HEALTH BENEFITS OF PEACHES AND PLUMS

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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; Vizzotto et al., 2006). 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 red-fleshed plums overlap the levels found in blueberry (Wang et al., 1996; Prior et al., 1998; Cevallos et al., 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, 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, 2007). 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).

The total content of phytochemicals and the antioxidant activity of fruits (cherries, blackberries, strawberries, and raspberries) also varies with the stage of maturity, post harvest handling, and growing/cultural conditions under which the fruit was produced (Gonçalves et al., 2004a; 2004b;

Wang and Lin, 2000; Serrano et al., 2005). The current work indicates this may also be true for peaches, nectarines, and plums. 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 and its changes through to senescence (Tomas-Berberan et al., 2001). These aspects of phytochemical development in stone fruit needs to be further studied to best manage these fruit to maximize their health benefits.

Phytochemical extracts of peach showed a weak antiproliferative activity in vitro (Sun et al., 2002); however, peach homogenates, in in-vivo tests, reduced induction of micronuclei in bone marrow cells by 43-50% confirming the protective effect of this extract. This effect may be due to a multitude of compounds present in the plant material (Edenharder et al., 2003). Initial work using the (3H) thymidine incorporation technique showed that methanolic extracts from red fleshed peach and plum inhibited the proliferation of T47D human breast cancer cells, Caco-2 human colon cancer cells, HTC rat hepatoma cancer cells, PC-3 prostate cancer cells and DS-19 mouse erythroleukemia cancer cells. More recent work in our laboratory has shown that methanolic extracts from a few peach and plum genotypes showed excellent antiproliferative activity on MDA-MB-435 estrogen-negative receptor breast cancer cell lines (Vizzotto, 2005). Current work is attempting to elucidate the mechanism and identify the specific phytochemicals responsible for this effect.

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. 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.

OBJECTIVES

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 short term objectives for this research are the following:

1. Determine the anti proliferation activity the methanolic extract of the phenolics of the specific varieties have on breast cancer cell lines (needs repetition).

2. Screen commercial stone fruit varieties for their antiproliferative activity in two cancer cell culture systems: colon cancer and prostate cancer (ongoing).
3. Determine the LDL oxidation inhibition that these extracts elicit (completed).
4. Determine the effect of fruit maturity on the total phenolic concentration, anthocyanin concentration, and antioxidant activity of 'Rich Lady" peach and "Black Splendor" plum (completed).

MATERIALS AND METHODS

Cancer cell proliferation and LDL oxidation surveys. Methanolic extracts of the frozen samples of the 26 stone fruit varieties collected in the summer of 2006 will be used in cancer cell line proliferation studies as well as the LDL oxidation studies.

Fruit maturity studies. Peach and plum fruit were harvested in California and were shipped in boxes via overnight mail to the Department of Horticultural Sciences at Texas A&M University and stored at 5° C until further use. The fruit were moved to room temperature (~18°C) and separated into the two groups of maturity stages according to their external firmness by hand assessment. Afterwards, the internal (flesh) firmness value (lb_f) was determined for an objective characterization of the maturity stage in both types of fruits. The ripening and over-ripening processes at room temperature of each group of peaches and plums was evaluated every other day by measuring the internal firmness of 5 fruits from each group. Then every fruit was cut, packaged and labeled in plastic bags, and stored at -20°C for further analysis.

Antiproliferation activity in cancer cell cultures (Objectives 1 and 2)

Cancer cell lines. Three breast cancer lines are used: MCF-7 (the estrogen-positive human breast cancer), MDA-MB-453 (estrogen-negative human breast cancer), and MCF-10A (non-cancerous breast cell line). These are cultured in Petri dishes using Dulbecco's modified Eagle's medium (DMEM) at 37˚C in a 5% CO2 atmosphere and supplemented differently depending on the cell line (see Vizzotto, 2005 for details).

Two human colon, colorectal adenocarcinoma cancer cell lines (HT-29 and Caco-2) and one human prostate carcinoma cell line (PC-3) will be used. The HT-29 is cultured in ATCC McCoy's medium with 1.5 mM L-glutamine-fetal bovine serum and Caco-2 is cultured in ATCC minimum essential medium (Eagle) with 2 mM L-glutamine and an adjusted Earle's BSS (Yi et al., 2005). The PC-3 line is grown in a RPMI medium 1640 (GIBCO) and supplemented with 10% FBS/1% penicillin/streptomycin. These cell lines are grown in an incubator at 37˚C with 5% CO_2. The medium will be changed 2-3 times per week.

Cell viability assay. Antiproliferation will be measured in the presence and absence of treatments by using MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] or methyl thiazol tetrazolium assay (Mosmann, 1983) based on its conversion to MTT-formazan. Cells are seeded in 96-well microtitre plates at a density of 5,000 to 10,000 cells per well depending on the cell line. After 12-24 h period of incubation to allow cell attachment, cells will be exposed to varying concentrations of peach and plum extracts for 24 h. The concentrations used for all the genotypes and fractions is based on total phenolic content and expressed as µg of chlorogenic acid/mL. After the appropriate incubation period, 100 µL of MTT (5 mg/ml) is added per well and incubated for 1 h at 37˚C. After incubation, MTT is aspirated and 100 µL of DMSO added to lyse the cells and dissolve the blue formazan crystals. Calculate cell viability according to the following equation.

$$\text{Cell viability (\%)} = \left[\frac{OD\, of\, cell\, culture\, with\, sample - OD\, of\, the\, medium}{OD\, of\, the\, cell\, culture\, without\, sample - OD\, of\, the\, medium} \right] x100$$

Where OD is the optical density measured by spectrophotometer at 555 excitation and 520 emission filters.

