Chemical composition, color, and antioxidant activity of three varieties of \textit{Annona diversifolia} Safford fruits

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\textbf{A B S T R A C T}

\textit{Annona diversifolia} is a Central America native fruit which has become an important crop because of its tasty flavor, high pulp content, nutritional value and antioxidant properties. Color, crude protein, crude fat, crude fiber, ash, minerals, pH, titratable acidity, and carbohydrate contents were determined in white, pink and deep pink varieties of \textit{A. diversifolia}. Our results show that the three studied varieties of \textit{A. diversifolia} could be considered as important sources of fiber, sugars, potassium and zinc. Color measuring was successfully used to objectively characterize the three \textit{A. diversifolia} varieties, resulting in an excellent agreement with visual observations. The antioxidant activity also was determined and compared. An in vitro study was conducted in terms of vitamin C, total phenols, total flavonoids, and radical scavenging activity. A strong correlation between total polyphenols and radical scavenging activity was observed. This paper reports for the first time the total flavonoid content of \textit{Annona} genus fruits. In addition, \textit{A. diversifolia} extracts possessed greater radical scavenging activity and similar total polyphenol content compared to those of other \textit{Annona} species reported elsewhere.

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\section{1. Introduction}

Plants of the family \textit{Annonaceae} are regarded as important economic crops worldwide (Liang et al., 2008). \textit{Annona diversifolia} is a native fruit of Southeastern Mexico, Guatemala and El Salvador and is considered as one of the most important subtropical fruits from warm weather locations. The tree can reach up to 8.5 m high. The trunk is usually no more than 23 cm thick, often branching from the ground to form three to six main stems. The fruit has an oval shape, with the largest specimens weighing from 500 to 900 g. Each tree produces an average of 50 fruits. The fruit surface is rough, with carpellary areas indicated by deeply incised lines. A short thick protuberance is attached to the areoles (Duke and duCellier, 1993). The color of the peel varies from pale green to deep pink. For the pale green peel fruits, the pulp is white; whereas for the pink peels, the pulp has a similar color than the peel; i.e., pink or deep pink. For the white pulp variety the flavor is sweet, very similar to that of sugar apple (\textit{A. squamosa}). In the case of the pink varieties, the flavor is more acid, resembling that of cherimoya. \textit{A. diversifolia} fruits are picked once a year, and are readily edible when the shell is open from the peduncle and the pulp becomes exposed (Fig. 1). The pulp of \textit{A. diversifolia} fruits is soft and has a pleasant taste.

\cite{cruz2000} reported the chemical composition and some physical characteristics for \textit{A. diversifolia} fruits from El Salvador. In the study a comparative analysis was made for different fruits of the genus \textit{Annona} (Cruz and Deras, 2000), which included \textit{A. diversifolia}. The authors obtained the proximate analysis and some morphological characteristics of this fruit; however, they did not specify which \textit{A. diversifolia} variety was studied.

Fruits of the genus \textit{Annona} contain a considerable amount of polyphenolic compounds (Roesler et al., 2006). These compounds are antioxidants and help to prevent diseases associated with oxidative stress, such as cancer, atherosclerosis and neurodegenerative diseases (Steinmetz and Potter, 1996; La Vecchia et al., 2001; Neto, 2007; Zibadi et al., 2007). A mechanism for antioxidant activity of natural compounds is the inhibition and suppression of the formation of reactive species either by inhibiting enzymes or by chelation of the trace elements involved in the formation of free radicals. Antioxidants also participate as free radical scavengers by regulating or protecting the endogenous antioxidant defense (Halliwell and Gutteridge, 1999).

The antioxidant activity of a plant food results from the interactions of complex biochemical structures. A number of parameters have been defined to characterize this activity, among which are: total polyphenols (TPP), total flavonoids (TF) and radical scavenging activity (RSA). TPP and RSA have been determined in some fruits of the genus \textit{Annona}; e.g., \textit{A. muricata} (Hassimotto et al., 2005), \textit{A. cherimolia} (Vasco et al., 2008) and \textit{A. squamosa} (Yan et al., 2006). RSA is
Fig. 1. Varieties of *A. diversifolia* fruit: (a) white, (b) pink and (c) deep pink.

an indicator of the functionality and antioxidant activity of a food. It has been related to the contents of TPP and TF in plant foods (Heim et al., 2002; Balasundram et al., 2006). Flavonoids and other phenolic compounds are highly reactive toward free radicals, so that quantification of these compounds is also indicative of antioxidant activity. 

