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Nutritional compositions in roots, twigs, leaves, fruit pulp, and seeds from pawpaw (*Asimina triloba* [L.] Dunal) grown in Korea

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Summary

Pawpaw (*Asimina triloba* L.) roots, twigs, leaves, fruit, and seeds were analyzed for their nutritional compositions. Seeds exhibited significantly higher levels of crude protein, lipid, fiber, and dietary fiber than those of the other parts. Sucrose in fruit was 9321.24 mg%, which was the highest among the samples. The total essential amino acid to total amino acid ratio was highest in the leaves, and the leaves contained the highest amount of potassium. The calcium content ranged between 8.15-153.41 mg%. Oleic and linoleic acids in seeds were 5905.11 and 8045.56 mg%, respectively, which were the highest among the pawpaw parts. The highest amount of linolenic acid was measured in the leaves, and β-carotene, vitamin C, and vitamin E were also the most abundant in the leaves. These results suggest that every part of pawpaw is a good source of an important food item. Additionally, this study provides basic data for improving the sitological value of pawpaw.

Abbreviations: AOAC, Association of Analytical Chemists; HPLC, high performance liquid chromatography; GABA, γ-aminobutyric acid; ICP, inductively coupled plasma spectrometer; FID, flame ionization detector; AA, amino acid; EAA, essential amino acid; NAA, nonessential amino acid; UFA, unsaturated fatty acid; SFA, saturated fatty acid.

Introduction

Asimina triloba [L.] Dunal (family: Annonaceae) is one species of *Asimina*, usually known as pawpaw. It is widely distributed from the southeastern areas of United States (Florida) to the eastern areas of Canada (Ontario). Although pawpaw is sometimes confused with papaya (*Carica papaya*), it is an entirely different species (Levine et al., 2015). Papaya is a tropical plant grown in tropical regions, but pawpaw can grow well in tropical regions as well as in humid microthermal climates. In Korea, pawpaw trees have approximately been cultivated since 2012 to the present day, and areas for cultivation have been on the rise as use of pawpaw as a food has increased. Almost all parts of pawpaw trees are attracting considerable attention for their economic value due to their broad use across diverse areas such as nutritional and medicinal fields.

In terms of its medicinal properties, pawpaw roots, twigs, and seeds contain a large amount of acetogenins, which are cancer cell inhibitors (Mclaughlin, 2008). Indeed, the extracts obtained from pawpaw twigs are used in a commercial product for the prevention of cancer (i.e., Paw Paw Cell-Reg). In addition, many researchers have studied the isolation and identification of acetogenins from pawpaw twig extracts (Gu et al., 1999; Mclaughlin, 2008). Mccage et al. (2002) demonstrated that herbal shampoo products that included pawpaw twig extracts could effectively remove head lice. Further, essential oil generated from pawpaw leaves has exhibited anticancer activity against breast and lung cancer cell lines (Farag, 2009). Moreover, pawpaw leaves are rich in various bioactive compounds such as phenolic acids and flavonoids (Pande and Akoh, 2010). Accordingly, various uses of pawpaw leaves have been employed, including infusing herbs in water. Pawpaw fruit has a sweet and sour taste like a mixture of pineapple, banana, and mango, and is popularly used as an ingredient for jam, wine, ice cream, and puree (Pomper and Layne, 2005). It is also a good source of amino acids, minerals, and vitamins (Templeton et al., 2003). Brannan et al. (2015) reported that pawpaw fruit contains a large amount of procyanidins, which have antioxidant effects, and Kobayashi et al. (2008) demonstrated that pawpaw fruit exhibits antioxidant activity.

Despite the importance of pawpaw as a nutritional and medicinal material, relatively little attention has been given to the nutritional composition of the roots, twigs, leaves, fruit, and seeds of the *A. triloba* variety of pawpaw grown in Korea. To this end, the objective of the present study was to investigate and compare the nutritional compositions of different parts of pawpaw cultivated in Korea. On the basis of these results, we seek to provide basic data for improving the sitological value of pawpaw and promote further studies on the nutritional compositions of other plants and their various components.

