Cardiovascular Health Benefits of Peaches and Plums

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ABSTRACT

We have confirmed that the phenolics concentration of the fruit extracts is well correlated to the antioxidant activity in peach, nectarine, and plum. Plums can contain 2 -15 times more phenolics and up to 26 times the antioxidant activity of either peaches or nectarines. *In fact, plums contain as much or even more antioxidant activity as the blueberry, a fruit touted as a super fruit rich in antioxidants.*

LDL oxidation inhibition, an important factor in the prevention of cardiovascular disease, varies widely with the peach, nectarine or plum variety assayed and the ability of the variety to inhibit LDL oxidation was not related to their antioxidant activity. This appears to be caused by the fact that LDL oxidation inhibition by phenolics involves multiple mechanisms such as radical scavenging and phenolic-LDL complexing.

Only two varieties (Arctic Pride and Spring Bright) had little to no ability to inhibit LDL oxidation. Excellent LDL oxidation inhibition was noted for one plum (Angeleno), two nectarines (Honey Blaze and Red Jim), and seven peaches (O'Henry, Spring Snow, Galaxy, Summer Sweet, Sweet Dream, Crimson Lady, and Elegant Lady). In contrast to the situation with antioxidant activity and total phenolics, there are multiple varieties of peaches and nectarines that are much better than most of the plums in their ability to inhibit the oxidation of LDL.

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). Orange-fleshed peaches have the carotenoids β-carotene and β- cryptoxanthin both which have vitamin A activity (Tourjee et al., 1998). Several hydroxycinnamates, flavan 3-ols and flavonols, predominantly chlorogenic acid, neochlorogenic acid, catechin, epicatechin, and quercetin 3-rutinoside, have been identified in peaches and

plums (Tomás-Barberán et al., 2001; Kim et al., 2003a). Plums contain large amounts of phytochemicals such as flavonoids and phenolic acids that may act as natural antioxidants in our diet (Wang et al., 1996), which in turn may provide health-promoting effects to consumers (Kim et al., 2003b).

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 some plums match and exceed the levels found in blueberry (Wang et al., 1996; Prior et al., 1998; Cevallos et al., 2003; 2005; Vizzotto et al., 2007). 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; Vizzotto et al., 2007). 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 cancer cell proliferation or LDL oxidation, suggesting that there is a specific phenolic compound or a class of phenolics that is responsible for the antiproliferative activity (Sun et al., 2002; current work at Texas A&M University).

Reduced levels of cardiovascular disease has also been shown associated with the consumption of plant foods rich in flavanoids 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 prunes (Stacewisz-Sapuntzakis et al., 2001; Donovan et al., 1998), processing peaches (Chang et al., 2000) and more recently with fresh market California peach, nectarines, and plums (current work at Texas A&M University), has shown that although these had the ability to inhibit LDL oxidation, their relative inhibition capacity varied considerably among the varieties assayed. Another critical component in the development of atherosclerosis is the proper circulation of human platelets. It has been found that any increase in the aggregation of platelets is associated with enhanced atherogenecity (Aviram, 1992; Aviram, 1995; Sinzinger, 1986).

The health benefits of fruits and other produce always seem to be in the news (Variyam and Golan, 2002). As the public becomes more aware of the health benefits of fruits and is being told to eat a colorful diet there is a potential to create a new market for cultivars specifically developed for their health benefits. Recent work has shown that carotenoids (orange/yellow pigments), anthocyanins (red pigments), and general phenolics (colorless) found in peaches have antioxidant properties that have protective properties against various pathological conditions such as inflammation, cancer, atherosclerosis, and other circulatory problems (Cevallos-Casals et al., 2005; Gil et al., 2002; Prior and Cao, 2000; Tomas-Barberan et al., 2001; Wargovich, 2000; Vizzotto et al., 2007). The

healthy-for-you marketing approach is being pursued aggressively to increase consumption of some fresh products but no stone fruit cultivars have been developed specifically for higher levels of these phytochemicals. Such “health enhanced” cultivars would provide a new product that could be sold fresh or processed (total crop or as an outlet for the cull fruit) into extracts that are natural sources of antioxidants, antimicrobials, and colorants (Byrne, 2005).

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., 2007). 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

OBJECTIVES

The long term objective of this research is to document the cardiovascular health benefits and the benefits of stone fruit consumption on the prevention of obesity. This three year project will involve one full time graduate student who will characterize stone fruit bioactive properties related to human LDL oxidation inhibition, human platelet aggregation inhibition, inhibition of adipogenesis in *in vitro* human fat cells, vasodilation properties and *in vivo* studies with obese Zucker rats. The yearly objectives are as follows:

The first year objectives for this research are the following:

1. Determine the total phenolic, and anthocyanin content as well as the antioxidant activity of a methanolic extracts of a range of peach, nectarine and plum varieties.

2. Determine the Human LDL oxidation inhibition properties that these extracts elicit.

MATERIALS AND METHODS

Plant materials

Twenty peach, nectarine and plum varieties were collected in California with the collaboration of the CTFA and Dr. David Ramming (USDA, ARS, Parlier, CA) at mature firm stage, packed in boxes and sent via overnight mail to Texas A&M University. Once the pits were removed, the samples were stored at -20 °C.

Total Phenolics Content

Total soluble phenolic content analysis was adapted from Swain and Hillis (23). The sample was homogenized with methanol. Tubes were capped and stored for 20-72 h at 4 °C. Extracts were centrifuged at 29 000g for 15 min. A 0.5 mL sample (0.5 mL water for the blank) was taken from the clear supernatant and diluted with 8 mL of nanopure water. A 0.5 mL aliquot of 0.25 N Folin-Ciocalteu reagent was added and allowed to react for 3 min; then, 1mL of 1 N Na_2CO_3 was added and allowed to react for 2 h. Spectrophotometric readings at 725 nm were taken. Total phenolics were expressed as mg chlorogenic acid equivalent/100 g fresh weight based on a standard curve.

Anthocyanin Content

Total anthocyanin content was adapted from Fuleki and Francis (1968) using the pH 1 method. A sample of 5 g was homogenized with 20 g of solvent (85:15, 95% ethanol:1.5N HCl). Tubes were stored for 24 h at 4°C. After centrifugation and filtration samples were added half its volume of hexane and shaken vigorously to remove carotenoids. Spectrophotometric readings at 535 and 700 ηm were taken. Anthocyanins were expressed as mg cyanidin 3-O-β-glucopyranoside (cyanidin 3-glucoside) equivalent per 100 g of fresh weight using a molar extinction coefficient of 20,941 and a molecular weight of 484.84.

Antioxidant activity

The DPPH assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20 °C until needed. The working solution was obtained by mixing 10mL stock solution with 45μL methanol to obtain an absorbance of 1.1 units at 515 nm using the spectrophotometer. Fruit extracts (150 μL) were allowed to react with 2850 μL of the DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. Results are expressed in μg TE/g fresh weight.