*A. diversifolia* fruits are relatively expensive in the local markets (approximately US$2.0 each fruit), mainly because of their tasty flavor and high pulp yield (approximately 50%). Three varieties of *A. diversifolia* were studied in this paper: white, pink and deep pink. In order to characterize the chemical composition and color of these fruits, we determined proximate composition, minerals, sugars and color expressed as L*, a* and b* parameters. In addition, the functional properties of these fruits were studied by assessing the antioxidant activity of the fruits expressed as TPP, TF and RSA.

2. Materials and methods

2.1. Chemicals

Sodium 2,6-dichloroindophenolate (DCP), metaphosphoric acid, Folín–Ciocalteu reagent, gallic acid, catechin, 2,6-di-t-butyl-4-methylphenol (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), lantanide oxide, d (+)-glucose, d (−)-fructose, sucrose were purchased from Sigma–Aldrich Inc. Sodium acetate (AcONa), acetic glacial acid (AcOH), HCl, HNO3, AlCl3, NaNO2, Na2CO3, NaOH, ethanol, vitamin C, water HPLC grade, EDTA, CsCl, Na and Mg standards were purchased from J.T. Baker. Zn and Ca standards were purchased from Merck, K standard from Buck Scientific, methanol HPLC grade was from EMD. All reagents and solvents were ACS grade, except when otherwise stated.

2.2. Plant material

The white, pink and deep pink fruits of *A. diversifolia* (6–10 kg) were purchased in local markets in Tuxtlá Gutierrez (Chiapas, Mexico) on September 2008. Only pest-free fruits with no physical damage were acquired. The fruits were washed and the pulp was separated manually from the seeds and the peel. Then, they were stored in glass flasks at −20 °C and protected from light prior to analysis.

2.3. Color quantification

A quartz cell was filled with 25 mL of homogenized pulp of *A. diversifolia* fruits. Color measurements were performed in a spectrophotometer colorimeter (Ultra HunterLabScanVis); using illuminant D65, 10° viewing angle and with an observation diameter of 1.25 cm. CIE color data (L*, a*, b*) were obtained in triplicate.

These parameters were used to calculate the hue angle $h_{ab}^{*}$ and the chromaticity value $C_{ab}^{*}$ with the following formulas:

$$h_{ab}^{*} = \tan^{-1} \left( \frac{b^{*}}{a^{*}} \right)$$

$$C_{ab}^{*} = (a^{*2} + b^{*2})^{0.5}$$

2.4. Proximate chemistry analysis

AOAC (1997) standard methods were used for the proximate analyses of *A. diversifolia* fruit pulps. Total carbohydrates were calculated as the mass balance difference (%) related to the other components; it was expressed as the nitrogen free extract (NFE). The contents of the primary and secondary metabolites are expressed in this work as a percent, grams of primary/secondary metabolite in 100 g of fresh pulp, except when otherwise stated.

2.5. Determination of pH and titratable acidity

The pH was measured with a Conductronic pH 10 potentiometer, calibrated with buffer solutions of pH=4 and 7, to 25 °C. Titratable acidity was determined by titration of the pulp juice with 0.05 N NaOH up to a pH of 9. The results were expressed as grams of citric acid per 100 g of fresh pulp. The pH was measured every 15 days during the period in which the pulp of each variety was kept frozen (October 2008–June 2009) to assess possible changes in the samples. Each measurement was performed in triplicate.

