HEALTH BENEFITS OF PEACHES AND PLUMS

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Abstract

Two-layer boxes of 10 peach, 10 nectarine, and 6 plum/pluot varieties were harvested in California and sent via overnight carrier to the laboratory at Texas A&M University over the time period of June 1st to mid September. Upon arrival the samples were processed and frozen until all the samples were collected at which time they were extracted and analyzed for total phenolic concentration, anthocyanin concentration, and antioxidant activity. In all three measurements there were clear differences among varieties and the antioxidant activity was well correlated with total phenolic concentration. Differences in the level of phenolics and anthocyanins seen in these samples as compared to a previous study indicate that other factors such as the cultural and environmental conditions under which the fruit is produced, the maturity state at harvest, and the post harvest handling protocols may affect the levels of fruit phytochemicals. These factors need to be further studied. This project will continue with the measurement of the antiproliferation activity in a breast cancer system and the inhibition of LDL oxidation of these 26 stone fruit varieties and should be finished by April of 2007.

Introduction

Fruits have long been promoted for their health benefits in preventing various cancers and age-related diseases (Prior and Cao, 2000; Wargovich, 2000). The phytochemicals reported in *Prunus* include carotenoids, anthocyanins, and phenolics (Weinert et al., 1990; Senter and Callahan, 1991; Tourjee et al., 1998; Gil et al., 2002; Cevallos et al., 2005). The antioxidant activity in both peaches and plums depends on the genotype tested. Some papers have reported that blueberry has the highest antioxidant activity among fruits; however, the levels found in red-fleshed plums overlap the levels found in blueberry (Wang et al., 1996; Prior et al., 1998; Cevallos et al., 2002; 2005). There is a good correlation between total phenolic compounds and antioxidant activity among peaches and plums (Cevallos et al., 2005; Gil et al., 2002; Vizzotto, 2005). Furthermore the contribution of phenolic compounds and anthocyanins to this antioxidant activity is much more important than the contribution of Vitamin C or carotenoids (Gil et al., 2002; Kim et al., 2003b; Chun et al., 2003; Vizzotto, 2005). Although there is a direct relationship between total phenolic and antioxidant activity there is no obvious linear relationship between either total phenolic content or total antioxidant activity and inhibition of cell proliferation, suggesting that there is a specific phenolic compound or a class of phenolics that is responsible for the antiproliferative activity (Sun et al., 2002).

Recent work in our laboratory has shown that methanolic extracts from a range of peach and plum genotypes showed antiproliferative activity on MDA-MB-435 estrogen-negative receptor breast cancer cell lines (Vizzotto, 2005). Current work is attempting to identify the specific phytochemicals responsible for this effect.

Reduced levels of cardiovascular disease has also been shown associated with the consumption of plant foods rich in flavonoids and other phenolic compounds which are obtained from fruits and vegetables (Prior and Cao, 2000; Wargovich, 2000). In the development of heart disease the prevention of low density lipoprotein (LDL) appears to be particularly important (Steinberg, 1989). LDL oxidation has been measured in a range of produce which indicated that fruits were a better source of phenol antioxidants than vegetables (Vinson et al., 2001). Work with eight processing peaches indicated that their relative LDL oxidation inhibition capacity varied five-fold among the varieties assayed (Chang et al., 2000) but nothing is known about the LDL oxidation inhibition capacity of fresh market peach, nectarine, or plum cultivars.

Little has been done to promote the health benefits of peaches, nectarines or plums as has been done with grapes, prunes, cranberries, cherries and many other crops. In part, this is due to the lack of specific information about the health benefits of the phytochemicals in these fruit. The ongoing project in the Department of Horticultural Sciences at Texas A&M University has been developing this information and has already screened about a hundred peach, nectarine, and plum genotypes with flesh colors ranging from white to yellow to orange to red for their anti-oxidant activity, total phenolics, and total anthocyanins (Cevallos et al., 2005; Vizzotto et al., in preparation). These studies found that the antioxidant activity of some plums overlapped that of blueberry, a small fruit touted for its high level of antioxidant activity. In addition, the group of phytochemicals best correlated with antioxidant activity were the phenolic acids. More recent work in our group also indicates the importance of the phenolic acids in the inhibition of breast cancer cell proliferation and on DNA methylation which is one of the mechanisms that control the cell cycle, an essential component of cancer development.