The ORAC procedure used an automated plate reader (KC4, Bio Tek, USA) with 96-well plates (Prior et al., 2003). Analyses were conducted in phosphate buffer pH 7.4 at 37 °C. Peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation at 485nm and emission at 520nm. The standard curve was linear between 0 and 50 mM Trolox. Results are expressed as μm TE/g fresh weight.

Antioxidant Activity Upon LDL Oxidation Evaluated By TBARS Assay

Isolation of LDL

Human plasma were obtained from Fisher Scientific Int. (Winnipeg, MB., Canada) in presence of 0.01%EDTA. LDL (1.019-1.063 g/L) was isolated by sequential density ultracentrifugation according to Schonfeld (1983). Briefly 2 mL of plasma was added to a centrifugation tube containing 4 mL NaCl (1.0063 g/L) and 30 μL of 1.5% (w/v) dithionitrobenzoic acid (Sigma Chemical, St. Louis, MO) and centrifuged at 40,000 rpm for 18 h at 4 °C, then 2 mL were discarded and corrected with 2 mL NaBr of 1.1416 g/L

for 18 h at 4 °C. After isolation, LDL was dialyzed in 0.01 M phosphate-buffered saline (PBS, pH 7.4) to removed EDTA and other interfering compounds. The protein content was measured using the Bradford reagent (Sigma Chemical, St. Louis, MO).

LDL Oxidation

LDL (75 μg/mL) was diluted in 0.01 M PBS pH 7.4 and incubated at 37 °C in presence of 5 mM AAPH for oxidation. The AAPH which is an inducer of the oxidation reaction was dissolved in PBS. A non-oxidized LDL sample, incubated in absence of AAPH constituted the blank control.

Protein Content

Protein content in purified fraction of LDL was quantified according to the Bradford method (Bradford 1976). A sample (50 μL) was taken and mixed with Bradford reagent (1500 μL) and finally read the absorbance at 595 nm in a spectrophotometer. The total protein concentration (mg/L) was expressed on the basis of a standard of BSA.

Thiobarbituric Acid Reactive Substances (TBARS)

Assay was performed according to the procedures of Wallin *et a*l. (1993). To each tube containing 0.55 mL of the incubated LDL (75 μg/mL, 37°C by 6 h) in the presence of 2,2'-Azobis(2-amidino-propane)-dihydrochloride solution AAPH (5 mM) was added 0.5 mL of 25% (w/v) trichloroacetic acid (TCA) to denature protein. After the samples had been centrifuged (10,000 rpm) at 10 °C for 30 min to remove pellets, 0.5 mL of 1% thiobarbituric acid (TBA) in 0.3% NaOH was added to the supernatant, and the mixed reagents reacted at 90- 95 °C in a water bath for 40 min. After completion of the reactions, samples were detected with excitation at 532 nm and emission at 600 nm in a Synergy HT 96-well fluorescence plate reader and the KC4 software (Bio-Tek® Instruments).

Percent inhibition (%Inhibition) of the formation of malonaldehyde was used as a parameter to compare antioxidant capacity. It is calculated according to the equation:

$$(\%\text{Inhibition}) = [(C - S)/C] \times 100$$

where *C* is the amount of malonaldehyde formed in the control (no antioxidant added) and *S* is the amount of malonaldehyde formed when antioxidant was present. The sample concentration that led to 50% inhibition, IC50, is used to compare the capacities of different antioxidants

Conjugated Dienes.

The formation of conjugated dienes was measured by determining the absorbance increase at 234 nm of the solution of LDL (100 μg protein/mL) in PBS incubated with 5 mM AAPH in the absence or presence of phenolic compounds from Salvadorians crops (5 uM CGAE). The absorbance was measured every 5 min for 420 min using a Synergy HT UV-VIS spectrophotometer, and the results were expressed as relative absorbance at 234 nm. The duration of the lag phase was defined as time (min) to the intercept of the tangent of the absorbance curve in the propagation phase with the baseline.

Statistical Analysis

For all the experiments three samples were analyzed and all the assays were carried out in triplicate. The results are expressed as mean values and standard error or standard deviation (SD). Results were processed by using the one-way variance analysis (ANOVA). Differences at $p < 0.05$ were considered to be significant. SPSS software (SPSS Inc. 2006) was used to run all the statistical analysis.

RESULTS AND DISCUSSION

Total phenolic content for different varieties of peach, nectarine and plums ranged from ~ 40-170, 50-120 and 350-650 mg chlorogenic acid/100g fw, respectively, while the total anthocyanin content ranged from ~ 1- 4.5, 0.5- 10 and 10 – 90 mg cyanindin 3-gly/100g fw, respectively (Tables 1a, 1b, 1c). Peach varieties that showed higher phenolic content included Galaxy, O'Henry and Spring Snow, while for nectarines, varieties high in phenolic content included Fire Pearl, June Pearl and Spring Bright. Angeleno and Black Splendor had the highest phenolic content among the plum varieties.

In relation to varieties high in anthocyanin content, these included Rich Lady, Red Jim and Black Splendor for peach, nectarine and plum fruit, respectively. Black Splendor plum was the only variety among the fruits studied that showed both high phenolic and anthocyanin content (~ 0.2 anthocyanin/total phenolic ratio).

The antioxidant activity based on the DPPH assay for peach, nectarine and plums ranged from ~ 450- 2300, 300 – 1200 and 2000 – 8000 ug Trolox/g fw, respectively (Tables 1a, 1b, 1c). The values obtained for plums are higher or similar to those reported previously for blueberries. In general, the varieties in each type of fruit with higher antioxidant activity followed similar trend to those observed in total phenolic content.

The specific antioxidant activity using the DPPH assay was calculated for all varieties and types of fruits studied (Tables 1a, 1b, 1c). The specific antioxidant activity expressed on phenolic basis, determines the antioxidant activity of the specific profile of phenolic compounds present in each variety tested. This means that fruits that contain a mixture of phenolics with higher specific antioxidant activity will have higher potency to scavenge free radicals. In contrast fruits that contain a mixture of phenolics with lower specific antioxidant activity will have lower potency to scavenge free radicals.

For peaches, nectarines and plums the specific antioxidant activity ranged from ~ 700 - 1400, 400 – 1200 and 625 – 1100 ug Trolox/mg chlorogenic acid, respectively. Sweet Dream, Arctic Star and Black Kat plum varieties were the fruits that showed higher specific antioxidant activity among peach, nectarine and plums, respectively. These fruits have profiles of phenolic compounds with potent antioxidant activity.

The antioxidant activity based on the ORAC assay for peach, nectarine and plums ranged from ~ 4 - 17, 4.5 – 11.5 and 15 – 62.5 uM Trolox/g fw, respectively (Tables 1a, 1b, 1c). In general, the varieties in each type of fruit with higher antioxidant activity using

the ORAC assay followed similar trend to those observed using the DPPH assay and in total phenolic content.