Materials and methods

Samples and reagents

Pawpaw trees (*A. triloba*) grown in identical conditions were purchased from a farm situated on the edge of Okchon, South Korea in September 2016. The climate of Okchon is as follows: an average annual temperature of 13.0°C, an average relative humidity of 66.7%, wind velocity of 1.9 m/s, and total annual rainfall of 1458.7 mm. Roots, twigs, and leaves were collected from 140-150 pawpaw trees aged 2 years old (height, 1-1.5 m). The fruit (length, 8-12 cm; weight, 150-300 g) was obtained from 25-30 pawpaw trees aged 8-10 years old. The roots, twigs, leaves, and fruit were washed dozens of times with water until all extraneous substances were removed. The samples were then drained and the fruits were peeled; seeds were then collected from the pawpaw fruit. All samples were cut into pieces and chopped using a chopper (TC-8B; Techon Co., Ltd., Bucheon, Korea) and stored at -70 °C until further use.

Free sugar standards (fructose, glucose, sucrose, maltose, and lactose), organic acid standards (malic acid, citric acid, oxalic acid, acetic acid, formic acid, tartaric acid, and succinic acid), an amino acid standard solution, fatty acid methyl ester, β-carotene, vitamins B_1, B_2, B_3, B_5, B_6, and B_{12}, ascorbic acid, and α-, β-, γ-, and δ-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mineral standards were obtained from AccuStandard (New Haven, CT, USA).

Determination of proximate composition and dietary fiber content

The proximate composition of the samples including moisture, crude ash, crude protein, crude lipid, and crude fiber was analyzed using

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procedures from the Association of Official Analytical Chemists (AOAC, 2005). The moisture content was analyzed using a dry oven (OF-22; Jeio Tech, Daejeon, Korea) at 105 °C, and the crude ash content was analyzed with a muffle furnace (JSMF-140T; JSR Inc. Laboratory, North Ringwood, Victoria, Australia) at 550 °C until a constant weight was attained. The crude protein content was determined by the Kjeldahl method using a Kjeltec® 2300 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). The crude lipid content was measured by ether extraction with a Soxtec system (Soxtec 1043; Foss Tecator AB, Höganäs, Sweden), and the crude fiber content was determined using an automatic fiber extractor (FIWE 6; Velp Scientifica, Usmate, Italy). The carbohydrate content was calculated as the weight of the entire sample less the moisture, crude ash, crude protein, and crude lipid. Determination of the dietary fiber content was conducted by an enzymatic-gravimetric method using a dietary fiber extraction instrument (1023 Filtration Module; Foss Tecator Co., Hillerød, Denmark).

Determination of free sugar and organic acid contents

The free sugar and organic acid contents were determined using high-performance liquid chromatography (Agilent 1100 series; Agilent Technologies, Palo Alto, CA, USA). Five grams of samples were blended with 10 times its weight of distilled water (w/v) and centrifuged at 8,000 rpm for 20 min (Supra-21K; Hanil, Incheon, Korea). The supernatant was transferred into a 50 mL volumetric flask and filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA). The filtrates were then used to analyze the free sugar and organic acid contents. To analyze the free sugars, 10 μL of each sample was injected into the HPLC system equipped with a carbohydrate analysis column (4.6 × 250 mm, Waters Co., USA). The free sugars were separated using an isocratic elution at a flow rate of 1.0 mL/min and a mobile phase of 80% acetonitrile. The elution peaks were detected using a refractive index (RI) detector. To analyze the organic acids, aliquots (20 μL) of each sample were injected into the HPLC system equipped with Shodex Rs Pak KC-811 column (8.0 × 300 mm; Shodex, Tokyo, Japan). The organic acids were separated using an isocratic elution at a flow rate of 1.0 mL/min and a mobile phase of 0.1% H_3PO_4. The elution peaks were detected using an ultraviolet (UV) detector at 210 nm.