The natural log of the remaining concentration (Ln C/Co) was calculated and plotted against the concentration (µg/ml of total phenolics). The first-order rate constant (k) was used to calculate the IC_{50} (concentration needed to reduce proliferation in 50%). $IC_{50} = Ln2 / k$, where C is the cell culture with sample, Co is the cell culture without sample and k is the first-order rate constant.

Antiproliferation assay. Cell growth determination will be performed using an electronic counter (Z1 Coulter). Five to ten thousand cells per well are seeded in 6-well plate and incubated for 24 h to allow cell attachment before exposure to varying concentrations of extracts. After the appropriate incubation period, the medium is aspirated and the cells rinsed with 1 ml of PBS (phosphate saline buffer). After incubation period of 5 min with 200 µL of trypsin, 800 µL of medium is added and the mix transferred to a vial previously filled with 19 mL of isotone II solution. Two readings were taken from each replication. The results were expressed as number of cells.

Inhibition of LDL oxidation (Objective 3)

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 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 (calculated as the chloride salt) obtained from a standard curve

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 mass.

Antioxidant Activity Upon LDL Oxidation Evaluated By TBARS Assay-Isolation of LDL. 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 standard of BSA.

Thiobarbituric Acid Reactive Substances (TBARS). Assay was performed according to the procedures of Camejo et al. (1992). 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:

(%Inhibition)= [(C - S)/C] x100

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.

The effect of maturity stage on phytochemical content (Objective 4)

Internal firmness was determined using a texture analyzer (TA-XT2, Texture Technologies Corp.), fitted with an 8-mm concave tip and the probe was driven with a crosshead speed of 10 mm sec^{-1}. A set of 10 and 5 fruits from each group (very firm and firm) was used to measure fruit firmness initially and during the ripening and over-ripening (senescence) processes. The fruit was peeled, and an average internal firmness was determined as the force recorded at 10.0 mm compression deformation force applied on two different and opposite equatorial spots of each fruit.

Total phenolic content, total anthocyanin content, and antioxidant activity were analyzed as previously described.

The total carotenoids content protocol was adapted from Talcott and Howard (1999). Under indirect light conditions, 2 g of frozen tissue was homogenized with 20 mL of acetone/ethanol (1:1) solution containing 200 mg/L BHT in a falcon tube until obtaining uniform consistency. After centrifugation for 20 min at 29,000 x g at 2°C, the supernatant was transferred to a 50-mL graduated cylinder and solvent added to a final volume of 50 mL. The solution was transferred to a plastic container with a screw cap. Twenty-five milliliters of hexane were added to the peach and plum samples and the container was shaken vigorously. The solution was left for 30 min to allow separation of the phases before 12.5 mL of nanopure water was added and the solution was shaken vigorously. Again, the phases were allowed to separate and the hexane phase was measured in a Spectrophotometer. The machine was zeroed using hexane as a blank solution and the measurements were taken in a quartz cuvette at 470 nm. Every time the measurements were above 0.7 AU, the samples were diluted with hexane and reanalyzed. The concentration of total carotenoids was estimated from a β-carotene (Sigma Chemical Co.) standard curve in terms of milligrams of β-carotene equivalent per 100 g of fresh tissue.

RESULTS AND DISCUSSION

Antiproliferation activity in cancer cell cultures (Objectives 1 and 2)
Extracts from the yellow fleshed peach 'Rich Lady' (RL) and of red fleshed plum 'Black Splendor' (BS) were evaluated on the estrogen-dependent MCF-7, the estrogen-independent MDA-MB-435 breast cancer cells and one non-cancerous breast cell line MCF-10A. The results showed that RL extract effectively inhibited the proliferation of the estrogen-independent MDA-MB-435 breast cancer cell line as compared to either the non cancerous breast line MCF-10A or the estrogen dependent breast cancer line MCF-7 respectively. In general BS extracts were less effective although they still affected the MDA-MB-435 to a greater degree than the other breast cancer cell line or the normal breast cell line. Thus subsequent screening was done with only the MDA-MB-435 estrogen-independent cell line.

Twenty-six commercial varieties were tested. The IC_{50} values found in peach extracts ranged from 110 mg/L to > 1200 mg/L. 'Spring Snow' and 'Rich Lady' showed high activity in suppressing the proliferation of MDA-MB-435 cells, with IC_{50} values of about 110 and 150 mg/L respectively. Among the nectarines varieties, IC_{50} values ranged from about 230 to > 1200 mg/L. 'Summer Fire' and 'Honey Blaze' were the most potent in suppressing the cell growth (IC_{50} between 230-250 mg/L). Finally, the IC_{50} values found for the plum varieties ranged from about 200 to 975 mg/L. 'Black Amber', 'Crimson Glo', 'Angeleno' and 'Friar' exerted the highest anti-tumor activity (IC_{50} ~ 200 mg/L). These tests are currently being repeated and then the screening with the colon and prostate cancer lines will begin once the new facility in Dr. Cisneros's laboratory obtains all the University approvals.

Inhibition of LDL oxidation (Objective 3)
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 .
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'. For plums, 'Angeleno' and 'Black Splendor' varieties had the higher phenolic content (Figure 1).