Thus far, most of the fruit genotypes tested have been breeding selections and not commercial varieties. Thus this proposal extends this work to important commercial peach, nectarine and plum varieties grown in California. The first step is to determine the total phenolics, total anthocyanins, and anti oxidant activity of 26 selected varieties. The methanolic extracts of these will then be tested for two other properties: ability to inhibit the proliferation of breast cancer cell lines as well as its ability to oxidize LDL, which is related to the avoidance of cardiovascular disease. This data will be also analyzed to determine whether fruit color, antioxidant activity or phytochemical levels are related to the breast cell antiproliferative or LDL oxidation inhibition activity.

The long range plan for this research is to further characterize these fruit varieties for their bioactive properties both relating to their effects on cancer proliferation and the development of cardiovascular disease. In parallel we will begin to investigate how various cultural, harvest and post harvest practices affect the levels of the phytochemicals and their bioactive properties of stone fruit as well as more in depth studies on the mechanisms involved in the inhibition of cancer cell proliferation.

Objectives

1. Determine the total phenolic, and anthocyanin content as well as the antioxidant activity of a range of California produced peach, nectarine and plum varieties (completed).
2. Determine the anti proliferation activity the methanolic extract of the phenolics of the specific varieties have on breast cancer cell lines (ongoing).
3. Determine the LDL oxidation inhibition that these extracts elicit (ongoing).

Plans & Procedures

Two layer boxes of 26 stone fruit varieties were be obtained from packing houses in California (Table 1) and sent via next day delivery to the Department of Horticultural Sciences. Upon arrival in the lab at Texas A&M University, the samples were stored at 2-5ºC until use (less than 5 days). For each variety a sample of uniform fruit had the stones removed and then divided into three groups. These were packaged separately and then frozen at -20ºC until analysis. The phytochemical and bioactive analyses began once all the samples had been collected. This project consists of three phases: phytochemical analyses, antiproliferation screening and LDL oxidation inhibition screening of the 26 varieties. Since the analysis began in September, this report will only cover the first phase of this study. The two other phases should be completed by April of 2007.

Total phenolics. Phenolics were quantified by the Folin-Ciocalteau method (Cevallos-Casals and Cisneros-Zevallos 2003). Five g of fresh tissue (flesh plus skin) was mixed with 25 mL of methanol in a conical screw-cap tube using a vortex mixer. These samples were stored overnight at 4°C and then centrifuged for 20 min at 29,000 g_n. at 2ºC in a centrifuge (Mod. J2-21, Beckman Instruments Inc.). A 0.5 mL aliquot from the prepared sample was diluted with 8 mL of nanopure water. At the same time, a blank containing 0.5 mL of methanol was diluted and analyzed. Each sample, and the blank, was combined with 0.5 mL of 0.25N Folin-Ciocalteau reagent, and allowed to react for 3 min and then 1mL 1N Na2CO3 was added. The reaction mixture was incubated for 2 h at room temperature and absorbance measured at 725 nm. If the measurements were above 0.6 absorbance unit (AU), the samples were diluted and reanalyzed. The concentration of total phenolics was estimated from a chlorogenic acid standard curve in terms of mg of chlorogenic acid equivalents.

Table 1. Stone fruit varieties collected for shipment to Texas A&M University in 2006 for an analysis of their phytochemical content and several bioactive properties.