2.6. Quantification of sucrose, glucose and fructose

Carbohydrate extraction was performed according to the method described by Ong et al. (2006). Briefly, 1 g of pulp was added to 20 mL of aqueous MeOH 85% (v/v), and was heated in a water bath at 80 °C under reflux for 30 min with stirring. The mixture was then filtered and the residue was thus re-extracted two more times with 15 mL of aqueous MeOH. The filtrate was evaporated in a rotary evaporator at 45 °C under vacuum until dryness. The residue, syrup, was dissolved in water, and filtered through 0.2 µm filters (Sartorius minisart). Each aqueous extract was obtained in triplicate. The separation and quantification was performed using an HPLC system comprised of a GBC LC1150 pump, a refractive index detector GBC LC1240, and a MetaCarb 87C Varian column with a Eppendorf CH-30 heater. The column was operated with an isocratic aqueous mobile phase containing 50 µg/mL of calcium EDTA at 85 °C. Sucrose, glucose and fructose were used as carbohydrate standards analytical grade. A calibration curve was obtained for each of the standards.
2.7. Quantification of minerals

The ashes obtained from proximate analysis were used for the determination of calcium, magnesium, sodium, potassium and zinc, according to the method described by Dürüst et al. (1997). A known amount of ashes was dissolved in 10 mL of HCl 20% (v/v), the solution was slowly boiled for 30 min. The solid-free solution was dissolved in water to a volume of 25 mL. Each sample was performed in triplicate. To avoid possible interferences in the determination of calcium and magnesium, lanthanum chloride was added both to the acid sample solution and to the standard solutions to a final concentration of 2000 µg/mL. Similarly, to avoid ionization in the determination of sodium and potassium, cesium chloride was added (2000 µg/mL). Absorbance acquisition was conducted in an atomic absorption spectrophotometer GBC 932 AA equipped with hollow cathode lamps (Table 1).

Recovery levels were determined by adding known amounts of calcium, magnesium and zinc before the acidic digestion. The average recovery was found to be 107 and 123%, indicating that the methods used for oxidation of organic matter and mineral quantification were reproducible.

2.8. Vitamin C quantification

2.8.1. Extraction procedure

Vitamin C extraction was performed according to Hernández et al. (2006). Briefly, 10 g of fruit pulp was mixed with 20 mL of extraction solution. Solution A [3 g metaphosphoric acid and 8 mL AcOH taken to 100 mL with H2O] in a home mixer for 2 min. The resulting mixture was centrifuged in an IEC HN-SII centrifuge at 4000 rpm for 5 min. Following this, the supernatant was separated and the pellet was thus extracted twice. The extracts were combined and filtered through cotton wool. The extracts of each variety were obtained in triplicate.

2.8.2. Vitamin C assay

Vitamin C quantification was carried out according to the method described by Dürüst et al. (1997). Absorbance readings were made at 520 nm in a UV/Vis spectrophotometer (Perkin-Elmer Lambda 35). This method consists of measuring the amount of DCPI reduced by the sample compared to that reduced by a vitamin C standard solution. Two absorbance measurements (L1 and L2) were performed as follows:

(a) L1 measurement: the spectrophotometer was adjusted to zero with deionized water. Then, 0.5 mL of Solution A was mixed with 0.5 mL sodium acetate buffer [4.5 g AcONa, 5H2O, 10.5 mL deionized H2O and 15 mL AcOH] and 4 mL of DCPI aqueous solution (12 µg/mL). The absorbance was measured following 15 s and logged as L1. Here, L1 is the absorbance of initial DCPI in all experiments.

(b) L2 measurement: the spectrophotometer was adjusted to zero with 0.5 mL of vitamin C standard aqueous solution (2 µg/mL) or sample solution, 0.5 mL of acetate buffer and 4 mL of deionized water. A solution was then prepared with 0.5 mL of vitamin C standard solution or sample solution, 0.5 mL acetate buffer and 4 mL DCPI (12 µg/mL). Following 15 s, the absorbance was measured and logged as L2. This value is the absorbance of the remaining DCPI after its reaction with vitamin C.

