

Impact of Fruit Maturity and Growing Season on Phenolic Phytochemicals and Antioxidant Activity Among Four Asian Plum Cultivars

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Abstract

Various factors, including environment, maturity, weather, and cultivars are known to impact the profile and concentration of phenolic phytochemicals in plums. Major anthocyanins and neochlorogenic acids were quantified in fruit among four cultivars (Methley, Obilnya, Shiro and Vanier) of Asian plum, *Prunus salicina*, at the partial-ripe and tree-ripe stages of maturity in 2014 and 2015. Anthocyanin concentration was greater with the later stage of maturity in every cultivar except 'Shiro' which did not have detectable anthocyanins. 'Obilnya' had the greatest anthocyanin concentration, followed by 'Methley' and 'Vanier'. Anthocyanin concentration was greater in 2015 than in 2014 in 'Methley' and 'Obilnya', but in 'Vanier', it was greater in 2014 in tree-ripe fruit. Cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside were detected in three cultivars, but their relative concentrations varied. Cyanidin 3-*O*-glucoside was predominant in 'Methley' in both years and in 'Vanier' partial-ripe fruit in both years, but cyanidin 3-*O*-rutinoside was the predominant anthocyanin in 'Obilnya' in both years and in 'Vanier' tree-ripe fruit only in 2014. Cyanidin 3-*O*-galactoside occurred in only 'Methley' and 'Obilnya' and was generally less than 25% of the total concentration. Large year-to-year variation in neochlorogenic acid, the predominant hydroxycinnamic acid (HCA), occurred in 'Shiro' and 'Vanier' fruit, but variation due to maturity was small in all four cultivars. Total phenolic concentration (TPC) was measured by the Folin-Ciocalteu method and antioxidant activity (AOA) by DPPH assay in only 2015. TPC was greater in tree-ripe than in partial-ripe plums of every cultivar. The relationship between TPC and AOA was curvilinear indicating a reduction in slope as TPC increased.

Grower selection of plum cultivars, in the northeastern USA and Canada, is largely based on horticultural traits such as yield and cold hardiness. In this region, 'Shiro' and 'Methley' plums have traditionally been selected despite relatively lower consumer appeal compared to other Asian cultivars (Myracle et al., 2018). As the demand for locally produced fruit increases, cultivar se-

lection has expanded to include ones with consumer appeal in addition to high yield. As consumers become more interested in the potential health benefits of fruits, cultivar selection may also be based on phytonutrient content and antioxidant activity (AOA), both of which are highly variable among plum cultivars (Fanning et al., 2014; Kaulmann et al., 2014).

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The phytonutrient content of Asian plum cultivars has been well documented (Kaulmann et al., 2014; Vizzotto et al., 2007; Fanning et al., 2014; Sahamishirazi et al., 2017), but information on how stage of maturity influences bioactive phenolics is not as well understood. Anthocyanins and AOA generally increase with advancing maturity (Bobrich et al., 2014; Kristl et al., 2011), but not consistently in all cultivars (Venter et al., 2014). When plums are grown close to market, fruit can be harvested at relatively later stages of maturity with the potential for greater quality but also greater potential for postharvest losses. Harvesting partially ripe fruit rather than mature but unripe may allow growers to market plums with greater antioxidant activity and adequate shelf-life for local sale. However, it is not known how this will impact the phytonutrient content and AOA.

Most studies have examined plum cultivars within one season, but a strong year effect occurs for total anthocyanins, total phenolics and AOA in Asian plums that is not consistent among all genotypes (Cuevas et al., 2015; Venter et al., 2014). Growing season can have a strong effect on the individual compounds that contribute to AOA in Asian plums grown in Spain (Cuevas et al., 2015), but has not been examined in plums grown in a cool, wet climate.

Anthocyanins contribute to AOA with a positively charged oxygen atom which makes anthocyanins effective hydrogen donors to an oxidizing agent (Ongkowijoyoa et al., 2018). Anthocyanin content varies considerably among fruit of different cultivars with concentrations that range from undetectable in yellow or green fruit to over 1000 mg • kg⁻¹ fresh weight in purple and red fleshed plums (Fanning et al., 2014; Vizzotto et al., 2007). *Prunus cerasifera* (cherry plum) fruit reportedly contains higher concentration of anthocyanins than European plums (Kaulmann et al., 2014), but fruit of yellow selections have undetectable anthocyanin content, indicating that wide genotypic

variation exists within *P. cerasifera* (Tešović et al., 2012; Wang et al., 2012) which has been used to breed Asian cultivars (Byrne, 1989). Three cultivars adapted to the north-eastern USA and Canada, Methley, Shiro and Obilnya, are derived from *P. cerasifera* and vary in flesh and peel color from pale yellow to dark purple.