The specific antioxidant activity using the ORAC assay was calculated for all varieties and types of fruits studied (Tables 1a, 1b, 1c). For peaches, nectarines and plums it ranged from ~ 5.5 - 12, 6.5 – 9.5 and 4 -9 uM Trolox/mg chlorogenic acid, respectively. Once again Sweet Dream and Black Kat plum varieties were the fruits that showed higher specific antioxidant activity among peach and plums, respectively. For nectarines Arctic Star as well as other 6 varieties (including Spring Bright) showed higher and similar specific antioxidant activity.

Table 1a. Phenolic content, anthocyanin content and antioxidant capacity of peaches.

Variety	Total phenolics[a]	Total Anthocyanins[b]	DPPH[C]	ORAC value[d]	Specific DPPH[e]	Specific ORAC[f]	%LDL oxidation inhibition [g]
Summer Sweet	43.2 ± 2.4 def	1.2 ± 0.1 b	778 ± 52 def	4.4 ± 0.4 f	1808 ± 200 a	13.6 ± 1.7 b	48.6 ± 4.0 abc
Sweet Dream	47.0 ± 4.0 f	1.1 ± 0.2 b	516 ± 92 f	5.9 ± 0.5 def	1093 ± 117 d	18.9 ± 4 a	43.1 ± 0.7 abc
Crimson Lady	54.7 ± 2.6 def	1.3 ± 0.2 b	804 ± 24 def	4.8 ± 0.3 ef	1478 ± 160 abcd	8.7 ± 0.1 cd	49.1 ± 3.4 ab
Rich Lady	62.3 ± 6.2 f	3.2 ± 0.6 a	693 ± 70 f	4.9 ± 0.4 ef	1111 ± 131 cd	7.8 ± 0.5 cd	38.7 ± 2.2 bcd
Elegant Lady	66.3 ± 4.1 ef	2.8 ± 0.2 ab	719 ± 99 ef	3.7 ±0.2 f	1089 ± 70 d	5.6 ± 0.5 de	42.6 ± 2.3 abcd
White Lady	82.0 ± 8.0 dce	1.3 ± 0.2 b	1229± 112 cde	7.5 ±0.5 cde	1501 ± 123 abc	9.2 ± 0.9 bcd	30.9 ± 1.7 d
Sugar Giant	83.4 ± 3.6 dc	1.0 ± 0.1 ab	1274 ± 78 dc	9.2 ± 0.2 bc	1533 ± 91 ab	11 ± 0.9 bc	37.9 ± 3.4 cd
O'Henry	129.6 ± 13.1 bc	2.8 ± 0.3 ab	1729± 196 bc	11.5 ± 1.2 b	1322 ± 114 bcd	8.8 ± 0.4 cd	50.0 ± 0.3 ab
Spring Snow	142.5 ± 3.7 ab	1.4 ± 0.2 b	2253 ± 147 ab	8.7 ± 0.4 bcd	1580 ± 35 ab	3 ± 0.3 e	54.3 ± 2.2 a
Galaxy	184.6 ± 7.4 a	1.3 ± 0.1 b	2670 ± 62 a	17.3 ± 1.0 a	1450 ± 71 abcd	9.4 ± 0.5 bcd	50.0 ± 2.1 a

[a]Expressed in mg CGA/100 g fresh tissue. [b] Expressed in mg Cyanidin-3-glucoside Equivalent/100 g Fresh tissue. [c]Expressed in μg trolox Equivalent/100 g fresh weight. [d]Expressed as μm Trolox Equivalent/g fresh tissue. [e]Expressed in μg trolox Equivalent/ mg CGA. [f]Expressed in μm Trolox Equivalent/ mg CGA. [g]Expressed in μM CGA. Values with the same letter are not statistically different at the 5% level.

Table 1 b. Phenolic content, Anthocyanin content and Antioxidant capacity of Nectarines.

Variety	Total Phenolics[a]	Total Anthocyanins[b]	DPPH[C]	ORAC value[d]	Specific DPPH[e]	Specific ORAC[f]	IC50[g]
Honey Blaze	53.37 ± 2.4 c	7.43 ± 0.8 ab	734.19 ± 57 de	4.93 ± 0.2 de	1387 ± 221 ab	9.3 ± 1.6 ab	41.8 ± 1.5 ab
Grand Pearl	72.38 ± 7.1 bc	0.97 ± 0.1 bc	765.7 ± 100 cde	4.75 ± 0.3 e	1068 ± 15 b	6.6 ± 0.52 b	11.8 ± 1.4 c
Arctic Pride	73.52 ± 10.1 bc	2.36 ± 0.4 bc	851.9 ± 120 bcd	5.38 ± 0.8 de	1153 ± 41 bc	7.3 ± 0.7 b	3.7 ± 1.4 d
Summer Bright	76.34 ± 10.2 ab	2.32 ± 0.1 bc	318.7 ± 19 e	6.97 ± 0.7 cde	401 ± 24 c	6.5 ± 1.2 b	38.5 ± 1.5 b
Summer Fire	83.30 ± 6.6 abc	2.80 ± 0.5 b	982.73 ± 88 bdc	7.60 ± 0.6 cd	919 ± 189 bc	9.1 ± 0.6 ab	14.9 ± 1.7 c
Red Jim	88.40 ± 4.2 bc	10.81± 1.8 a	1013.1 ± 100 bcd	6.35 ± 0.2 cde	1143 ± 77 b	7.2 ± 0.2 b	48.8 ± 2.1 a
Arctic Star	100.84 ± 8.8 bc	2.65 ± 0.3 bc	1266.25 ± 90 abc	9.01 ± 0.4 bc	1810 ± 51 a	12.8 ± 3.4 a	39.1 ± 1.5 b
Spring Bright	108.47 ± 6.9 ab	2.48 ± 0.5 bc	1215.9 ± 130 abc	10.79 ± 0.5 ab	1123 ± 89 b	10.0 ± 1.2 ab	1.2 ± 1.0 d
June Pearl	115.29 ± 7.2 ab	1.16 ± 0.01 bc	1633 ± 276 a	10.92 ± 0.3 ab	1410 ± 92 ab	9.5 ± 0.6 ab	34.3 ± 2.1 b
Fire Pearl	121.38 ± 9.0 a	0.64 ± 0.1 c	1115.98 ± 113 ab	4.93 ± 0.2 a	1236 ± 47 ab	9.8 ± 0.2 ab	50.0 ± 0.2 a

[a]Expressed in mg CGA/100 g fresh tissue. [b] Expressed in mg Cyanidin-3-glucoside Equivalent/100 g Fresh tissue. [c]Expressed in μg trolox Equivalent/100 g fresh weight. [d]Expressed as μm Trolox Equivalent/g fresh tissue. [e]Expressed in μg trolox Equivalent/ mg CGA. [f]Expressed in μm Trolox Equivalent/ mg CGA. [g]Expressed in μM CGA. Values with the same letter are not statistically different at the 5% level.

Table 1 c. Phenolic content, anthocyanin content and antioxidant capacity of plums.

