Health Benefits of Peach, Nectarine and Plums

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Abstract

Peaches, nectarines, and plums are the most important stone fruit in the world, but little is known about their effect on human health. Over 100 genotypes of peaches and plums of a range of flesh colors (white, yellow, red) were analyzed for their total phenolics, carotenoid, and anthocyanin contents and antioxidant activity. In general, the level of total phenolics was well correlated with the antioxidant activity. The levels changed dramatically among the stone fruit varieties examined indicating that varieties with improved levels of these phytochemicals could be developed. Selected plums and red fleshed peaches had equal or greater phenolics and antioxidant activity than blueberries. Antiproliferative assays with three breast cell lines indicated that peach and plum phytochemicals inhibited the cell proliferation for estrogen-receptor negative MDA-MB-435 breast cancer cells but not the estrogen positive breast cancer MCF-7 line or the normal breast cell MCF-10A line. Proliferation studies with MBA-MD-435 cells and assays to measure the LDL oxidation inhibition activity with the extracts from 26 peach, nectarine and plum cultivars indicated a range of effectiveness. Although there is a strong correlation between total phenolic and antioxidant activity, there is no obvious linear relationship between either total phenolic content or total antioxidant activity with either bioactivity measured, suggesting that there are distinct mechanisms other than a reactive oxygen species scavenging mechanism that are responsible for these bioactivities.

INTRODUCTION

Fruits have long been promoted for their capacity in preventing various cancers and age-related diseases (Prior and Cao, 2000; Wargovich, 2000). The phytochemicals reported in *Prunus* include carotenoids, anthocyanins, and phenolic acids (Weinert et al., 1990; Senter and Callahan, 1991; Tourjee et al., 1998; Gil et al., 2002; Cevallos et al., 2006). The antioxidant activity in both peaches and plums depends on the genotype tested. Some papers have reported that blueberries have the highest antioxidant activity among fruits; however, the levels found in plums and red-fleshed peaches overlap the levels found in blueberries (Wang et al., 1996; Prior et al., 1998; Cevallos et al., 2006).

There is a good correlation between total phenolic compounds and antioxidant activity among peaches and plums (Cevallos et al., 2006; Gil et al., 2002; Vizzotto, 2005, 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., 2003; Chun et al., 2003; Vizzotto, 2005).

There is evidence to suggest that phenolics and particularly anthocyanidins and anthocyanins can inhibit chemical carcinogenesis (Edenharder et al., 1995; Yoshimoto et al., 1999; Hagiwara et al., 2001; Hou, 2003). Phytochemical extracts of peach showed a weak antiproliferative activity in vitro (Sun et al., 2002). Plums were reported to have a high content of catechins (Pascual-Teresa et al., 2000), which suppress the growth and induce apoptosis in human prostate cancer DU145 cells (Kampa et al., 2000; Chung et al., 2001), breast cancer (Damianaki et al., 2000), and endothelial cell proliferation

(Sartippour et al., 2001).

Reduced levels of cardiovascular disease is also 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 oxidation of low density lipoprotein (LDL) appears to be particularly important (Steinberg, 1989). The results of LDL oxidation measurements in a range of produce indicated that fruits were a better source of phenolic antioxidants than vegetables (Vinson et al., 2001). Work with eight processing type (canning clingstone) 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.

MATERIALS AND METHODS

Fruit of over 100 genotypes of peaches, nectarines, and plums including both breeding lines and commercial varieties were obtained from packing houses or breeding plots in California, Texas, and Georgia (Cevallos et al., 2006; Vizzotto et al., 2007). Upon arrival in the lab at Texas A&M University, the fruit was visually inspected, the stones removed, and tissue (flesh plus skin) samples were frozen at –80°C until analyzed. Six to twelve fruits at the firm ripe stage were chosen from each genotype. Three replicates, each using two to four fruits, were used and they were packaged separately.