The predominant anthocyanins present in Asian and *P. cerasifera* plums are cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside (Tešović et al., 2012; Venter et al., 2014) with cyanidin 3-*O*-galactoside, acetylated cyanidin 3-*O*-glucoside, cyanidin 3-*O*-xyloside and one other unidentified anthocyanin occurring in small quantities in *P. cerasifera* (Wang et al., 2012). The relative AOA of cyanidin 3-*O*-glucoside is greater than cyanidin 3-*O*-galactoside (Wang et al., 1997) and slightly greater than cyanidin 3-*O*-rutinoside (Baskan et al., 2015). Because anthocyanins vary in how much they contribute to AOA, identifying and quantifying them may contribute to our understanding of the AOA in plums.

Hydroxycinnamic acids are another source of AOA in plums (Kaulmann et al., 2014; Mitrović et al., 2019; Mubarak et al., 2012; Usenik et al., 2008) due to the presence of hydrogens in the aromatic ring which can be donors to an oxidizing agent. Among Asian plum selections, neochlorogenic acid is the predominant HCA in fruit of most cultivars, while chlorogenic acid is the predominate one in others (Mubarak et al., 2012). Neochlorogenic acid concentration is comparatively low in *P. cerasifera* fruit and some of its hybrids with *P. salicina*, a trait that may differentiate it from other Asian plums (Jaiswal et al., 2013) and may impact their AOA, as well.

The objective of this research was to quantify year-to-year and stage of maturity effects on the dominant anthocyanins and neochlorogenic acid in fruit of four Asian plum cultivars, three of which are derived from *P. cerasifera*.

Table 1. Harvest dates, and precipitation and average temperature for the 30 days prior to harvest of four cultivars of Asian plum in two seasons.

Cultivar	Harvest date		Precipitation (mm)		Temperature (°C)	
	2014	2015	2014	2015	2014	2015
Methley	4 Aug.	1 Aug.	115	50	20.4	23.0
Obilnya	18 Aug.	9 Aug.	87	18	27.7	23.3
Shiro	19 Aug.	9 Aug.	87	18	19.0	23.3
Vanier	1 Sept.	3 Sept.	99	42	20.6	23.4

Materials and Methods

Plum trees were grown at the University of Maine Agricultural and Forest Experiment Station in Monmouth, ME, located at 44.231 N and -77.068 W latitudinal and longitudinal coordinates, respectively. Fruit from four cultivars, Methley, Obilnya, Shiro and Vanier, were harvested at the partial-ripe and tree-ripe stages of maturity from early Aug. to mid Sept. in 2014 and 2015 (Table 1). Photographs of these cultivars are shown in Table 2. Maturity was subjectively assessed by gently squeezing fruit, and by skin color, and according to past experience of these two traits. ‘Methley’ skin color changed from green to pink and then purple. ‘Shiro’ skin color changed from green to pale green and yellow. ‘Obilnya’ became dark purple before it ripened, so maturity was based on flesh softening. ‘Vanier’ skin color changed from yellow to red. ‘Shiro’ and ‘Methley’ firmness at harvest was lower than ‘Obilnya’ and ‘Vanier’ (Table 3), indicating that one standard could not be applied to all cultivars. Both stages were harvested on the same day. Fruit firmness was measured on 10 fruit per cultivar and stage on two peeled sides of each fruit and using a hand-held penetrometer with an 8 mm tip (Fruit Hardness Tester model 803, General Specialty Tools and Instruments, Secaucus, NJ). Soluble solids concentration (SSC) was measured using filtered juice from a pooled sample of 5 to 10 fruit. The samples were prepared by blending pitted plums for 5 s, then centrifuged (5804R; Eppendorf, Hamburg, Germany) for 10 min at 3234 x g. The centrifuged samples were

filtered through Whatman #4 filter paper (GE Healthcare Lifesciences, Chicago, IL) in a Buchner funnel with an applied vacuum. Samples were stored at -20 °C until analysis. Soluble solids concentration was measured with a digital refractometer (PAL-3 model, Atago, Bellevue, WA).

