, CA). All fertilizers were applied at the recommended nitrogen rate (56 kg/ha N) and the fertilizer treatments were arranged in a completely randomized block design with three replicate plots per treatment.

Berries were hand-harvested three times during the 2012 and 2013 growing seasons (July 6-17 and June 24-July 9, for 'Obsidian' in 2012 and 2013, respectively; Aug. 10-24 and Aug. 1-15 for 'Triple Crown' in 2012 and 2013, respectively) at approximately one week intervals, referred to as the "Early Harvest", "Middle Harvest", and "Late Harvest", respectively. Depending on the weather conditions, the exact harvest date varied year-by-year. Approximately 16 berries from each plot were placed o each date into hinged Polyethylene Terephthalate (PETE) clamshell containers (Pactiv, LLC. Lake Forest, IL). The filled containers were then placed into open-topped cardboard boxes, stored at 4±1 ^{o}C and 85% RH, and sampled at days 0, 2, 4, 6 and 0, 2, 5, 8, 10 for 'Obsidian' in 2012 and 2013, respectively, and at days 0, 4, 10, 12 (±1) and 0, 4, 8, 10, 12 for 'Triple Crown' in 2012 and 2013, respectively. Sampling discontinued when more than half of the berries in a given container decayed. On each sampling day, one randomly-selected container from each replicate was removed from storage and analyzed for physiochemical and antioxidant properties.

Weight loss during storage

Weight loss of fruit during refrigerated storage was measured using the modified method of Joo et al. (2011). The weight of the fruit in each clamshell was measured on day 0 and each subsequent sampling date. The results were recorded as percentage of weight change at each sampling date divided by the initial weight of the fruit.

Fruit decay and leakage

Fruit decay and leakage were evaluated following the procedures described by Civello et al. (1997) with some modifications. Briefly, individual fruits were gently taken out of the clamshell containers and inspected visually for mold and/or extensive damage (defined as having < three ruptured/crushed contiguous drupelets or < five ruptured/crushed drupelets overall, either of which rendered a berry "decayed"). Non-decayed fruits were tested for leakage by transferring them to a standard "letter size" (215.9 mm x 279.4 mm) sheet of white printer paper and gently rolled so that all berry surfaces were exposed to the paper. Juice stains on the paper rendered a fruit "leaking". Decay rate was calculated as the percentage of berries in a container that were decayed, while leakage was calculated as the percentage of non-decayed berries in a container that were leaking.

Fruit moisture content, pH, titratable acidity, and total soluble solids content

Moisture content (MC) was measured by oven-drying two individual berries (~10 g) from each clamshell at 105 ºC (Duan et al. 2011). The samples were dried to constant weight, and MC was calculated as the percentage of weight loss.

Fruit pH and titratable acidity (TA) were determined using the methods from Fisk et al. (2008). Two individual berries from each clamshell were used for the measurements. The berries were mixed with distilled water at 1:9 (w/w) ratio, and blended for 1 min using a 12-speed homogenizer (Osterizer, Jarden Corp., Mexico). The mixture was filtered through qualitative filter paper to remove insoluble material.

The filtrate was assayed for pH using a pH meter (Corning 125, Corning Science Products, Medfield, MA, USA), and TA was determined by titration to an endpoint of pH 8.2 with a standardized 0.1 N aqueous NaOH solution. TA values were calculated based on the assumption that malic acid was the predominant acid.

Fruit firmness

Five non-decayed berries from each clamshell were individually measured on each sampling date for firmness using methodology originally developed by Joo et al. (2011) with modification to better approximate the conditions of the non-destructive subjective manual test commonly used by growers (Perkins-Veazie et al. 1997). Briefly, berries were placed on their side, and the force (N) required to compress the berry 5% of its total thickness was measured using a texture analyzer (Model TA-XT2, Texture Technologies Corp. Scarsdale, NY, USA) fitted with a 25 kg load cell and a 50 mm cylindrical probe.

Fruit extraction for antioxidant assays

Four berries were taken from each clamshell, combined according to the treatment group to give a total of 12 berries per treatment, and rapidly frozen by immersion in liquid nitrogen. The frozen samples were then pulverized under liquid nitrogen using a 1-liter blender (Waring laboratory Science, Torrington, CT) fitted with a specialized lid to allow for pressure release.

Samples of pulverized berry powder (15 g) were subjected to a modified ultrasound-assisted sequential extraction procedure developed in the laboratory (Wu et al. 2010). Briefly, a given sample was extracted first using acidified acetone (0.1 mL/L HCl), then twice with a 3:7 of water:acidified acetone solution, with each extraction involving a fixed time ultrasound treatment (90, 300 and 300 s, respectively). After centrifuging, supernatants were decanted and pooled together for partitioning with 150 mL of chloroform, vortexing thoroughly, and centrifuging to separate the two phases for removing any lipophilic components. The aqueous phase was then decanted and evaporated to remove residual organic solvents using a rotary evaporator (Roto-vap, Brinkman Instruments, Westbury, NY). Extract volume was standardized to 150 mL using deionized (DI) water and 1.5 mL aliquots of the standardized solutions were stored at -80 ºC until the time of assay.

Juice extraction for sugar profiling

A modified procedure from Qian (2006) was used to prepare aqueous berry extracts. Briefly, ~35 g of the pulverized berry powder that was not used for the antioxidant assays were mixed in glass jar with DI water equal to one-half the mass of the sample. The jars were fitted with lids and immersed in a boiling water bath for 20 min to inactivate enzymes. The resultant juice/slurry was centrifuged to remove solids and decanted into clean polypropylene bottles for storage at -25 °C until the time of assay. Extracts were prepared from berries harvested during the 2012 season only in order to avoid the variation inherent to the first fruiting of blackberries.

Total phenolic content

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu colorimetric method as described by Singleton et al. (1999). Briefly, aqueous extracts were diluted until their absorbance value was less than 1.2, and 0.5 mL aliquots of this diluted sample were added to tubes containing 7.5 mL of DI water and 0.5 mL of Folin-Ciocalteau reagent. After vortexing to mix, solutions were allowed to react for 10 min before the addition of 3 mL of 20% sodium carbonate solution. The resultant mixture was vortexed and then placed into a 40 °C water bath for 20 min. Following the heat treatment, samples were plunged into a 0 °C ice/water bath until they reached room temperature. Absorbance of the samples at 765 nm was measured using a spectrophotometer (Model UV160U, Shimadzu Corporation, Kyoto, Japan). These values were used to calculate gallic acid equivalents based upon the equation of a standard curve prepared the same day using solutions of gallic acid (0, 150, 200, 250 ppm). Assays were performed in triplicate, and values were reported as mg gallic acid equivalents (GAE)/g fresh weight (FW).

Radical scavenging activity

The refined colorimetric assay method relying on the reduction of the stable free radical 2,2-diphenyl-1-picryhydrazyl (DPPH) (Brand-Williams et al. 1995) was used to determine radical scavenging activity (RSA). Briefly, 1.5 mL of freshly prepared DPPH solution in methanol (0.09 mg/mL) was added to disposable cuvettes

containing 0.75 mL of diluted fruit extract, mixed and allowed to react at room temperature for 5 min. Absorbance at 517 nm was measured and used to calculate ascorbic acid equivalents (AAE) based upon the equation of a standard curve prepared the same day using ascorbic acid solutions (0, 100, 200, 300, and 400 ppm). Assays were performed in triplicate, and values were reported as mg AAE/g FW.

Total monomeric anthocyanins

The spectrophotometric method based upon pH-induced changes in absorbance (Giusti and Wrolstad (2001) was used to assay total monomeric anthocyanins (TMA). Briefly, for each sample, aliquots of extract were placed into two disposable cuvettes, diluted with either a standardized sodium acetate buffer (pH 4.5) or a standardized potassium chloride buffer (pH 1.0) and allowed to equilibrate for at least 15 min at room temperature. Optical absorbance was measured at both 510 nm and 700 nm, with the former value being selected based upon the predominant anthocyanin in blackberries, cyanadin-3-glucoside (Siriwoharn et al. 2004; Fan-Chiang and Wrolstad 2005). Absorbance values were then used to calculate concentration of monomeric anthocyanins (expressed as mg TMA/g FW) in the fruit using the Beer-Lambert-Bouguer law according to Eq. (1).

$$TMA\left(\frac{mg}{g\ FW}\right) = \frac{[(A_{510\ nm}-A_{700\ nm})_{pH1.0}-(A_{510\ nm}-A_{700\ nm})_{pH4.5}]\times 449.2\frac{g}{mol}\times DF\times 1000\frac{mg}{g}}{26900\frac{L}{cm\cdot mol}\times 1\ cm}\times\frac{1L}{100\ g\ FW} \quad (1)$$

where DF was dilution factor. Each extract was assayed three times.

Oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) was measured using the fluorescent method described by Cao et al. (1993). The method was adapted for use in a 96-well microplate fluorometer (SpectraMax Gemini XS, Molecular Devices, Foster City, CA). Briefly, three 30 μL aliquots of each extract (diluted as necessary) were dispensed into the wells of a pre-warmed microtiter plate along with 200 μL of a pre-warmed β-phycoerythrin solution. Microtiter plates were incubated at 37 °C for 1 h, after which 70 mL of 2,2‘-Azobis(2-amidinopropane) dihydrochloride (AAPH) was added to initiate the reaction. Fluorescence of β-phycoerythrin was induced by excitation at 485 nm and was measured at 585 nm every 2 min for 2 h. Proprietary software (SoftMax Pro 5.4.5, Molecular Devices, LLC, USA) was used to calculate the antioxidant capacity based upon positive changes to the area under the curve as compared to curves generated using a series of standardized Trolox solutions (0, 10, 20, or 40 μmol/L). Results were expressed as μmol Trolox equivalent (TE)/g FW.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was assayed using the automated colorimetric method developed by Benzie & Strain (1996). Duplicate aliquots of 40 μL were taken from each extract and dispensed into the wells of a pre-warmed microtiter plate along with 300 μL of pre-warmed FRAP reagent (a mixture of 83% 300 mmol/L acetate buffer, 3.5% 10 mmol/L tri(2-pyridil)-s-triazine, and 3.5% 20 mmol/L Iron (III) chloride). Plates were incubated at 37°C for 15 min and then

measured for absorbance at 550 nm using a microplate absorbance reader (SpectraMax 190, Molecular Devices, Foster City, CA). Proprietary software (SoftMax Pro 5.4.5, Molecular Devices, LLC, USA) was used to calculate antioxidant power from the measured absorbance values based upon a standard curve generated from a series of standardized Trolox solutions (0, 62.5, 125, 250, or 500 mmol/L Trolox). Values were reported as μmol Trolox equivalent (TE)/g FW.

Sugar profile

A high-pressure liquid chromatography (HPLC) system, consisting of a quaternary pump, solvent degasser, auto-sampler, column heater, and refractive index detector (Series 1200, Agilent Technologies, Santa Clara, CA) fitted with a 300 mm long ligand exchange column and an appropriate guard column (Hi-PLex pB, Varian, Inc., Palo Alto, CA) was used to determine the sugar profile of the fruit. Aliquots of 2 mL were taken from each of the juice extracts prepared above, passed through a 0.47 μm syringe filter, and placed into a standard 2 mL screw cap auto-sampler vial. Vials were loaded into the HPLC system, and separated using the following parameters: ultra-pure water as mobile phase, flow rate of 0.7 mL/min, column temperature of 70 °C, injection volume of 15 μL, detector temperature of 35 °C, and total run time of 45 min. All samples were assayed in triplicate, and the concentrations of the three major sugars (fructose, sucrose, and glucose) were calculated based upon standard curves constructed using a series of pure sugar solutions (0.9375, 1.875, 3.75 and 7.5 g/100 mL of each).

Experimental design and statistical analysis

A completely randomized design was employed in this study with the principle effects being fertilizer treatment and harvest date. Data were analyzed for statistical significance via multi-way analysis of variance (ANOVA) with least significant difference (LSD) *post hoc* testing as appropriate, using statistical software (SAS v9.2, The SAS Institute, Cary, NC). Results were considered to be different if $\alpha<0.05$.

Results

Weight loss during storage

Table 3.1 presents the weight loss of fruits for the 2013 harvest (Data for the 2012 harvest were not presented due to equipment faults encountered that year). While the overall trend for increased weight loss along with increased storage time was expected, examining the data revealed an interesting difference between the two cultivars. Specifically, weight loss of 'Obsidian' was not affected by harvest date, but affected by the fertilizer source, while 'Triple Crown' showed the opposite response. In 'Obsidian', PPL resulted in the modest reduction in weight loss compared with the other two fertilizers (0.59-2.4% vs. 0.70-2.66% loss over 10 d of storage), while in 'Triple Crown' the late harvest showed an increase in weight loss compared with the earlier harvests (1.46-3.78% vs. 1.01-2.93% loss over 12 d of storage).

Fruit decay and leakage

Fruit decay and leakage were unaffected by fertilizer source but were significantly influenced by harvest date. Fig. 3.1 and Fig. 3.2 presented the mean measures across all three fertilizer treatments for the 2012 and 2013 harvest, respectively. Decay rates for the two cultivars ranged from 5.56-54.86% in 2012, and 0.74-46.89% in 2013, with 'Triple Crown' having higher decay rate in 2012 and 'Obsidian' having higher decay rate in 2013 (Figs. 3.1 and 3.2). In the former case, it should be noted that 'Triple Crown' was stored twice as long as 'Obsidian' fruit. In general, decay rates increased with prolonged storage. 'Triple Crown' also tended to have a slightly higher leakage rates in both years than 'Obsidian'.

Fruit moisture content

Fruit moisture content (MC) was similar between the cultivars (Table 3.2). Early harvest 'Obsidian' fruit decreased from 72% to 69% after 6 d of storage in 2012, while late harvest 'Obsidian' fruit decreased from 70% to 63% after 5 d of storage in 2013 and then increased to 67% after 10 d.

Fruit pH and titratable acidity

The effects of harvest date and storage time on pH and TA for the two harvest years are shown in Fig. 3.3 and Fig. 3.4. In 2012, juice pH was similar between cultivars and among storage times, while TA was higher in 'Obsidian' than in 'Triple Crown' and decreased during storage in early and late harvest fruit from both cultivars

(Fig. 3.3). For ‘Obsidian’, harvest date affected both TA and pH in 2012, with Juice pH was also lower in early harvest ‘Obsidian’ fruit than in middle harvest fruit, while TA was higher. ‘Triple Crown’ had a higher initial pH in the late harvest fruits compared with the early harvest, but these differences disappeared during storage, while the pH of early and middle harvest fruit increased by 5.7% and 6.8%, respectively, after 12 d of storage. Similar TA trends were observd in ‘Triple Crown’.

In 2013, ‘Obsidian’ had a higher initial (day 0) pH and TA than ‘Triple Crown’, and after 10 d of storage, TA of early harvest ‘Obsidian’ fruit was slightly higher than TA of late harvest fruit (Fig. 3.4). Juice pH of early harvest fruit decreased and TA increased after 10 d of storage, and the late harvest fruit underwent a slight (~2.97%) increase in TA during the first 5 d of storage, but not thereafter. TA of middle harvest fruit also decreased significantly at 10 d of storage, going from 0.96% to 0.67%. For ‘Triple Crown’ fruit, the initial pH of the middle harvest was significantly higher than that of the early and late harvest, but late harvest fruit had a significantly higher TA initially than fruit from the other two harvest dates. Middle and late harvest fruit also underwent significant increases in pH (16% and 17.1%, respectively) over the 12 d of storage, which was also accompanied by a profound decrease in TA (29.4% and 23.0%, respectively).

