Phenolic Content and Antioxidant Capacity of Pawpaw Fruit (Asimina triloba L.) at Different Ripening Stages

Hideka Kobayashi1, Changzheng Wang2,4, and Kirk W. Pomper3
Human Nutrition Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601

Abstract. Pawpaw [Asimina triloba (L.) Dunal], a native species of the eastern United States, bears the largest edible fruit of all indigenous trees. Chemoprotective properties of fruits have been partly attributed to phenolics such as gallic acid and chlorogenic acid, and the phenolic content generally correlates with antioxidant capacity for various kinds of fruits. Despite many reports of commonly available fruits, little information is available on phenolic content or antioxidant capacity for currently underused fruits. The objectives of this study were to determine the phenolic content (PC) and antioxidant capacity (AC) in fruit of two pawpaw cultivars at different stages of ripening. Sample extraction of pawpaw was achieved by adding acetone (2 mL/g of sample) to the pulp of ‘PA-Golden (#1)’ and advanced selection 1-23, and then vortexing (30 s) and sonicating (15 min.) the sample and solvent before centrifuging it (15 min) twice at 2987 g. Folin-Ciocalteu assay and ferric reducing/antioxidant power assay were used for the estimation of PC and AC, respectively. PC and AC tended to decrease with ripening of fruit. The highest AC was found in the semiripe ‘PA-Golden (#1)’ puree (22.06 μmol TE/g fresh weight), whereas the puree of ripe fruit contained the lowest AC (17.04 μmol TE/g fresh weight), about a 23% decrease. In contrast, the greatest PC and AC were observed in intermediate fruits for 1-23. A positive correlation was found between PC and AC of fruit of ‘PA-Golden (#1)’ (r = 0.62) and 1-23 (r = 0.82). These results suggest that phenolic components of pawpaw pulp have a major effect on AC, as reported for other fruits and vegetables. The relatively high AC found in pawpaw pulp may motivate more health-conscious people to consume pawpaw fruit. The diversity in PC and AC of pawpaw cultivars emphasizes the need for additional screening to identify cultivars with high AC and health-promoting potential.

Recently, much attention has been paid to understanding the roles of fruits and vegetables in the promotion of human health and prevention of chronic diseases. Epidemiological studies have repeatedly shown the apparent association between high fruit intake and the lower incidence of chronic diseases (Ness and Powles, 1997; New et al., 2000). The chemoprotective properties of fruits have been partly attributed to phenolics such as gallic acid and chlorogenic acid, and the phenolic content (PC) generally correlates with antioxidant capacity (AC) for various types of fruits (Olsson et al., 2004; Sun et al., 2002). Phenolic antioxidants can scavenge free radical species, inhibit free radical formation, and prevent damage to cellular components, and subsequent cellular damage or death (Sun et al., 2002).

Despite many reports of commonly available fruits such as blueberry and apple on their PC and AC, little information is available for currently underused fruits. These underused fruits may contain a significant amount of phytochemicals or even unique compounds that are health-promoting. Their AC may be comparable or even superior to that of the more extensively studied fruits. For example, a study conducted at the USDA Human Nutrition Center found that blueberry had the greatest AC of all 40 fresh fruits and vegetables examined (Cao et al., 1996). The strong AC and other evidence of health-promoting properties have helped blueberry become a popular commercial fruit crop (Butkus and Pliszka, 1993, Haddad et al., 2001; McNulty et al., 2003; Rossi et al., 2003; Schmidt et al., 2004; Weiguang et al., 2006).

Pawpaw [Asimina triloba (L.) Dunal] is a small tree found in the temperate woodlands of the Eastern United States that bears the largest edible fruit of all native species (Kral, 1960; Pomper and Layne, 2005). Efforts to popularize pawpaw started in the early part of the last century through domestication and development of superior cultivars (Peterson, 2003). The interest in pawpaw waned over the years, possibly because of perishability (Agriculture, Cooperative State Research, Education, and Extension Service Agreement no. KYX-2003-01971) with Kentucky State University.

1Co-Investigator of Human Nutrition.
2Principal Investigator of Human Nutrition
3Principal Investigator of Horticulture and Curator, USDA National Clonal Germplasm Repository for Asimina species.
4To whom reprint requests should be addressed: e-mail changzheng.wang@ksu.edu

Received for publication 13 June 2007. Accepted for publication 13 Aug. 2007.

This research was supported by U.S. Dept. of Agriculture, Cooperative State Research, Education, and Extension Service Agreement no. KYX-2003-01971 with Kentucky State University.
weight of both the tube and the samples was recorded. Acetone was added at 2 mL/g of sample. After the addition of acetone, samples were thawed in the solvent to prevent oxidation, vortexed for 30 s, and then sonicated for 15 min. The samples were then centrifuged twice at 2980 g to obtain supernatant for analysis of PC and AC.

All chemical reagents were purchased from Sigma (St. Louis, MO) unless otherwise mentioned. The PC was quantified by a modified protocol of Singleton and Rossi (1965) for 96-well plates (Dicko et al., 2005). Absorbance of the mixture was measured at 760 nm with a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA). After initial evaluation of the reaction kinetics for up to 150 min, the absorbance reading at 90 min was selected to determine the total PC, expressed in milligrams of gallic acid equivalent per 100 grams of fresh weight (mg GAE/100 g fresh weight).