All remaining fruits were freeze-dried whole during a seven-day step down cycle

Table 2. Photos of peel and flesh for four cultivars of Asian plum.








Methley		
Obilnya		
Shiro		Photo not available.
Vanier		

Table 3. Fruit firmness (N) at harvest in four cultivars of Asian plum at the partial ripe (PR) and tree-ripe (TR) stages of maturity in two seasons.

Cultivar	2014		2015	
	PR	TR	PR	TR
Methley	8.7	6.0	6.2	3.1
Obilnya	22.7	19.4	18.5	11.3
Shiro	6.7	4.0	11.7	6.8
Vanier	26.1	13.2	17.4	14.9

starting at $-30\text{ }^{\circ}\text{C}$ and ending at $23\text{ }^{\circ}\text{C}$. The freeze drier was a VirTis Ultra35EL (Millrock Technology, Kingston, NY). A pooled sample of six plums of each cultivar, including peel and flesh with the pit removed, were ground into powder using a mortar and pestle with liquid-nitrogen. The plum powders were stored at $-23\text{ }^{\circ}\text{C}$.

Reagents and Chemicals. Chlorogenic acid (*trans*-3-*O*-caffeoylquinic acid), cyanidin 3-*O*-glucoside chloride, cyanidin 3-*O*-galactoside chloride, cyanidin 3-*O*-rutinoside chloride, cyanidin chloride, 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH reagent), gallic acid, neochlorogenic acid (*trans*-5-*O*-caffeoylquinic acid), quercetin, reagent grade formic acid, and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, MO). Sodium bicarbonate, HPLC grade methanol and acetonitrile, and certified ACS grade ethyl acetate and hydrochloric acid were purchased from Fisher Scientific (Waltham, MA). Nanopure water from a Barnstead Ultrapure water system (Thermo-Fisher Scientific, Waltham, MA) was used for extraction and in the HPLC mobile phase.

Extraction of Anthocyanins and Hydroxycinnamic Acids. Freeze-dried plum powder (1 g) was extracted with 10 mL of acidified methanol (MeOH:H₂O:37 % HCl, 85:12.3:2.7, v/v) for anthocyanins, and with 10 mL of non-acidified 85 % aqueous methanol for HCA. The sample was vortexed for 20 s and placed in an ultrasonic bath for 30 min at $21\text{ }^{\circ}\text{C}$. The extracts were kept at room temperature for 1 hr and centrifuged for 15 min at 362 x g . The supernatant was re-

moved, and the residue was extracted two additional times. The supernatants were pooled, and methanol was removed under vacuum. The final volume of aqueous extract was measured. The extract was passed through a $0.45\text{ }\mu\text{m}$ nylon membrane syringe filter (Phenomenex, Torrance, CA) and analyzed immediately after extraction. Each pooled plum sample was analyzed as three separate extracts (replicates), and each replicate was measured three times.

Identification and Quantification of Anthocyanins and Hydroxycinnamic Acids. Plum extracts were analyzed using an Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA) coupled with a diode array detector (DAD, model G1315A) and a mass selective detector (MSD, model G2445A) operating in electrospray ionization (ESI) mode. Instrumental operation and data analysis were performed using the Agilent LC-MSD Chemstation software. Anthocyanins and HCAs were detected as absorbance at 520 nm and at 320 nm respectively. Identification of most anthocyanins and HCAs were performed by the comparison of chromatographic retention times in UV-*vis* spectral data with known standards. Compounds for which there were no available standards were tentatively identified on the basis of their MS/MS fragmentation by mass spectrometry (MS), and relative retention times with respect to known compounds. Anthocyanins and neochlorogenic acid were quantified using a separate calibration curve with an external standard for each phenolic compound.