Fruit firmness

No significant differences in firmness were seen between berries grown with different fertilizer types, but differences were seen among the different harvest date in

both years. Fig. 3.5 presents the mean values across all three fertilizer types. In general, ‘Obsidian’ had higher firmness than ‘Triple crown’ for both harvest years, with initial values of the former ranging from 1.28-2.23 N in 2012 and 0.84-0.99 N in 2013, while the initial values of the latter ranged from 1.06-1.90 N and 0.65-0.87 N in the same years. In both cultivars, firmness values tended to be higher in 2012 harvest than in 2013, and the effect of storage showed variation among the two cultivars and harvest years, with most experiencing the expected stability or decline during storage, but some, specifically early and middle harvest ‘Obsidian’, seeing increases in firmness by 4 d of storage. While this effect was present in both harvest years, it was more pronounced (but also had greater variation) in 2012.

Sugar profiles

Figs. 3.6 and 3.7 illustrate the sugar profiles of fruit from 2012 harvest during refrigerated storage for ‘Obsidian’ and ‘Triple Crown’, respectively.

For ‘Obsidian’, initial total sugar concentration ranged from 14.7-18.7 g/100 g FW, with berries from the SM fertilizer treatment having the highest values in the early and middle harvests, and berries from the PPL treatments having the highest values in the late harvest (Fig. 3.6). ‘Triple Crown’ fruit had a higher initial total sugar concentration than that of ‘Obsidian’, ranging from 22.14 to 28.41 g/100 g FW, with berries from the SM treatment again having the highest values in the middle harvest, and berries from the PPL and FE treatments having the highest values in early and late harvest, respectively (Fig. 3.7). Shortly after storage, sugar content of both cultivars

tended to increase slightly, which was then followed by either a leveling off or a slight decline.

Sucrose comprised a minor fraction (2-3% for ‘Obsidian’ and 0-7.4% for ‘Triple Crown’) of the total sugars on all harvest dates, while fructose represented the major fraction at each harvest in ‘Triple Crown’ and early harvest in ‘Obsidian’. However, glucose dominated the sugar fraction during the middle and late harvestsin ‘Obsidian’. During storage, the ratio of sucrose to fructose remained fairly consistent across all treatments and harvests in both of the cultivars, while the amount of sucrose was cultivar specific.

Phenolic content and antioxidant capacity

Antioxidant analysis was only performed on fruits from 2012 harvest. Table 3.3 presents the initial (day of harvest) TPC and TMA values for both cultivars, while the three measures of antioxidant capacity (DPPH, ORAC, and FRAP) are presented in Table 3.4. In general, ‘Obsidian’ fruits had higher antioxidant content, with values ranging from 3.31-4.85 mg GAE/g for TPC and 2.28-3.51 mg/g for TMA, than ‘Triple Crown’ fruits, which ranged from 2.71-4.39 mg GAE/g and 1.89-2.65 mg/g, and a similar trend was seen in the measures of antioxidant capacity, with ‘Obsidian’ having higher values than ‘Triple Crown’ in DPPH (8.44-10.84 mg AAE/g vs. 6.61-7.89 mg AAE/g), ORAC (289.52-763.73 μMol TE/g vs. 253.09-467.42 μMol TE/g) and FRAP (596.05-791.71 μMol Fe2+/g vs. 489.67-646.68 μMol Fe2+ /g)).

Examining the measures of a given harvest date and fertilizer combination found that ‘Triple Crown’ fruit had lower measures of both content and capacity than the corresponding combination for ‘Obsidian’, with the sole exception being the ORAC values for the early harvest which were 4.4-61.4% higher in ‘Triple Crown’, depending on fertilizer source.

The effect of refrigerated storage was quite erratic, with some samples showing increases in both amount of antioxidant contents (TPC, TMA) as well as antioxidant capacity (DPPH, ORAC, FRAP), while others showed marked decreases in the same measurements. Fig. 3.8 presents the relative changes in TPC and TMA and Fig. 3.9 reports the relative change in DPPH, ORAC and FRAP. In all cases, the relative change was calculated using Eq. (2).

$$Relative\ value_{day\ X}\ (\%) = \frac{Mean\ Measurment\ at\ Day\ X}{Initial\ Mean\ measurment} \quad (2)$$

Discussion

Fruit weight loss during storage

Fruit weight loss during refrigerated storage was similar between ‘Obsidian’ and ‘Triple Crown’ and comparable to weight loss in ‘Navaho’, ‘Cheyenne’, and ‘Shawnee’ blackberry stored under the same conditions (Perkins-Veazie et al. 1996). However, weight loss in the present study was affected by fertilizer source in ‘Obsidian’ and by harvest date in ‘Triple Crown’. The effect of harvest date on weight loss was likely due primarily to differences in berry maturity, whereas the effect of fertilizer source on weight loss is more complex. (Gutser et al. 2005) suggested that

different fertilizers cause different effects on the rate of nutrient release and found that animal manure has the most immediate effect on nutrient availability, resulting in a sharp "spike" in release of nitrogen. In the present study, PPL fertilizer was applied only one time while FE fertilizer was applied four times during plant development, with the last application occurring during flowering that perhaps provided the plants with additional nutrients during berry formation and growth which later allowed the fruits to maintain higher metabolic rates during storage and lead to higher weight loss.

Storage conditions also directly impact fruit weight loss. Temperature and relative humidity play an important role, with lower values of the former generally reducing fruit respiration and transpiration and lower values of the latter increasing the drying rate. (Ben-Yehoshua 1987) reported more weight loss than observed in the present study (as high as 6%) when blackberries were stored for 5 d at room temperature. Higher losses (3.3-13.8%) were also seen by (Basiouny 1995), but those berries were kept in open bags in the refrigerator.

Decay and leakage

Decay of blackberries is usually caused by fungi. Kidd et al. (2003) reported that *Botrytis cinerea* is the primary fungal disease of ripe blackberry fruit, and the fungi can grow continuously at low temperatures (Joo et al. 2011). Based on the USDA standards of grades for blackberries (United States Department of Agriculture 1928), 'Obsidian' berries were marketable for only 2 d, while 'Triple Crown' berries were marketable for about 4 d. (Perkins-Veazie et al. 1996) similarly reported that

blackberry decay rates exceeded 35% in ‘Cheyenne’ and ‘Shawnee’ after 7 d of refrigerated storage. However, later work by the same group found less than 12% decay after 7 d of refrigerated storage for ‘Shawnee’ and ‘Navaho’ (Perkins-Veazie et al. 1999a), which was lower than the results in this study. ‘Obsidian’ and ‘Triple Crown’ have about a 1-month harvest period in June-July and August-September, respectively (Finn et al. 2005). Weather conditions at the time of fruit harvest directly impact fruit ripeness, mold growth, and some other physicochemical properties. Rain at harvest can especially cause significant fruit decay, while high temperature and high UV index may result in more fruit leakage and a loss of firmness. To maintain the uniform fruit quality for the fresh market, growers usually picked the fruit at 2-3 different times during the harvest season (Fisk et al. 2008).

Moisture content, pH and titratable acidity of fruit

Fruit MC is an important physicochemical parameter used for assessing the quality of fresh fruit (Yan et al. 2008). The decrease in MC seen in 2012 in ‘Obsidian’ berries was probably due to the high metabolism activity for early harvest fruit due to a larger fraction of under-ripe fruit. A similar reason might also explain the decline in MC seen during storage of ‘Obsidian’ fruit in 2013 harvest, as all ripe and nearly ripe fruit were removed from the plants after the middle harvest by the farm owner. This could result in the berries picked the subsequent week being slightly under ripe or having only been ripe for a short period of time. The slight increase in MC observed during the latter part of storage for late harvest ‘Obsidian’ fruit in 2013 was likely due to

slight changes in relative humidity in the storage environment due to either the other products stored in the cooler or higher levels of relative humidity in the outside air. Either could result in a relative humidity higher than the water activity of fruit, causing the fruit to absorb moisture from the environment. A similar phenomenon was reported by Barth et al. (1995) during storage of 'Chester' blackberries. For 'Triple Crown', the observed differences between the early and middle harvest and the late harvest in 2012, which only appeared after 4 d of storage might be because the elevated UV index and high temperatures at early and middle harvests (28-34 °C with a UV index of 8) resulted in damage to the fruit, which would then allow more water loss during storage.

During postharvest storage, fruit continue to metabolize, which consumes starch and acid and causes an increase in pH and a decrease in TA (Duan et al. 2011; Ali et al. 2012). TA decreased during storage in both 'Obsidian' and 'Triple Crown', which was also reported in conventionally-grown blackberries (Perkins-Veazie et al. 1999; Joo et al. 2011). Perkins-Veazie et al. (1996) reported that TA decreases by 60% between the mottled and the shiny black stage and by 40% between the shiny and dull black stage. High TA values in our study for early harvest fruit might be due to under-ripe fruit. The pH values of the two blackberry cultivars in this study were within the range of eleven conventionally-grown blackberry cultivars (Reyes - Carmona et al. 2005). Woods et al. (2006) reported that pH increased during storage in conventionally-grown 'Triple Crown' blackberry, as well as other cultivars, probably

owning to the binding of pectin from fruit cell wall to polyphenols (Ozawa et al. 1987).

Firmness

Fruit softening involves a series of chemical reactions including physiological and biochemical changes caused by cell wall hydrolysis and pectin degradation (Tucker 1993; Duan et al. 2011; Mworia et al. 2012). At the same time, enzymatic transformations of cell wall pectin and the bonding of calcium to pectic acid polymers could potentially lead to fruit hardening during storage (Eaves et al. 1972). In the present study, both increases in firmness shortly after storage and decreases in overall firmness during storage were observed at levels consistent with previous studies (Eaves et al. 1972; Perkins-Veazie et al. 2000; Joo et al. 2011).

The firmness of fruit harvested in 2012 was significantly greater than 2013 for both cultivars and ‘Obsidian’ had significantly higher firmness than ‘Triple Crown’ during both years. ‘Obsidian is a trailing cultivar, and those typically produce firmer fruit than semi-erect cultivars like ‘Triple Crown’ (Finn et al. 2005). The firmness of ‘Obsidian’ fruit harvested in 2012 showed great variation in comparison with that of 2013 fruit, with the 2012 middle harvest ‘Obsidian’ fruit showing significantly higher firmness than early and late harvest fruit after 2-4 d of storage and reached the highest firmness at 4 d. The variation in firmness was likely due to the variance of ripeness between individual berries, as less ripe fruit is typically firmer than that of ripe or over-ripe fruit (Perkins-Veazie et al. 1996). The firmness of early harvest ‘Obsidian’

fruit also appeared to increase slightly between 2-4 d of storage, while late harvest fruit showed decreases in firmness with storage. These trends were somewhat surprising, as berries would normally be expected to further ripen during storage, resulting in decreased firmness, as was seen in the 2012 late harvest of 'Obsidian'. One possible explanation is that berries become more fragile as they ripen, increasing their risk of microbial spoilage. Thus the berries which were the softest at harvest may have become decayed by day 2 or day 4, excluding them from firmness testing.

The firmness of 'Triple Crown' in 2012 showed a slightly different trend. While the late harvest fruit also significantly decreased during storage, no significant change in firmness was seen in the middle harvest fruit. Late harvest fruit showed significantly higher initial firmness than the early and middle harvest fruit, possibly due to damage from the high temperature and UV index for those harvests. Early harvest 'Triple Crown' fruit firmness increased at 4 d of storage, but then showed decreasing trend during the rest of storage, which could be explained as the defense reaction to environment change protected the fruit initially, but the high decay and leakage during prolonged storage caused severe damage to the fruit tissues, thus softening fruit. Firmness of late harvest fruit decreased significantly at 4 d of storage (~40% reduction), but no further decrease in the rest of storage ($P<0.05$). Similarly, the increased fruit decay and leakage caused cell damage of fruit, and the metabolism process during storage made the fruit physical compressive property weak.

In 2013, 'Obsidian', late harvest fruit showed significantly lower firmness compared with early and middle harvest fruit, potentially due to the high temperature

causing fruit softening or the inclusion of some overripe fruit in the samples, but no significant variation was seen during storage for individual harvests. However, examining the mean values across all three 2013 ‘Obsidian’ harvests showed a significant decrease (from 0.93 N to 0.81 N) by 8 d of storage. Unlike ‘Obsidian’ cultivar, early harvest ‘Triple Crown’ fruit in 2013 harvest showed significantly higher initial firmness than late harvest fruit, again, likely due to the degree of berry ripeness. Firmness also decreased significantly during storage, with the firmness of middle harvest fruit decreasing by 23.87% and that of the early harvest by 22.92% after 10-12 d of storage. These decreases in firmness generally agreed with the findings of Perkins-Veazie et al. (2000) that commercially grown (CG) ‘Navaho’ blackberries lost 36% firmness during refrigerated storage and Joo et al. (2011) that CG ‘Chester’ blackberries underwent a 35% decrease in firmness after 12 d of refrigerated storage.

Sugar profiles

The observed effects of fertilizer on total sugar content varied depending on cultivar, with ‘Obsidian’ fertilized with SM having the highest initial values in the early and middle harvests, and those fertilized with PPL having the highest in the late harvest. ‘Triple Crown’ SM fertilized berries also had the highest initial values in the middle harvest, but FE resulted in higher initial levels during the late harvest and PPL in the early. These fertilizer effects were hardly surprising, given that the three regimens likely have different rates of nitrogen release/absorption, and multiple studies have shown a relationship between available nitrogen and fruit sugars in such

diverse fruit as strawberries, tomatoes, chokeberries, dates, and grapes, as well as potential influence of other trace minerals (Wang and Lin 2002; Beckles 2012; Skupien and Oszmianski 2007; Christensen et al. 1994; Al-Kharusi et al. 2009).

Examining individual sugars, the initial relative values agreed with previously published data by Fan-Chiang (1999) and Kafkas et al. (2006) for other CG blackberry varieties. The observed shift in predominant sugar during the 'Obsidian' harvest might be explained as fruit harvested early in the season would be more likely to be under-ripe, thus less sweet. However, not only did the early harvest 'Obsidian' fruit generally contained greater overall amount of sugar, but they also showed higher proportions of fructose, which strongly implied that they would be perceived as sweeter, given that fructose is roughly twice as sweet as glucose, and 1.7 times as sweet as sucrose (Shallenberger 1963; Fontvieille et al. 1989). In contrast, the 'Triple Crown' fruit followed a more predictable pattern, with the middle and late harvests having higher initial values of both total sugars and fructose.

The effect of storage on the sugar profile tended to follow a trend of increasing slightly shortly after storage and then either leveling off, or declining slightly. Of the five harvest/treatment combinations that did not follow this trend, four were fertilized with PPL (early and late harvest 'Obsidian' and middle and late harvest 'Triple Crown') and one was fertilized with FE (late harvest 'Triple Crown'). These harvest/treatment combinations showed marked (as much as 30%) increases in total sugars, which could be explained by a combination of postharvest ripening and the degradation of anthocyanins during storage, the latter of which released previously

bound sugars (Giusti and Wrolstad 2001), while the differences in fertilizer effect were likely related to the effects of different rates of nitrogen release on the average maturity of the harvested berries which would in turn show different trends in post-harvest ripening.