The difference L1 – L2 indicates the amount of vitamin C present in the calibration-standard/sample. To construct a calibration curve, a series of vitamin C calibration standards were prepared with concentrations ranging from 4 to 10 µg/mL. The calibration curve was constructed by plotting L1 – L2 values in terms of the calibration standard vitamin C concentrations (µg/mL). All solutions were prepared the same day using deionized water. Standard and sample measurements were performed in triplicate.

2.9. Total polyphenols quantification (TPP)

2.9.1. Extraction procedure

The extraction was performed according to the method described by Roesler et al. (2006). Briefly, 2 g of pulp were mixed with 25 mL of purified and degassed ethanol 90% (v/v) in a home blender for 1 min. The mixture was filtered through cotton wool. Extracts for each variety were thus obtained in triplicate. A small volume of this extract was used to determine radical scavenging activity (RSA).

2.9.2. Total polyphenols assay (TPP)

TPP quantification was performed with the method of the Folin–Ciocalteu reagent (Erçil et al., 2008). Briefly, a mixture was prepared comprising 0.1 mL of sample extract, 4.6 mL of deionized, degassed water, and 0.1 mL of the Folin–Ciocalteu reagent. The mixture was left to rest for 3 min in the absence of light; then, 0.3 mL of Na2CO3 aqueous solution (2%, w/v) was added. Following this, the mixture was stirred and nitrogen was injected into the reaction tubes. After 30 min of incubation at 40 °C, the absorbance was measured at 760 nm in a UV/Vis spectrophotometer (Perkin-Elmer Lambda 35). Gallic acid was used as a spectrophotometric standard with concentrations ranging from 25 to 150 µg/mL. TPP content in the fruit sample extracts was determined with the calibration curve, and expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight. Standard and sample measurements were performed in triplicate.

2.10. Total flavonoids quantification (TF)

2.10.1. Extraction procedure

1 g of pulp was mixed with 5 mL of methanol. The mixture was sonicated in a sonicator-degasser (Branson 8510) at room temperature during 30 min. The resulting mixture was centrifuged at 3000 rpm for 30 min. Following this, the supernatant was separated and the pellet was thus extracted twice. The extracts were pooled into a single flask and were then filtered through cotton wool. The extracts of each variety were obtained in triplicate.
2.10.2. Total flavonoid assay (TF)

This determination was carried out according to the method described by Lee et al. (2003), adapted to a microplate reader Biotek ELX-808. Briefly, 0.1 ml of extract, 2 ml of deionized water and 0.15 ml of NaNO₂ aqueous solution (5%, v/v) were mixed for 5 min by vortexing. Then, 0.15 ml of AIC₃₂ aqueous solution (10%, v/v) was added and the mixture was vortexed for 1 min. Following this, 1 ml of 1 M NaOH was added and stirring was resumed for 1 min. The samples and standards were then measured in the microplate reader at 490 nm. A calibration curve was performed using catechin standards with concentrations ranging from 100 to 500 µg/ml. The content of TF in the fruit sample extracts was determined with the calibration curve, and expressed as milligrams of catechin equivalents (CE) per 100 g of fresh weight. Standard and sample measurements were performed in triplicate.

2.11. Radical scavenging activity quantification (RSA)

RSA is expressed by the IC₅₀ parameter, which is defined as the fresh weight concentration required to decrease the initial concentration of DPPH radical (in vitro) by 50%.

2.11.1. Extraction procedure

Fruit extracts were obtained using the method described in the previous section for TPP extraction. Ethanolic extracts of each A. diversifolia variety were prepared for RSA determination with pulp concentrations ranging from 500 to 3000 µg pulp/ml.

2.11.2. RSA assay

The RSA was determined by the DPPH assay according to the method reported by Roessler et al. (2006). To evaluate the reduction of the DPPH radical, 2500 µl of sample extract were mixed with 2500 µl of DPPH ethanolic solution (0.004%, w/v). A fresh solution of DPPH was used for every RSA determination to prevent DPPH degradation by oxygen and light. The mixture was incubated at room temperature in the dark and stirred for 30 min. This procedure was also applied to ascorbic acid, BHT and gallic acid, which were employed as controls of the test. A negative control was prepared with no extract using 90% aqueous ethanol for baseline correction. Sample IC₅₀ data were obtained by plotting the decrease of the DPPH absorbance (%) at 517 nm as a function of the pulp extract concentration (Yu et al., 2002). All measurements were performed in triplicate.