Variety	Total Phenolics[a]		Total Anthocyanins[b]		DPPH[c]		ORAC value[d]		Specific DPPH[e]		Specific ORAC[f]		%LDLoxidation inhibition[g]
Blackamber	352.91 ± 16	c	14.08 ± 1.6	b	2294.32 ± 36	cd	33.21 ± 1.5	b	653 ± 59	cd	9.41 ± 1.3	a	32.1 ± 2.7 b
Black Kat	354.32 ± 30	bc	21.49 ± 0.8	b	4834.18 ± 321	b	33.35 ± 0.9	b	1370 ± 93	a	7.0 ± 1	ab	30.8 ± 2.4 b
Crimson Glo	365.37 ± 15	bc	9.09 ± 1.6	b	3401.27 ± 338	d	15.53 ± 1.7	c	464 ± 16d	c	4.25 ± 0.6	b	19.1 ± 2.8 c
Friar	368.83 ± 16	bc	16.19 ± 3.0	b	4021.19 ± 168	bc	31.68 ± 3.4	b	1090 ± 17	ab	8.54 ± 1.0	a	28.2 ± 1.5 b
Black Splendor	464.91 ± 39	ab	88.29 ± 10.6	a	4883.07 ± 494	bc	40.03 ± 2.5	b	850 ± 89	bc	9.51 ± 1.6	a	32.9 ± 4.6 b
Angeleno	664.74 ± 25	a	20.68 ± 2.4	b	8135.50 ± 337	a	55.80 ± 2.8	a	1223.6 ± 20	ab	8.5 ± 1.1	a	50.0 ± 0.3 a

[a]Expressed in mg CGA/100 g fresh tissue. [b] Expressed in mg Cyanidin-3-glucoside Equivalent/100 g Fresh tissue. [c]Expressed in μg trolox Equivalent/100 g fresh weight. [d]Expressed as μm Trolox Equivalent/g fresh tissue. [e]Expressed in μg trolox Equivalent/ mg CGA. [f]Expressed in μm Trolox Equivalent/ mg CGA. [g]Expressed in μM CGA. Values with the same letter are not statistically different at the 5% level.

Linear correlation analysis indicated high correlations ($r^2 > 0.78$) between total phenolic content and antioxidant activity using the ORAC assay for group of varieties in each type of fruit (Figure 1). Similarly, high correlations were found between total phenolic content and antioxidant activity using the DPPH assay ($r^2 > 0.72$) (Figure 2).

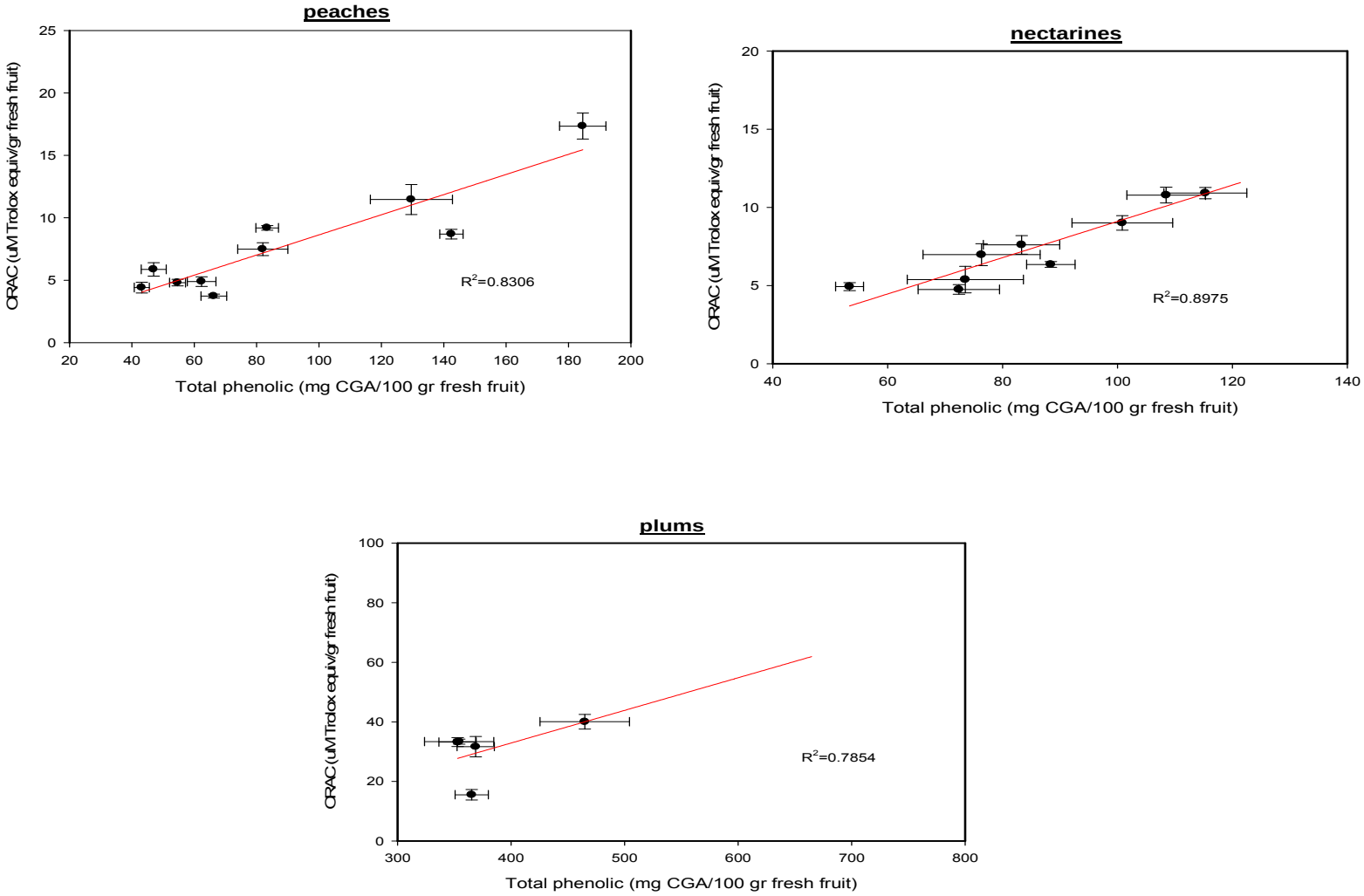

Figure 1. Phenolic content-Antioxidant activity (ORAC) linear correlations for different peach, nectarine and plum varieties.