Date	Variety	Fruit size (gm)	Soluble solids (%)
6/01	Spring Snow	150	13
6/06	Crimson Lady	155	11
6/13	Arctic Star	140	15
6/22	June Pearl	160	19
6/22	Rich Lady	180	15
6/22	Honey Blaze	170	20
6/22	Galaxy	200	15
6/23	Spring Bright	160	15
6/23	Black Splendor plum	130	13
7/06	Blackamber plum	66	14
7/06	White Lady	260	14
7/14	Crimson Glo	125	16
7/14	Sugar Giant	210	13
7/18	Summer Sweet	180	14
7/18	Elegant Lady	240	15
7/18	Summer Bright	180	15
7/18	Sweet Dream	260	15
7/27	Fire Pearl	150	12
8/03	Friar plum	110	12
8/03	Summer Fire	140	12
8/17	Red Jim	170	13
8/21	O'Henry	150	12
8/21	Arctic Pride	190	14
8/31	Black Kat plumcot	90	15
9/10	Angeleno plum	90	14

Total anthocyanins. Total anthocyanin content analysis was adapted from Fuleki and Francis (1968) by measuring the absorbance of extracts at pH 1 (Cevallos-Casals and Cisneros-Zevallos, 2003) after removing carotenoids with hexane. Anthocyanins are expressed as mg cyanidin 3-glucoside equivalents/100 g fresh or dry weight, using a molar extinction coefficient of 25 965 M^{-1} cm^{-1} and a molecular weight of 449 g/mol (Abdel-Aal & Hucl, 1999).

Antioxidant activity. Antioxidant activity was quantified by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical method (Brand-Williams et al., 1995). Five g of fresh tissue (flesh plus skin) was homogenized with 25 mL of methanol in a conical screw-cap tube using a vortex mixer. The samples were stored overnight at 4°C and then centrifuged for 20 min at 29,000 g_n. at 2ºC in a centrifuge (Mod. J2-21, Beckman Instruments Inc.). Before running the reaction, the

spectrophotometer is blanked with methanol, and DPPH was diluted with methanol from a mother solution to reach an absorbance of 1.1 AU at 515 nm. Then 150 µL of sample was combined with 2850 µL of the DPPH solution. The samples and the blank were allowed to react for 24 h after which the absorbance was measured in a quartz cuvette at 515 nm. If the absorbance was below 0.2 AU the samples were diluted with methanol and reanalyzed. The antioxidant activity was estimated as equivalents of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) by comparison to a standard curve.

Results and Discussion

First phase: Chemical analysis and preparation of the extracts. A total of 10 peach, 10 nectarine and 6 plum/pluot varieties were obtained from California packing houses (Table 1) in two layer tray boxes beginning on the 1st of June until mid September. Upon arrival in the laboratory at Texas A&M University, the samples were photographed, weighed, checked for soluble solids and then frozen. After all the samples were collected, the phenolics were extracted and analysis of their phytochemical content and bioactive properties was initiated.

Phytochemical concentrations. Among the California peach and nectarine varieties assayed in this study the total phenolics concentration ranged from ~45 to ~175 mg chlorogenic acid/100 g fresh weight for the peaches and nectarines and ~380 to ~640 mg chlorogenic acid/100 g fresh weight for the plums (Table 2 and Figure 1). Thus as previously shown with other varieties, there are significant differences in the total phenolics among peach, nectarine, and plum varieties. Within these commercial varieties there is a 3-4 fold difference in total phenolics among peach and nectarines and a two fold difference in total phenolics among the plums. In addition, the general level of total phenolics among the plums is greater than the yellow and white fleshed peach and nectarine genotypes. As compared to the previous work in the laboratory, the levels of total phenolics is a little lower (Table 2).

The anthocyanin concentration among the white and yellow fleshed peach/nectarine varieties was similar to the levels previously reported but among the plums the levels in the present study were lower (~15 to ~95 mg cyanidin 3-glucoside/100 g fresh tissue) than the previous study (~2 to ~375 mg cyanidin 3-glucoside/100 g fresh tissue) (Vizzotto et al., 2006) (Table 3). As expected, the greatest concentration of anthocyanins is in the Black Splendor plum, the only red-fleshed stone fruit in this study (Figure 2).