2.12. Statistical analysis

All data were reported as means ± standard deviation of three samples. An analysis of variance (ANOVA) was performed to assess the statistical significance of the differences between samples. In addition, Duncan’s multiple range method was used for comparison of means, considering a confidence level of 95% (p < 0.05). Statistical analyses were made with Design Expert 6.0 computer software. Linear correlations were performed to assess the relationships between variables following Pearson’s method.

Table 2

<table>
<thead>
<tr>
<th>Variety</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Hue (h°)</th>
<th>Chroma (C°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>65.1</td>
<td>0.23</td>
<td>17.8</td>
<td>82.6</td>
<td>17.9</td>
</tr>
<tr>
<td>Pink</td>
<td>58.1</td>
<td>0.67</td>
<td>10.7</td>
<td>58.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Deep pink</td>
<td>47.4</td>
<td>18.9</td>
<td>0.5</td>
<td>358.5</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Values in the same column are significantly different (p < 0.05) according to the method of Duncan’s multiples ranges.

3. Results and discussion

3.1. Color and proximate chemical composition

The CIE color value L* of a sample indicates its lightness. As expected from visual observations (Fig. 1), the opaqueness of the sample increased (L* decreasing) in the following order for the A. diversifolia varieties: white, pink and deep pink (Table 2). The a* and b* values indicate the intensity of red and yellow in the pulp, respectively. Similarly to the L* results, the red color intensity increased from the white to the deep pink variety, whereas a reduction in the yellow color intensity was observed for the varieties (Table 2). These results also agreed with visual observations. The hue angle h° is an indicator of the tone of each variety. Using this color parameter, the white variety indicated a yellow color with h° = 85.7°; the rose variety, displayed an orange tone (h° = 58.0°); and the deep pink, resulted in a red color (h° = 358.5°). The chromacity value C° indicated that the white and deep pink varieties are perceived as more intense than the pink fruit.

For the proximal analyses, significant differences (p < 0.05) were obtained for the white variety compared to the other two fruits in the protein, fat and crude fiber content. On the other hand, the three fruits were statistically different in their content of nitrogen-free extract (NFE) (Table 3). No significant differences were encountered for the ash and moisture content assays.

The protein in the white variety, 1.14%, is similar to that reported by Cruz and Deras (2000) of 1.31% of fresh weight. The pink and deep pink varieties showed crude protein contents slightly lower than the white variety with values of 0.92 and 0.89%, respectively.

Similarly values of crude fat were determined for the three varieties (0.20–0.31%) compared to the values reported by Duke and DuCellier (1993) of 0.2%. Additionally, the concentration of this component in the fruits of A. diversifolia is within the range described for fruits of the genus Annona (Duke and DuCellier, 1993; Cruz and Deras, 2000; Diaz, 2004; Roessler et al., 2006).

Table 3

Proximate composition, pH, and titratable acidity of three varieties of Annona diversifolia fruits.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Carbohydrate content</th>
<th>Ash</th>
<th>Moisture</th>
<th>pH</th>
<th>Titratable acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>1.14 ± 0.01a</td>
<td>0.31 ± 0.01a</td>
<td>4.44 ± 0.16a</td>
<td>13.55 ± 0.19a</td>
<td>0.94 ± 0.01a</td>
<td>79.61 ± 0.02a</td>
<td>4.99 ± 0.02a</td>
<td>0.16 ± 0.004a</td>
</tr>
<tr>
<td>Pink</td>
<td>0.92 ± 0.01b</td>
<td>0.22 ± 0.03b</td>
<td>0.63 ± 0.02b</td>
<td>18.36 ± 0.06b</td>
<td>0.92 ± 0.03a</td>
<td>78.94 ± 0.06a</td>
<td>5.19 ± 0.05b</td>
<td>0.17 ± 0.001b</td>
</tr>
<tr>
<td>Deep pink</td>
<td>0.89 ± 0.03b</td>
<td>0.20 ± 0.03b</td>
<td>0.66 ± 0.01b</td>
<td>20.25 ± 0.06c</td>
<td>0.89 ± 0.01a</td>
<td>77.11 ± 0.02a</td>
<td>4.79 ± 0.06c</td>
<td>0.34 ± 0.002c</td>
</tr>
</tbody>
</table>