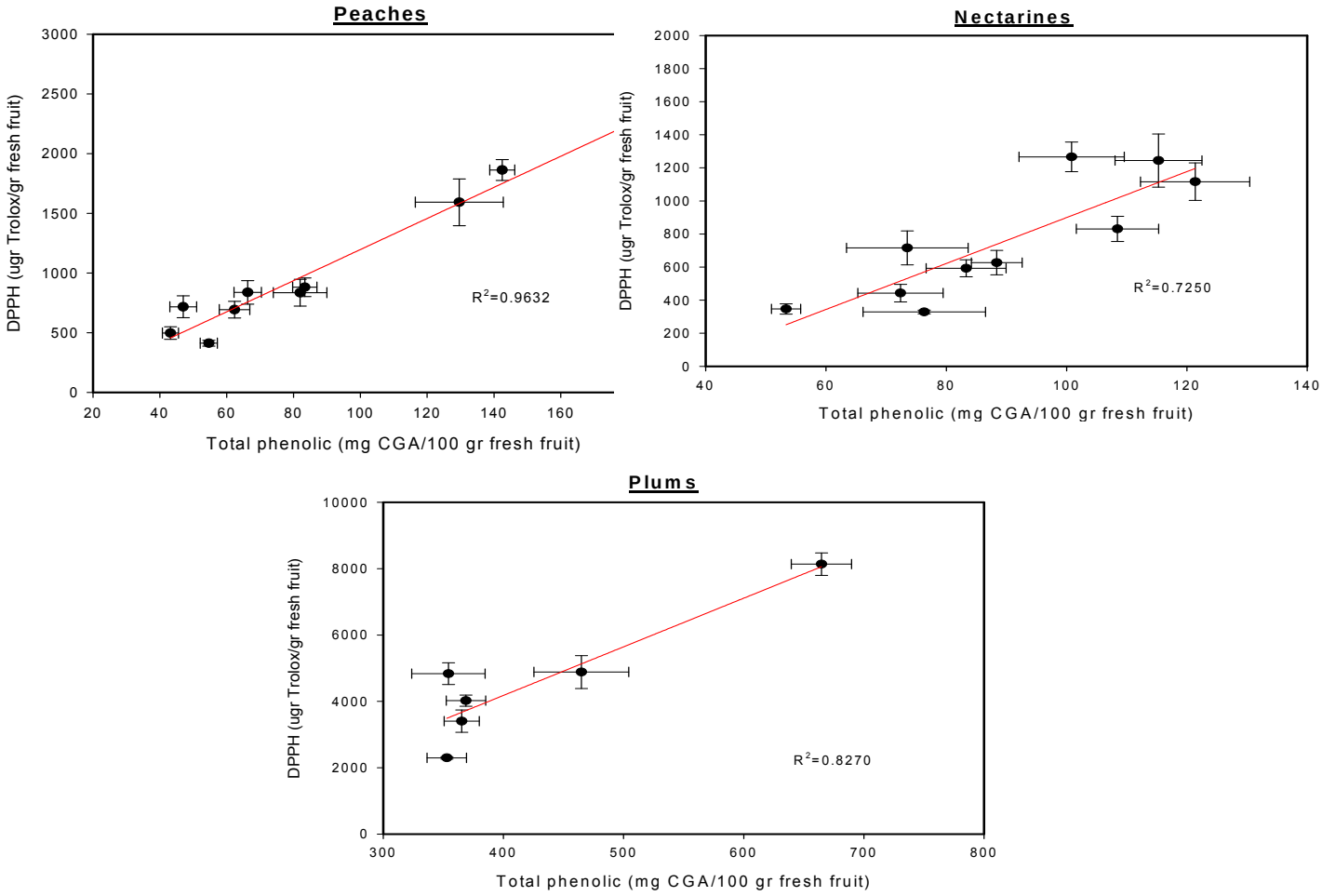

Figure 2. Phenolic content-Antioxidant activity (DPPH) linear correlations for different peach, nectarine and plum varieties

Next, we characterized the effects of stone fruit phenolic compounds on the inhibition of Human LDL oxidation. Galaxy peach, Fire Pearl nectarine and Angeleno plum were initially selected due to their high phenolic and antioxidant content (Figure 3). The IC50 values were obtained from curves of inhibition of LDL oxidation and concentration of phenolic compounds. The IC50 values represent the concentration of phenolic compound that induces a 50% inhibition of LDL oxidation. The IC50 values for the selected nectarine, peach and plum varieties were ~ 9.3, 11.7 and 13.7 µM chlorogenic acid, respectively. These IC50 values or phenolic concentrations were used to screen the inhibition of LDL oxidation for different varieties in each type of fruit studied.

Results indicated that the inhibition of Human LDL oxidation in different peach varieties ranged from ~ 30 – 55% (Table 1a), while in nectarines it ranged from ~ 1 – 50% (Table 1b). , and for different plum varieties the inhibition of Human LDL oxidation ranged from ~ 19 – 50% (Table 1c). This large variation in LDL oxidation inhibition is related to the type of phenolic compounds present in each type of fruit variety studied.

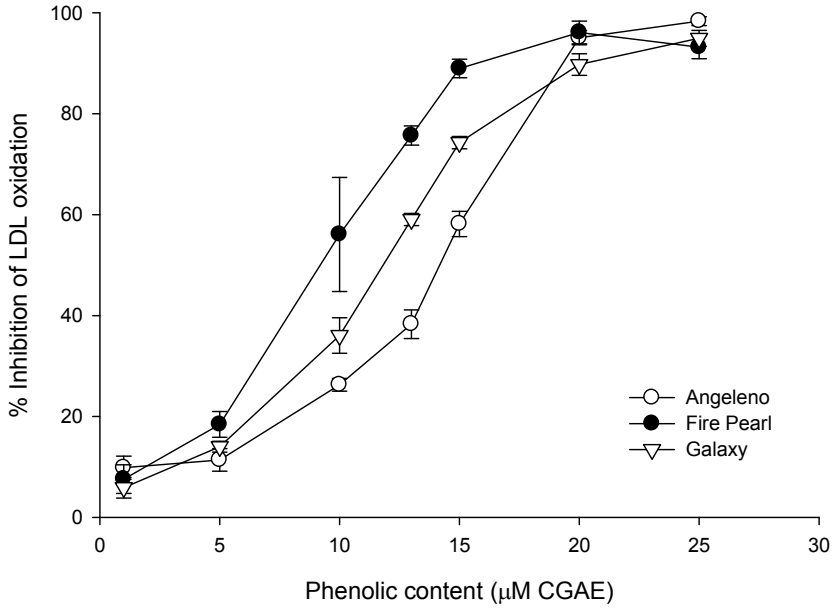

Figure 3. Effect of LDL incubation with Angeleno plum, Galaxy and Fire Pearl crude extracts on the susceptibility of LDL to oxidation. *LDL (100 μg protein/ mL) was incubated with 5 mM AAPH at 37 °C for 6 hrs with phenolic content (1-25 μM CGA). LDL oxidation was measured by TBARS assay. Results (means ±S.D of three experiments) are expressed as a percentage from control LDL that was not supplemented with the phenolic content from specific crude extract.

We observed that in peaches, Spring Snow and Galaxy varieties showed the highest inhibition, while White Lady variety the lowest. In nectarines, Fire Pearl and Red Jim varieties showed highest inhibition, while Spring Bright and Arctic Pride the lowest. Finally, in plums, Angeleno and Black Splendor showed highest inhibition while Crimson Glo the lowest.

