Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties

Bolivar A. Cevallos-Casals a, David Byrne a, William R. Okie b, Luis Cisneros-Zevallos a,*

a Department of Horticultural Sciences, Room 202, Texas A & M University, College Station, TX 77843-2133, USA
b USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008-7066, USA

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Abstract

Fourteen red-fleshed plum (Prunus salicina Erhr. and hybrids) and eight peach [Prunus persica (Batsch) L.] genotypes were characterized for their total phenolic and anthocyanin contents. Selected rich phenolic genotypes showed high antioxidant activity, stable colour properties and good antimicrobial activity. Results indicated positive correlations between phenolic compounds ($r^2 = 0.83$) and antioxidant activity for both types of fruit. Colorants prepared from an anthocyanin rich plum genotype showed similar hue to that of synthetic colorant FD&C red 3 and higher stability than a commercial red grape colorant with respect to time, temperature and pH. Additionally, a selected rich phenolic plum genotype exhibited strong antimicrobial activity against Salmonella Enteritidis and Escherichia coli O157:H7. This study proposes that selection of crops high in phenolic compounds can be related to enhanced functional properties and opens the possibility of breeding fruits with targeted functional properties for the fresh produce and processing market.

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Keywords: Anthocyanins; Phenolics; Peach; Plum; Antioxidant activity; Antimicrobial activity; Color stability

1. Introduction

Increasing recent interest in nutraceuticals and functional foods has led plant breeders to initiate selection of crops with higher than normal phenolic antioxidant contents such as blueberries (Prior et al., 1998), potatoes (Reyes, Miller, & Cisneros-Zevallos, 2004), and others (Shim, Park, Lee, & Shetty, 1999; Yoshinaga, Tanaka, & Nakatani, 2000). Similarly, the Prunus Breeding Program at Texas A&M University and the USDA Stone Fruit Breeding Program at Byron, GA, are working at developing red-fleshed peaches [Prunus persica (Batsch) L.] and plums (Prunus salicina Erhr. and hybrids) with high levels of beneficial phenolic compounds for the fresh produce and processing market. All these programmes aim to set the baseline for establishing breeding efforts, with the intention of adding value to fruits and vegetables with respect to the level and diversity of health benefit properties that crops could impart. However, for achieving this goal, information regarding the quantity and functional properties of the phenolic compounds present is needed.

Anthocyanins and other phenolic compounds are responsible for many health benefits (Davidson & Brauen, 1981; Duthie, Duthie, & Kyle, 2000; Harborne & Williams, 2000; Moline, Bukharovich, Wolff, & Phillips, 2000; Naidu, 2000; Okuda, 1997; Wang, Cao, & Prior, 1997; Wang et al., 1999). Anthocyanins have been identified as key contributing compounds to antioxidant activity in vitro and in vivo (Igarashi, Takanashi, Makino, & Yasui, 1989; Tsuda, Horigo, & Osawa, 1998; Tsuda, Ohshima, Kawakishi, & Osawa, 1994; Wang et al., 1997). Antioxidants are needed for preventing...
degenerative reactions produced by reactive oxygen and nitrogen species in vivo and lipid peroxidation in foods (Cevallos-Casals & Cisneros-Zevallos, 2003). Phenolics have also been found to be natural antimicrobial compounds, which are important for increasing the shelf life of food and inhibiting the growth of pathogenic microorganisms (Bowles & Juneja, 1998; Davidson & Branen, 1981; Naidu, 2000; Payne, Rico-Munoz, & Davidson, 1989; Sofos, Beuchat, Davidson, & Johnson, 1998). In addition, anthocyanins may serve as natural colorant sources and even potential substitutes of synthetic food colorants due to their attractive orange, red and blue hues (Cevallos-Casals & Cisneros-Zevallos, 2004; Francis, 1989; Mazza & Miniati, 1993).