Chemical Analyses

The total phenolics were extracted from five grams of frozen tissue (flesh plus skin) homogenized with 25 ml of methanol in a conical screw-cap tube using a vortex mixer. Phenolics were quantified by the Folin-Ciocalteau method (Cevallos et al., 2006; Vizzotto et al., 2007). The concentration of total phenolics was estimated from a chlorogenic acid standard curve in terms of mg of chlorogenic acid equivalents. 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. The anthocyanins were extracted from five grams of frozen tissue (flesh plus skin) homogenized with 15 ml of 95% aqueous ethanol:1.5 N HCl solution (85:15) in a conical screw-cap tube using a vortex mixer. Samples were stored overnight at 4°C and then centrifuged for 15 min at 29,000 g at 2ºC (Vizzotto et al., 2007). 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 and Hucl, 1999). Antioxidant activity of the phenolic extract was quantified by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical method (Brand-Williams et al., 1995). The antioxidant activity was estimated as equivalents of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma Chemical Co., St Louis, MO) by comparison to a standard curve.

Cancer Cell Lines

Cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Three breast cell lines were used initially: 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% CO_2 atmosphere and supplemented differently depending on the cell line (see Vizzotto, 2005 for details). Methanolic extracts from the yellow fleshed peach 'Rich Lady' and the red fleshed plum 'Black Splendor' were used for cell proliferation studies of all three cell lines. The screening of antiproliferative activity of the extracts from 26 commercial varieties of peach, nectarine, and plums was done only on the MDA-MB-435 cell line.

Cell Viability Assay

Antiproliferation was estimated in the presence (up to 1,000 mg chlorogenic acid equiv./L) and absence of fruit extracts 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 and measured by optical density with a spectophotometer at 555 excitation and 520 emission filters. The natural log of the remaining concentration 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%) (Vizzotto et al., 2007).

LDL Oxidation

Plasma was 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). Antioxidant activity upon LDL oxidation was evaluated with the thiobarbituric acid reactive substances (TBARS) assay (Chang et al., 2000; Frankel et al., 1992). Percent inhibition of the formation of malonaldehyde was used as a parameter to compare antioxidant capacity. The sample concentration that led to 50% inhibition, IC_{50}, was used to compare the capacities of different peach, nectarine, and plum extracts.

RESULTS AND DISCUSSION

Among the selections and the California peach and nectarine varieties assayed in this study, the total phenolics concentration ranged from ~45 to ~371 mg chlorogenic acid/100 g fresh weight for the yellow and white fleshed peaches and nectarines, ~228 to ~1260 mg chlorogenic acid/ 100 g fresh weight for red fleshed peaches and ~380 to ~898 mg chlorogenic acid/100 g fresh weight for the plums (Fig. 1). Thus as previously shown, 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 and red fleshed peaches is greater than that found in the yellow and white fleshed peach and nectarine genotypes. The level of phenolics in the blueberry sample was comparable to that measured in red fleshed peaches and selected plum cultivars.

The anthocyanin concentration among the white and yellow fleshed peach/nectarine varieties was similar in both the selections and commercial cultivars (~0.5 to ~10 mg cyanidin 3-glucoside/100 g fresh tissue) and as expected much higher in all of the red fleshed peaches and plums (~45 to ~ 375 mg cyanidin 3-glucoside/100 g fresh tissue) (Fig. 2). The content of anthocyanins in the blueberry sample was similar to that found in some of the red fleshed peach and plum samples but was much higher than that found in any of the white or yellow fleshed peaches, nectarines, or plum selections or varieties (Fig. 2).

As was observed in previous studies (Cevallos et al., 2006; Vizzotto et al., 2007), the total phenolics but not anthocyanin content was well correlated with antioxidant activity ($r = 0.79$ to 0.96). The selections and varieties of peaches, nectarines, and plums differed significantly in antioxidant activity (Fig. 3). The antioxidant activity seen in the red fleshed peach selections and yellow and red fleshed plums was similar to that measured in the blueberry and was higher than that seen among the white or yellow fleshed peaches or nectarines. The antioxidant activity of a few of the white and yellow fleshed peach and nectarine selections and varieties approached that of blueberry (Fig. 3).