These lower levels of total phenolics and anthocyanins as compared to the concentrations reported by Vizzotto et al. (2006) are, in part, due to differences in the varieties that were assayed (Vizzotto et al., 2006) but probably also in the stage of maturity, post harvest handling, and growing/cultural conditions under which the fruit was produced. . The only report with peaches, nectarines, and plums looked at the changes of ripening fruit picked at the firm ripe stage but not the effect of picking fruit at different maturity stages (Tomas-Berberan et al., 2001).. Research with cherries (another stone fruit), blackberry, raspberry, and strawberry has shown differences in phenolics and especially anthocyanin levels in response to storage, ripening stage of harvest, and the year of harvest (Gonçalves et al., 2004a; 2004b; Wang and Lin, 2000; Serrano et al., 2005). These aspects of phytochemical development in stone fruit needs to be further studied to best manage these fruit to maximize their health benefits.

Table 2. Total phenolics concentration (mg chlorogenic acid equiv/100 g fresh weight) in stone fruit in two studies.

	Current work	Vizzotto et al., 2006
Peach/nectarine		
White flesh	~70 - ~175	137 - 371
Yellow flesh	~45 - ~120	158 - 250
Red flesh	--	228 - 1260
Plums	~380 - ~640	182 - 898

Table 3. Total anthocyanin concentration (mg cyanidin 3-glucoside/100 g fresh weight) in stone fruit in two studies.

	Current work	Vizzotto et al., 2006
Peach/nectarine		
White flesh	~0.5 - ~2.0	1.5 – 6.8
Yellow flesh	~1.0 - ~10.0	1.5 – 5.0
Red flesh	--	45 – 266
Plums	~15.0 - ~95.0	2.4 – 375

As was seen in previous studies, the total phenolics concentration was well correlated with antioxidant activity whether it was measured by the DPPH or the ORAC method (Table 4). Thus the varieties differed significantly in antioxidant activity (Figure 3). Among the peaches, Galaxy, Spring Snow and O'Henry had the highest antioxidant levels (~1,600 to 2,200 DPPH ug Trolox/g fresh weight) and Crimson Lady and Summer Sweet the lowest (~400 to ~500 DPPH ug Trolox/g fresh weight) and among the nectarines the highest antioxidant activity was in the varieties Arctic Star, June Pearl and Fire Pearl (~1,000 to ~1,200 DPPH ug Trolox/g fresh weight) and the lowest were in the varieties Summer Bright and Honey Blaze (~300 to ~400) DPPH ug Trolox/g fresh weight). The antioxidant activity in the plums was higher than that seen among the peaches or nectarines with a range from ~2,200 to ~8,000 DPPH ug Trolox/g fresh weight. All these are comparable to the previous study.

Table 4. Correlations (R^2) between total phenolic concentration and antioxidant activity as measured by the DPPH and ORAC methods.

	DPPH	ORAC
Peach	0.96	0.83
Nectarine	0.73	0.90
Plum	0.83	0.79

Summary

There were clear differences among the 10 peach, 10 nectarine, and 6 plum/pluot varieties in their content of phenolics and anthocyanins as well as their anti oxidant activity. As has been previously reported the antioxidant activity was well correlated with total phenolic concentration. In general the plums had greater levels of phenolics, anthocyanins, and antioxidant activity as compared to the peaches or nectarines. Differences in the level of phenolics and anthocyanins seen in these samples as compared to a previous study indicate that other factors such as the cultural and environmental conditions under which the fruit is produced, the maturity state at harvest, and the post harvest handling protocols may affect the levels of fruit phytochemicals. These factors need to be further studied. The second part of this project, the measurement of the antiproliferation activity in a breast cancer system and the inhibition of LDL oxidation of these 26 stone fruit varieties should be finished by April of 2007.

The long term objective of this research program is to document the health benefits of stone fruit consumption and to understand the management and other conditions to maximize these health benefits in the stone fruit produced for consumption. The specific objectives are the following:

1 Characterize the phytochemistry and the bioactive properties of these phytochemicals related to both the cancer and cardiovascular disease development.
2. Characterize the variability of the phytochemicals and their bioactive properties as it relates to genetics (varieties) and the environment (cultural management, harvest maturity, post harvest management and climatic/edaphic conditions).