These benefits, among others, make anthocyanin and other phenolic compounds an interesting target for breeding programmes. We propose, in this study, that peach and plum selections, rich in phenolic and anthocyanin compounds, will yield fruits with enhanced functional properties such as antioxidant, colorant and antimicrobial properties.

The specific objectives of this study were (1) to determine the anthocyanin and total phenolic contents, and antioxidant properties of red-fleshed peach and plum genotypes, and (2) to investigate the colorant and antimicrobial properties of selected phenolic rich peach and plum genotypes.

2. Materials and methods

2.1. Materials

Eight red-fleshed peach and 14 red-fleshed plum selections from the USDA Stone Fruit Breeding Program at Byron, GA were used in this study. Fruit samples were collected in July 2000 and shipped overnight to Texas. The endocarp and seed were immediately removed and samples were frozen at −20 °C. DPPH, Folin-Ciocalteu reagent and Mueller-Hinton broth were purchased from Sigma Chemical Co. (St. Louis, Mo., USA), while McIlvaine buffer from Lab Chem Inc. (Pittsburgh, PA, USA). Antho-Red Grape concentrate (03880, LOT HH861) was supplied by Warner Jenkinson (St. Louis, Missouri, USA). *Escherichia coli* O157:H7 (ATCC® 43895) was donated by Dr. Elsa Murano (Food Microbiology Laboratory at Texas A&M University, College Station, Texas), while *Salmonella enterica* subsp. *enterica* serotype Enteritidis (ATCC® 13076) was purchased from Key Scientific Products Co. (Round Rock, TX, USA).

Six fruits from each genotype were chosen, at random, for measuring anthocyanin content, total phenolics and antiradical activity. Three replicates, each using two fruits, were analyzed. Additionally, plum fruits were divided into flesh (mesocarp) and skin (exocarp) sections to determine the level of active compounds in these structural parts. All results were expressed on fresh weight basis of the flesh.

2.2. Total phenolics and anthocyanin content

Total soluble phenolic content of methanolic extracts was assayed as described by Cevallos-Casals and Cisneros-Zevallos (2003). Total phenolics were expressed as mg chlorogenic acid equivalent (CGA)/100 g fresh or dry weight, based on a standard curve. Total anthocyanin content was adapted from Fuleki and Francis (1968) by measuring the absorbance of extracts at pH 1, as described by Cevallos-Casals and Cisneros-Zevallos (2003). Hexane was added to peach and plum samples for removing any carotenoids present. Anthocyanins were expressed as mg cyanidin 3-glucoside equivalents/100 g fresh or dry weight, using a molar extinction coefficient of 25,965 M⁻¹cm⁻¹ and a molecular weight of 449 g/mol (Abdel-Aal & Hucl, 1999).

2.3. Antiradical activity and kinetic assay

Antiradical activity of phenolic compounds was adapted from Brand-Williams, Cuvelier, and Berset (1995). The same methanol extract as for phenolics was used. A total of 150 μl of sample (equivalent methanol volume to control) reacted with 2850 μl DPPH (98.9 μM in methanol) in a shaker covered with aluminum foil at 25 °C. Readings at 515 nm were taken at 15 min. The change in absorbance was used and results were expressed as trolox equivalents from a standard curve. Readings at 15 min were used for calculation of the relative antiradical capacity (RAC), which indicates the antiradical capacity of the sample compared to trolox for a specific reaction time (for example, 15 min).

Second-order antiradical kinetic determinations were adapted from Espin, Soler-Rivets, Wickers, and García-Viguera (2000) using DPPH and methanolic extracts, as described by Cevallos-Casals and Cisneros-Zevallos (2003). The second-order rate constant (k₂) was obtained by having the antiradical compound (phenolics) in larger amounts than the DPPH, thus forcing the reaction to behave as first-order. From these reactions, pseudo-first-order rate constants (k₁) were obtained. The k₁ was linearly dependent on antiradical concentration, and from the slope of these plots, k₂ was determined.