In initial work the crude methanolic extracts from the yellow fleshed peach 'Rich Lady' (RL) and of red fleshed plum 'Black Splendor' (BS) were evaluated for their antiproliferative effects on estrogen-dependent MCF-7 and 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 cells as compared to either the non cancerous breast cells

MCF-10A or the estrogen dependent breast cancer cells MCF-7, respectively. In general, BS extracts were less effective although they still affected the MDA-MB-435 cells to a greater degree than the other breast cancer cells or the non-cancerous breast cells. 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, nectarine, and plum extracts ranged from 110 mg/L to > 1200 mg/L, 230 to > 1200 mg/L and 200 to 975 mg/L, respectively (Fig. 4). 'Spring Snow' and 'Rich Lady' showed highest activity in suppressing the proliferation of MDA-MB-435 cells, with IC_{50} values of about 110 and 150 mg/L, respectively.

The IC_{50} value or the concentration of phenolic compounds that induces a 50% inhibition of LDL oxidation were calculated for the California commercial stone fruit varieties. The inhibition of human LDL oxidation in different peach varieties ranged from ~ 30-55%, in nectarines it ranged from ~ 0-50% and in plum varieties the inhibition of human LDL oxidation ranged from ~ 20-50% (Fig. 4). This large variation in LDL oxidation inhibition could be 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.

There is not a consistent relationship between the antiproliferative activity for breast cancer cells, or LDL oxidation inhibition with total phenolics, antioxidant activity or specific antioxidant activity. Since there is no apparent correlation, the antioxidant properties of phenolic compounds from stone fruits are probably not the only mechanism by which phenolics or other compounds inhibit these bioactive properties. Thus our results confirm that selecting or screening varieties based solely on antioxidant activity is misleading and does not represent the fruits' 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 are cost effective.

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Figures

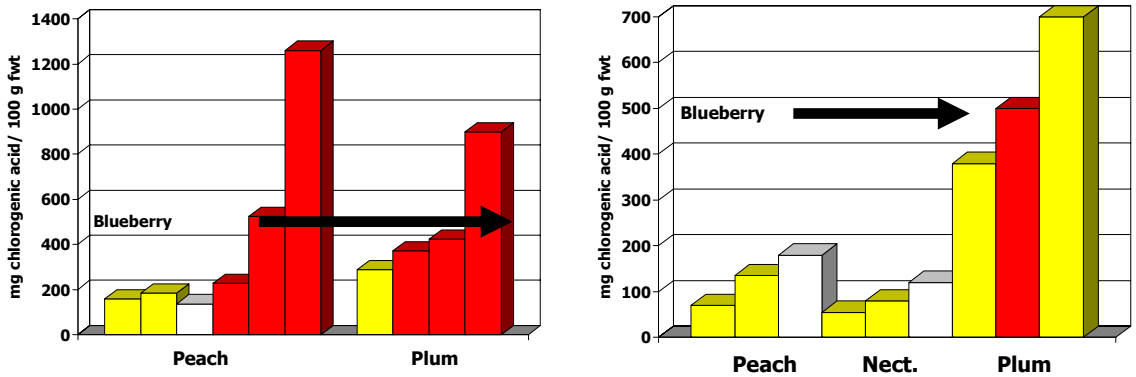

Fig. 1. Total phenolic content of peach, nectarine, and plum selections (right) and California commercial varieties (left) as compared to blueberry. White, gray and black bars indicate white, yellow, and red fleshed fruit respectively.