The varieties with the highest anthocyanin content 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 (Figure 2). The values obtained for plums are higher or similar to those reported previously for blueberries (Vizzotto et al., 2007; Cevallos et al., 2006).

The specific antioxidant activity using the DPPH assay was calculated for all varieties and types of fruits studied. The specific antioxidant activity expressed on phenolic basis, determines the antioxidant activity of the specific profile of phenolic compounds present in each variety tested. For peaches, nectarines and plums it ranged from ~ 700 -1400, 400 – 1200 and 625 – 1100 ug Trolox/mg chlorogenic acid, respectively. 'Sweet Dream', 'Arctic Star' and 'Black Kat' varieties were the fruits that showed higher specific antioxidant activity among peach, nectarine and plums, respectively.

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. 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. 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'

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.

Linear correlation analysis indicated high correlations ($r^2 > 0.78$) between total phenolic content and antioxidant activity using the ORAC assay or the DPPH assay ($r^2 > 0.72$) for varieties within each type of fruit. This is consistent with previous work with stone fruit (Vizzotto et al., 2007).

Figure 1. Total phenolic content in selected California commercial varieties of peaches, nectarines and plums.
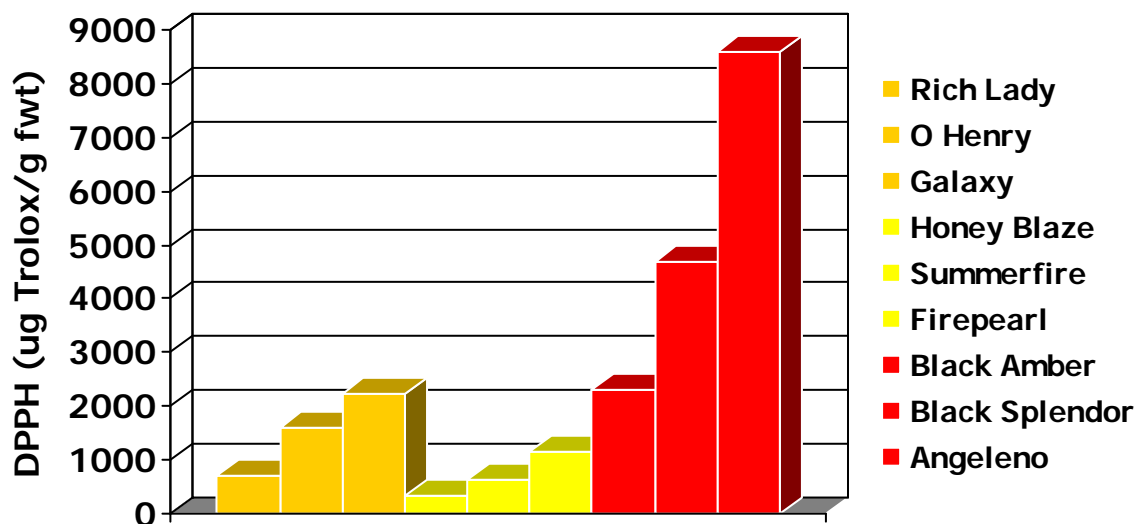

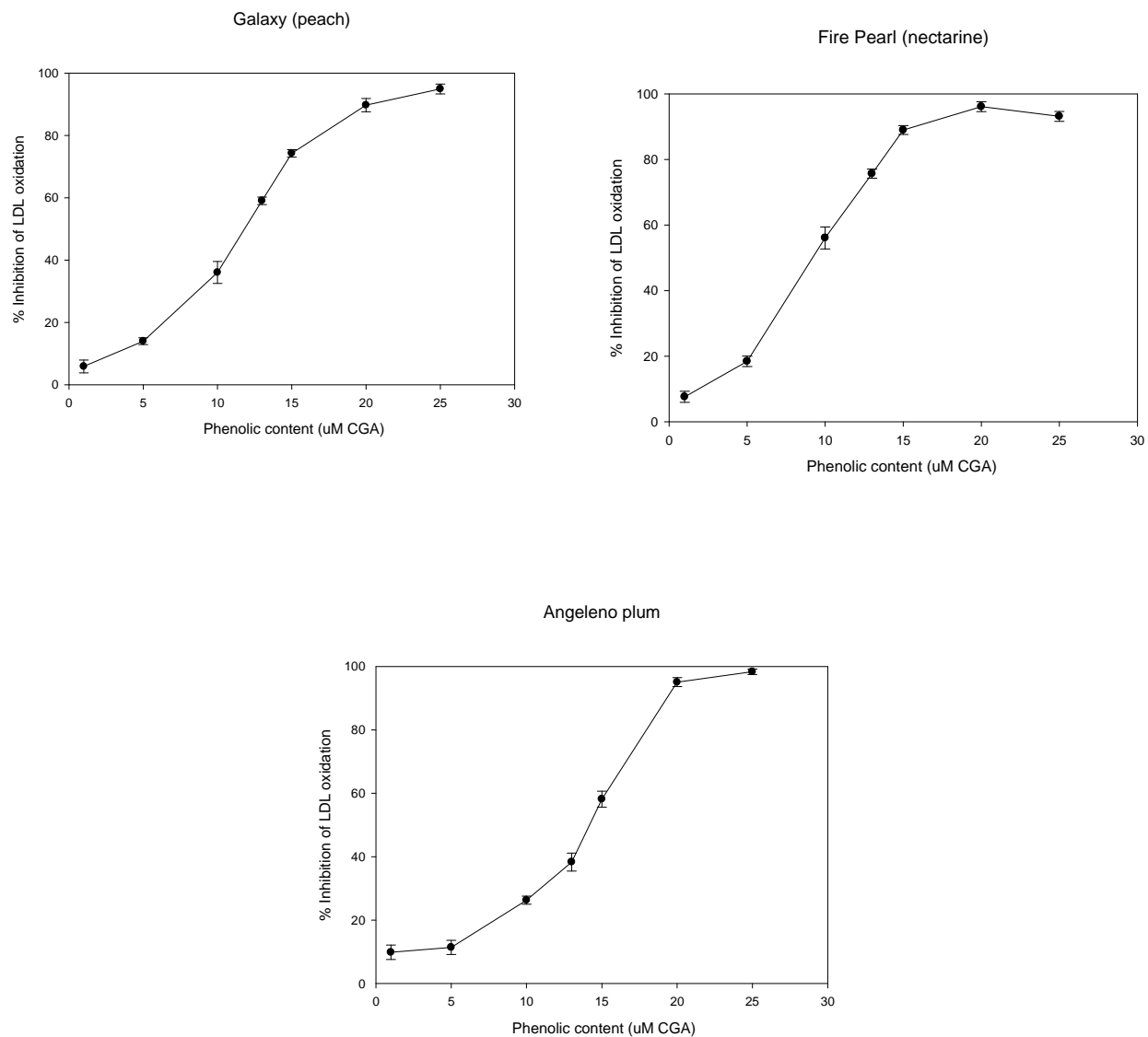Figure 2. Antioxidant activity of selected California varieties of peach, nectarine and plum.