Determination of amino acid content

The amino acid contents were determined using an amino acid analyzer (Biochrom 30; Pharmacia Biotech, Stockholm, Sweden). Samples for analysis of 18 amino acids were hydrolyzed with 6 N HCl at 110 °C for 22 h in test tubes filled with nitrogen. The hydrolysate was concentrated on a rotary evaporator (R-210; Buchi, Flawil, Switzerland) and diluted with 50 mL of distilled water including 10 mL of dilution buffer. Samples for analysis of taurine and γ-aminobutyric acid (GABA) were prepared identically by the same pre-treatment method used for the free sugar and organic acid analysis. Each sample was filtered through a 0.45 μm membrane filter (Millipore) and loaded onto a cation-exchange column (11 ± 2 μm). The flow of the ninhydrin reagent (pH 3.20-6.45) was set to 25 mL/h, and the sample injection volume was 20 μL.

Determination of mineral content

Each sample was dissolved in a HNO_3/H_2O_2 mixture (9:1) and digested for 30 min using a microwave digestion system (Ethos TC Digestion Lab Station 5000; Milestone Inc., Monroe, CT, USA). After digestion, the solution from each sample was passed through a filter paper (No. 5A; Whatman International Ltd., Maidstone, UK). An inductively coupled plasma (ICP) spectrometer (Perkin Elmer Co., Shelton, CT, USA) was used to measure the contents of seven elements (Ca, Cu, Fe, K, Mg, Na, and Zn). The operating conditions of the ICP instrument were as follows: 1.4 kW reflected power, 10 L/min argon plasma gas flow rate, 0.2 L/min auxiliary gas flow rate, and 0.55 L/min nebulizer gas flow rate. Selenium was determined by ICP-mass spectrometry; these analysis conditions were as follows: 1.4 kW reflected power, 9.6 V lens voltage, 18 L/min plasma flow, 1.5 L/min auxiliary gas flow rate, and 0.92 L/min nebulizer gas flow rate.

Determination of fatty acid content and composition

Fatty acids were measured by the method of Jang et al. (2016) with some modification. Ten grams of each sample were extracted with ether, and the extracted fat (25 mg) was dissolved in 0.5 N NaOH-methanol (2 mL) and converted to its fatty acid methyl esters using 14% BF_3-methanol (2 mL). The fatty acid contents were determined by gas chromatography (Agilent 6890N/5975 MSD series; Avondale, PA, USA) coupled with an SP™ 2560 column (100 m × 0.25 mm; Supelco Inc., Bellefonte, PA, USA). The column oven temperature was programmed as follows: initial temperature was 170 °C (held for 15 min); increased to 180 °C at 1 °C/min (held for 15 min); further increased to 245 °C at 3 °C/min with a final holding time of 13 min. The injector and detector (FID, flame ionization detector) temperatures were 250 °C and 285 °C, respectively. Helium was used as a carrier gas at a flow rate of 0.75 mL/min. The fatty acid contents were calculated using a FID conversion factor.

Determination of β-carotene content

β-Carotene was determined by the AOAC method (2005). Samples (1 g) were dissolved in 30 mL of ethanol and 1 mL of 10% of pyrogallol. Then, 3 mL of KOH was added to the sample solution and the mixture was boiled under reflux for 30 min. The sample was allowed to cool to 20 °C, and 30 mL of distilled water were added in order to collect the ether layer. The ether layer was then dehydrated by Na_2SO_4 and evaporated. n-Hexane (20 mL) was added to the dehydrated product, which was used as the test solution. The test solution was injected into an Agilent 1100 series HPLC system coupled with a Nova-Pak silica column (3.9 × 150 mm; Waters Co., Milford, MA, USA). The mobile phase consisted of n-hexane and isopropyl alcohol (99:1, v/v) at a flow rate of 1.0 mL/min with an injection volume of 20 μL. The β-carotene content was expressed as μg per 100 g of fresh weight.

Determination of vitamin B complex content

Each sample (1 g) was weighed and extracted by 20 mL of a 75 mM ammonium formate solution (pH 7.0) for 1 h. The extracts were centrifuged at 3,000 rpm for 15 min, and the supernatant was filtered through a 0.45 μm membrane filter (Millipore). The filtrate was injected into an HPLC-tandem mass spectrometer (Agilent 1200 series; Agilent Technologies, Santa Clara, CA, USA) equipped with a Luna C_{18} column (3.0 × 150 mm; Phenomenex, Torrance, CA, USA). The mobile phase A was 5 mM ammonium formate in distilled water, and mobile phase B was 5 mM ammonium formate in methanol. The gradient system was programmed as follows: 0 min (10% B); 0-8 min (45% B); 8-15 min (10% B). The flow rate was 0.3 mL/min, and the temperature was 35 °C. The content of vitamin B was expressed as μg per 100 g of fresh weight.