Total Phenolic Content (TPC) and Antioxi-

idant Activity. Fruits were analyzed for TPC and free radical scavenging ability in 2015 with two replicates of each stage of fruit maturity and cultivar. The TPC and AOA of the plum samples were evaluated using an extract from freeze-dried plum powder (1 g) dissolved in 10 mL of 85% aqueous methanol acidified with 1% citric acid. Samples were extracted 5 times using polytron homogenizer for two minutes, then centrifuged (5804R; Eppendorf, Hamburg, Germany) at 3224 x g for 10 minutes. The supernatant from each of the five extractions was collected and pooled. Samples were vacuum concentrated (Eppendorf Vacufuge plus, Hamburg, Germany) at room temperature to a volume of less than 10 mL. All samples were brought to a final volume of 10 mL with the same extraction solvent, then stored at -20 °C until the assays were performed.

Total phenolic concentration was measured using Folin-Ciocalteu reagent as described by Veliloglu et al. (1998) with minor modifications. Aliquots (20 µL) of sample extracts and 90 µL of Folin-Ciocalteu reagent were added to a 96-well plate and allowed to incubate at room temperature for 5 min. Sodium bicarbonate solution (90 µL of 6 g • 100 mL⁻¹ solution) was added to the wells and the plates were incubated in the dark at room temperature for 90 min. The absorbance was read at 750 nm in a UV plate reader (Biotek ELx 800, Winooski, VT). The samples for the TPC assay were diluted to fit the linear range of the assay (0.2–1.2 absorbance units). A standard curve was generated using gallic acid at concentrations of 0, 25, 50, 100, 150, 200 and 250 µg • mL⁻¹. Results were expressed as gallic acid equivalents (GAE). Antioxidant activity, also referred to as antioxidant capacity, was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, modified from Duymus et al. (2014). Equal amounts of 0.3 mM DPPH solution (150 µL) and the diluted samples (150 µL) were loaded to a 96-well plate. The mixture was incubated in the dark at room temperature for 30 min and the ab-

sorbance was read at 515 nm with a Biotek plate reader (ELx800, Winooski, VT, U.S.). A mixture of DPPH solution (150 µL) and water (150 µL) was used as the negative control for this assay. Scavenging percentage was calculated with the following formula:

$$\text{Scavenging \%} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

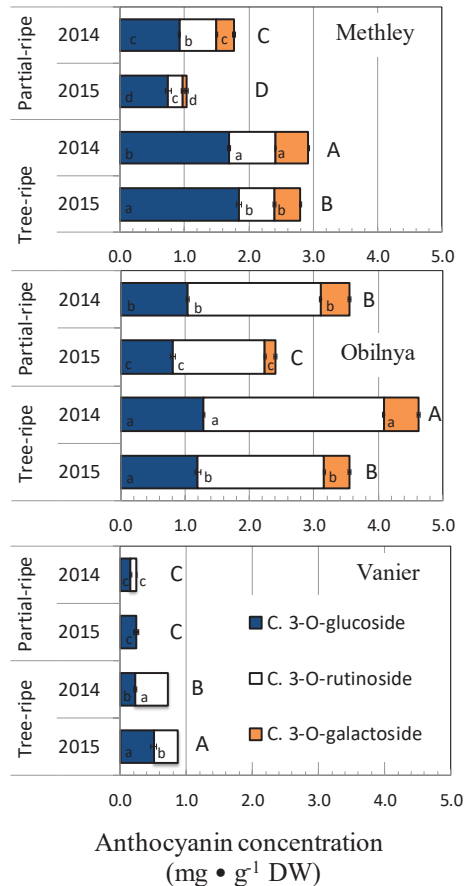


Figure 1. Anthocyanin concentration in fruit of three plum cultivars harvested at the partial-ripe and tree-ripe stages of fruit maturity in 2014 and 2015. Each bar is a mean ± se of 3 replicates. Within each cultivar, bars with the same letter are not significantly different ($P \leq 0.05$). Capital letters indicate means separation for total anthocyanin and small case letters for each type of anthocyanin.

Scavenging activity of each sample was measured at five different concentrations to calculate IC_{50} values, which is the concentration of the sample required to scavenge 50% of the DPPH free radicals. Gallic acid was used as the positive control. Measurements were made in 2015 only, and all samples were replicated twice.

Statistical Analysis. The orchard was planted as a randomized block design with two to five single-tree replications of each cultivar, but fruit from the different replications were pooled for chemical analysis and analyzed as a completely randomized design with three replications and of anthocyanins and hydroxycinnamic acids, and two replications of TP and AOA. The main effects of stage of maturity, year, cultivar and their interactions were analyzed using PROC GLIMMIX MIXED SAS procedures (Version 9.4) with means separation by Tukey's Test and Slice ($P \leq 0.05$). The RSREG procedure was used to estimate the regression equation of TPC and AOA.