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. The IC_{50} values were obtained from curves of LDL oxidation inhibition and concentration of phenolic compounds (Figure 3). The IC_{50} values represent the concentration of phenolic compound that induces a 50% inhibition of LDL oxidation. The IC_{50} values for the selected peach, nectarine and plum varieties were ~ 9.3, 11.7 and 13.7 uM chlorogenic acid, respectively. These IC_{50} values or phenolic concentrations were used to screen the inhibition of LDL oxidation for different varieties in each type of fruit studied.

The inhibition of Human LDL oxidation in different peach varieties ranged from ~ 30 – 55%, in nectarines it ranged from ~ 0 – 50% and in different plum varieties the inhibition of Human LDL oxidation ranged from ~ 20 – 50%. This large variation in LDL oxidation inhibition is related to the type of phenolic compounds present in each type of fruit variety studied. It is likely that the specific profiles influence the response. We observed that in peaches, 'Spring Snow' and 'Galaxy' varieties showed the highest inhibition, while the 'White Lady' variety the lowest. In nectarines, 'Fire Pearl' and 'Red Jim' varieties showed the highest inhibition, while 'Spring Bright' and 'Artic Pride' the lowest. Finally, in plums, 'Angeleno' and 'Black Splendor' showed higher inhibition while 'Crimson Glo' the lowest.

There is not consistent relationship between our results of the antiproliferative activity for breast cancer cells, or LDL oxidation inhibition with those obtained for total phenolics, antioxidant activity or specific antioxidant activity. 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. The possible mechanisms would have to be explored and studied in future work.

Our results confirm that selecting or screening varieties based solely on antioxidant activity is misleading and does not represent the fruit's ability for specific bioactivity (in this case Human LDL oxidation inhibition and antiproliferative activity towards breast cancer cells). Thus it is important to screen varieties using the appropriate bioassays targeting the specific bioactivity searched for. One big challenge is to find bioassays that could be cost effective and efficient in yield and time output.

Figure 3. Obtained curves of % inhibition of Human LDL oxidation for different peach, nectarine and plum varieties. Selection of varieties was based on highest total phenolic content and antioxidant capacity in order to obtain IC50 values in the LDL oxidation assay.

The effect of maturity stage on phytochemical content (Objective 4)

'Rich Lady' Peach- The carotenoid content in 'Rich Lady' peaches increased substantially (~200%) during the first four days at 18 C corresponding to the ripening process, while the internal (flesh) firmness decreased rapidly during this period from an initial pre-ripe stage (FRP) (~13 lb_f) until the fruit reached the fully-ripe stage at a firmness value of ~ 1 lb_f. The minimum firmness value for "ready to eat" peach fruit is ~ 2 lb_f (Crisosto et al. 2007). A slight increase in the carotenoid content (~60% compared to day 0) was observed between days 4 and 12 of storage corresponding to the over-ripening process. Fruit firmness during this period did not change and remained at ~ 1 lb_f (Figure 1). Carotenoid development occurs as the chloroplast is transformed into chromoplast during ripening, resulting in new synthesis of various carotenoids that are not present in green fruit (Abbie et al., 2005). ß-Carotene synthesis in peach fruit (Katayama et al., 1971) during the ripening process has been reported previously. However, no previous work has been reported for the over-ripening process or senescence period.
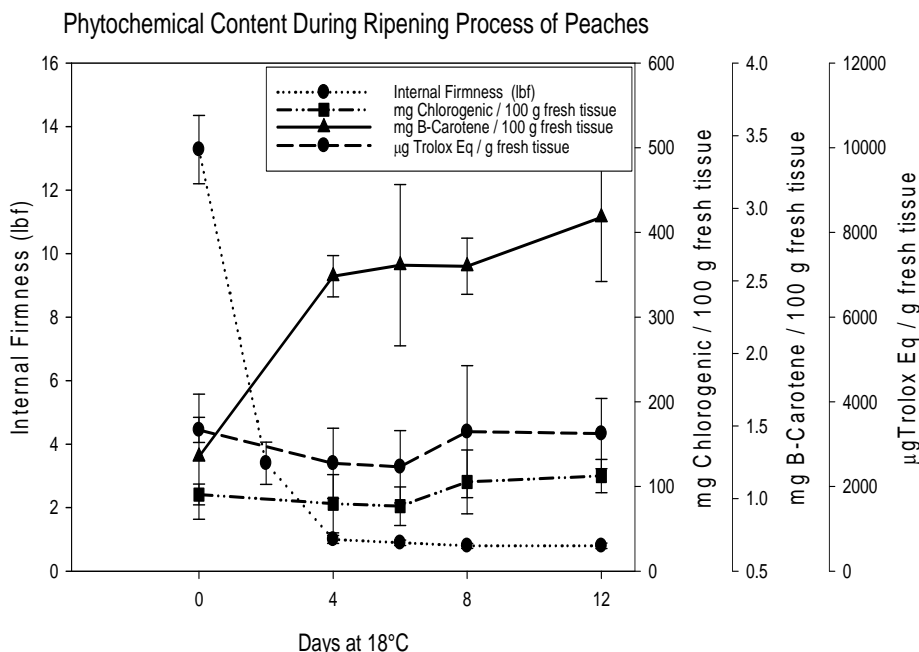