Determination of vitamin C content

The sample (5 g) was extracted adding 5% meta-phosphoric acid (50 mL) for 40 min and filtered through a 0.45 μm membrane filter (Millipore). The filtrate was injected into an Agilent 1200 series HPLC system equipped with a Shiseido Capcell Pak C_{18} column (4.6 × 250 mm; Shiseido, Tokyo, Japan). The mobile phase was 0.05 M

KH_2PO_4 and acetonitrile (99:1, v/v), and the flow rate was 0.9 mL/min. The eluate was detected using a UV detector at 254 nm. The content of vitamin C was expressed as mg per 100 g of fresh weight.

Determination of vitamin E content

Sample preparation for vitamin E determination was performed in the same way as that for β-carotene. The samples were analyzed using an Agilent 1100 series HPLC system equipped with a Nova-Pak silica column (Waters Co.). The mobile phase was n-hexane and isopropyl alcohol (99:1, v/v), and the flow rate was 0.5 mL/min. The content of vitamin E was expressed as mg per 100 g of fresh weight.

Statistical analysis

All experiments were performed in triplicate, and the results were analyzed using the Statistical Package for Social Sciences Version 10.0 (SPSS Inc., Chicago, IL, USA). For multiple comparisons of normally distributed data, parametric one-way analysis of variance was used. The significance of differences between data was calculated by Duncan's multiple range test, with $P<0.05$ being considered as statistically significant.

Results and discussion

Proximate composition and dietary fiber

The proximate composition and dietary fiber content of different parts of pawpaw tree (*A. triloba*) differed significantly ($P<0.05$, Tab. 1). The moisture content these various parts ranged from 28.48% (seeds) to 84.98% (roots). The highest value of crude ash was observed in the leaves, which was significantly different from the other pawpaw tree parts ($P<0.05$). Pawpaw seeds exhibited significantly higher levels of crude protein, crude lipid, crude fiber, carbohydrate, and dietary fiber than those of the roots, twigs, leaves, and fruit ($P<0.05$). In particular, the dietary fiber content of seeds (46.77%) was more than 15 times the dietary fiber of fruit (3.03%). Fruit had significantly lower levels of crude ash, crude protein, crude lipid, crude fiber, and dietary fiber than those of the other parts ($P<0.05$). This result is consistent with that of a previous study of papaya (*Carica papaya*), in which the fruit pulp showed a low crude protein compared to the leaves and seeds (Nwofia et al., 2012). However, many reports proposed that all the proximate composition and dietary fiber content may differ according to several factors such as growing season, cultivars, and climatic conditions (Jang et al., 2011; Nwofia et al., 2012; Rohloff et al., 2015). Therefore, the present study suggests that the proximate composition and dietary fiber content of pawpaw tree can depend on their different parts, as well as these other factors.

Free sugars

The free sugar content in various parts of pawpaw tree is given in Tab. 2. Fructose, glucose, and sucrose showed the highest levels in the fruit at 1691.35 mg%, 2148.20 mg%, and 9321.24 mg%, respectively. These free sugars in the fruit had levels higher than at least twice and up to 16 times the free sugars contained in the roots, twigs, leaves, and seeds. In particular, the sucrose content in the fruit was the highest among the free sugars, which was ~9% of the fruit weight. Maltose and lactose were not detected in any part of pawpaw tree. Fructose, glucose, and sucrose were observed as the main free sugars of pawpaw, and their amounts differed between parts.

Tab. 1: Proximate composition and dietary fiber content in various parts of *Asimina triloba*.