When we compare the % LDL oxidation inhibition values with the specific antioxidant activity using ORAC (Figure 4a, 4b, 4c) we observe no clear trend. Since there is no apparent correlation, it is likely that antioxidant properties of phenolic compounds from stone fruits would not be the only mechanism by which phenolics inhibit Human LDL oxidation. An alternative to the radical scavenging properties of phenolics, may include a phenolic-LDL interaction which may inhibit or block the radical effects on oxidizing LDL (e.g., by reducing the oxidized tocopherol present in the LDL and reconstituting its antioxidant properties). The possible mechanism of inhibition of LDL oxidation was explored using the kinetic trend of conjugated dienes formation on LDL.

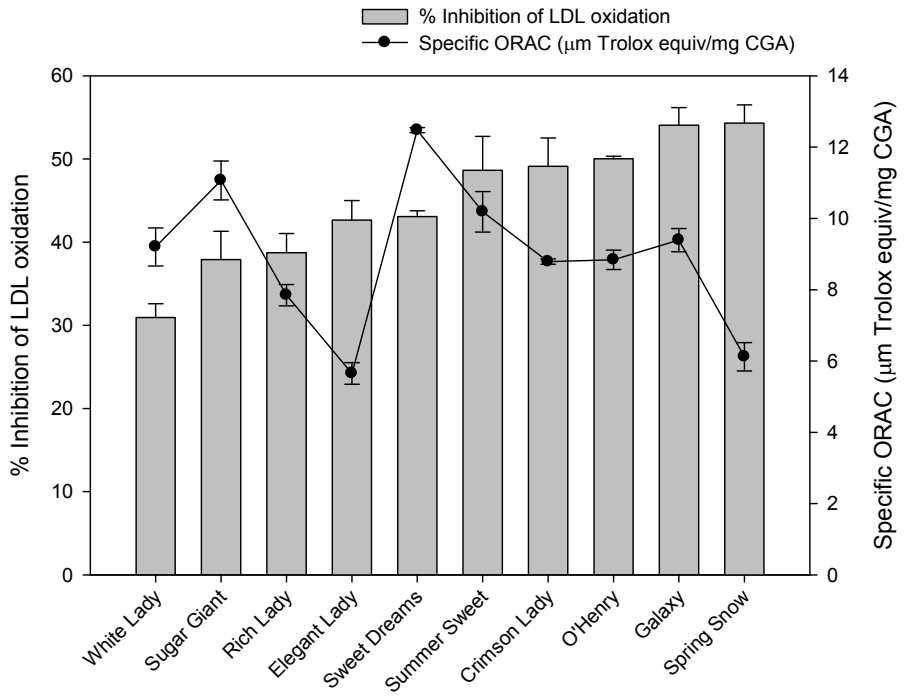

Figure 4a. Correlations of % Inhibition of LDL oxidation –Specific ORAC value for different peaches varieties.

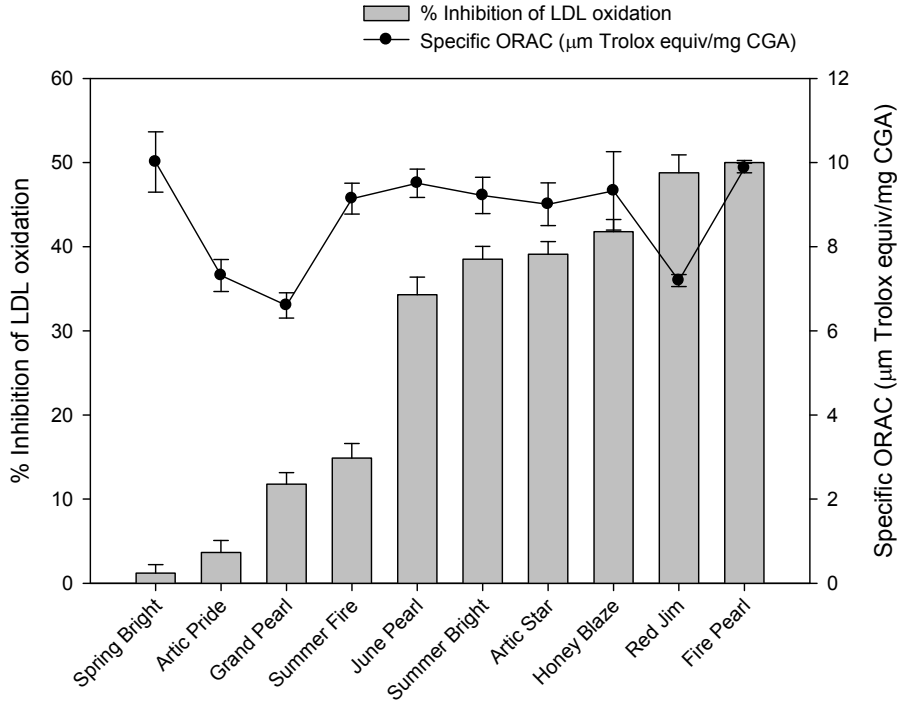

Figure 4b. Correlations of % Inhibition of LDL oxidation –Specific ORAC value for different nectarines varieties

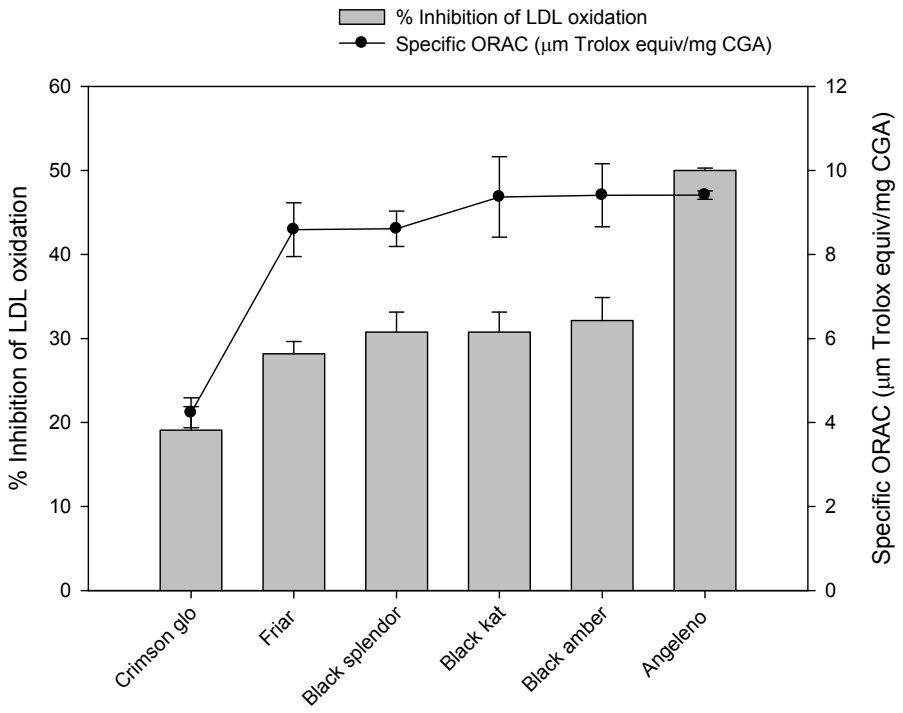

Figure 4c. Correlation of % Inhibition of LDL oxidation –Specific ORAC value for different plum varieties

In order to understand the mechanism of LDL oxidation, we used chlorogenic acid as a standard since previous work reported that chlorogenic acid is an abundant compound present on peaches and nectarines (Figure 5). In the case of evaluate the mechanism of plums, an anthocyanin (cyanidin-3-glucoside) was used as standard to elucidate the mechanism of LDL oxidation (Figure 6). In addition, we included quercetin since many stone fruits have been reported to contain quercetin derivatives (Figure 7). In general, the phenolic profiles of the fruits studied would contain different proportions of these compounds.