Figure 4. Phytochemical antioxidant changes in 'Rich Lady' peach fruit during the ripening and over-ripening processes.

In contrast, no differences in phenolic content and antioxidant capacity were observed during the ripening and over-ripening processes (Figure 4). The phenolic content change in peach fruit agrees with previous peach work (Tomas-Barberan et al., 2000) for firm ripe versus ripened fruit.

Soluble solid content increased slightly during the over-ripening process (after day 4), while the pH remained constant during both the ripening and over-ripening periods (Figure 5).
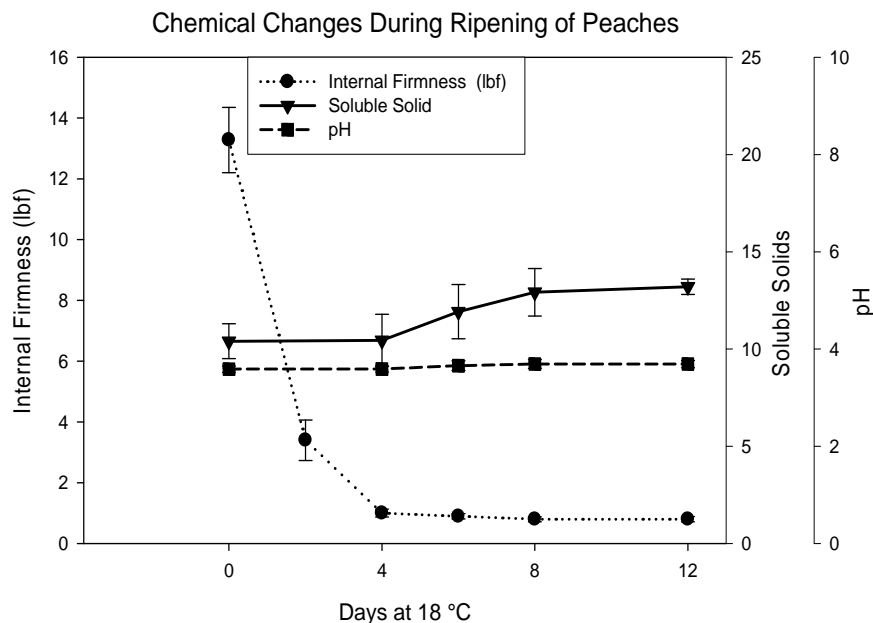
Chemical Changes During Ripening of Peaches

Figure 5. Soluble solids and pH changes in 'Rich Lady' peach fruit during the ripening and over-ripening processes**.**

The carotenoid content during the ripening process in 'Rich Lady' peach fruits increased in samples harvested at both very firm (~13 lb$_f$) pre-ripe fruit (FRP) and the firm (~ 3 lb$_f$) "ready to eat" fruit (RTE) maturity stages. Although more significant differences were observed in FRP compared to RTE fruit, both groups showed a similar trend and rates in the change of flesh firmness and carotenoid content during the ripening process. The flesh firmness decreased rapidly while the carotenoid content increased during the ripening period (Figure 6).
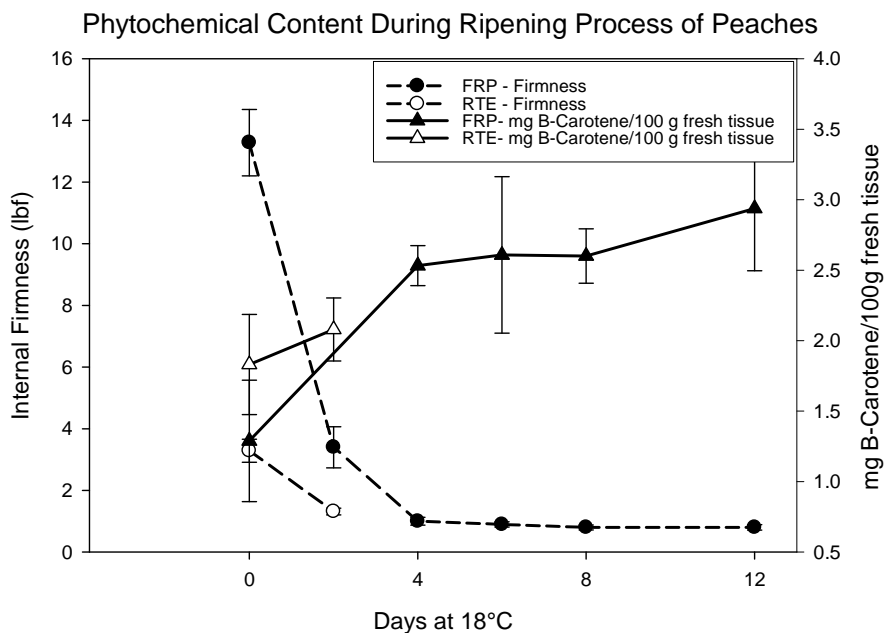
Phytochemical Content During Ripening Process of Peaches