Looking at individual sugars, the cultivar specific response to storage is quite noticeable. Except in the early harvest, 'Obsidian' berries had decreased sucrose levels as storage progressed, most likely due to sucrose hydrolysis due to metabolic processes in the fruit. 'Triple Crown' berries, on the other hand showed the opposite relationship, except for the SM fertilized middle harvest berries, with the early harvest showing undetectable levels of sucrose across all storage period, while the middle and late harvests actually showed significant increases in sucrose levels. While few studies have examined the effects of cold storage on the sugar profile of blackberries, other CG fruit were shown to undergo reductions in relative sucrose levels, including pears (Akhavan and Wrolstad 1980), strawberries (Cordenunsi et al. 2005), and peaches (Robertson et al. 1992), and the downward trend observed in 'Obsidian' was similar to the findings for raspberries and blackberries from Ali et al. (2011). Explaining the increases in sucrose seen in the early harvest 'Obsidian' and middle/late harvest 'Triple Crown' fruit was more difficult, particularly in the latter as it was so pronounced. One possibility was that these fruits were slightly under mature when picked, and underwent ripening during storage, as was explained for the changes in antioxidant measures. This could have resulted in an increase in sucrose metabolism, as was observed in strawberries (Cordenunsi et al. 2005) and peaches (Robertson et al.

1992), with the latter showing differences in behavior based upon degree of fruit ripeness.

Initial measures of antioxidants

The values generally fall within the ranges reported for CG berries from the same two cultivars by Siriwoharn et al. (2004) and Moyer et al. (2001). While the purpose of the current work was not to compare the two cultivars directly, it is important to note that for all TPC, TMA and DPPH measurements, 'Triple Crown' fruit showed lower values overall compared with 'Obsidian' berries. Further, with the exception of the early harvest ORAC values (Table 3.4), the measures of a given harvest date and fertilizer combination for 'Triple Crown' berries were also lower than the corresponding combination for 'Obsidian' except ORAC value in early harvest 'Triple Crown'. The overall trend agreed with the previous findings from Moyer et al. (2001), which showed similar results for CG berries of the two cultivars. Regarding the different behaviors seen in the early harvest ORAC, the explanation could be the differences in berry maturity between the samples taken during the two early harvests, as previous studies on CG berries have shown that ORAC values increase over 40% during the transition of fruit from under-ripe to overripe (Siriwoharn et al. 2004).

The data also revealed a complex interplay between fertilizer type and harvest date. As it was expected, harvest date had a significant effect on the antioxidant properties of the fruit, and the effect varied depending on the fertilizer applied. In general, middle harvest fruit showed the highest TPC, TMA and DHHP values in both

cultivars, excepting the FE samples and the TMA of ‘Triple Crown’ which both showed increased values during the late harvest date. These results were hardly a surprising, as harvests from later in the season were more likely to include ripe or overripe fruit than earlier harvest, and an increase in anthocyanins, the primary red/blue/purple pigments in fruit (Kähkönen et al. 2003), have been observed in a variety of CG berry and non-berry fruit as they become more mature (Ingalsbe et al. 1965; Sellappan et al. 2002; Siriwoharn et al. 2004). Furthermore, the observed differences in FE fertilized samples were likely due to the differences in application regimen mentioned above. In contrast, the effect of harvest date on FRAP and ORAC values varied depending on the cultivar (Table 3.4), with late harvest fruit showing lower values compared to the early harvest among the ‘Triple Crown’ berries (except for the FRAP of SM samples which showed the highest value in the late harvest), while the ‘Obsidian’ berries showed the lowest ORAC values, but the highest FRAP values in the early harvest. This could be caused by a variety of factors, including the above mentioned nutrient stress, as well as climate/weather conditions, as the middle/late harvest of ‘Obsidian’ and the early/middle harvest of ‘Triple Crown’ both experienced higher temperatures and greater sunlight. This increase in sunlight exposure was important, as one of the principle reasons hypothesized for the existence of plant phenolics is protection from photo-oxidation, i.e., the plant produces antioxidant phenolic compounds in order to quench radicals generated by exposure to UV (Close and McArthur 2002). Hence, fruit exposed to higher levels of UV would register lower overall values in assays that rely upon the quenching of radicals, as the

activity of the compounds would already have been depleted quenching the ROS from UV exposure.

The trends observed among the fertilizer treatments were likely best explained by two factors: differences in application methods and differences in soil/plant responses to the fertilizers. Regarding application method, while all fertilizers were applied at the same rate, there were two different application schedules - SM and PPL, being pelletized products, were applied a single time, while FE was applied in four intervals, the final of which occurred shortly prior to the beginning of the harvest season, after the plants had bloomed (Fernandez-Salvador et al. 2013). This application schedule likely provided the plants receiving FE fertilizer a more uniform amount of nutrients, allowing for greater reserves during fruit development and maturation. In addition to the application schedules, the properties of the individual fertilizer types might also play a role, as it has been shown that fertilizer source and form can have a profound effect on short-term availability of nutrients in the soil, with composted fertilizers having the smallest immediate effect, animal slurries having some of the highest, and legume meals falling somewhere in between (Gutser et al. 2005). Since it is well-known that plants respond to various stresses in complex ways, typically involving the use of reactive oxygen species (ROS) as mechanisms for signaling various types of stress, from drought to pollutants to excess UV to diseases (Reddy et al. 2004; Dat et al. 2000), it is possible that if the nitrogen release rates of the various fertilizer types result in a dearth of available nutrients, this stress would be similarly signaled, affecting the antioxidant content of the resulting fruit. Such behavior has been seen in

multiple plant species, with increased fertilization, particularly prior to flowering, reducing the levels of various antioxidants in fruit (Bryant et al. 1987; Iason et al. 1993; Close and McArthur 2002).

Effect of storage on antioxidants

Storage had a very erratic effect on both the antioxidant content and capacity, with many harvest/fertilizer treatments showing increases in either or both during storage. While these increases might be counter-intuitive, this was not the first time such trends have been noticed. Wu et al. (2010) found similar trends in the CG blackberry cultivars they examined, Kalt et al. (1999) also observed increases in various CG small fruit, including raspberries, and Ali et al. (2011) reported increases in ellagic acid in late-harvest CG blackberries and raspberries, as well as increases in anthocyanins and total phenolics in raspberries. While metabolic mechanisms were indicated in these rises, there was some debates over what initiated the process, with possibilities ranging from normal ripening of potentially under-mature fruit, to the breakdown of other fruit components (notably organic acids) creating additional carbon skeletons to feed the pathways that synthesized phenolic compounds (Kalt et al. 1999; Wu et al. 2010). In addition, it was also possible that the stimulus for the production is related to the aforementioned plant responses to stress (Dat et al. 2000), as it could be expected that refrigeration at 4 °C would create temperature stresses in the summer fruit, and such behavior was noted in CG tomatoes, watermelons, apples, strawberries, and

mangoes (Pérez-Ilzarbe et al. 1997; Rivero et al. 2001; Zhao et al. 2006; Cordenunsi et al. 2005).

Conclusion

In organically grown blackberries, the use of different fertilizers had virtually no significant effect on the physicochemical properties of 'Obsidian' and 'Triple Crown' blackberry fruit at the time of harvest and during the refrigerated storage, but did have a profound effect on the measures of antioxidant content, antioxidant capacity and the composition of sugars. Furthermore, these measures were also affected by differences in blackberry cultivar, harvest date, and storage time, with the interaction between the factors showing great complexity. Despite this, several general observations could be made, namely that with respect to both antioxidant measures and sugar profiles, fertilization with either a fish emulsion/fish hydrolysate or soy-meal based fertilizers was preferable to the use of processed poultry litter, that during storage ripening tended to increase the relative levels of fructose at the expense of decreasing sucrose content and that while the middle harvest had higher initial levels of phenolic compounds, including anthocyanins, their antioxidant capacity followed less predictable trends, particularly in the 'Triple Crown' cultivar. Further, organically grown 'Obsidian' fruit may be marketed for fresh consumption within 4 d after harvest while 'Triple Crown' fruit can be extended for 8 d due to its lower rates of decay and leakage. While further study is needed to elaborate the mechanisms involved and how well these findings can be applied over other cultivars, it is entirely likely that

fertilizer regimen might be able to be used to maximize the healthful properties of blackberries, and potentially in other fruit, while having a negligible effect on traditional physicochemical measures of quality.

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Table 3.1 Fruit weight loss (%) during refrigerated storage of 'Obsidian' and 'Triple Crown' blackberries hand-harvested in 2013†

	Storage (d)	Fish Emulsion/ Hydrolysate Blend	Soy Meal	Processed Poultry Litter
Obsidian‡	2	0.70 ± 0.16Aa	0.73 ± 0.25Aa	0.59 ±0.07Ab
	5	1.33 ± 0.16Ba	1.40 ± 0.27Ba	1.18 ± 0.10Ba
	8	2.05 ± 0.16CDa	2.18 ± 0.18Ca	1.93 ±0.10Cb
	10	2.51 ± 0.17Da	2.66 ± 0.27Da	2.40 ± 0.18Db
	Storage (d)	Early Harvest	Middle Harvest	Late Harvest
Triple Crown§	4	1.01 ± 0.13Aa	1.06 ± 0.13Aa	1.46 ± 0.21Ab
	8	1.87 ± 0.18Ba	1.96 ± 0.18Ba	2.60 ± 0.32Bb
	10	2.34 ± 0.22Ca	2.36 ± 0.21Ca	3.14 ± 0.38Cb
	12	2.75 ± 0.25Da	2.93 ± 0.22Da	3.78 ± 0.43Db

† Mean values ± SD, n=9. Within a given cultivar, values followed by the same capital letters (A-D) in the same column and those followed by the same lowercase letters (a-d) in the same row are not significantly different ($\alpha \leq 0.05$);
‡ Harvest date had no significant effect in this cultivar;
§ Fertilizer treatment had no significant effect in this cultivar.

Table 3.2 Changes in fruit moisture content (%) during refrigerated storage of 'Obsidian' and 'Triple Crown' blackberries hand-harvested in 2012 and 2013†

		Obsidian				Triple Crown		
Year	Storage (d)	Early Harvest	Middle Harvest	Late Harvest	Storage (d)	Early Harvest	Middle Harvest	Late Harvest
2012	0	71.91 ± 2.47Aa	71.75 ± 4.91Aa	73.72 ± 1.34Aa	0	70.25 ± 2.54Aa	68.09 ± 3.25Aa	69.89 ± 1.96Aa
	2	71.39 ± 2.69Aa	71.64 ± 3.24Aa	73.51 ± 2.92Aa	4	68.82 ± 3.54Aab	67.55 ± 1.58Ab	70.54 ± 2.94Aa
	4	71.44 ± 3.34Aa	71.19 ± 3.01Aa	72.59 ± 2.42Aa	10	68.17 ± 2.76Aab	66.02 ± 3.22Ab	70.78 ± 2.16Aa
	6	68.78 ± 3.59Ab	72.90 ± 3.61Aa	73.49 ± 4.99Aa	12	68.31 ± 2.12Aab	66.30 ± 1.75Ab	69.65 ± 3.07Aa
2013	0	72.34 ± 1.53Aa	69.47 ± 4.53Aa	70.19 ± 4.84ABa	0	68.96 ± 3.41Aa	68.64 ± 4.10Aa	69.15 ± 3.33Aa
	2	73.75 ± 2.26Aa	73.26 ± 3.48Aa	71.38 ± 6.17Aa	4	71.20 ± 2.73Aa	71.41 ± 3.76Aa	69.71 ± 2.66Aa
	5	72.16 ± 2.30Aa	72.98 ± 4.39Aa	63.11 ± 5.91Cb	8	72.02 ± 3.02Aa	68.87 ± 2.04Aab	68.59 ± 3.99Ab
	8	72.68 ± 2.08Aa	70.69 ± 5.50Aa	66.42 ± 4.34Bb	10	68.47 ± 3.19Aab	71.69 ± 3.52Aa	66.32 ± 3.85Ab
	10	72.59 ± 1.64Aa	70.05 ± 4.10Aab	67.34 ± 2.98ABb	12	69.41 ± 2.56Aa	69.23 ± 2.69Aa	67.44 ± 2.38Aa

†Mean ± S.D. (n=9). Within a given cultivar, values followed by the same capital letters (A-B) in the same column or the same lowercase letters (a-b) in the same row within each cultivar are not significantly different ($\alpha \leq 0.05$).

Table 3.3 Total phenolic content and monomeric anthocyaninsin fresh 'Obsidian' and 'Triple Crown'blackberries hand-harvested in 2012†

		Fish Emulsion/ Hydrolysate Blend	Soy Meal	Processed Poultry Litter
TPC (mg GAE/g)‡				
Obsidian	Early Harvest	A 3.31 ± 0.08 a	B 3.86 ± 0.10 a	B 4.05 ± 0.21 a
	Middle Harvest	A 4.21 ± 0.02 a	B 5.55 ± 0.15 b	A 4.30 ± 0.15 b
	Late Harvest	A 4.85 ± 0.23 c	B 4.59 ± 0.06 c	C 3.54 ± 0.11 c
Triple Crown	Early Harvest	A 3.03 ± 0.07 a	B 3.76 ± 0.14 a	C 3.42 ± 0.06 a
	Middle Harvest	A 2.96 ± 0.07 a	B 3.81 ± 0.09 a	C 4.39 ± 0.12 b
	Late Harvest	A 3.55 ± 0.18 b	B 2.89 ± 0.05 b	C 2.71 ± 0.05 c
TMA (mg/g) ‡				
Obsidian	Early Harvest	A 2.28 ± 0.11 a	A 2.51 ± 0.14 a	A 2.45 ± 0.11 a
	Middle Harvest	A 3.16 ± 0.20 b	B 3.51 ± 0.24 b	C 3.10 ± 0.19 b
	Late Harvest	A 3.17 ± 0.22 b	B 3.11 ± 0.21 c	B 2.56 ± 0.16 a
Triple Crown	Early Harvest	A 1.89 ± 0.00 a	A 1.93 ± 0.08 a	A 2.08 ± 0.08 a
	Middle Harvest	A 2.39 ± 0.08 b	B 2.10 ± 0.03 b	B 2.14 ± 0.04 a
	Late Harvest	A 2.65 ± 0.02 b	A 2.57 ± 0.03 c	B 2.57 ± 0.05 b

TPC: total phenolic content, TMA: total monomeric anthocyanins

† Mean values ± S.D, n=3. Values preceded with the same capital letters (A-C) within the same row of a given table are not statistically different ($\alpha \leq 0.05$).

‡ Within a given cultivar, values followed with the same lowercase letters (a-c) within the same column of a given table are not statistically different ($\alpha \leq 0.05$).