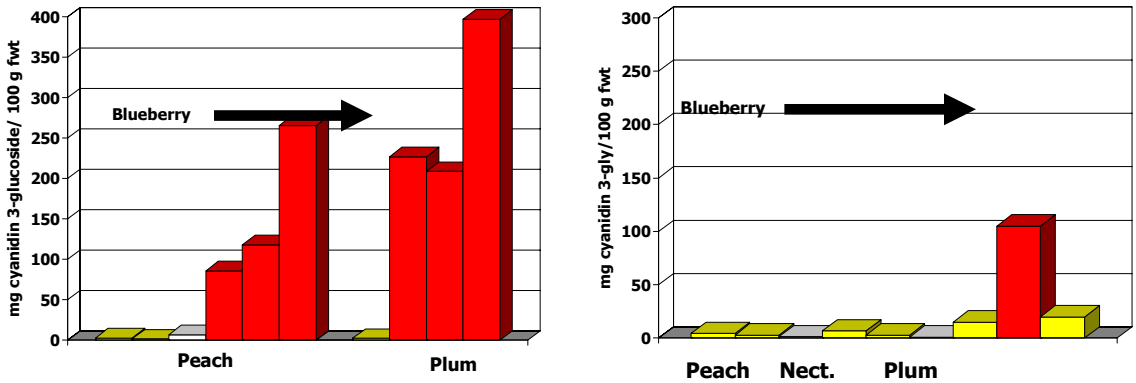

Fig. 2. Total anthocyanin content of peach, nectarine, and plum selections (right) and California commercial varieties (left) as compared to blueberry. White, gray and black bars indicate white, yellow, and red fleshed fruit respectively.

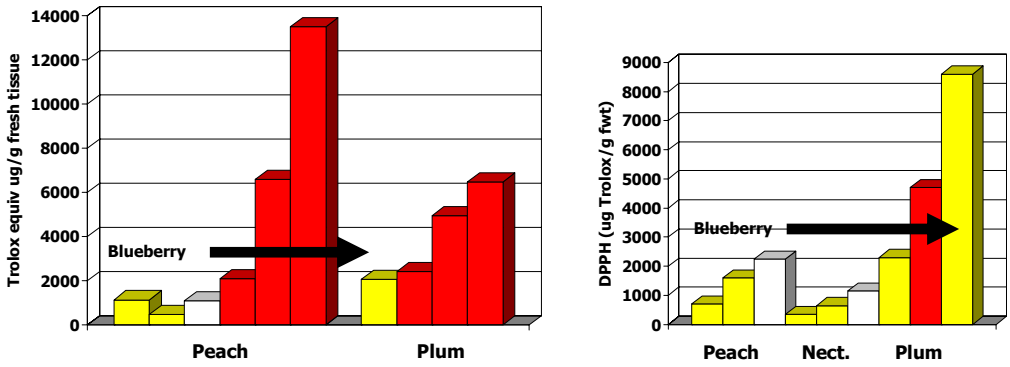

Fig. 3. Total antioxidant activity (DDPH method) of peach, nectarine, and plum selections (right) and California commercial varieties (left) as compared to blueberry. White, gray and black bars indicate white, yellow, and red fleshed fruit respectively.

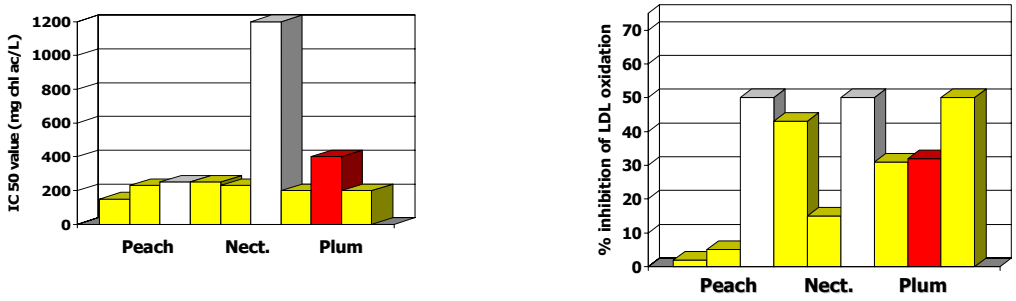

Fig. 4. Inhibition of MBA-MD-435 breast cancer cell proliferation (right) and percent inhibition of LDL oxidation (left) of California commercial peach, nectarine, and plum varieties. White, gray and black bars indicate white, yellow, and red fleshed fruit respectively.