Determinations of k₁ were conducted in triplicate, with five different extract concentrations per sample. Fitting of the experimental data to obtain k₁ was done by using an exponential decay (single, 2 parameter) equation generated by Sigma Plot 2.01 (1994). The specific reaction conditions between DPPH and samples were as follows, 86 μM DPPH and plum extract (0.380–0.476 mg phenolics/ml); 82 μM DPPH and peach extract (0.279–0.332 mg phenolics/ml).
2.4. Chromaticity and colour stability

Plum slices from six fruit were steam-blanch cured at 100 °C for 10 min, quenched in an ice-water bath, and homogenized with nanopure water in an Ultra- Turrax homogenizer. Denaturation of degrading enzymes was confirmed by measuring peroxidase (POX) activity, as described by Cevallos-Casals and Cisneros-Zevallos (2003). Tube contents were filtered and centrifuged for 15 min at 29,000 g. Then, methanol was evaporated from filtered samples in a vacuum concentrator. Samples were re-diluted with sterile nanopure water to the desired concentrations. Final solutions were filtered with 0.20 µm filters into sterile glass vials.

2.5. Antimicrobial activity

Fruits were homogenized with methanol in an Ultra- Turrax homogenizer (IKA Works, Inc., Wilmington, NC, USA) to uniform consistency. Tube contents were filtered with cheesecloth and centrifuged for 15 min at 29,000 g. Then, methanol was evaporated from filtered samples in a vacuum concentrator. Samples were re-diluted with sterile nanopure water to the desired concentrations. Final solutions were filtered with 0.20 µm filters into sterile glass vials.

2.5.2. Spread plate assay

Bacterial solutions of 10^3 cfu/ml were prepared in 0.1% peptone water. The actual number of cells was determined on Trypticase Soy Agar (TSA) plates. Then, 100 µl of the extract-bacteria solution (100 µl of the water-bacteria solution as control) was spread-plated on TSA plates. Plates were incubated at 35 °C and colonies were counted at 0, 12, 24 and 48 h.

2.5.3. Absorbance assay

Bacteria were diluted in double strength Mueller-Hinton broth to concentrations of 10^3 and 10^5 CFU/ml. One hundred microlitres extract (100 µl sterile water as control) were placed in a well with 100 µl of the diluted bacteria inoculum. Other controls used were extracts incubated with sterile water without the bacteria, for verifying any change in absorbance of the extract. Absorbances at 630 nm were taken for a period of 48 h in a plate reader. Turbidity readings were related to bacterial growth.

2.6. Analysis of variance (ANOVA)

One-way ANOVA was performed using the SAS Statistical Analysis System v8.1 (SAS Institute Inc., Cary, NC, USA). Means were compared by Duncan’s multiple range test at \( \alpha = 0.05 \).

3. Results and discussion

3.1. Total phenolic and anthocyanin contents

Results showed a total phenolic content ranging from 298 to 563 mg CGA/100 g for plums and 100 to 449 mg CGA/100 g for peaches. The anthocyanin content in plums ranged from 33 to 173 mg/100 g, which in general was significantly higher than the anthocyanin content in peaches (6–37 mg/100 g) (Fig. 1). The phenolic content observed in the Prunus salicina varieties in our study is higher than that previously reported for Prunus domestica varieties (160–300 mg/100 g, Los, Wilska, & Pawlak, 2000) and for other commercial varieties (14–109 mg/100 g, Gil, Tomás-Barberán, Hess-Pierce, & Kader, 2002; 125–373 mg/100 g, Kim, Chun, Kim, Moon, & Lee, 2003). Additionally, the phenolic contents of the plum varieties were comparable to those of blueberries reported previously (292–672 mg CGA/100 g, Cevallos-Casals & Cisneros-Zevallos, 2004).

Plums showed a 3- to 4-fold higher phenolic concentration in the skin than in the flesh. Similarly, the
antocyanin concentration in the skin was 3- to 9-fold higher than in the flesh (Table 1). Even though the exocarp is a concentrated source of phenolic compounds, it only represents 7–9% of the fruit weight. Thus, the total distribution of phenolic compounds in skin and flesh per fruit is ~30% and 70%, respectively.