Proximate composition in g/100 g and titratable acidity in mg citric acid/100 g. Average of three replicates ± standard deviation. Different letters in the same column indicate that combination means are significantly different (p < 0.05) according to the method of Duncan’s multiples ranges.
of sugar production is closely related to the peptization of some of the constituents of crude fiber, such as dextrines, starch, among others. NFE reports for other Annona species range from 15.5 to 29% (Duke and duCellier, 1993; Cruz and Deras, 2000; Díaz, 2004; Roesler et al., 2006). In this study, the pink and deep pink varieties were within that range, while the white variety was slightly lower.

The ash and moisture contents for the three varieties were statistically similar (p<0.05) since the fruit varieties were grown in the same region with similar soil characteristics, rainfall, average temperature and altitude. Additionally, the fruits of A. diversifolia were harvested during the same season. The ash content for the three varieties ranged from 0.89 to 0.94%, whereas the corresponding moisture was 77.11–79.61%. The ash content of the fruits of A. diversifolia was within the range reported for other Annona genus fruits, from 0.5 to 1.4%. Similarly, humidity for the A. diversifolia varieties were comparable with the values reported for Annona genus fruits, 60–83% (Duke and duCellier, 1993; Cruz and Deras, 2000; Díaz, 2004; Roesler et al., 2006).

3.2. pH and titratable acidity

The pH of fruit juice remained constant during storage. The pH of the three varieties was statistically different (p<0.05). The deep pink variety was the most acidic with a pH of 4.79, Table 3. The pH of the pulp can determine consumer preference for one particular variety, as well as pulp susceptibility to enzymatic browning. Consistent with the pH results, the titratable acidity was higher for the deep pink variety, 0.34%, compared to the other two varieties which only contained half of this concentration. Consequently, the less acidic varieties (white and pink) will be more susceptible to browning by exposure to air.

3.3. Minerals

Since, in general, fruits are characterized by a high content of potassium (deMan, 1999); A. diversifolia high potassium contents were expected. Potassium was the mineral with highest concentration in the three varieties, with no significant differences among varieties (p<0.05), Table 4. The average concentration of this mineral for the three varieties was 0.344%. The Food Drug Administration (FDA, 1993) defines that foods that provide greater than 20% of the recommended daily intake of any mineral are considered as high contribution sources. For an adult, the recom-mended daily intake of potassium is 1000 mg/day. In this account, 100 g of A. diversifolia pulp provides 34% of potassium required by an adult.

The fruits of A. diversifolia in this study were not characterized as having high sodium contents. The pink and deep pink varieties, had sodium concentrations of 2.96 and 3.18 mg/100 g, respectively. The white variety had the lowest sodium concentration, 2.15 mg/100 g, which was statistically different from the other two varieties.

Calcium was the second most abundant mineral. The pink variety presented the highest amount of this mineral with 22.93 mg/100 g, followed by the deep pink and white varieties with 14.17 and 0.86 mg/100 g, respectively. These values were significantly different for the three varieties (p<0.05). The low calcium concentration in the white variety can be attributed to the fact that the tree of this variety produces considerably more fruits than those produced by the pink and deep pink trees; consequently, a lower calcium concentration was expected in the fruits of this variety. Additionally, the growth rate of the white variety fruits is faster than those of the other two varieties, resulting in low concentrations of calcium (Saure, 2005; Moreno-Velásquez et al., 2008).