Table 3.4 Antioxidant capacity at harvest for cultivars 'Obsidian' and 'Triple Crown', 2012 harvest

		Fish Emulsion/ Hydrolysate Blend	Soy Meal	Processed Poultry Litter
DPPH (mg AAE/g) †‡				
Obsidian	Early Harvest	A 8.44 ± 0.75 a	A 8.77 ± 0.52 a	A 7.78 ± 0.21 a
	Middle Harvest	A 9.94 ± 1.04 a	A 10.84 ± 0.13 b	A 9.26 ± 0.15 b
	Late Harvest	A10.01 ± 0.31 a	A 9.20 ± 0.90 a	A 8.98 ± 0.11 c
Triple Crown	Early Harvest	A 6.96 ± 0.29 a	A 6.63 ± 1.18 a	A 6.68 ± 0.06 a
	Middle Harvest	A 6.61 ± 0.28 a	A 7.61 ± 0.58 a	A 7.98 ± 0.12 b
	Late Harvest	A 7.70 ± 0.68 a	A 6.80 ± 0.47 a	A 6.63 ± 0.05 c
ORAC (µMol TE/g) †*#				
Obsidian	Early Harvest	A 289.52 ± 22.57 a	B 427.86 ± 27.86 a	B 392.54 ± 0.11 a
	Middle Harvest	A 380.48 ± 9.79 b	B 501.69 ± 0.51 b	A 416.80 ± 0.19 b
	Late Harvest	A 763.73 ± 18.39 c	B 717.15 ± 55.05 c	C 591.50 ± 0.16 a
Triple Crown	Early Harvest	A 467.42 ± 36.00 a	A 446.88 ± 5.89 a	A 427.55 ± 0.08 a
	Middle Harvest	A 362.66 ± 12.78 b	B 403.50 ± 12.65 b	A 387.89 ± 0.04 a
	Late Harvest	A 253.09 ± 5.69 c	A 268.04 ± 24.43 c	A 253.71 ± 0.05 b
FRAP (µMol Fe2+/g)‡*#				
Obsidian	Early Harvest	A 753.51 ± 0.06 a	A 791.71 ± 0.00 a	A 767.75 ± 6.60 a
	Middle Harvest	A 596.05 ± 60.21 b	B 727.32 ± 11.85 b	A 649.29 ± 2.05 b
	Late Harvest	A 709.10 ± 6.39 a	A 680.87 ± 34.27 b	B 622.75 ± 5.66 b
Triple Crown	Early Harvest	A 521.53 ± 4.35 a	B 588.15 ± 3.04 a	A 534.38 ± 1.06 a
	Middle Harvest	A 489.67 ± 3.06 b	B 548.21 ± 13.17 b	C 600.98 ± 9.18 b
	Late Harvest	A 646.48 ± 13.73 c	B 574.66 ± 10.10 a	B 559.62 ± 5.37 c

DPPH: radical scavenging activity by the 2,2-diphenyl-1-picryhydrazyl colorimetric method; ORAC: Oxygen radical absorbance capacity; FRAP: Ferric reducing antioxidant power

† Mean values ± S.D, n=3

‡ Mean values ± S.D, n=2.

* Values preceded with the same capital letters (A-C) within the same row of a given table are not statistically different ($\alpha \leq 0.05$).

Within a given variety, values followed with the same lowercase letters (a-c) within the same column of a given table are not statistically different ($\alpha \leq 0.05$).

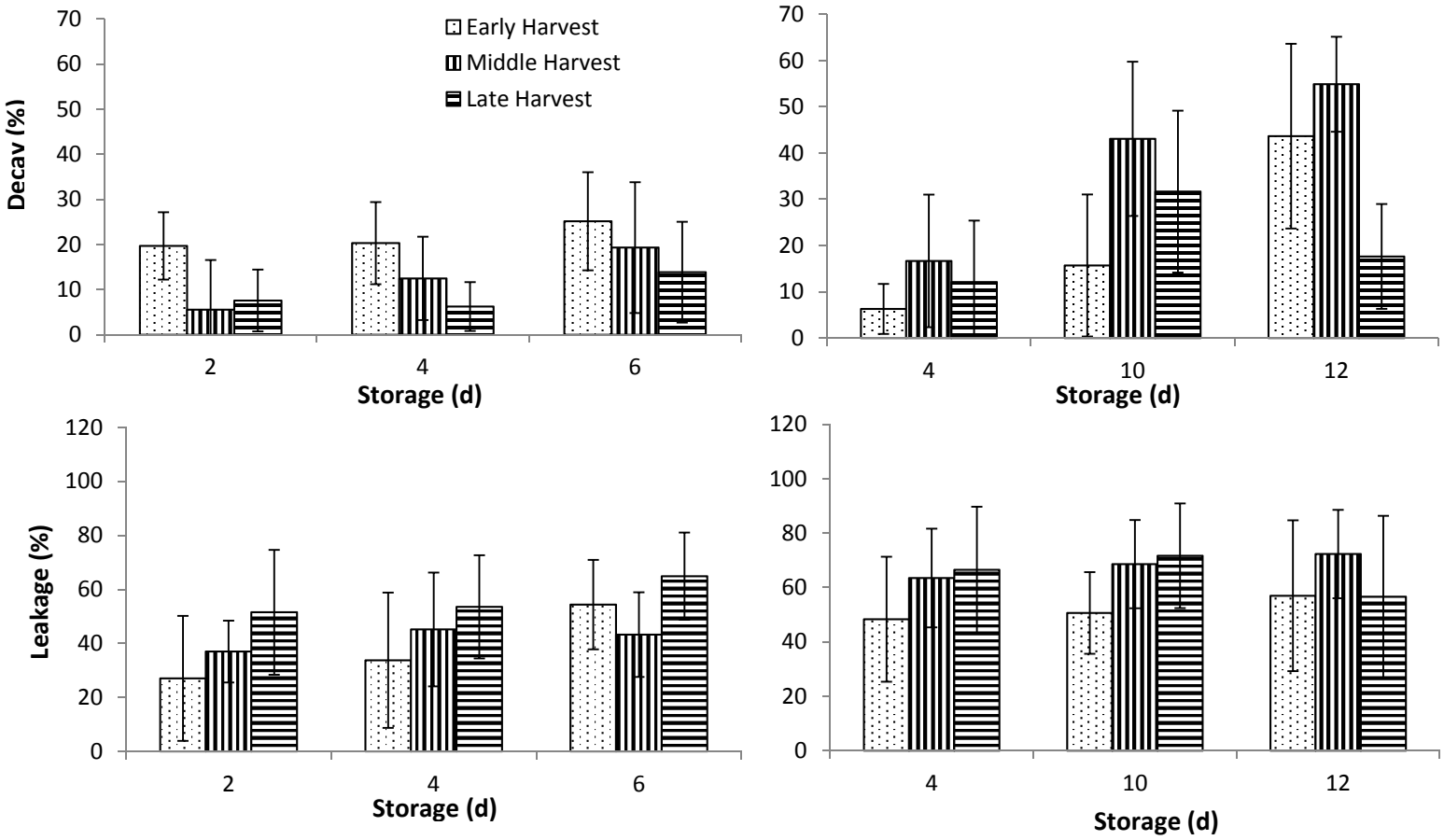

Fig. 3.1 Effects of harvest date and refrigerated storage on the decay and leakage rate of two blackberry cultivars: 'Obsidian' (left side) and 'Triple Crown' (right side), 2012 harvest.

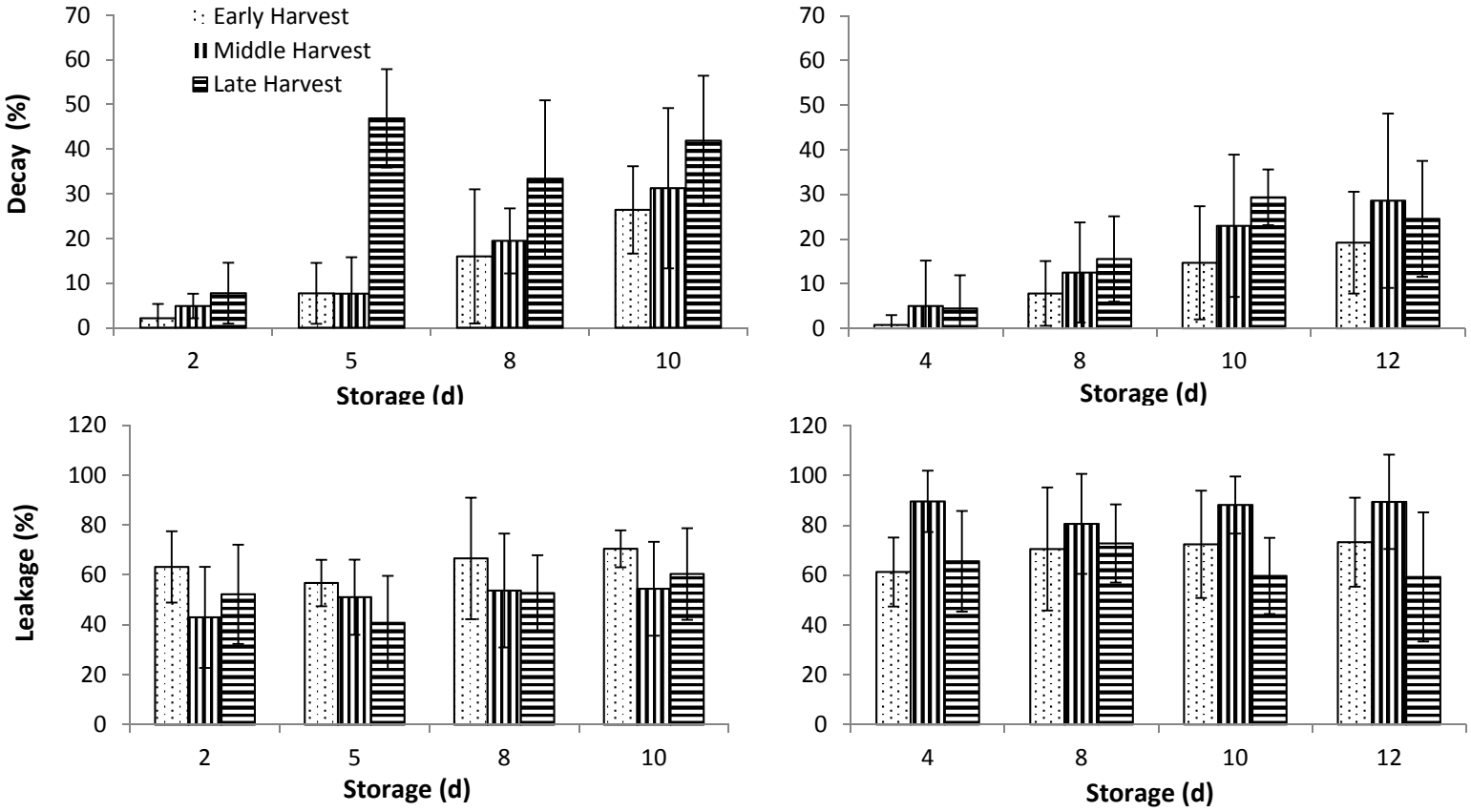

Fig. 3.2 Effects of harvest date and refrigerated storage on the decay and leakage rate of two blackberry cultivars: 'Obsidian' (left side) and 'Triple Crown' (right side), 2013 harvest.

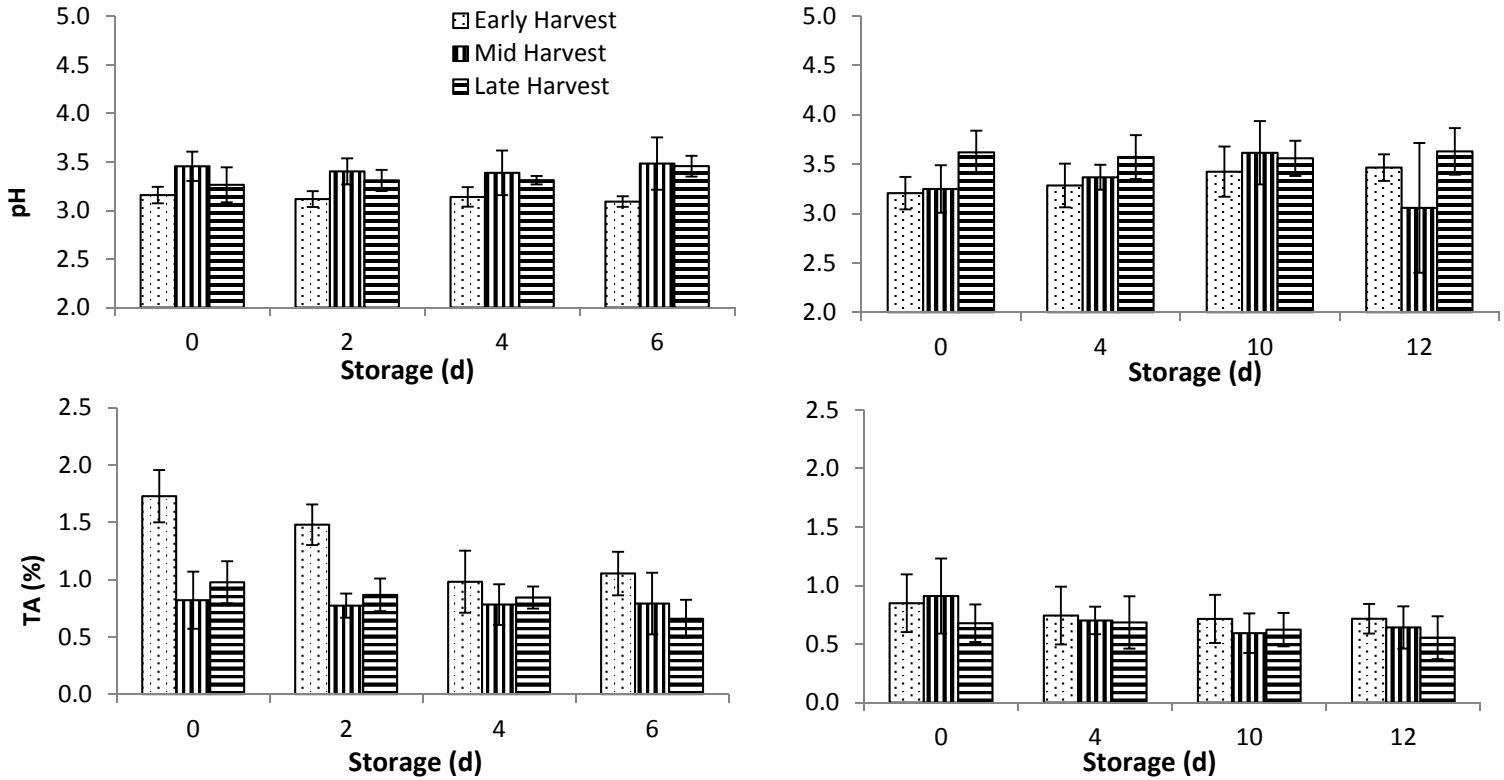

Fig. 3.3 Effects of harvest date and refrigerated storage on pH and titratable acidity of two blackberry cultivars: 'Obsidian' (left side) and 'Triple Crown' (right side), 2012 harvest.