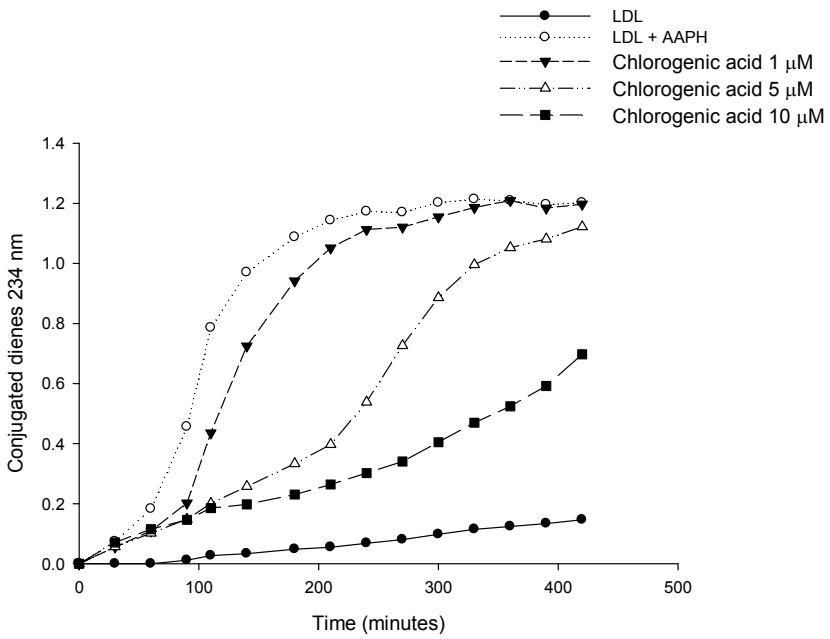

Figure 5. Effect of Chlorogenic acid on AAPH- mediate conjugated dienes formation on
LDL. *LDL (100 μg protein/ mL) was incubated with 5 mM AAPH at 37 °C and LDL oxidation was determined by continuous monitoring the absorbance at 234 nm in absence (LDL + AAPH), or presence of 1,5 and 10 μM of the specific phenolic compound.

Figure 5 indicates that chlorogenic acid has multiple mechanism of inhibition of LDL oxidation; one of the mechanisms is the radical scavenging effect, since a prolongation of the lag phase occurred. On the other hand, a decrease on the propagation rate of peroxidation and a decrease of the maximal rate of absorbance (OD) of accumulation of oxidation products was observed at higher phenolic concentrations, compared to a positive control (absence of antioxidants) indicating that chlorogenic acid can react with the peroxyl radicals to stop the propagation chain and with the alkoxyl radicals to inhibit the breakdown of the hydroperoxides and the formation of aldehydes (Pinchuck and Lichtenberg, 2002; Frankel, 2005) or alternatively indirectly by reconstituting the antioxidant power of the native tocopherol present in the LDL . Anthocyanins exhibit a similar trend as chlorogenic acid (Figure 6), while quercetin showed a trend of an extended prolongation of the lag phase with higher phenolic concentration (Figure 7) indicating a predominantly scavenging mechanism.

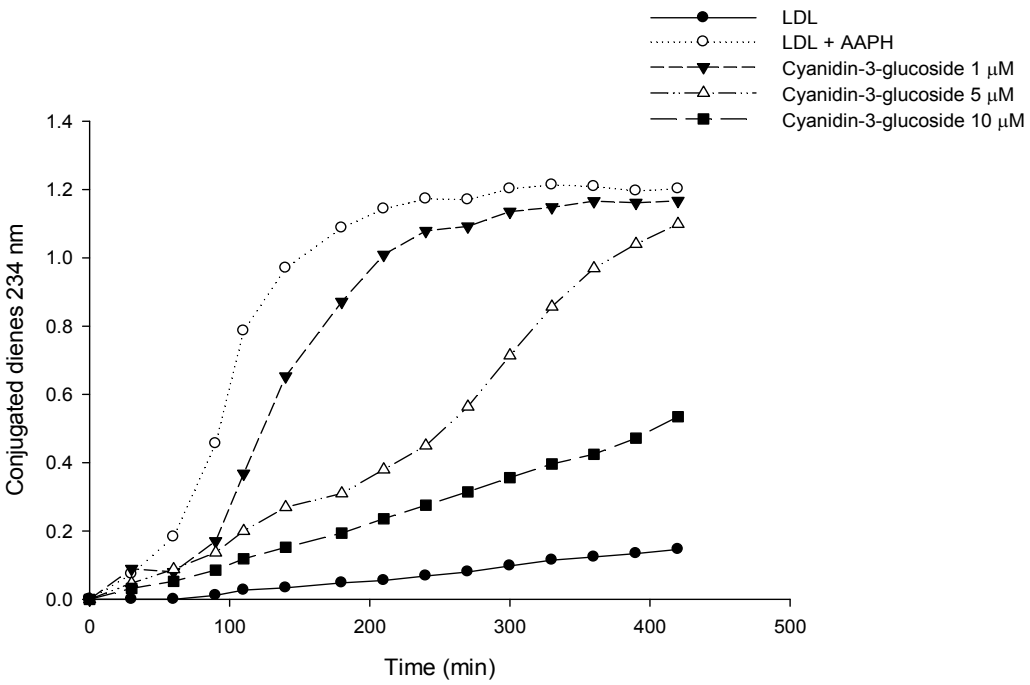

Figure 6. Effect of anthocyanin-3-glucoside on AAPH- mediate conjugated dienes formation on LDL. *LDL (100 μg protein/ mL) was incubated with 5 mM AAPH at 37 °C and LDL oxidation was determined by continuous monitoring the absorbance at 234 nm in absence (LDL + AAPH), or presence of 1,5 and 10 μM of the specific anthocyanin compound.