Figure 6. Carotenoid changes in 'Rich Lady' peach fruit during the ripening process at two initial maturity stages

'Black Splendor' Plum- Total anthocyanin content increased substantially (~100%) during the first 4 days of ripening at room temperature of this red-fleshed plum, when the fruit firmness decreased from an initial pre-ripe stage of ~ 8 lb_f to a fully ripe stage of ~1 lb_f. Ready to eat plum fruit is considered in the range of 2 - 3 lb_f of flesh firmness (Crisosto et al. 2007). During the over-ripening period. between days 4 and 15, the anthocyanin accumulated up to ~ 230% in relation to day 0 (Figure 4).

This result indicated this red-fleshed plum fruit continued synthesizing anthocyanins during senescence and beyond the ripening period. These results confirm previous studies that observed increasing anthocyanin content during the ripening of plum fruits at 20°C (Manganaris et al. 2007). However, previous studies did not characterize anthocyanin synthesis during the over-ripening process or senescence period.
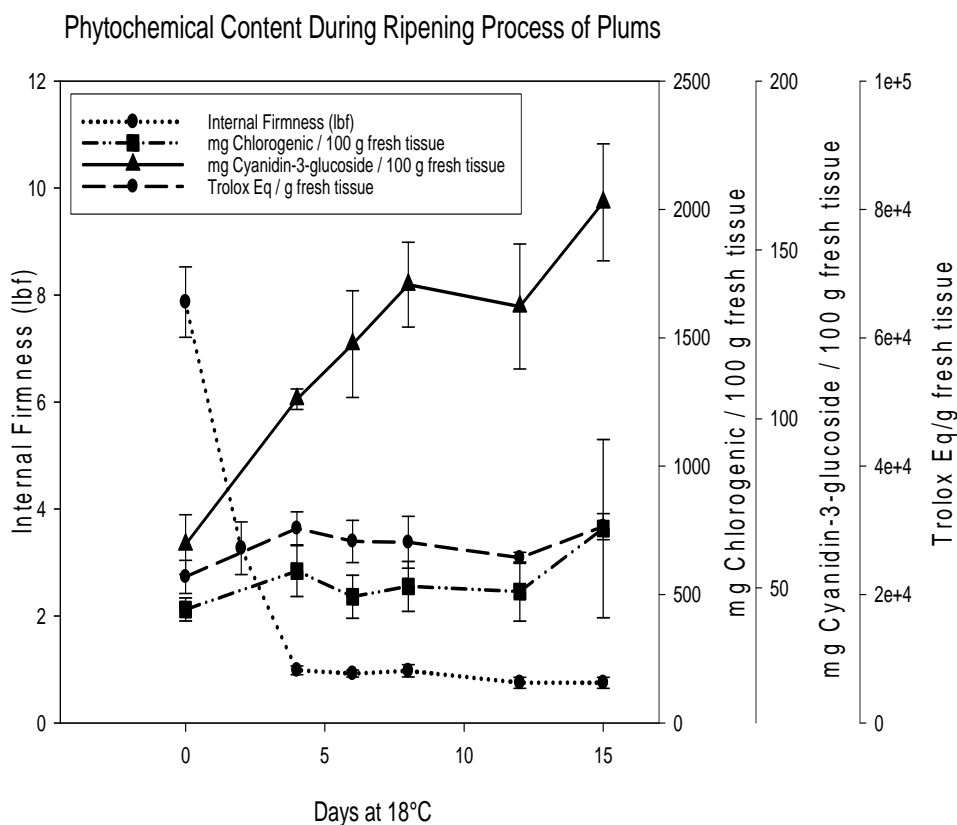

Figure 7. Phytochemical antioxidant changes in 'Black Splendor' plum fruit during the ripening and over-ripening processes.

In the present study phenolic content did not significantly change during the ripening and over-ripening periods for the 'Black Splendor" plum (Figure 7). In a previous study by Senter et al (1992), phenolic compounds evaluated only during the ripening process showed no significant changes. This group observed that the degree of polymerization of proanthocyanidins, as indicated by vanillin/proanthocyanidin ratios, did not change. In the present study, the antioxidant activity mimicked the phenolic content and did not show major changes during the ripening and over-ripening processes in plum fruit. The overall antioxidant capacity was strongly

correlated with the phenolics content, but not with the anthocyanin content. The reason is that anthocyanin only represents a small fraction of the total phenolic content in plum fruit (~0.2) and thus has little contribution to the total antioxidant activity of the fruit. Similar observations were reported previously for stone fruit (Tomas-Barberan et al., 2000; Vizzotto et al., 2007) and strawberry fruit (Ferreyra et al., 2007).