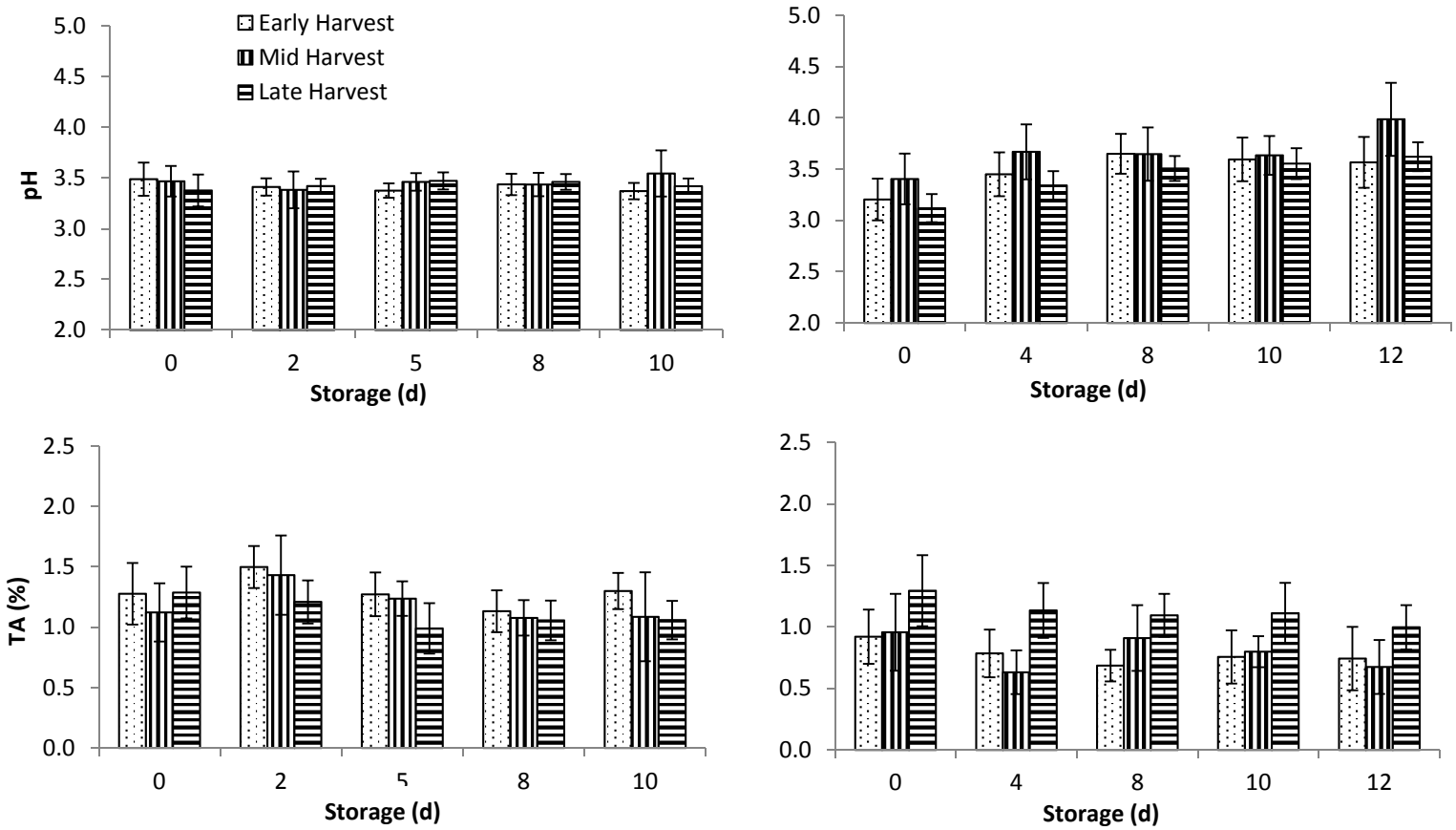

Fig. 3.4 Effects of harvest date and refrigerated storage on pH and titratable acidity of two blackberry cultivars: 'Obsidian' (left side) and 'Triple Crown' (right side), 2013 harvest.

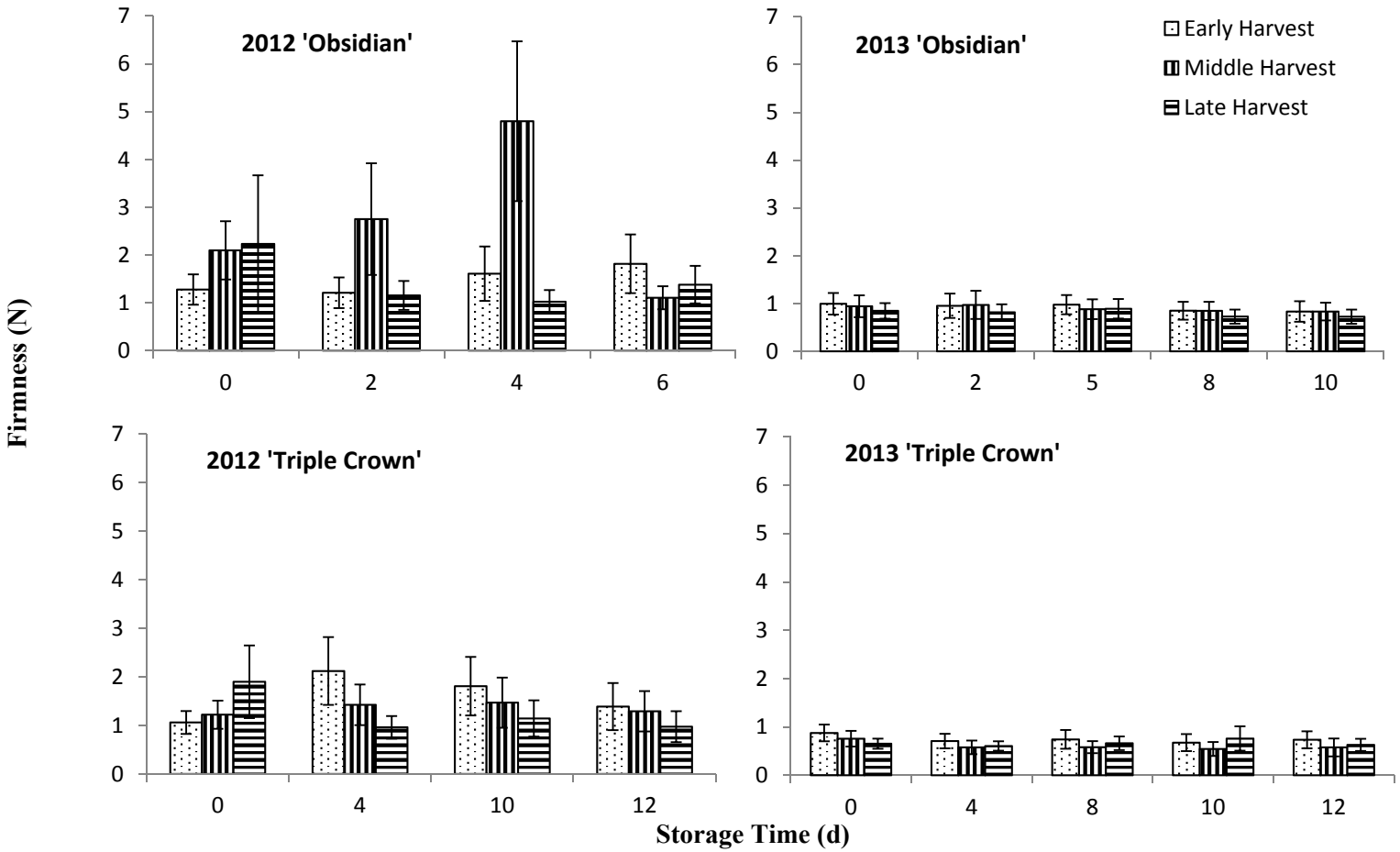

Fig. 3.5 Effect of different harvest date and refrigerated storage on the firmness of two blackberry cultivars: 'Obsidian' and 'Triple Crown' at 2012 and 2013 harvest.

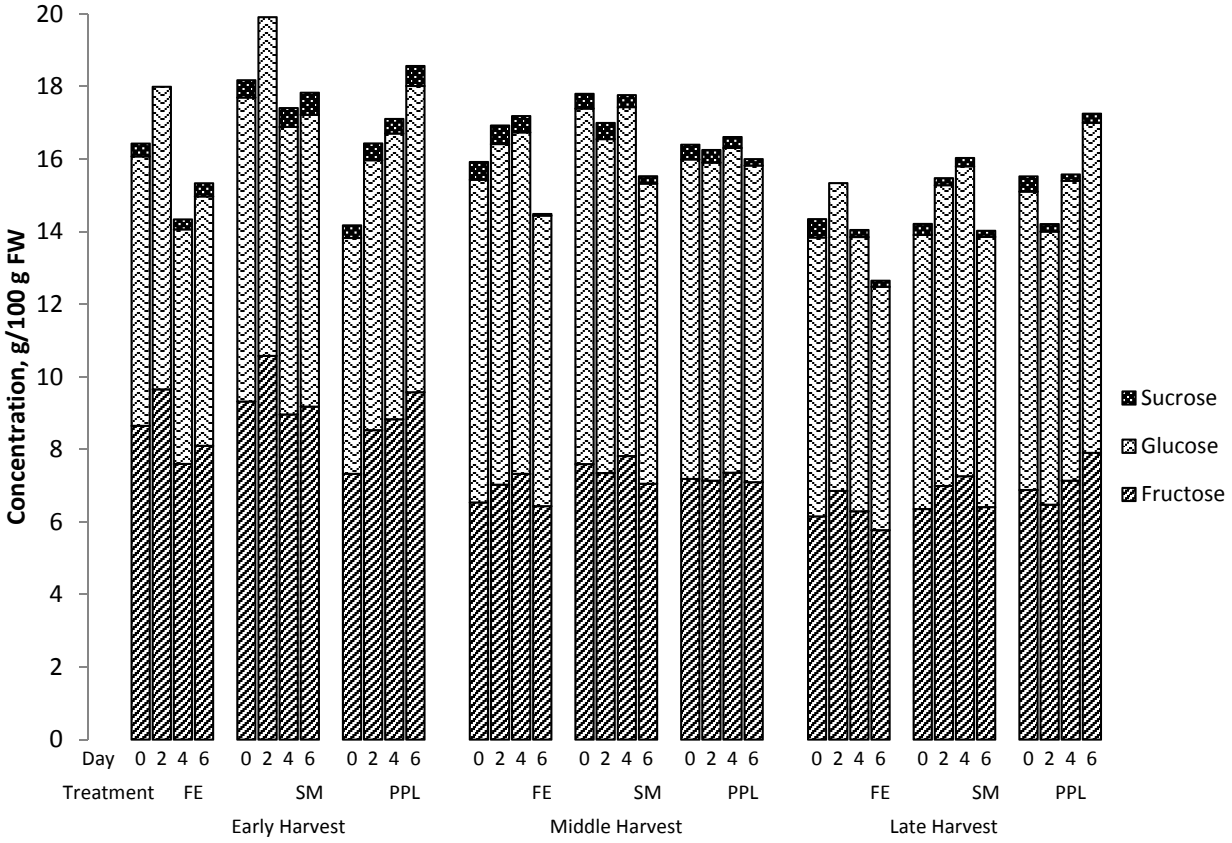

Fig. 3.6 Sugar profile of 'Obsidian', expressed in total concentration, 2012 harvest. FE: blend of fish emulsion and fish hydrolyzate, SM: soy meal; PPL: processed poultry litter.

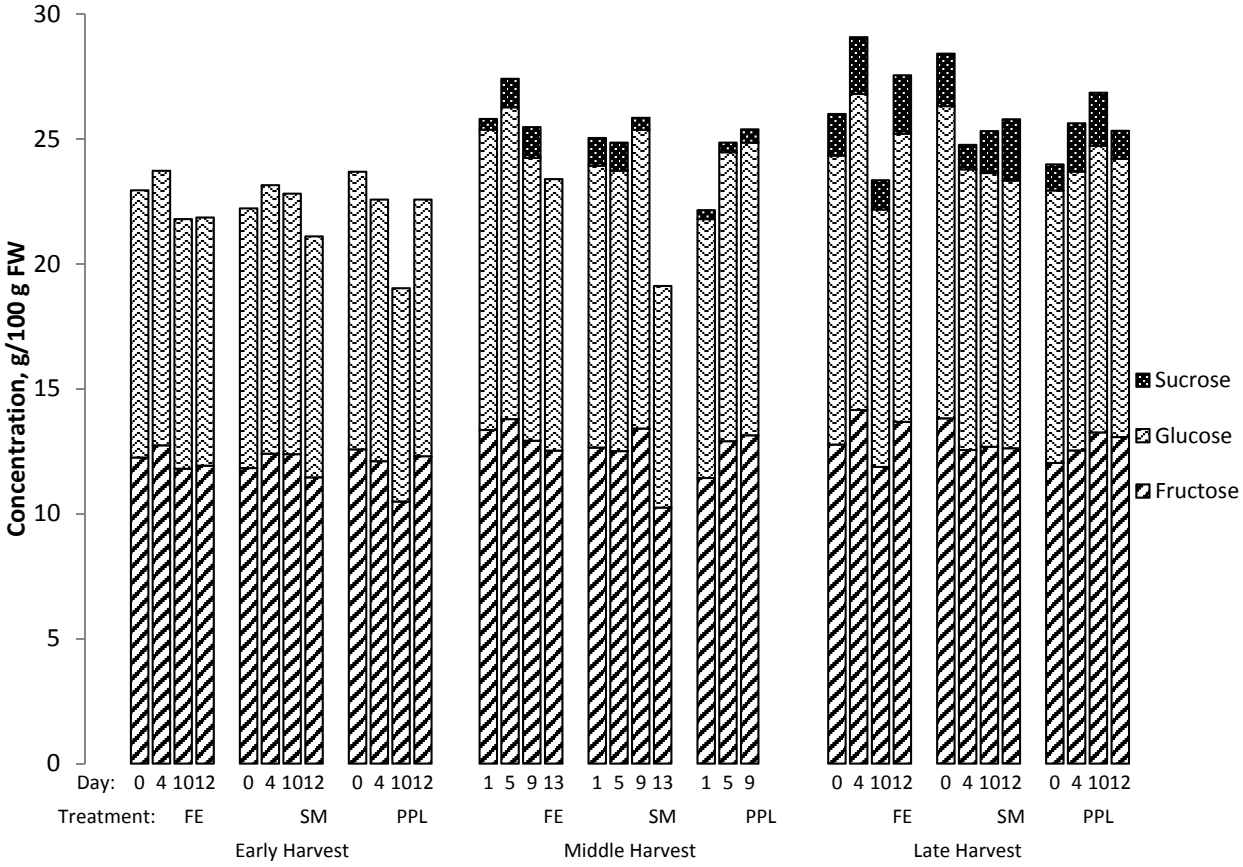

Fig. 3.7 Sugar profile of 'Triple Crown', expressed in total concentration, 2012 harvest. FE: blend of fish emulsion and fish hydrolyzate, SM: soy meal; PPL: processed poultry litter.