Results and Discussions

Fruit Maturity. In 2014, mean fruit firmness for all cultivars at the partial-ripe stage was 15.8 ± 2.3 N, and at the tree-ripe stage was 10.4 ± 1.5 N (Table 3). Soluble solids concentration (SSC) was 11.2 ± 0.4 %, the same for both stages of maturity. In 2015,

partial-ripe fruit firmness was 13.6 ± 0.8 N, and SSC was 10.7 ± 0.3 %. Tree-ripe fruit firmness was 9.1 ± 0.7 N, and SSC was 11.1 ± 0.4 %.

Anthocyanins. Anthocyanins were not detected in 'Shiro' fruit, which have yellow-green peel and flesh. Total anthocyanin concentration in colored plums varied among years, stages of maturity and cultivars with interactions among these three factors (Table 4). Total anthocyanin concentration was greater in 2014 than in 2015 in 'Methley' and 'Obilnya' at both stages of fruit maturity (Fig. 1). The large difference in anthocyanin concentration between 2014 and 2015 in 'Obilnya' may be attributed to the light crop in this year compared to 2015. Crop load in the other cultivars was consistent from year to year. In 'Vanier', anthocyanin was greater in 2015 than in 2014 in tree-ripe fruit, but similar in both years in partial-ripe fruit. In both years, tree-ripe fruit had greater total anthocyanin concentration in all three cultivars. 'Obilnya' had the highest concentration of anthocyanins in both years, higher than 'Methley' which developed red flesh first near the pit and progressively outward toward the peel becoming completely red when tree-ripe. In contrast, 'Obilnya' had red coloring near the peel and none near the pit (Table 2). The greater concentration in 'Obilnya' plums may be attributed to their thicker peel

Table 4. Type 3 *P*-values for the effects of cultivar, harvest maturity (partial-ripe and tree-ripe), year (2014 and 2015) and their interactions on anthocyanins in 'Methley', 'Obilnya' and 'Vanier' plums, and on neochlorogenic acid in these and 'Shiro' plums.

	Total	Cyanidin	Cyanidin	Cyanidin	Neochlorogenic
Cultivar	anthocyanin	3- <i>O</i> -glucoside	3- <i>O</i> -rutinoside	3- <i>O</i> -galactoside	acid
Cultivar (C)	0.0001	0.0001	0.0001	0.0001	0.0001
Year (Y)	0.0001	0.9258	0.0001	0.0001	0.0205
Maturity (M)	0.0001	0.0001	0.0023	0.0001	0.2001
C x Y	0.0001	0.0001	0.0001	0.0001	0.0001
C x M	0.0001	0.0001	0.0001	0.0001	0.0001
Y x M	0.0022	0.0001	0.7621	0.0002	0.0008
C x Y x M	0.0427	0.0266	0.0033	0.0001	0.0001

which was noticeably more difficult to grind after freeze drying compared with ‘Methley’. The two major anthocyanins, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside were detected in all three cultivars. Cyanidin 3-*O*-galactoside was detected only in ‘Methley’ and ‘Obilnya’, both of which were derived from *Prunus cerasifera* which is reported to have this anthocyanin (Wang et al., 2012). Cyanidin 3-*O*-glucoside was predominant in ‘Methley’ in both years and stages of maturity (52% and 73% of the total in partial-ripe fruit, and 58% and 66% in tree-ripe fruit in 2014 and 2015, respectively). In partial-ripe fruit of ‘Vanier’, cyanidin 3-*O*-rutinoside was the predominant anthocyanin (64% and 100% of the total in 2014 and 2015, respectively), and was the major anthocyanin in tree-ripe fruit (58%) in 2015, but not 2014 (32%). Cyanidin 3-*O*-rutinoside was the predominant anthocyanin in ‘Obilnya’ fruit in both stages of maturity and in both years (55% to 61%). Some selections of *P. cerasifera* plums were reported previously to have cyanidin 3-*O*-rutinoside as the predominant anthocyanin (Wang et al., 2012). Cyanidin 3-*O*-(6"-acetyl)-glucoside, which was reported previously in Asian plums (Tomás-Barberán et al., 2001), was found in trace amounts in all three cultivars.