The three varieties showed significant differences in the magnesium content, with increasing-order concentrations (mg/100 g) as follows: white, 8.00; pink, 12.71; and deep pink, 14.01. With regards to trace minerals, only zinc was detected and quantified in the three varieties of A. diversifolia, the concentrations of this mineral were significantly different between varieties. The pink variety had the highest concentration of this mineral, 0.15 mg/100 g, whereas the white and deep pink varieties showed 0.13 and 0.10 mg/100 g, respectively (Table 4). It is noteworthy that zinc is an essential mineral for the metabolism of carbohydrates, lipids and proteins.

Additionally, we assessed the relatedness between ash and mineral concentrations, using Pearson correlations (Table 5). Concentrations of magnesium and sodium presented the only significant correlation (p<0.05), with a high r value of 1.00. This result indicates that the magnesium concentration in the pulp is directly proportional to the sodium concentration. Despite the fact that no works in the literature have reported a correlation between sodium and magnesium in fruits, this correlation can be attributed to the plant metabolism requiring proportional amounts of these minerals.

3.4. Sucrose, glucose and fructose

Sucrose, glucose and fructose were detected and quantified in the pulp of the three A. diversifolia varieties, Table 6. The deep pink

Table 4

<table>
<thead>
<tr>
<th>Variety</th>
<th>Microelement</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>K</td>
<td>Na</td>
<td>Mg</td>
<td>Na</td>
</tr>
<tr>
<td></td>
<td>347.80 ± 8.75a</td>
<td>2.15 ± 0.22a</td>
<td>0.86 ± 0.04a</td>
<td>8.00 ± 0.26a</td>
</tr>
<tr>
<td>Pink</td>
<td>335.95 ± 7.11a</td>
<td>2.96 ± 0.05b</td>
<td>22.93 ± 0.51b</td>
<td>12.71 ± 0.22b</td>
</tr>
<tr>
<td>Deep pink</td>
<td>347.40 ± 10.00a</td>
<td>3.18 ± 0.07b</td>
<td>14.17 ± 0.58b</td>
<td>14.01 ± 0.44c</td>
</tr>
</tbody>
</table>

Mineral content in mg/100 g. Average of three replicates ± standard deviation. Different letters in the same column indicate that means are significantly different (p<0.05) according to the method of Duncan’s multiples ranges.

Table 5

<table>
<thead>
<tr>
<th>Ash</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>Zn</th>
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<td></td>
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<td>0.50</td>
<td>0.82</td>
<td>0.91</td>
<td>0.82</td>
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<tr>
<td>Ca</td>
<td>0.50</td>
<td>1.00</td>
<td>0.82</td>
<td>0.91</td>
<td>0.82</td>
</tr>
<tr>
<td>Mg</td>
<td>0.82</td>
<td>1.00</td>
<td>1.00</td>
<td>0.91</td>
<td>0.82</td>
</tr>
<tr>
<td>Na</td>
<td>0.91</td>
<td>0.82</td>
<td>1.00</td>
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<td>0.91</td>
</tr>
<tr>
<td>K</td>
<td>0.89</td>
<td>0.82</td>
<td>0.34</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Zn</td>
<td>0.68</td>
<td>0.29</td>
<td>0.32</td>
<td>0.09</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Linear correlation coefficients (r) between total ash and mineral contents according to the Pearson analysis.

The value in bold is statistically significant (p<0.05).

Table 6

<table>
<thead>
<tr>
<th>Variety</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>7.59 ± 0.06</td>
<td>3.19 ± 0.06</td>
<td>4.09 ± 0.23</td>
</tr>
<tr>
<td>Pink</td>
<td>6.79 ± 0.32</td>
<td>2.48 ± 0.04</td>
<td>3.01 ± 0.06</td>
</tr>
<tr>
<td>Deep pink</td>
<td>9.33 ± 0.31</td>
<td>3.54 ± 0.08</td>
<td>4.81 ± 0.09</td>
</tr>
</tbody>
</table>

Sugar composition of the three varieties of Annona diversifolia fruits. Sugar composition in g/100 g. All values are significantly different (p<0.05).
variety had the highest concentrations of sucrose, glucose and fructose with 9.33, 3.54 and 4.81%. The three varieties had significant differences in the content of these sugars (p < 0.05).