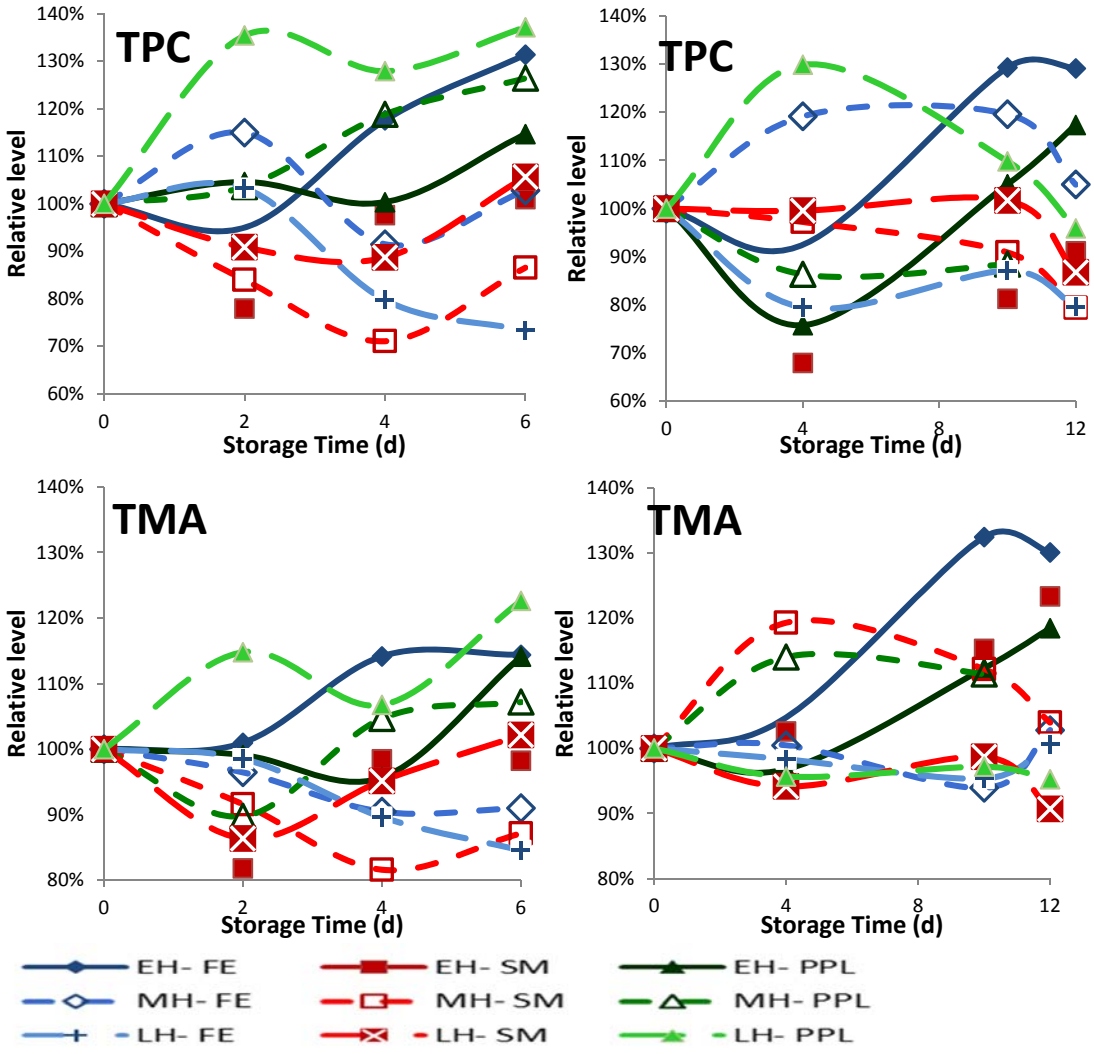

Fig. 3.8 Relative phenolic content and monomeric anthocyanins during storage of two blackberry cultivars: 'Obsidian' (left) and 'Triple Crown' (right), 2012 harvest. FE: blend of fish emulsion and fish hydrolyzate, SM: soy meal; PPL: processed poultry litter.

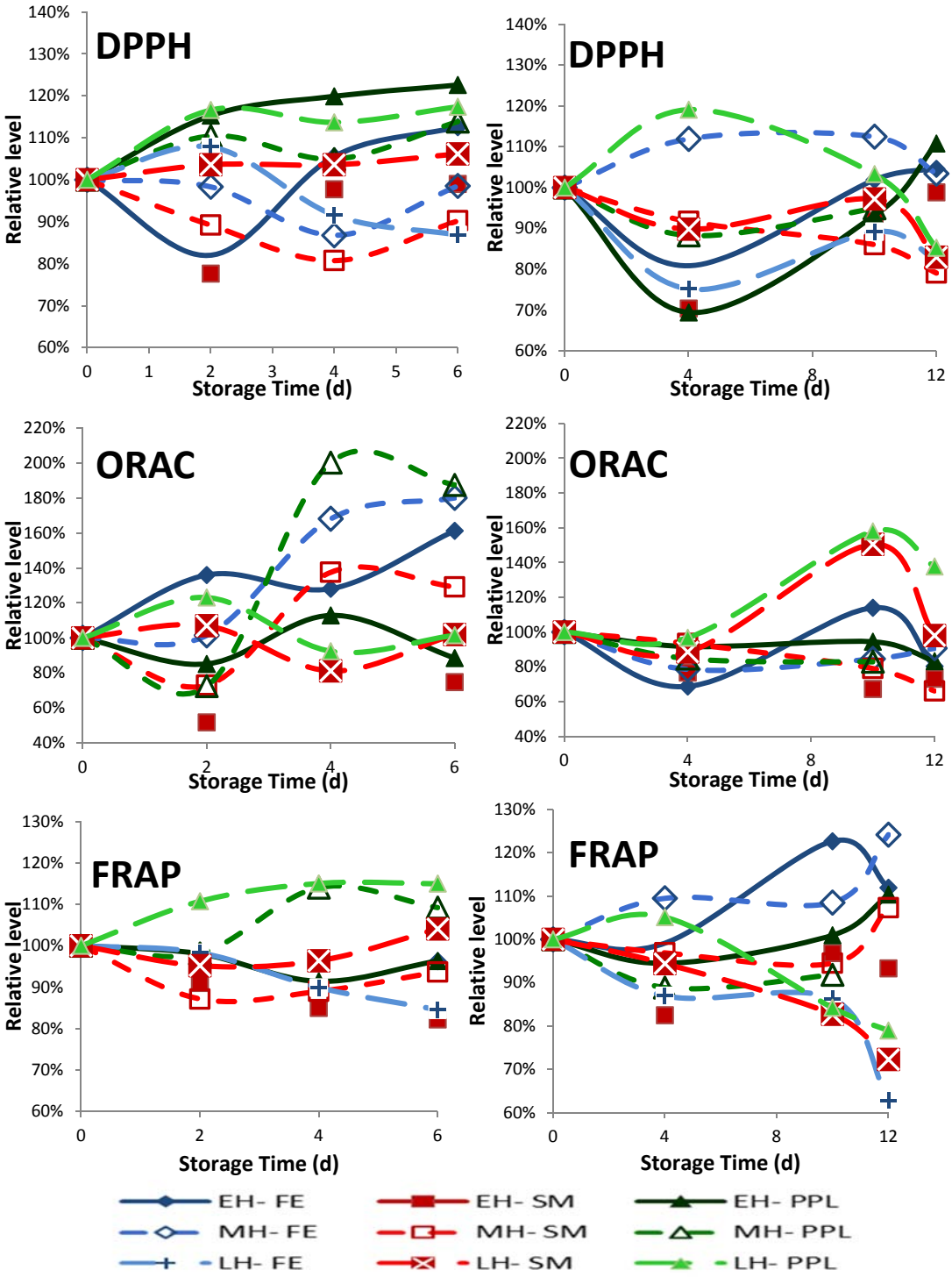

Fig. 3.9 Relative antioxidant potential by three methods (DPPH, ORAC and FRAP) during storage of two blackberry cultivars: 'Obsidian' (left) and 'Triple Crown' (right), 2012 harvest. FE: blend of fish emulsion and fish hydrolyzate, SM: soy meal; PPL: processed poultry litter.

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CHAPTER 4

Influence of Weed Management Practices, Harvest Date and Duration of Freezer Storage on the Physicochemical and Antioxidant Properties of Organic Processed Blackberries

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ABSTRACT

A study was conducted to determine the effects of different weed management strategies on fruit quality and freezer storage in certified organic blackberry. Fruit were machine-harvested in 2012 from two cultivars of trailing blackberry, 'Marion' and 'Black Diamond'. Both cultivars were grown in plots that were non-weeded, hand-weeded, or covered with landscape fabric (weed mat). The fruit was collected weekly at three times during the harvest season (early, middle, and late harvest) and stored at -20 ºC for up to 9 months in a dark freezer. Weed management had no effect on the physicochemical properties of the fruit, including pH, titratable acidity (TA), and total soluble solid content (TSS), but had various effects on the nutraceutical properties. Fruit from the hand-weeded treatment had more total phenolics and monomeric anthocyanins during the early and middle harvest in both cultivars than fruit from the hand-weeded or weed mat treatments. Fruit quality also differed between cultivars. 'Black Diamond' contained more total phenolics, on average, than 'Marion', but had 10% less total phenolics and 24% less total monomeric anthocynins than 'Marion' when plants were grown with weed mat. Fresh 'Marion' blackberries, on the other hand, had 13% greater oxygen radical absorption capacity (ORAC) and 19% greater ferric reducing ability of plasma (FRAP) than 'Black Diamond' blackberries at harvest. Non-weeded treatment 'Marion' and 'Black Diamond' fruit in early and middle harvest showed significantly increasing total phenolic content during frozen storage. This study provided valuable information about the effect of organic production system on physicochemical and nutraceutical properties of blackberries.

Keywords: Blackberry, organically grown, weed management, harvest date, frozen storage, antioxidant property

Introduction

Organic blackberry production has increased to more than 2500 ha around the world, of which 200 ha was in the United States in 2008 due to the high demanding of organic products (Strik and Finn 2011). It is predicted that the organic blackberry production will continue to increase in the next 10 years due to market demands (Strik and Finn 2011). Many studies have reported the physicochemical and antioxidant properties of conventionally grown blackberries (Lopez-Medina et al. 2000; Siriwoharn et al. 2004; Takeda et al. 2002; Fan-Chiang and Wrolstad 2005), but very little information is available on fruit quality of organic blackberries, especially machine-harvested trailing types.

Weed management is an important factor for plant and fruit production (Pritts and Kelly 2001; Barney et al. 2007). Although blackberry is fairly tolerant of weeds and can be grown commercially without weed control, weed management increases growth and production (Harkins et al. 2013). While numerous herbicides are available for conventional blackberries, hand weeding or landscape fabric, often referred to as weed mat, is typically used for weed control in organic production systems (USDA 2011b). Several studies have reported that weed mat is en effective method for controlling weeds in conventional and organic orchard and blueberry systems (Granatstein and Mullinix 2008; Julian et al. 2012), and it is more effective in terms of growth and yield than hand weeding in trailing blackberry (Harkins et al. 2013, 2014). However, it is unknown how these different weed management strategies affect the fruit quality.

Fresh blackberry fruit are highly perishable and easily damaged during harvest due to their soft tissue and fragile skin. Postharvest storage is also limited as a result of rapid mold growth and high rates of fruit water loss (Joo et al. 2011). Freezing is the most common method of preserving blackberries. The total volume of frozen blackberries produced in the United States in 2012 was approximately 111.5 million pounds. Unlike the fresh berries, frozen blackberries have a long shelf life and retain very good quality. Sousa et al. (2007) reported that there was no difference in sensory firmness between frozen and fresh blackberries. Türkben et al. (2010) found that phenolic compounds such as caffeic acid and quercetin remained at about the same level as fresh blackberries after 6 months of frozen storage, while González et al. (2003) reported that total anthocyanins in frozen Spanish wild blackberry was about the same as fresh blackberries after 12 months of frozen storage.

The objective of the present study was to examine the effect of three weed management strategies, including no weeding, hand weeding, and weed mat on fruit quality in frozen organic blackberries. The fruit were machine-harvested from a certified organic planting and included two common cultivars of trailing blackberry, ‘Marion’ and ‘Black Diamond’.

Material and Methods

Fruit materials

Fruit were machine-harvested using an over-the-row rotary harvester (Littau Harvesters Inc., Stayton, OR) in July 2012 from a certified organic planting of 'Marion' and 'Black Diamond' blackberry located at the North Willamette Research and Extension Center in Aurora, Oregon, USA. Both cultivars were either non-weeded (NW), hand weeded (HW), or covered with weed mat (WM). Five replicates of each treatment were arranged in a split-plot design with cultivar as main plots and weed management treatments as subplots. See (Harkins et al. 2013) for further details on the experimental design and establishment and management of the planting. Blackberries were collected three times during the harvest period at 1 week intervals, which corresponded to early, middle, and late harvest dates. Once picked, the fruit was immediately packed on ice and transferred back to the Food Science building at Oregon State University in Corvallis, OR. The fruit was then put carefully into Ziploc plastic storage bags and frozen and stored at -20 ºC in a dark freezer. Three random subsamples from each treatment were analyzed for physicochemical and antioxidant properties after 0, 6, and 9 months of storage.

Fruit pH, titratable acidity, and total soluble solids

Fruit pH, titratable acidity (TA) and total soluble solids (TSS) were measured following the methods outlined by Fisk et al. (2008). Two individual fruit from each plastic bag were mixed with 9 times of fruit weight of distill water, and blended for 1

min using a 12-speed homogenizer (Osterizer, Jarden Corp., Mexico). The mixture was filtered through filter paper (Whatman International Ltd., Maidstone, England) to remove fat, pectin and other chunk tissues. The filtrate was measured for pH using a digital pH meter (Corning Science Products, Medfield, MA), and TSS using a Kyoto Electronics RA-250HE refractometer (Kyoto Electronics Manufacturing Co., Ltd., Japan). TA was quantified by titrating with 0.1 N aqueous NaOH (Alfa Aesar, Ward Hill, MA) until reaching final pH of 8.2 and reported as percent malic acid equivalent on the fresh weight basis of the fruit.

Fruit extraction for antioxidant assays

The extraction procedure of Wu et al. (2010) was employed with slight modifications. Briefly, fruit samples were quickly frozen with liquid nitrogen, and pulverized into powders using stainless steel blender (Waring Laboratory Science, Torrington, CT). A 15 g of sample powder were extracted by 100% acidified acetone (0.1 mL/L HCl) and then 70% acidified acetone solution (0.1 mL/L HCl) twice. A fixed time ultrasound treatment (90, 300 and 300 s, respectively) was applied after acetone added and then centrifuged (International Equipment Co., USA) at 10,000 g for 15 min. The supernatants were collected and combined together with 150 mL of chloroform and centrifuged to separate the two phases for removing lipophilic components. The aqueous phase was concentrated using a rotary evaporator (Brinkman Instruments, Westbury, NY) at 40 °C. Final extract solution was diluted to 150 mL using deionized (DI) water and stored at -80 ^{o}C until the time of assay.

Analysis of total phenolic content (TPC), radical scavenging activity (RSA) and total monomeric anthocyanins (TMA)

TPC was determined using the Folin-Ciocalteu assay as described by (Singleton and Rossi 1965). A 0.5 mL of properly diluted extracts were mixed with 7.5 mL of DI water and 0.5 mL of Folin-Ciocalteu reagent, conditioned at room temperature for 10 min, and then incubated under 40 °C water bath for 20 min with the addition of 3 mL of 20% sodium carbonate solution. The samples were transferred into a 0 °C ice bath for about 3 min until reached room temperature. Absorbance was measured spectrometrically at 765 nm (Model UV160U, Shimadzu Corporation, Kyoto, Japan). A series of gallic acid solution (0, 150, 200, and 250 ppm) were also prepared and measured for the absorbance as standard curve. Results were expressed as mg gallic acid equivalents (GAE)/g fresh weight (FW).