Phenolic Acids. Neochlorogenic acid (*trans* 5-*O*-caffeoylquinic acid) was the predominant HCA in the four Asian cultivars in this study, which was consistent with results reported for other cultivars of Asian plum (Jaiswal et al., 2013; Noratto et al., 2009). Concentration varied by year and cultivar, but not maturity, and interactions among these three factors were significant (Table 4). In ‘Obilnya’ and ‘Shiro’, concentrations were lower in 2014 than in 2015 at both maturity stages, but in ‘Vanier’, the concentration was greater in 2014 than in 2015 (Fig. 2). In ‘Methley’, concentration was greater in 2014 than in 2015 in partial-ripe fruit, but in tree-ripe fruit it was similar in both years. The effect of year was inconsistent among cultivars and stage of maturity. Environmental varia-

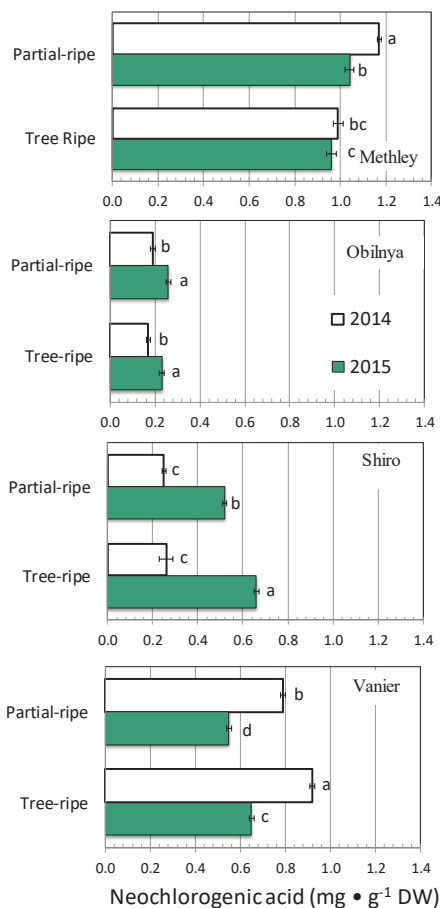


Figure 2. Concentration of neochlorogenic acid in fruit of four plum cultivars harvested at the partial- and tree-ripe stages of fruit maturity in 2014 and 2015. Each bar is a mean \pm se of 3 replicates. Within each cultivar, bars with the same letter are not significantly different ($P \leq 0.05$).

tion between the different ripening dates and seasons may account for the cultivar inconsistency. Less rainfall occurred prior to ‘Shiro’ and ‘Obilnya’ harvest than before harvest of the other two cultivars. Temperatures in 2014 were cooler, and the temperature range was wider than in 2015 for the 30 days prior to harvest (Table 1). It was also noted that the cultivars, ‘Shiro’ and ‘Vanier’, with lower concentrations of anthocyanins showed

greater year to year variation in HCA, but this trend should be verified with studies that include a larger number of cultivars.

Other HCA derivatives, such as chlorogenic acid (*trans* 3-*O*-caffeoylquinic acid), *p*-coumaroylquinic acids, and caffeoylshikimic acids were also detected in all four cultivars as minor phenolic acids, and they were reported previously in Asian plums (Jaiswal et al., 2013). However, feruloylquinic acid, methyl caffeoylquinic acid, and *p*-methoxycinnamoylquinic acid, which were observed in Asian and European plums (Jaiswal et al., 2013), were not detected in the present study. Hydroxybenzoic acids were not detected.

Total Phenolic Content and Antioxidant Activity. Total phenolic concentration (TPC), which is a measure of all phenolics including anthocyanins and HCAs, varied with cultivar and fruit maturity with a significant interaction in 2015 (Table 5). Total phenolic concentration was greater in tree-ripe fruit compared to partial-ripe fruit in all cultivars. In partial-ripe fruit, 'Methley' had the greatest TPC and 'Shiro' had the lowest TPC. In tree-ripe fruit, 'Vanier' had the greatest TPC, while 'Obilnya' and 'Methley' had intermediate TPC, and 'Shiro' the lowest TPC. The cultivar with no detectable anthocyanins, 'Shiro', had the lowest TPC, but the cultivar

with the highest anthocyanins, Obilnya, had intermediate TPC.