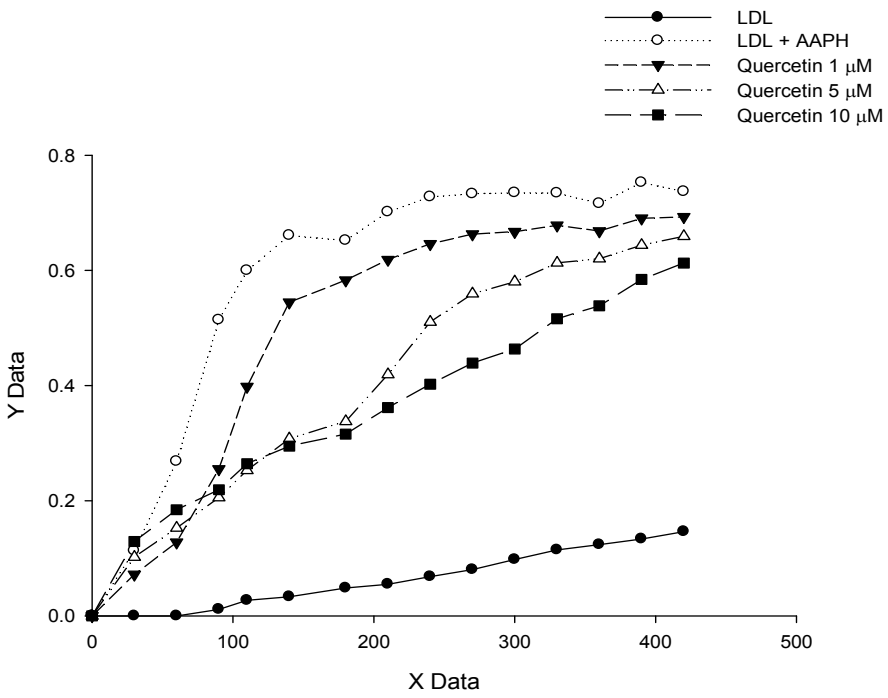

Figure 7. Effect of quercetin on AAPH- mediate conjugated dienes formation on LDL.

*LDL (100 μg protein/ mL) was incubated with 5 mM AAPH at 37 °C and LDL oxidation was determined by continuous monitoring the absorbance at 234 nm in absence (LDL + AAPH), or presence of 1,5 and 10 μM of the specific quercetin compound.

Similar kinetics behavior was observed for Galaxy and Fire Pearl (peach and nectarine, respectively) of an extended lag phase for the phenolic concentrations studied suggesting a scavenging mechanism predominating. For Angeleno plum there was an extended lag phase and a decrease in the overall maximal rate of absorbance indicating a dual mechanism (Figure 8).

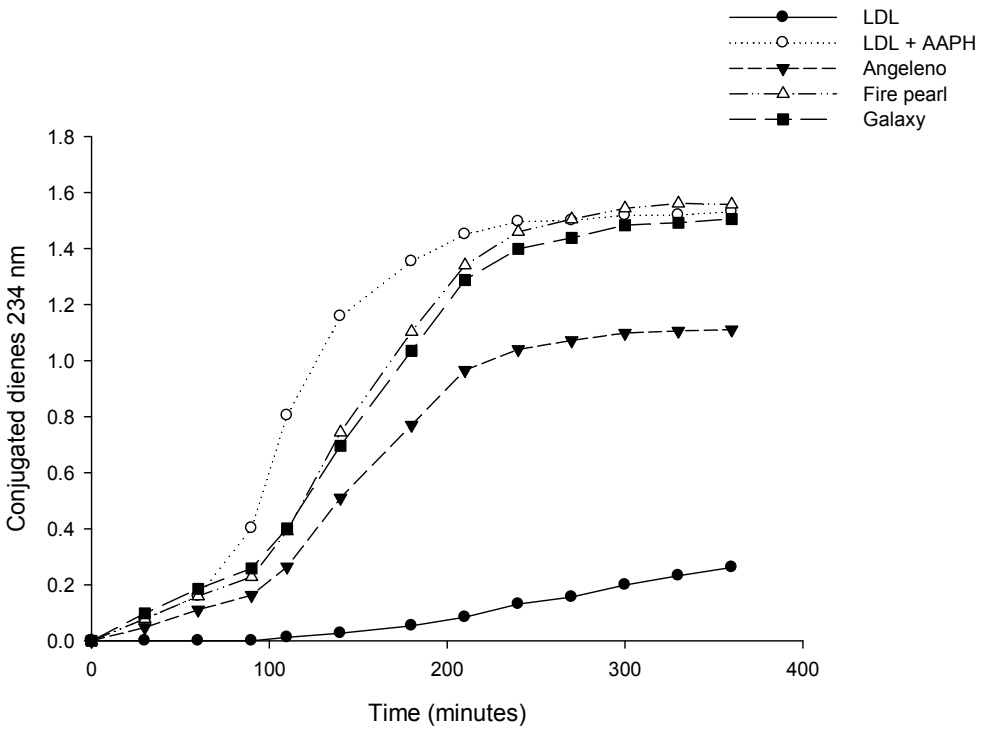

Figure 8. Effect of phenolic compounds from Angeleno plum, Fire Pearl and Galaxy crude extracts on AAPH- mediate conjugated dienes formation on LDL. *LDL (100 μg protein/ mL) was incubated with 5 mM AAPH at 37 °C and LDL oxidation was determined by continuous monitoring the absorbance at 234 nm in absence (LDL + AAPH), or presence of 5 μM of the specific crude extract.

Several studies have shown that phenolic compounds can reduce the oxidation of LDL in vitro and can inhibit atherosclerosis in animals. However, various phenolic compounds can react differently in LDL and their effectiveness varies greatly according to their concentration. For example, chlorogenic acid and quercetin at concentrations of 1 and 10 μM, show different kinetic trends (Figures 5, 7). It is important to note that the mechanism of the inhibition of LDL oxidation is too complex to be defined only by the lag phase and maximal absorbance kinetics or accumulation of hydroperoxydes since the condition of the kinetics depends of the lipid oxidation inducer and the bulk of the phenolic compounds presence on the crude extracts. In addition, the presence of endogenous enzymatic antioxidant systems add to the complexity playing an important role in biological systems, which in the LDL in vitro assays are not taken into account. Nevertheless, the data obtained from the kinetics assay of conjugated dienes using standard compounds provides valuable information of potential mechanisms responsible for the *in vitro* inhibition of LDL oxidation, such as in the present study. However, other techniques need to be applied in order to obtain a more complete picture of the role phenolic compounds play on the inhibition of LDL oxidation.

CONCLUSIONS

In summary we have shown in this study that phenolics from peach, nectarines and plums have high phenolic content and antioxidant activity. These phenolic compounds exhibit human LDL oxidation inhibition which is an important factor in the prevention of cardiovascular disease.
Furthermore, we have shown that by using a LDL in vitro assay we can screen different varieties of stone fruits to select those with higher LDL oxidation inhibition properties. Results indicate that type of fruit and variety show large variation, very likely due to the specific phenolic profiles present.

Interestingly, the specific antioxidant activity (which measures the radical scavenging properties of the specific phenolic profiles present in the fruit) did not correlate necessarily with the %LDL oxidation inhibition. This could be explained based on the fact that LDL oxidation inhibition by phenolics involves multiple mechanisms such as radical scavenging and phenolic-LDL complexing. It is likely that the specific phenolic profiles present in the fruit determine the predominant mechanism involved.

Overall, it seems that antioxidant assays are not appropriate for variety screening when searching for human LDL oxidation inhibition. We recommend the use of LDL oxidation inhibition assays for this purpose due to the multiple mechanisms involved in the process.

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