Components (% fresh weight)	Roots	Twigs	Leaves	Fruit	Seeds
Moisture	84.98±0.11^a	61.86±0.38^d	75.47±0.04^c	79.14±0.03^b	28.48±0.11^e
Crude ash	1.24±0.02^b	0.90±0.04^d	1.51±0.02^a	0.38±0.01^e	1.01±0.02^c
Crude protein	1.97±0.02^d	2.20±0.08^c	5.18±0.01^b	1.51±0.14^e	9.18±0.04^a
Crude lipid	0.75±0.02^d	0.93±0.02^c	1.38±0.01^b	0.36±0.02^e	16.68±0.09^a
Crude fiber	6.37±0.02^c	23.45±0.35^b	5.77±0.04^d	2.47±0.03^e	38.63±0.06^a
Carbohydrate	11.05±0.14^e	34.12±0.25^b	16.47±0.04^d	18.61±0.11^c	44.66±0.21^a
Dietary fiber	10.06±0.23^d	30.80±0.26^b	11.81±0.30^c	3.03±0.12^e	46.77±0.77^a

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test ($P<0.05$).

Tab. 2: Free sugar content in various parts of *Asimina triloba*.

Components (mg% fresh weight)	Roots	Twigs	Leaves	Fruit	Seeds
Fructose	193.20±12.70^d	331.68±36.53^c	740.75±79.77^b	1691.35±29.66^a	238.58±29.27^d
Glucose	207.45±27.79^d	430.34±76.84^c	660.33±120.06^b	2148.20±74.14^a	131.76±20.66^d
Sucrose	n.d.	n.d.	n.d.	9321.24±122.38^a	1067.61±47.91^b
Maltose	n.d.	n.d.	n.d.	n.d.	n.d.
Lactose	n.d.	n.d.	n.d.	n.d.	n.d.

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test ($P<0.05$).
n.d. = not detected.

Sweetness is closely related to free sugar, and its intensity depends on the free sugar composition. LEE et al. (2013) showed that the free sugar content of tropical fruit was consistent with the trend of °Brix. In addition, fructose has a sweetness ~70% higher than sucrose, and the sweetness of glucose is only ~50% of the sweetness of sucrose (MOSKOWITZ, 1970). According to ANDERSON (1986), the free sugar content depends on climate conditions including temperature, rainfall, relative humidity, and the amount of light. Increases of sucrose and sucrose synthases are related to metabolic adaptations by low temperature, and water stress was been shown to increase the free sugar level in leaves (ACKERSON, 1981; GUY et al., 1992). Therefore, it is expected that the free sugar content and composition of pawpaw tree may differ according to the cultivation environment and that the intensity of sweetness by the free sugars and their composition will vary between pawpaw constituents.

Organic acids

Malic acid and citric acid were the most abundant organic acids in all samples, with their highest levels measured in pawpaw roots. However, oxalic acid, acetic acid, formic acid, tartaric acid, and succinic acid were not detected in the roots (Tab. 3). The twigs and leaves only contained malic acid, citric acid, and formic acid. The malic acid and citric acid levels in the twigs were higher than those in the leaves; however, the formic acid content in the twigs was lower level than that in the leaves. Fruit contained various types of organic acids such as malic acid, citric acid, oxalic acid, acetic acid, and formic acid, and their content was 30.62, 8.65, 37.17, 61.59, and 43.29 mg%, respectively. The major organic acids identified in the seeds were malic acid, citric acid, and oxalic acid, and, in particular, citric acid, which was the most abundant organic acid measured at 66.27 mg%.

Organic acids including malic acid, citric acid, oxalic acid, and succinic acid play an important role in the tricarboxylic acid cycle, which is the metabolic pathway to produce energy viz. oxidation of pyruvate in the mitochondria of animals and plants (YANG et al., 2014). Malic acid participates in photosynthesis, and citric acid and oxalic acid have been reported to be involved in diverse metabolic pathways such as the transportation of cations, detoxification of metal substances, and resistance of anaerobic stress in roots (MUCHA et al., 2005). In addition, organic acids found in the metabolites of plants or various plant products are important indicators of biological and physiological metabolism and fermentation processes (CHUNG et al., 2000). Organic acids have also been widely used as food additives in the production of fruit and vegetable beverages, drinks, and juices (PEREIRA et al., 2013). Finally, the addition of these acids to food imparts distinct sensory characteristics such as color, flavor, and aroma and affects their tissue and biological stability (CHUNG et al., 2000). Therefore, it is likely that each part of pawpaw trees can be effectively utilized on account of their distinct organic acid content and composition.