The main anthocyanins identified in *P. salicina*, the principal species in the genetic background of the studied plums, have been cyanidin 3-glucoside, cyanidin 3-rutinoside and cyanidin 3-xylosylglucosides (sambubioside) (Ahn, 1973; Draetta, Iaderoza, Baldini, & Francis, 1985; Ishikura, 1975; Itoo, Matsuo, Noguchi, & Kodama, 1982). The main anthocyanin identified in *Prunus persica* (peach) has been cyanidin 3-glucoside with contribution of cyanidin 3-rutinoside (Hsia, Luh, & Chichester, 1965; Ishikura, 1975; Van Blaricom & Senn, 1967).

Apart from anthocyanins, 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 (Kim et al., 2003; Tomás-Barberán et al., 2001).

### 3.2. Antioxidant activity and kinetics

The obtained RAC values varied from 1254 to 3244 μg trolox/g for plums and from 440 to 1784 μg trolox/g for peaches (Fig. 1). There was a positive correlation between phenolic content and RAC for the plum and peach genotypes studied, suggesting that phenolic compounds are responsible for the antioxidant activity (Fig. 2). A slightly higher correlation was obtained with

![Graph showing total anthocyanins, total phenolics, and RAC of new plum and peach genotypes](image)

Fig. 1. Total anthocyanins, total phenolics, and RAC of new plum and peach genotypes. Peach genotypes are indicated with dotted bars. Data indicates mean ± SD of three replicates. Each replicate came from two different fruit.

### Table 1

<table>
<thead>
<tr>
<th>Plum genotype</th>
<th>Section</th>
<th>Anthocyanin content</th>
<th>Phenolic content</th>
<th>% of total crop weight</th>
<th>% anthocyanin distribution</th>
<th>% phenolic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>BY96M777</td>
<td>Skin</td>
<td>872 ± 96</td>
<td>2385 ± 14</td>
<td>7.2</td>
<td>43.7</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td>Flesh</td>
<td>87 ± 13</td>
<td>430 ± 43</td>
<td>92.8</td>
<td>56.3</td>
<td>69.9</td>
</tr>
<tr>
<td>BY94M1945</td>
<td>Skin</td>
<td>344 ± 12</td>
<td>2394 ± 37</td>
<td>9.3</td>
<td>29.3</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>Flesh</td>
<td>85 ± 4</td>
<td>562 ± 5</td>
<td>90.7</td>
<td>70.7</td>
<td>69.6</td>
</tr>
</tbody>
</table>

Data indicate means ± SD of three replicates. Each replicate came from two different fruit.

**a-c** Means within a column with the same superscript letter are not significantly different (*p* > 0.05).

d In mg cyanidin-3-glucoside equiv/100 g wet basis.

e In mg CGA/100 g wet basis.

f Total weight does not include the endocarp and seed.

g Values indicate the contribution of the section to the total weight of the sample excluding endocarp and seed.
While the peach still represents a lower content with \( \sim 46\% \) the antioxidant activity of blueberry. However, when comparison is made by antioxidant kinetics (AK), peach shows higher AK than blueberry and plum, indicating that peach phenolic compounds may have faster reaction kinetics against radical species (DPPH radicals used in this study). Antioxidant kinetic studies are important because they indicate how much an antioxidant reduces the rate of oxidation (Shi & Niki, 1998). When compared to the kinetic rates of common antioxidants used in foods, the \( k_2 \) of peach obtained in this study was slightly lower than that of BHA (0.42) and higher than that of BHT (0.005), reported in previous work (Espin et al., 2000).