No significant changes in pH and soluble solids in plums were observed during the ripening and over-ripening processes (Figure 8). Similar results have been reported previously for plum fruits during a ripening period of 7 days at 20°C (Manganaris et al. 2007).
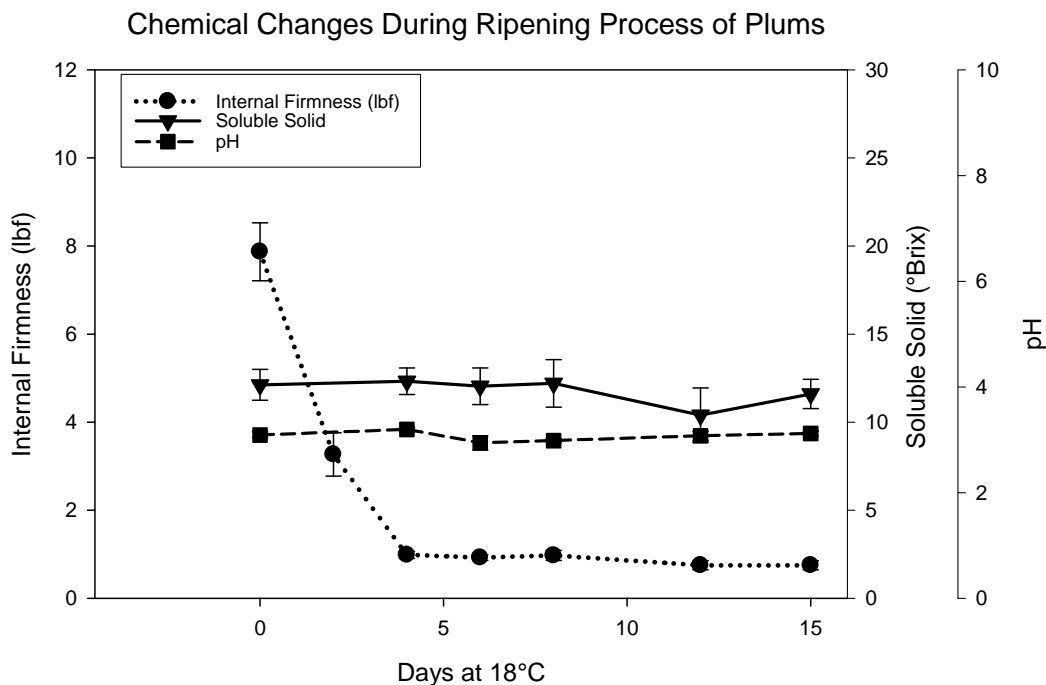

Figure 8. Soluble solid and pH changes in 'Black Splendor' plum fruit during the ripening and over-ripening processes.

Finally, we compared the anthocyanin changes in plum fruits harvested at two maturity stages (Figure 9). The flesh firmness for both the very firm (~ 8 lb$_f$) FRP fruit and firm (~2.lb$_f$) RTE fruit decreased rapidly while the anthocyanin content increased during the ripening period.
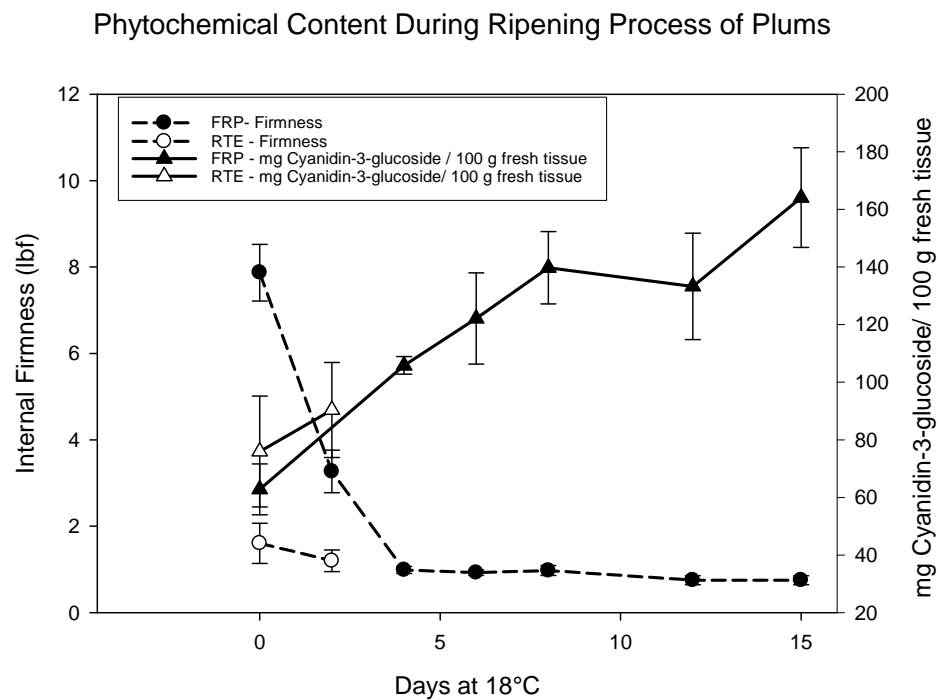
Phytochemical Content During Ripening Process of Plums

Figure 9. Anthocyanin changes in 'Black Splendor' plum fruit during the ripening and over-ripening processes at two initial maturity stages

LITERATURE REVIEW

Abbie, J. F., Del Pozo-Insfran, D., Lee, J. H., Sargent, S. A., and Talcott, S. 2005. Ripening-induced chemical and antioxidant changes in bell peppers as affected by harvest maturity and postharvest ethylene exposure. HortScience. 40: 732-736.

