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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 °C 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 anthocyanins 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 an 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 °C 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 °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-picrylhydrazyl (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).

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

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 μ L of fruit extract (diluted as necessary) was added into each well with 200 μ L of a pre-warmed β -phycoerythrin solution and 70 μ L of 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH). The mixture was homogenized and incubated at 37 $^{\circ}$ 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 μ mol/L) were prepared to subtract the area under the curve of the blank. Results were expressed as μ mol 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 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, titratable 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 $-12\text{ }^{\circ}\text{C}$.

Titrateable 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 °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[†]

Storage	Cultivar	Harvest Date	Weed Management		
			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).

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