RSA was determined using 1,1-diphenyl-2-picryhydrazyl (DPPH) method (Brand-Williams et al. 1995). A 1.5 mL of DPPH-methanol solution (0.09 mg/mL) was mixed with 0.75 mL of diluted blackberry extract to react at room temperature for 10 min. Absorbance was measured at 517 nm, and a series of ascorbic acid solutions (0, 100, 200, 300, and 400 ppm) were also prepared and the absorbance was recorded as standard curve. Results were expressed as mg ascorbic acid equivalents (AAE)/g FW.

TMA was measured by pH differential method (Giusti and Wrolstad 2001). The extracts were diluted with either a 0.4 M sodium acetate buffer (pH 4.5) or a 0.025 M potassium chloride buffer (pH 1.0) and allowed to equilibrate for 15 min at room

temperature. The absorbance was measured at both 510 nm and 700 nm according to the predominant anthocyanin in blackberries, cyanadin-3-glucoside (Siriwoharn et al. 2004; Fan-Chiang and Wrolstad 2005). Results were expressed as mg cyanadin-3-glucoside equivalent/g FW using the following equation (Giusti and Wrolstad 2001).

$$TMA\left(\frac{mg}{g\ FW}\right) = \frac{[(A_{510\ nm}-A_{700\ nm})_{pH1.0}-(A_{510\ nm}-A_{700\ nm})_{pH4.5}]\times 449.2\frac{g}{mol}\times DF\times 1000\frac{mg}{g}}{26900\frac{L}{cm\cdot mol}\times 1\ cm}\times\frac{1L}{100\ g\ FW}$$

where DF was dilution factor.

Oxygen radical absorbance capacity (ORAC)

ORAC was measured as described by Dudonne et al. (2009) with slight modification using a 96 well microplate fluorometer (SpectraMax Gemini XS, Molecular Devices, Foster City, CA). A 30 µL of fruit extract (diluted as necessary) was added into each well with 200 µL of a pre-warmed β-phycoerythrin solution and 70 µL of 2,2‘-Azobis(2-amidinopropane) dihydrochloride (AAPH). The mixture was homogenized and incubated at 37 °C for 1 h. Fluorescence was measured at 485 nm and 585 nm, respectively every 2 min up to 2 h. The area under the curve was used to calculate the antioxidant capacity using proprietary software (SoftMax Pro 5.4.5, Molecular Devices, LLC, USA). A series of standardized Trolox solutions (0, 10, 20, or 40 µmol/L) were prepared to subtract the area under the curve of the blank. Results were expressed as µmol Trolox equivalent (TE)/g FW.

Ferric reducing antioxidant power (FRAP)

FRAP was assayed according to the method developed by Benzie & Strain (1996)

with modifications. A 40 µL of fruit extract was transferred into the wells with 300 µL of pre-warmed FRAP reagent which consist of 300 mmol/L acetate buffer, 10 mmol/L tri(2-pyridil)-s-triazine (TPTZ), and 20 mmol/L $FeCl_3 \cdot 6H_2O$ at ratio of 10:1:1. The mixtures were homogenized for 3 min and then incubated at 37 °C for 15 min. Absorbance was measured at 550 nm using a microplate absorbance reader (SpectraMax 190, Molecular Devices, Foster City, CA). A series of Trolox solutions (0, 62.5, 125, 250, or 500 mmol/L Trolox) were used as standards. Results were reported as µmol Trolox equivalent (TE)/g FW using proprietary software (SoftMax Pro 5.4.5, Molecular Devices, LLC, USA) to calculate antioxidant power.

Experimental design and statistical analysis

Fruit cultivars (‘Marion’ and ‘Black Diamond’) were used as main plots and three weed management strategies as subplots using split plot design (Harkins et al. 2013). All the quality parameters were measured in triplicate. ANOVA (analysis of variance) and GLM (general linear model) were applied for data analysis using SAS 9.2 (SAS Institute, Cary, NC, USA). Statistical differences were conducted by using LSD (least significant difference) at $P<0.05$.

Results and Discussion

Fruit pH, titritable acidity, and total soluble solids

Fruit pH was often similar between ‘Marion’ and ‘Black Diamond’ (Table 4.1). However, at day 0, ‘Marion’ blackberries from the early harvest had a higher pH than

those from the late harvest in the NW treatment and a higher pH than those from the middle harvest in the HW treatment. ‘Black Diamond’ blackberries, on the other hand, had a higher pH at the middle harvest than at the early or late harvest in the NW and WM treatments. Fruit pH increases with fruit ripening (Tosun et al. 2008), indicating the fruit from the treatments with higher pH was riper than from the other treatments at harvest. During storage, fruit pH in some treatments such as early and middle harvest ‘Marion’ blackberries from the HW and the WM treatments, respectively, and early harvest ‘Black Diamond’ blackberries from the NW and HW treatments and late harvest ‘Black Diamond’ blackberries from the WM treatment increased by an average of 6-7% after 9 mo ($P < 0.05$). Similar results were reported by (Sahari et al. 2004) reported similar results in frozen strawberries, which increased in pH by up to 4% after 90 d of storage at -12 ºC.

Titratable acidity (TA) of the fruit was also similar between ‘Marion’ and ‘Black Diamond’ and was unaffected by weed management when averaged across all three harvest dates (Table 4.2). However, like pH, TA sometimes differed between harvests within several of the weed management treatments. For example, NW and HW ‘Marion’ fruit from the early harvest had higher TA at day 0 than fruit in those treatments from middle and late harvests, while middle harvest ‘Black Diamond’ fruit had lower TA than early harvest fruit in each weed management treatment and late harvest fruit in the WM treatment. Again, riper fruit contain less acid (Perkins-Veazie et al. 1996), indicating fruit with lower TA values were riper. During storage, HW ‘Marion’ fruit from the early harvest had higher TA after 6 mo than WM ‘Marion’ fruit

and decreased by up to 15% after 9 mo ($P < 0.05$). 'Marion' fruit from the WM treatment, on the other hand, had little change in TA even after 9 mo of frozen storage.

Total soluble solids (TSS) in the fruit were also similar between cultivars, but in this case, fruit from the middle harvest in both cultivars had lower TSS in the HW treatment than in the NW treatment at day 0 (Table 4.3). Hand weeding may have increased fruit size and fruit water content relative to no weeding and therefore increased TSS during the middle harvest (Harkins et al. 2013). 'Marion' blackberries from the middle harvest also had lower TSS than those from the early and late harvests in the HW and WM treatments at day 0. During storage, 'Marion' fruit from the late harvest of the WM treatment had higher TSS after 6 mo than those from the middle harvest, probably due to riper fruit in the late harvest. However, TSS in stored 'Black Diamond' fruit was similar among harvest dates and unaffected by weed management. Difference in the TSS content during storage between the two cultivars might be due to fruit maturity, weather conditions during harvest, and physical damage caused by the machine harvester (Takeda and Peterson 1999; Perkins-Veazie et al. 2000b).

Total phenolic content (TPC) and total monomeric anthocyanin (TMA)

TPC and TMA values during frozen storage for 'Marion' and 'Black Diamond' fruit are reported in Fig. 4.1. Generally, 'Black Diamond' fruit showed significantly higher TPC and TMA values than Marion fruit ($P<0.05$) at the harvest. 'Marion' fruit from non-weeded plots showed the lowest TPC and TMA values at the harvest. This result was not surprising because weeds in the production field competed with

blackberry plant absorbing essential nutrients causing less antioxidant levels of blackberry fruit. For both cultivars, all samples in early harvest showed the lowest TPC and TMA values compared with samples from late harvest at the harvest. Moreover, late harvest ‘Marion’ fruit showed significantly higher TPC and TMA values than early harvest fruit, probably due to more ripened or overripe fruit in late harvest as mentioned above, and overripe fruit may contain higher phenolic compounds which is confirmed by other researchers (Sellappan et al. 2002; Siriwoharn et al. 2004). During storage, early harvest ‘Marion’ fruit showed increase in TPC and TMA after 6 mo of storage, and then continued increase at the end of 9 mo of storage except HW fruit. However, late harvest ‘Marion’ and ‘Black Diamond’ fruit showed decrease in TPC and TMA values during the first 6 mo of storage which was not surprising because ripe and overripe fruit might lose resistance to quality deterioration during storage (Perkins-Veazie et al. 1999).

Radical scavenge activity (RSA), Oxygen radical absorbance capacity (ORAC) and Ferric reducing antioxidant power (FRAP)

RSA, ORAC and FRAP values for ‘Marion’ and ‘Black Diamond’ fruit at the harvest are shown in Fig. 4.2. ‘Black Diamond’ fruit showed significantly higher RSA values than ‘Marion’ fruit ($P<0.05$) which is consistent with the trend of antioxidant contents reported above. ‘Black Diamond’ fruit from weed mat plots in early harvest showed significantly lower RSA value than samples from middle and late harvest ($P<0.05$) which is also consistent with the trend of the lower TPC and TMA in early

harvest samples. ‘Marion’ in all three weed management strategies from early harvest showed lower RSA values than samples in middle and late harvests ($P<0.05$) which completely made sense that samples in early harvest were less ripe than middle and late harvests. There was no significant difference in ORAC values between ‘Marion’ and ‘Black Diamond’ fruit ($P>0.05$) while FRAP value of ‘Marion’ cultivar was significantly higher than that of ‘Black Diamond’ cultivar ($P<0.05$). Late harvest fruit showed the highest ORAC and FRAP values for both cultivars except HW ‘Black Diamond’ fruit. This might be due to fruit ripeness in different harvest time, early and middle harvest fruit contain more under ripe fruit, and antioxidant capacity of less ripe fruit was lower than ripe fruit (Siriwoharn et al. 2004). Similar results were reported by other researchers (Moyer et al. 2002; Reyes-Carmona et al. 2005).

Considering antioxidant content and antioxidant capacity, samples treated by weed mat method showed high antioxidant capacity with relatively low antioxidant contents. This could be explained by signaling mechanisms for a variety of stresses (Reddy et al. 2004). Plant will synthesize phenolic compounds to eliminate the stresses. One of stress to elicit this signaling is the reduction of availability of water and nutrients which are necessity plant compete with weeds (Harkins et al. 2013). Due to less competition with weeds, samples treated by weed mat underwent less stress and then less phenolic compounds synthesizing which is consistent with findings in this research.

Conclusion

The study indicated that weed management is an effective means to improve fruit quality in processed organic blackberries. Harvest date and frozen storage could also affect the quality of fruit. 'Black Diamond' fruit in middle harvest and 'Marion' fruit in late harvest showed higher pH, lower TA and relatively high TSS when compared with fruit harvested at other times. Total phenolic content, total anthocyanin content and radical scavenging activity were greatly affected by weed management strategy, harvest date and frozen storage time, and varies between the fruit cultivars. Frozen storage at -20 $^{\circ}$C was a feasible way to preserve the physicochemical and antioxidant quality of blackberry fruit, even after 9 months of storage. Further studies are needed to determine how weed management affects plant development and other blackberry cultivars. Sensory evaluation of processed fruit was also needed to make better selection of proper cultivar with proper harvest date for processed market.

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Table 4.1 pH values of 'Marion' and 'Black Diamond' fruit during frozen storage in 2012 harvest[†]

			Weed Management		
Storage	Cultivar	Harvest Date	NW[††]	HW	WM
0 month	Marion	Early Harvest	3.13 ± 0.08 Ab[*]	3.04 ± 0.05 Ab	3.16 ± 0.17 Aa
		Middle Harvest	3.28 ± 0.06 Aab	3.52 ± 0.36 Aa	3.18 ± 0.06 Aa
		Late Harvest	3.40 ± 0.16 Aa	3.35 ± 0.19 Aab	3.26 ± 0.19 Aa
	Black Diamond	Early Harvest	3.06 ± 0.02 Cb	3.09 ± 0.01 Ba	3.24 ± 0.01 Ab
		Middle Harvest	3.59 ± 0.08 Aa	3.20 ± 0.64 Aa	3.47 ± 0.16 Aa
		Late Harvest	3.25 ± 0.23 Ab	3.25 ± 0.19 Aa	3.14 ± 0.09 Ab
6 months	Marion	Early Harvest	3.15 ± 0.04 Ab	3.08 ± 0.04 Ab	3.11 ± 0.07 Ab
		Middle Harvest	3.30 ± 0.07 Aa	3.50 ± 0.21 Aa	3.37 ± 0.11 Aa
		Late Harvest	3.38 ± 0.08 Aa	3.35 ± 0.11 Aa	3.31 ± 0.03 Aa
	Black Diamond	Early Harvest	3.11 ± 0.04 Bb	3.07 ± 0.04 Bb	3.27 ± 0.04 Ab
		Middle Harvest	3.67 ± 0.23 Aa	3.23 ± 0.12 Ba	3.65 ± 0.04 Aa
		Late Harvest	3.23 ± 0.10 Ab	3.39 ± 0.16 Aa	3.26 ± 0.06 Ab
9 months	Marion	Early Harvest	3.26 ± 0.07 Aa	3.23 ± 0.07 Aa	3.22 ± 0.09 Ab
		Middle Harvest	3.31 ± 0.08 Aa	3.37 ± 0.08 Aa	3.40 ± 0.07 Aa
		Late Harvest	3.31 ± 0.05 Aa	3.33 ± 0.11 Aa	3.34 ± 0.06 Aab
	Black Diamond	Early Harvest	3.22 ± 0.10 Ab	3.31 ± 0.09 Ab	3.32 ± 0.10 Ab
		Middle Harvest	3.61 ± 0.07 Aa	3.52 ± 0.07 Aa	3.60 ± 0.03 Aa
		Late Harvest	3.37 ± 0.08 Ab	3.41 ± 0.07 Aab	3.38 ± 0.07 Ab

[†] Values were reported by mean ± S.D., n=3.

[††] NW, no weeding, HW, hand weeding; WM, weed mat

* Means followed by the same capital letters (A-B) in the same row within each cultivar were not significantly different (P>0.05); Means followed by the same lowercase letters (a-b) in the same column within each cultivar were not significantly different (P>0.05).