3.3. Colour stability and chromaticity

Selection of plum genotypes high in anthocyanin content may be related to colorant properties, such as colour stability and chromaticity. Colour stability evaluation can be done by comparing with grape extracts, considered a common source of food commercial anthocyanins (Malien-Aubert, Dangles, & Amiot, 2001). For example, a selected plum rich in anthocyanins (BY94M1945) shows higher colour retention than grape extracts when stored at different pHs and temperatures (Table 3). At 25 °C for 138 days, colour losses, for pH 1 and 3 plum extracts, were 21% and 23%, compared to colour losses of 30% and 31% for grape extracts, respectively. Similarly, at 99 °C for 2 h at pHs 1 and 3, colour losses were 17% and 35% for the plum extract, while colour losses were 69% and 30% for grape extracts. Additionally, red grape extracts were completely clear at pH 4 and 5, whereas plum extracts still maintained red coloration (data not shown). Regarding browning, both extracts experienced an increase in \( A_{420 \text{ nm}} \) with time (Table 3). In general, colour degradation may be due to anthocyanin polymerization, the presence of sugars and their degradation products (Duhard, Garnier, & Megard, 1997).

### Table 2

<table>
<thead>
<tr>
<th>Crop</th>
<th>Dry matter (%)</th>
<th>Anthocyanin content</th>
<th>Phenolic content</th>
<th>RAC</th>
<th>AK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet basis</td>
<td>Dry basis</td>
<td>Wet basis</td>
<td>Dry basis</td>
<td></td>
</tr>
<tr>
<td>Plum BY94M1945</td>
<td>11.1</td>
<td>125( \pm ) 4</td>
<td>563( \pm ) 6</td>
<td>3244( \pm ) (1,100 )</td>
<td>0.12</td>
</tr>
<tr>
<td>Peach BY94P7552</td>
<td>18.0</td>
<td>36( \pm ) 5</td>
<td>5074( \pm ) 50</td>
<td>29225( \pm ) (1,100 )</td>
<td>0.30</td>
</tr>
<tr>
<td>Blueberry(a)</td>
<td>16.5</td>
<td>276( \pm ) 25</td>
<td>1675( \pm ) 31</td>
<td>1784( \pm ) (1,100 )</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data indicate means ± SD of three replicates. Each replicate came from two different fruit. Data for AK were conducted in triplicate with five different extract concentrations per sample.

\( a-c \) Means within a column with the same superscript letter are not significantly different (\( p > 0.05 \)).

\( d \) In mg cyanidin-3-glucoside equiv./100 g.

\( e \) In mg CGA/100 g.

\( f \) In \( \mu \)g trolox equiv./g.

\( g \) Second-order rate constant (\( k_2 \)) in (g/l)\(^{-1}\) s\(^{-1}\), \( r^2 \) denotes the linear fitting of five different sample concentrations to their respective \( k_1 \) constants.

\( h \) Data for dry matter, anthocyanin content, phenolic content and AK of blueberry were taken from Cevallos-Casals and Cisneros-Zevallos (2003).
99 °C was chosen to reflect a severe heat treatment during thermal food processing operations (e.g., blanching, pasteurization, cooking). Chromaticity evaluation was done by comparing with synthetic colorants red #3 and red #40, considered common commercial red colorants (Rodrıǵuez-Saona, Giusti, & Wrolstad, 1998). For example, the selected plum rich in anthocyanins, formed extracts with similar hue to red #3 and slightly lower hue than red #40, at similar tinctorial strengths at pH 3 (Table 4). Lightness was similar and chroma lower than both synthetic colorants. Changing conditions of pH and tinctorial strength may aid in adjusting the chromaticity of the natural extracts to those of the synthetic colorants (Rodrıǵuez-Saona et al., 1998).

### 3.4. Antimicrobial activity

Selection of plum genotypes high in phenolic content may also be related to antimicrobial properties. The antimicrobial evaluation can be done against human pathogens of significant importance to the food industry (Davidson & Parish, 1989). For example, a selected plum rich in anthocyanins, formed extracts with similar hue to red #3 and slightly lower hue than red #40, at similar tinctorial strengths at pH 3 (Table 4). Lightness was similar and chroma lower than both synthetic colorants. Changing conditions of pH and tinctorial strength may aid in adjusting the chromaticity of the natural extracts to those of the synthetic colorants (Rodrıǵuez-Saona et al., 1998).