Antioxidant activity (AOA) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in which the concentration of the sample necessary to decrease the DPPH concentration by 50 % was determined and reported as the IC₅₀ value (Table 5). Thus, a lower IC₅₀ indicates greater antioxidant activity. As expected, fruits that were high in TPC were also high in AOA. There was a curvilinear relationship between AOA and TPC with R² = 0.98 (Fig. 3) indicating a decreasing slope as total phenolic concentration increased. This suggests that when the TPC is low, a small increase in TPC has a greater effect on AOA than when TPC is high. In previous studies, a linear relationship has been observed between AOA and TPC in different plum cultivars with only one stage of maturity (Gil et al., 2002; Cevallos-Casals et al., 2005). However, measuring TPC in both stages of maturity may result in more widely distributed TPC, causing the curvilinear relationship in this study.

Although there have been mixed results regarding correlations between AOA and anthocyanin content or hydroxycinnamic acid content (Huang and Prior, 2016; Nowicka et al., 2016), in this study, AOA was not di-

Table 5. Total phenolic concentration and IC₅₀ values of antioxidant activity (concentration of the sample required to scavenge 50% of the DPPH free radicals) in cultivars of partial-ripe (PR) and tree-ripe (TR) plums in 2015.

Cultivar	Total Phenolics (mg • kg ⁻¹ dry weight)		IC ₅₀ (mg • mL ⁻¹)	
	PR	TR	PR	TR
Methley	11.4 a ²	10.9 b	1.05 d	1.14 b
Obilnya	10.3 b	10.8 b	1.08 b	0.98 c
Shiro	4.9 c	8.2 c	2.12 a	1.41 a
Vanier	10.1 b	13.0 a	1.06 b	0.83 d
<i>Cultivar</i>	0.0001		0.0001	
<i>Maturity</i>	0.0001		0.0001	
<i>Cultivar x maturity</i>	0.0001		0.0001	

²Means separation within stage of maturity by the LSmeans slice option. Means within a column followed by the same letter are not significantly different, 5% level.

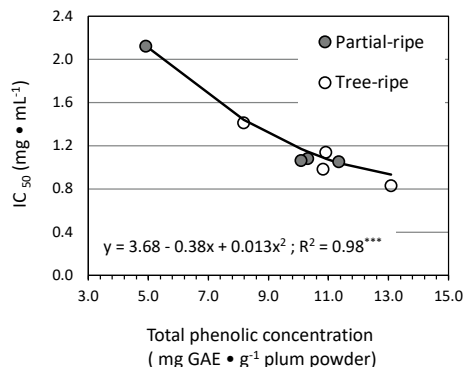


Figure 3. The relationship between total phenolic concentration as gallic acid equivalents (GAE) and antioxidant activity (IC_{50}) in four Asian plum cultivars and two stages of fruit maturity in 2015. IC_{50} is the concentration of sample required to scavenge 50% of the DPPH free radicals. Each point is a mean of two replications. Standard error bars are hidden by symbols.

rectly related to concentration of anthocyanins or phenolic acids. Other phenolic phytochemicals detected in plums, which include flavan-3-ols, and flavonols, also contribute to antioxidant capacity (Poonam et al., 2011). Venter et al. (2014) reported that plums with high flavan-3-ol content had high AOA. Additional AOA likely comes from non-phenolic compounds such as vitamins and carotenoids which were not taken into account in the present study.

The present study provides meaningful information regarding the variation in phenolic composition, particularly anthocyanins and hydroxycinnamic acids, among four Asian plum cultivars grown in Maine, USA. Concentrations of anthocyanins were strongly dependent on cultivar and stage of maturity, whereas concentration of HCA was strongly dependent on cultivar and year. ‘Obilnya’ and ‘Vanier’, two cultivars with good adaptation to the Northeast, were higher in AOA than two more predominant cultivars, Shiro and Methley. Future studies involving the identification and quantification of flavonols and flavan-3-ols in a greater number of

plum species will provide a comprehensive view of the differences in phenolic profiles and the relationship between phenolic profiles and antioxidant activities. In addition, this may also offer a better understanding of phenolic phytochemicals and their impact on human health.

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