Table 4.2 Titratable acidity (TA, %) values of 'Marion' and 'Black Diamond' fruit during frozen storage in 2012 harvest[†]

			Weed Management		
Storage	Cultivar	Harvest Date	NW[††]	HW	WM
0 month	Marion	Early Harvest	1.44 ± 0.22 Aa[*]	1.53 ± 0.04 Aa	1.28 ± 0.42 Aa
		Middle Harvest	0.85 ± 0.28 Ab	0.64 ± 0.16 Ac	0.85 ± 0.07 Aa
		Late Harvest	0.93 ± 0.08 Ab	1.01 ± 0.18 Ab	1.10 ± 0.32 Aa
	Black Diamond	Early Harvest	1.32 ± 0.17 Aa	1.08 ± 0.16 Aa	1.13 ± 0.08 Aa
		Middle Harvest	0.63 ± 0.06 Ab	0.77 ± 0.10 Ab	0.63 ± 0.14 Ab
		Late Harvest	0.99 ± 0.36 Aab	1.04 ± 0.41Aab	1.23 ± 0.27 Aa
6 months	Marion	Early Harvest	1.28 ± 0.20ABa	1.54 ± 0.09 Aa	1.10 ± 0.17 Ba
		Middle Harvest	0.74 ± 0.15 Ab	0.70 ± 0.25 Ab	0.84 ± 0.11 Aa
		Late Harvest	0.97 ± 0.10 Ab	0.85 ± 0.09 Ab	0.98 ± 0.18 Aa
	Black Diamond	Early Harvest	1.32 ± 0.09 Aa	1.17 ± 0.03 Ba	1.10 ± 0.12 Ba
		Middle Harvest	0.68 ± 0.02 Ab	0.70 ± 0.11 Ab	0.66 ± 0.13 Ab
		Late Harvest	1.03 ± 0.22 Aa	1.02 ± 0.15 Aa	1.14 ± 0.12 Aa
9 months	Marion	Early Harvest	1.18 ± 0.08ABa	1.30 ± 0.08 Aa	1.13 ± 0.06 Ba
		Middle Harvest	0.73 ± 0.03 Ac	0.67 ± 0.10 Ac	0.77 ± 0.04 Ab
		Late Harvest	0.96 ± 0.10 Ab	0.88 ± 0.07 Ab	0.87 ± 0.08 Ab
	Black Diamond	Early Harvest	1.15 ± 0.10 Aa	1.08 ± 0.07 Aa	1.12 ± 0.08 Aa
		Middle Harvest	0.60 ± 0.03 Ac	0.63 ± 0.04 Ab	0.61 ± 0.09 Ab
		Late Harvest	0.92 ± 0.07 Ab	0.96 ± 0.11 Aa	0.98 ± 0.08 Aa

[†] Values were reported by mean ± S.D., n=3.

[††] NW, no weeding, HW, hand weeding; WM, weed mat

* Means followed by the same capital letters (A-B) in the same row within each cultivar were not significantly different (P>0.05); Means followed by the same lowercase letters (a-b) in the same column within each cultivar were not significantly different (P>0.05).

Table 4.3 Total soluble solids (TSS, %) values of 'Marion' and 'Black Diamond' fruit during frozen storage in 2012 harvest[†]

			Weed Management		
Storage	Cultivar	Harvest Date	NW[††]	HW	WM
0 month	Marion	Early Harvest	12.50 ± 1.32Aa[*]	12.83 ± 1.61Aa	11.33 ± 0.58Aa
		Middle Harvest	10.83 ± 1.26Aa	9.17 ± 0.29Bb	9.33 ± 0.58ABb
		Late Harvest	10.67 ± 1.53Aa	12.00 ± 1.00Aa	12.33 ± 0.58Aa
	Black Diamond	Early Harvest	9.67 ± 2.08Aa	8.33 ± 0.58Aa	9.00 ± 2.00Aa
		Middle Harvest	10.50 ± 0.50Aa	7.83 ± 1.61Ba	9.50 ± 0.87ABa
		Late Harvest	9.50 ± 0.87Aa	8.83 ± 0.29Aa	10.00 ± 1.00Aa
6 months	Marion	Early Harvest	11.67 ± 1.61Aa	11.33 ± 0.58Aa	10.17 ± 0.58Aab
		Middle Harvest	10.83 ± 1.04Aa	9.17 ± 0.76Ab	9.17 ± 0.58Ab
		Late Harvest	10.33 ± 0.76Aa	10.67 ± 0.29Aa	11.17 ± 0.76Aa
	Black Diamond	Early Harvest	9.33 ± 1.44Aa	7.50 ± 0.87Aa	8.00 ± 1.32Aa
		Middle Harvest	9.83 ± 1.04Aa	7.50 ± 0.50Ba	9.33 ± 1.26Aa
		Late Harvest	9.83 ± 0.76Aa	8.67 ± 1.04Aa	9.67 ± 1.04Aa
9 months	Marion	Early Harvest	11.00 ± 0.50Aa	10.83 ± 1.04Aa	10.67 ± 1.04Aab
		Middle Harvest	10.67 ± 0.76Aa	10.50 ± 1.32Aa	10.17 ± 0.29Ab
		Late Harvest	10.33 ± 0.76Aa	10.50 ± 1.00Aa	11.33 ± 0.76Aa
	Black Diamond	Early Harvest	9.17 ± 1.04Aa	8.83 ± 0.29Aa	9.33 ± 0.76Aa
		Middle Harvest	8.83 ± 0.76Aa	8.50 ± 0.87Aa	9.67 ± 0.76Aa
		Late Harvest	9.33 ± 0.58Aa	8.50 ± 0.87Aa	9.50 ± 0.50Aa

[†] Values were reported by mean ± S.D., n=3.

[††] NW, no weeding, HW, hand weeding; WM, weed mat

* Means followed by the same capital letters (A-B) in the same row within each cultivar were not significantly different (P>0.05); Means followed by the same lowercase letters (a-b) in the same column within each cultivar were not significantly different (P>0.05).

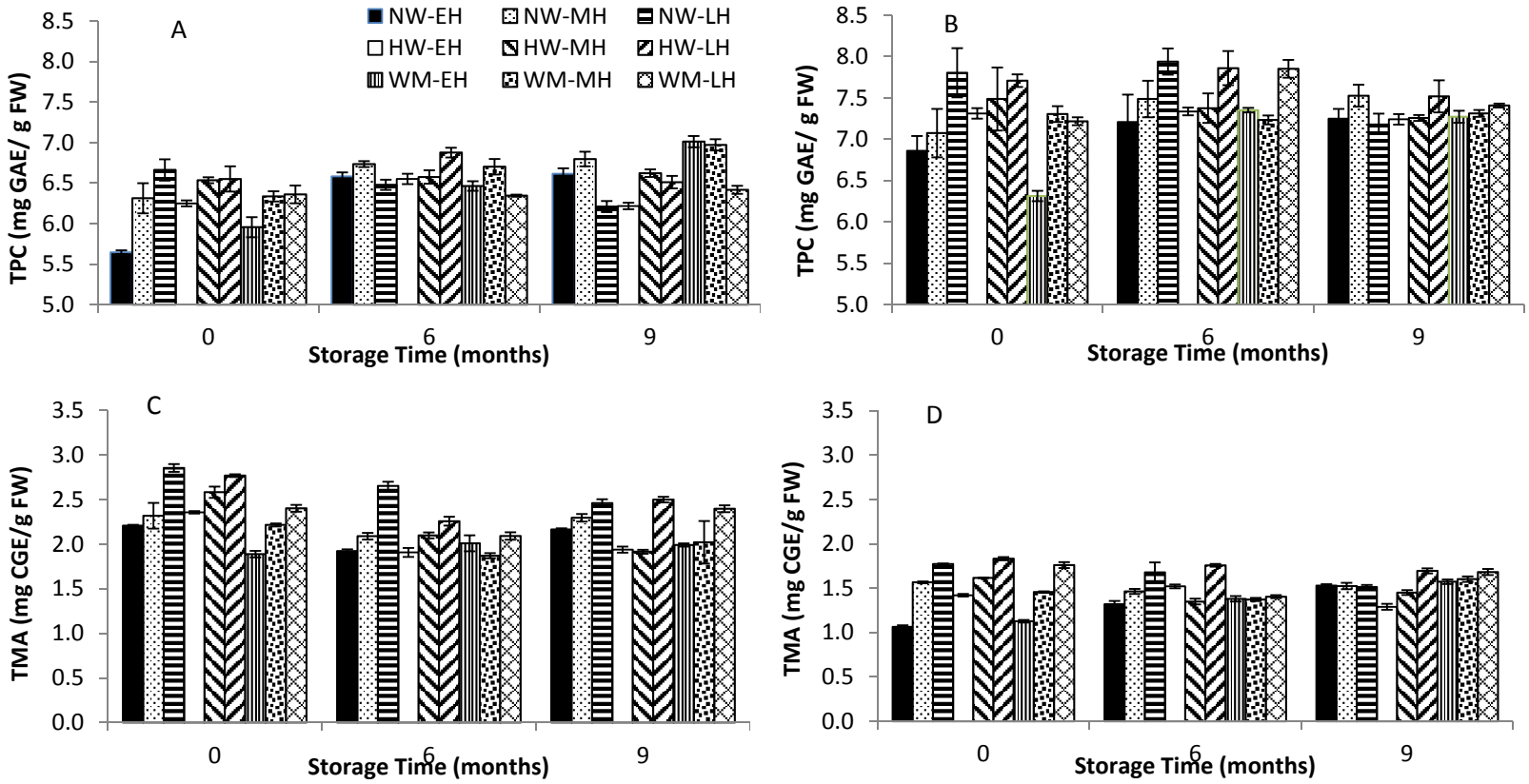

Fig. 4.1 TPC (total phenolic content) of 'Marion' (A) and 'Black Diamond' (B), TMA (total monomeric anthocyanins) of 'Marion' (C) and 'Black Diamond' (D) during storage in 2012 harvest. No significant difference of RSA during storage. NW: no weeding, HH: hand weeding, and WM: weed mat. GAE, gallic acid equivalent; AAE, ascorbic acid equivalent; CGE, cyanindin-3- glucoside equivalent.

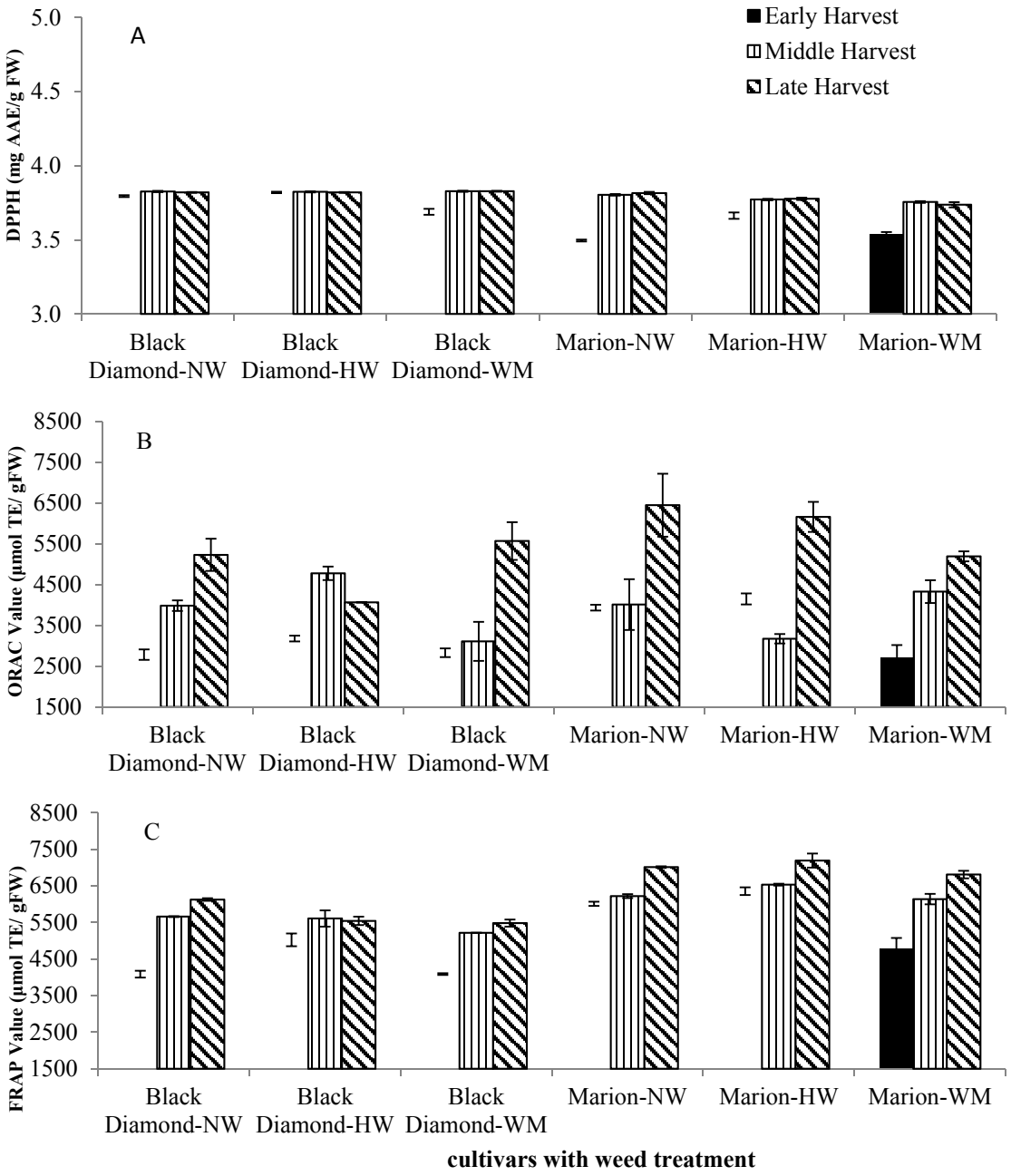

Fig. 4.2 Antioxidant capacity at harvest by radical scavenging activity (DPPH) method (A), ORAC method (B) and FRAP method (C) for 'Marion' and 'Black Diamond' blackberry with different weed management right after harvest in 2012. NW: non-weeding; HW: hand-weeding; WM: weed mat.

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CHAPTER 5

General Conclusion

This thesis research investigated the impact of different organic fertilizer and harvest date on the physicochemical properties and antioxidant capacities of two hand-picked organically grown blackberry fruit during refrigerated storage as well as the impact of different weed management strategies and harvest date on quality change of two machine harvested organically grown blackberry fruit during frozen storage.

The results showed that different organic fertilizers evaluated in this study have no significant effect on the physicochemical properties of 'Obsidian' and 'Triple Crown' blackberry fruit at the time of harvest and during the refrigerated storage, but antioxidant capacities of 'Triple Crown' fruit treated by fish emulsion/fish hydrolysate or soy-meal based fertilizers increased during refrigeration storage. Generally, titratable acidity, pH and total soluble solids of 'Obsidian' and 'Triple Crown' fruit were complexly affected by fruit cultivar, harvest date and storage time, and based on the results from this study, recommended storage time at 4±1 ºC and 85% RH is 4 d after harvest for 'Obsidian' cultivar and 8 d for 'Triple Crown' cultivar.

This study also demonstrated that freeze processing effectively retain fruit quality without significant change of physicochemical properties. Physicochemical and antioxidant properties of both 'Marion' and 'Black Diamond' cultivars were affected complexly by weed management strategy and harvest date. Middle harvest fruit of both cultivars had lower titratable acidity than fruit in early and late harvest, and 'Marion' fruit in middle harvest showed lower total soluble solids when compared

with early and late harvest ones. Weed management resulted in higher content of phenolic compounds and anthocyanins of ‘Marion’ and ‘Black Diamond’ blackberry fruit compared with no weed treated fruit. The total phenolic content and antioxidant capacity of organically grown blackberries were well maintained during frozen storage at -25 ºC for up to 9 months.

In the future study, more blackberry cultivars should be investigated since the physicochemical and antioxidant properties during post-harvest refrigerated and frozen storage varied significantly among those four cultivars studied. Different organic fertilizers and weed management strategies showed various effects on the quality characteristics of blackberry fruit. Therefore, more investigation on the impact of the organic fertilizers and weed management strategies on the fruit development mechanisms are necessary. Moreover, the correlation between the physicochemical properties and antioxidant capacity of the fruit needs to be further studied.