**Table 3**

<table>
<thead>
<tr>
<th>pH</th>
<th>λmax (nm)</th>
<th>Absorbance (a.max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>514</td>
<td>0.84 (0.29)</td>
</tr>
<tr>
<td>3</td>
<td>516</td>
<td>0.47 (0.20)</td>
</tr>
</tbody>
</table>

Values in parentheses show the absorbances of extracts at 420 nm, an indicator of browning.

### 3.4. Antimicrobial activity

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**Table 4**

<table>
<thead>
<tr>
<th>Colorant</th>
<th>pH</th>
<th>L</th>
<th>Hue</th>
<th>Chroma</th>
<th>Tinctorial strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red 40</td>
<td>3</td>
<td>72</td>
<td>39</td>
<td>70</td>
<td>9.0</td>
</tr>
<tr>
<td>Red 3</td>
<td>3</td>
<td>71</td>
<td>25</td>
<td>73</td>
<td>7.2</td>
</tr>
<tr>
<td>Plum</td>
<td>3</td>
<td>71</td>
<td>23</td>
<td>53</td>
<td>10.6</td>
</tr>
</tbody>
</table>

* Data for red 40 and red 3 taken from Cevallos-Casals and Cisneros-Zevallos (2004).

Salmonella Enteritidis than against *Escherichia coli* O157:H7.

Both, the absorbance assay and the spread plate assay, showed a lag, log and stationary phase within 50 h. The selection of the assay may be related to the resemblance to the food matrix where the antimicrobial is intended to be used. In the absorbance assay, antimicrobials interact with microorganisms in a liquid matrix, as opposed to a semi-dry environment in the spread plate technique.

It has been shown that phenolic compounds, including anthocyanins, have antimicrobial activity (Beuchat & Golden, 1989; Davidson & Branen, 1981). Chlorogenic acid (3-caffeyl-quinic) has been shown to have strong antimicrobial activity (Davidson & Branen, 1981), and is usually present in plums in high amounts. The active portion of chlorogenic acid, according to Grodzinska-Zachwieja and Kahl (1966), is caffeic acid, a hydroxycinnamic acid. Several hydroxycinnamic acid derivatives have been found to have antimicrobial effects against several microorganisms, including *E. coli* (Baranowski, Davidson, Nagel, & Branen, 1980; Leifertova, Hejtmankova, Hlava, Kudrnacova, & Santavy, 1975; Valle, 1957). The general mechanism of antimicrobial activity may involve a reaction with the cell membrane or inactivation of essential cellular enzymes or a combination of the two (Davidson & Branen, 1981). Previous studies have suggested that the reactive portion of antimicrobial phenolic compounds may be the free hydroxyl group (Prindle & Wright, 1977).

In conclusion, through breeding programmes, crops may constantly be improved to have improved functional properties. The selection of crops rich in phenolic compounds, with enhanced antioxidant, antimicrobial and colorant properties, would be a first step. In this study, different assays were used to characterize these three major phenolic properties in new peach and plum genotypes. The resulting selected fruits, rich in phenolic compounds and functional properties, may be used for the fresh produce and processing market. For the latter, fruit extracts or juices may have GRAS (Generally Recognized As Safe) status and may be potential food ingredients for protecting food and consumers and for imparting colour.
Fig. 3. Effect of plum BY96M777 extracts on the growth of *Escherichia coli* O157:H7 and *Salmonella* Enteritidis with two different assays: (A) absorbance assay and (B) spread plate assay. *a,b*Initial microbial concentrations (CFU/mL): *a*10^3, *b*10^5. *c*Plum concentrations in mg CGA/mL solution. *d*Plum concentrations in mg CGA/plate. Data indicates mean ± SD of three replicates with three repetitions for each replicate.

### References