The AC of pawpaw extract was quantified by ferric reducing/antioxidant power (FRAP) assay (Benzie and Strain, 1999), adapted for 96-well plates (Firuzi et al., 2005). Working FRAP solution was freshly made by mixing 15 mL of acetate buffer (300 mM) and 1.5 mL of 2,4,6-tripyridyl-s-triazine (10 mM) and FeCl₃/C₁₆H₂O solution. Acetate buffer and FRAP solution were warmed to 37 °C before adding to the well of the plate. Absorbance of the mixture was measured at 595 nm with the aforementioned microplate reader at 37 °C. The antioxidant power was expressed in micromoles of Trolox (a water-soluble analog of vitamin E) equivalent per gram of fresh weight (µM TE/g fresh weight). The absorbance readings at 60 min were used after preliminary experiments.

Each pulp sample was analyzed three times. The results were analyzed using two-way analysis of variance followed by the least significant difference test for mean comparison. Regression analysis was used to examine the relationship between PC and AC for both genotypes. All statistical analyses were performed using the statistical software package PC-SAS, version 8E (SAS Institute, Cary, NC).

Overall, ‘PA-Golden (#1)’ had significantly greater hardness, lower SSC, and higher PC and AC than the advanced selection 1-23 (Table 1). The SSC tended to increase with ripening more for ‘PA-Golden (#1)’ than for the advanced selection 1–23. This was in agreement with others, who observed that SSC gradually increased during ripening in various types of fruits, including muskmelon/muskmelon (Corzo and Gomez, 2004; Villanueva et al., 2004), cherry (Yoon et al., 2006), mango (Jha et al., 2006), and nectarine (Aubert et al., 2003). The increase in SSC may be from water loss during ripening (Corzo and Gomez, 2004) or degradation of starch and subsequent conversion into sucrose, glucose, fructose, and galactose (Villanueva et al., 2004). Considering that no change in water content was observed in pawpaw pulp (data not shown), starch catabolism and subsequent conversion into mono-

saccharide and disaccharides are the possible cause of the increase in SSC in pawpaw pulp. PC and AC tended to decrease with ripening of fruit. The highest AC was found in the semiripe ‘PA-Golden (#1)’ puree (22.06 µmol TE/g fresh weight), whereas the puree of ripe fruit contained the lowest AC (17.04 µmol TE/g fresh weight), about a 22.8% decrease. In contrast, the greatest PC and AC were observed in intermediate fruit for 1-23. Of all

<table>
<thead>
<tr>
<th>Clone and fruit maturity</th>
<th>Penetration force (N)</th>
<th>Soluble solid content (°Brix)</th>
<th>Phenolic content (mg GAE/100 g FW)</th>
<th>Antioxidant capacity (µmol TE/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'PA-Golden (#1)'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unripe</td>
<td>12.31 a</td>
<td>15.10 b</td>
<td>123.06 a</td>
<td>21.43 a</td>
</tr>
<tr>
<td>Semiripe</td>
<td>1.04 b</td>
<td>15.78 ab</td>
<td>106.22 b</td>
<td>22.06 a</td>
</tr>
<tr>
<td>Ripe</td>
<td>0.72 b</td>
<td>16.73 a</td>
<td>98.42 b</td>
<td>17.04 b</td>
</tr>
<tr>
<td>1-23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unripe</td>
<td>1.63 a</td>
<td>18.25 a</td>
<td>71.41 a</td>
<td>16.94 b</td>
</tr>
<tr>
<td>Semiripe</td>
<td>0.65 b</td>
<td>18.55 a</td>
<td>76.23 a</td>
<td>19.88 a</td>
</tr>
<tr>
<td>Ripe</td>
<td>0.44 c</td>
<td>18.65 a</td>
<td>64.11 b</td>
<td>15.57 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different within the same column (P < 0.05).

Fig. 1. Correlations between phenolic content and antioxidant capacity of puree of ‘PA-Golden (#1)’ (A) and 1-23 (B).
correlation coefficients obtained, only PC and AC showed a high correlation for both cultivars (Fig. 1). These results suggest that phenolic components of pawpaw pulp have a major effect on AC, as reported for other fruits and vegetables (Wu et al., 2004).

Compared with other common fruits, PC and AC of pawpaw were equivalent or even superior in some cases. Although PC of pawpaw fruit examined in this study was lower than that of cranberry, apple, red grape, and strawberry, it is comparable or superior to other fruits such as lemon, peach, orange, banana, pear, pineapple, and grapefruit, as reported by Sun et al. (2002). Blueberry is well known for its AC, but it can vary depending on cultivars, storage duration (Conner et al., 2002), and place of production (Taruscio et al., 2004). The PC and AC of pawpaw fruit are comparable to those of at least some blueberry cultivars.

In conclusion, the relatively high AC found in pawpaw pulp may motivate more health-conscious people to consume pawpaw fruit. The diversity in PC and AC between pawpaw cultivars emphasizes the need for additional screening to identify cultivars with high AC and health-promoting potential.

Literature Cited