Figure 1. Total phenolic concentration for 10 peach, 10 nectarine, and 6 plum varieties harvested in California.

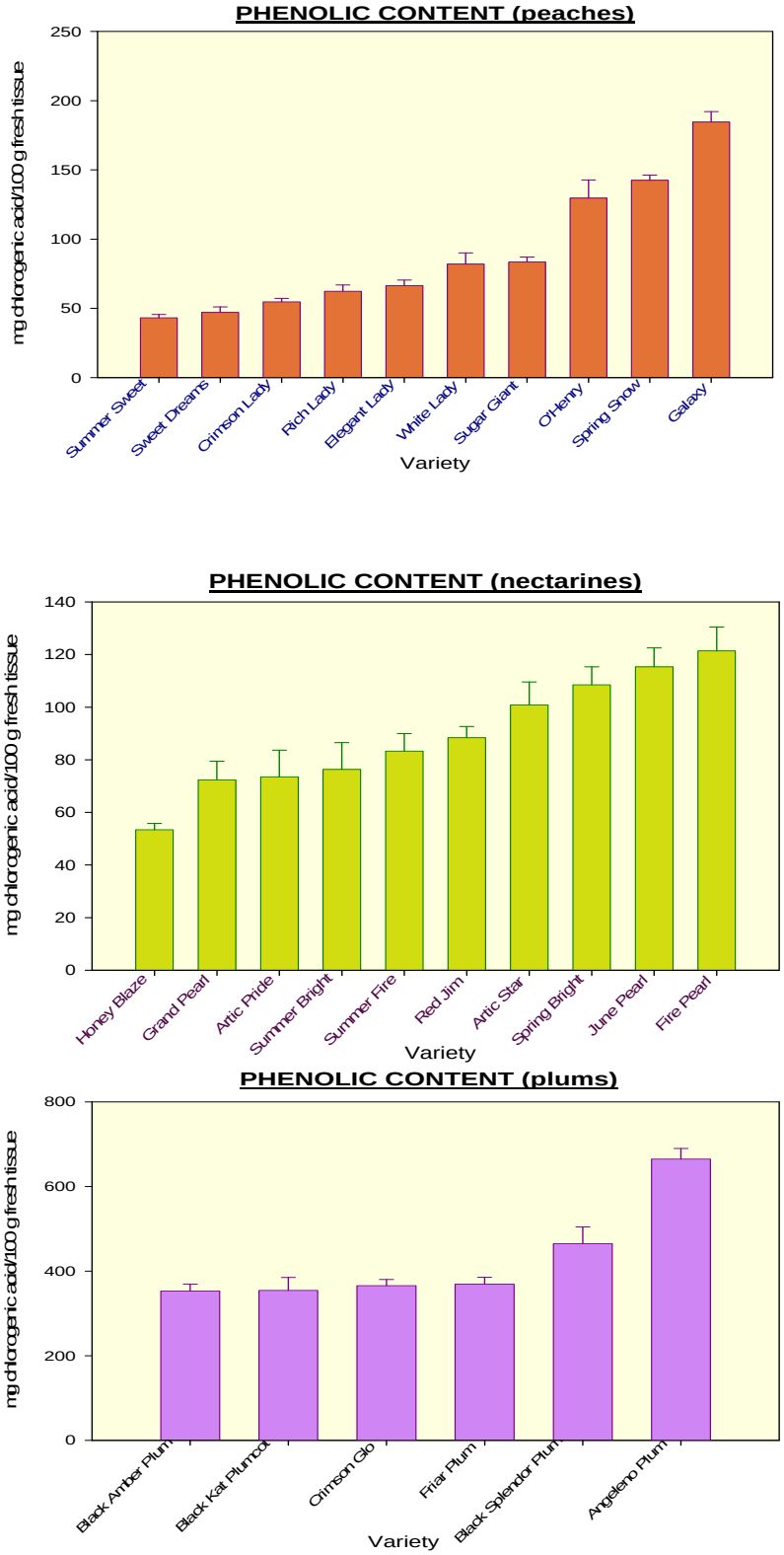


Figure 2. Anthocyanin content of 10 peach, 10 nectarine, and 6 plum varieties harvested in California.