Depending on their chemical structure, sugars have different sweetening power. Sucrose is used as a reference for sweetening power with a value of 1. Based on this, fructose has a value of 1.8 and glucose of 0.7 (Badui, 1993). Accordingly, the sweetness of the A. diversifolia fruit pulps is attributed mainly to sucrose and fructose as they represent 51.0–55.3% and 24.5–27.5% of the total sugars detected, respectively.

3.5. Vitamin C

The results showed that the white variety has the highest vitamin C concentration with 2.38 mg/100g (Table 7). The pink and deep pink varieties were not significantly different between them, whereas the white variety was different than the former two (p < 0.05). Vitamin C concentrations determined in this study for A. diversifolia were lower than those reported in the literature: 14 mg/100g (Duke and duCellier, 1993); and 8.2 and 8.6 mg/100g for white and pink varieties, respectively (Moreno-Velásquez et al., 2008). Additionally, vitamin C content in A. diversifolia is on average lower than those reported in other Annona species; e.g., A. cherimolia, 4–6 mg/100g (Vasco et al., 2008); A. muricata, 4 mg/100g (Duke and duCellier, 1993); and A. squamosa, 15–35 mg/100g (Andrade et al., 2001).

3.6. Total polyphenols

The deep pink and white varieties presented the highest concentrations of TPP with 0.171 and 0.170%, respectively; which showed no significant differences between them (p < 0.05) (Table 7). The pink variety had the lowest TPP concentration (0.129%) and was significantly different to the other two fruits. These concentrations are similar to those reported in the literature for A. muricata, 0.120% (Hassimoto et al., 2005); and A. squamosa, 0.165–0.175% (Yan et al., 2006).

3.7. Total flavonoids

Both white and deep pink varieties showed the largest total flavonoids content, 0.152 and 0.143%, respectively (Table 7). No significant differences between the varieties were established (p < 0.05). The pink variety presented the lowest concentration of TF, 0.107%, which was significantly different to those of the other two fruits. This is the first report in the literature of total flavonoids content for Annona genus fruits.

3.8. Radical scavenging activity

Lower IC50 pulp concentrations indicate better radical scavenging activity of the sample. The varieties with best RSA were white and deep pink with an IC50 of 1715 and 1702 µg/mL, respectively (Table 7). The pink variety with an IC50 of 1998 µg/mL showed significant differences with the other two. Comparing these results with those reported for red-brown and green varieties of A. squamosa, with IC50 of 3900 and 4600 µg/mL, respectively (Yan et al., 2006), we found that the RS activity of A. diversifolia is more than 100% greater. None of the extracts were as effective DPPH radical scavengers as the standard controls, vitamin C, gallic acid and BHT (Table 7).

3.9. Correlations between antioxidants compounds and radical scavenging activity

In order to assess the degree of relatedness between RSA and the concentrations of vitamin C, TPP and TF, we conducted a Pearson correlation. There was a high linear correlation between TPP and RSA (r = −1.00), with the minus sign indicating an inverse correlation. TF and RSA also showed a good correlation, r = −0.969; however, the data lie outside the confidence interval (p < 0.05). These results suggest that in addition to flavonoids, other phenols significantly contribute to the RSA of A. diversifolia fruits. On the other hand, no correlation was found between vitamin C and RSA.

4. Conclusions

Our results show that the three varieties of A. diversifolia could be considered as important sources of fiber, sugars, potassium, zinc and biologically active components with antioxidant activity, such as flavonoids. Color measuring was successfully used to objectively characterize the three A. diversifolia varieties, resulting in an excellent agreement with visual observations. A strong correlation between total polyphenols and radical scavenging was observed. A. diversifolia extracts possessed greater radical scavenging activity and similar total polyphenol content compared to those of other Annona species reported elsewhere. Since high concentrations of total flavonoids were encountered in all three varieties, a further study of the chemical structure of the flavonoids present in these fruits would be greatly beneficial for the characterization of the functional properties of this species. The results reported in this paper may contribute to the appreciation of the nutrimental and functional value of A. diversifolia fruits.

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References