Brand-Williams, W.; Cuvelier, M. E.; Berset, C. 1995**.** Use of a free-radical method to evaluate antioxidant activity. Food Sci Technol-Leb. 28, 25-30.

Cevallos-Casals, B., D. Byrne, W. R. Okie, and L. Cisneros-Zevallos. 2006. Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. Food Chem. 96: 273-280.

Chun, O.K., D. Kim, H.Y. Moon, H.G. Kang, and C.Y. Lee. 2003. Contribution of individual polyphenolics to total oxidant capacity of plums. J. Agr. Food Chem. 51:7240-7245.

Crisosto, C. H., Mitcham, E. J., and Kader, A. A. 2007. Product Facts. Available in: http://postharvest.ucdavis.edu/Produce/Producefacts/index.shtml (last review: 11/30/2007)

Ferreyra, R. M., Viña, S. Z., Mugridge, A., and Chaves A. R. 2007. Growth and ripening season effects on antioxidant capacity of strawberry cultivar Selva. Science Horticulturae. 112 : 27-32.

Fuleki, T. and F.J. Francis. 1968. Quantitative methods for anthocyanins 1. Extraction and determination of total anthocyanin in cranberries. Food Sci. 33:72–77.

Gil, M., F. Tomas-Barberan, B. Hess-Pierce, and A. kader. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin A contents of nectarine, peach, and plum cultivars from California. J. Agric. Food Chem. 50:4976-4982.

Gonçalves, B., A. Landbo, D. Knudsen, A. Silva, J. Moutinho-Pereira, E. Rosa, and A. Meyer. 2004a. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (Prunus avium L). J. Agric. Food Chem. 52:523-530.

Gonçalves, B., A. Landbo, M. Let, A. Silva, E. Rosa, and A. Meyer. 2004b. Storage effects the phenolic profiles and antioxidant activities of cherries (*Prunus avium* L.) on human low-density lipoproteins. J. Sci. Food Agric. 84:1013-1020.

Katayama, T. Nakayama, T. H. Lee, T. H. Chichester, C. O. 1971. Carotenoid transformations in ripening apricots and peaches. Journal of food science. 36 : 804-806.

Kim, D.O, S.W. Jeong, and C.Y. Lee. 2003b. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 81:321-326.

Manganaris, G. A., Vicente, A. R., Crisosto, C. H., and Labavitch, J. A. 2007. Effect of dips in a 1-methylcyclopropene-generating solution on 'Harrow Sun' plums stored under different temperature regimes. J. Agric. Food Chem. 2007, 55, 7015-7020

Marin, A., Ferreres, F., Tomas-Barberan, F. A., and Gil M. 2004. Characterization and quantitation of antioxidant constituents of sweet pepper (*capsicum annuum* l.). J. Agric. Food Chem. 2004. 52: 3861-3869

Prior, R.L., and G. Cao. 2000. Antioxidant phytochemicals in fruits and vegetables: diet and health implications. HortScience 35:588-592.

Senter, S.D., and A. Callahan. 1991. Variability in the quantities of condensed tannins and other major phenols in peach fruit during maturation. J. Food Sci. 56:1585-1587.

Senter, S. D., and Fobes, W. R. 1992. Variations in Proanthocyanidins of Japanese-Type Plums During Maturation and Storage J Sci Food Agric. 1992. 60: 11-14

Serrano, M., F. Giullén, D. Martínez-Romero, S. Castillo, and D. Valero. 2005. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. J. Agric. Food Chem. 53:2741-2745.

Sun, J., Y-F. Chu, X. Wu, and R.H. Liu. 2002. Antioxidant and proliferative activities of common fruits. J. Agric. Food Chem. 50:7449-7454.

Swain, T. and Hillis W. E. 1959. The phenolic constituents of Prunus domestica. J. Sci. Food. Agr. 10, 63-58.

Talcott, T. S. and Howard, R. L. 1999. Phenolic autoxidation is responsible for color degradation in processed carrot puree. J. Agr. Food Chem. 47:2109–2115.

Tomás-Barberán FA, Gil MI, Cremin P, Waterhouse AL, Hess-Pierce B, Kader AA. 2001. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J Agric Food Chem 49:4748-60.

Tourjee, K.R., D.M. Barrett, M.V. Romero, and T.M. Gradziel. 1998. Measuring flesh color variability among processing clingstone peach genotypes differing in carotenoid composition. J. Amer. Soc. Hort. Sci. 123:433-437.

Vizzotto, M., L. Cisneros, W. R. Okie, D. W. Ramming, and D. H. Byrne. 2007. Large variation found in the phytochemical content and antioxidant activity of peach and plum germplasm. J. Amer. Soc. Hort. Sci., 132: 334-340

Wang, H., G. Cao, and R.L. Prior. 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44(3):701-705.

Wang, S. Y., and H-S. Lin. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. 48:140-146.

Wargovich, M.J. 2000. Anticancer properties of fruits and vegetables. HortScience 35:573-575.

Weinert, I., J. Solms, and F. Escher. 1990. Diffusion of anthocyanins during processing and storage of canned plums. Lebensm. Wiss. Technol. 23:396-9.