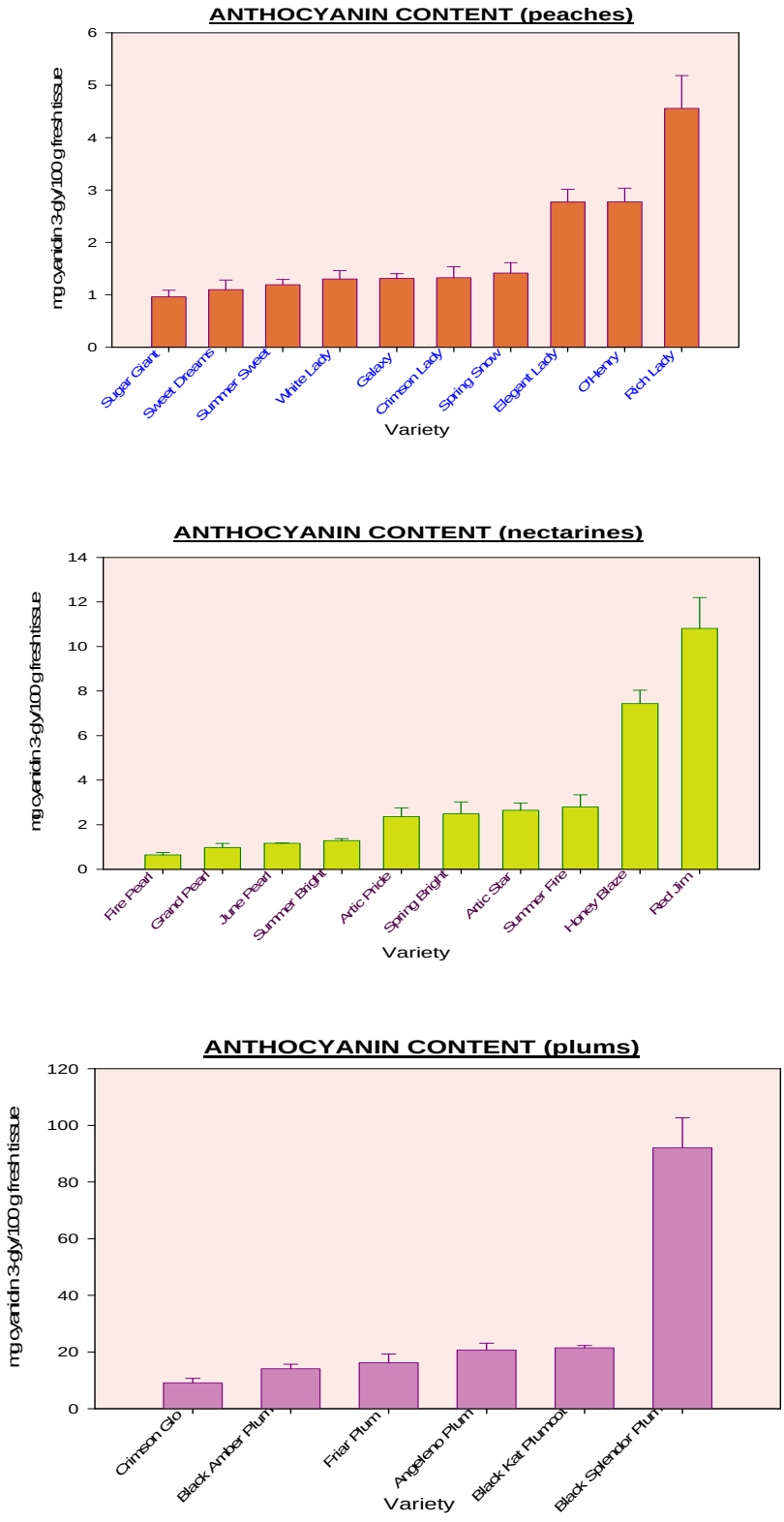
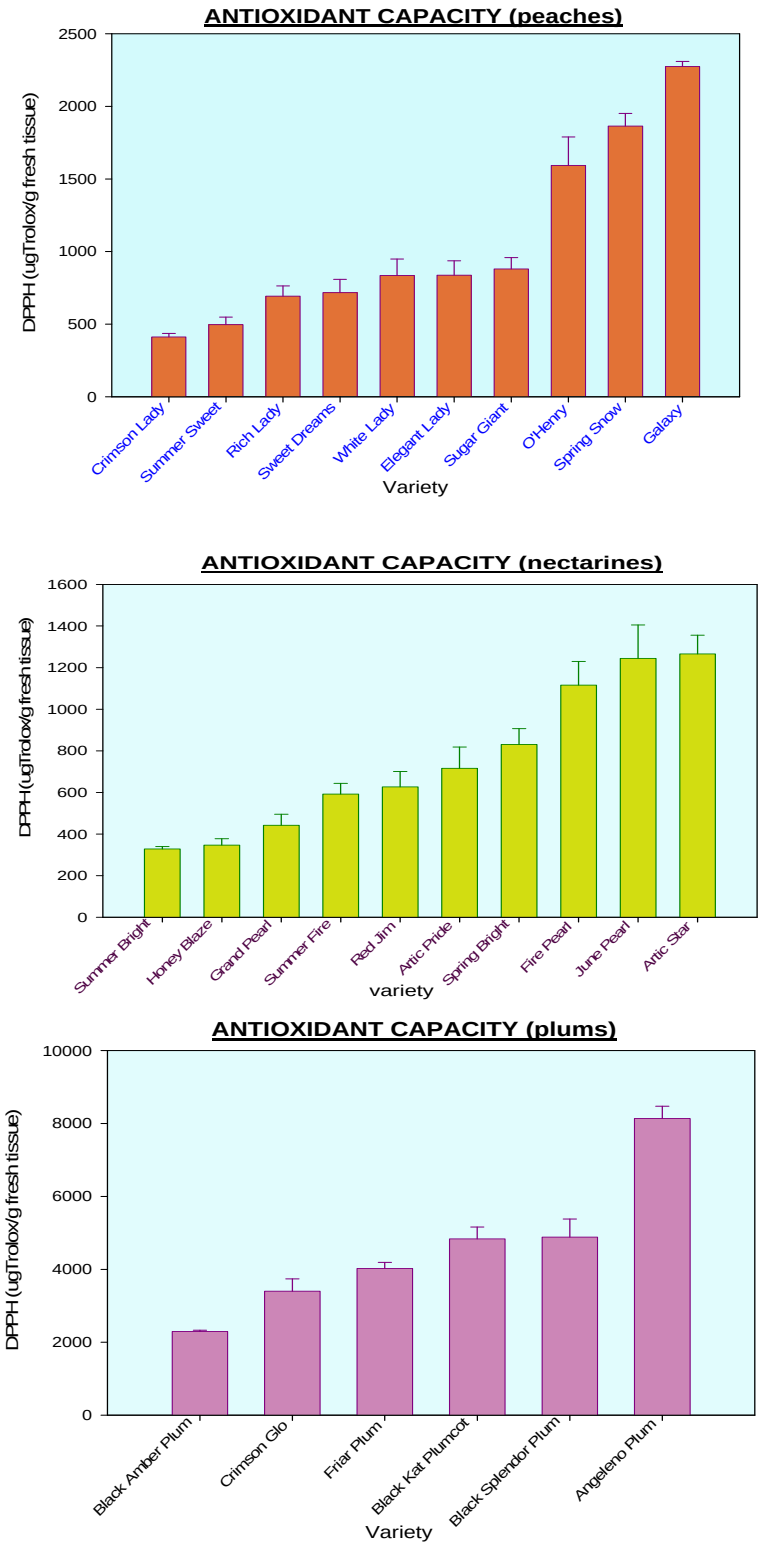


Figure 3. Antioxidant activity as measured by the DPPH procedure of 10 peach, 10 nectarine, and 6 plum varieties harvested in California.



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