Amino acids

The amino acid contents in pawpaw tree are displayed in Tab. 4. The contents of the total amino acid (AA), total essential amino acid (EAA), total nonessential amino acid (NAA), and total extra amino acid of the seeds were significantly higher than those of the roots, twigs, leaves, and fruit ($P<0.05$). However, the EAA ratio of seeds to AA was the third largest (leaves > twigs > seeds > fruit > roots). The contents of AA, EAA, NAA, total extra amino acid, and the ratio of fruit to AA were conspicuously lower than those of the other parts ($P<0.05$), which is consistent with previous studies that found that most of the amino acids contained in the seeds of okra (*Hibiscus esculentus*) and marula (*Sclerocarya birrea*) had higher levels than those in the fruit (GLEW et al., 1997). Most of the amino acids were identified evenly in all samples, but taurine was not detected in all parts of pawpaw. The twigs, leaves, and seeds contained methionine, but the roots and fruit did not. Tryptophan and arginine were not detected in the fruit, and proline was not detected in the twigs and leaves. In addition, cysteine was observed only in the seeds. The main amino acids detected in the pawpaw roots and fruit were proline, accounting for more than 38% and 25% of the entire amino acid profile, respectively. Aspartic acid, glutamic acid, and histidine were the major amino acids detected in the twigs, and leucine, aspartic acid, and glutamic acid were main amino acids found in the leaves. Aspartic acid, glutamic acid, and arginine were the major amino acids measured in seeds; glutamic acid had the highest level at 1396.27 mg%. Overall, it is clear that the amino acid content and composition in pawpaw tree vary according to the parts of the tree. However, the most prolific amino acids of pawpaw, except in fruit, were aspartic acid and glutamic acid, the contents of which closely matched in the different tree sections. Aspartic acid and glutamic acid have an umami taste, and their mixture creates a tremendous synergic effect (DERMIKI et al., 2013). Proline was observed as the most plentiful amino acid in roots and fruit. This amino acid is closely related to radical scavenging activity; previous *in vitro* studies found that it contributed to free radical scavenging (KAUL et al., 2008). Therefore, these results suggest that the distinct components of pawpaw tree have various nutritional and functional properties.

Tab. 3: Organic acid content in various parts of *Asimina triloba*.

Components (mg% fresh weight)	Roots	Twigs	Leaves	Fruit	Seeds
Malic acid	297.09±3.67^{a}	159.79±0.87^{b}	74.42±0.98^{c}	30.62±1.92^{d}	31.25±0.55^{d}
Citric acid	252.03±2.74^{a}	113.53±0.45^{b}	48.91±1.88^{d}	8.65±0.23^{e}	66.27±0.82^{c}
Oxalic acid	n.d.	n.d.	n.d.	37.17±0.34^{b}	56.73±0.40^{a}
Acetic acid	n.d.	n.d.	n.d.	61.59±0.92	n.d.
Formic acid	n.d.	41.48±0.67^{b}	201.80±1.55^{a}	43.29±1.59^{b}	n.d.
Tartaric acid	n.d.	n.d.	n.d.	n.d.	n.d.
Succinic acid	n.d.	n.d.	n.d.	n.d.	n.d.

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test ($P<0.05$).
n.d. = not detected.

Tab. 4: Amino acid content in various parts of *Asimina triloba*.

Amino acids (mg% fresh weight)		Roots	Twigs	Leaves	Fruit	Seeds
Essential amino acid	Threonine	46.14±1.23^{cd}	61.43±6.27^{c}	170.05±8.96^{b}	23.86±2.62^{d}	278.95±28.69^{a}
	Valine	34.84±5.27^{c}	45.10±4.61^{c}	142.60±3.90^{b}	24.09±2.97^{c}	288.97±27.94^{a}
	Methionine	n.d.	22.00±9.00^{c}	81.06±7.93^{a}	n.d.	70.85±3.57^{b}
	Isoleucine	23.17±3.23^{c}	29.24±3.65^{c}	98.89±11.53^{b}	12.69±0.23^{c}	208.20±19.02^{a}
	Leucine	70.61±5.44^{c}	85.57±5.10^{c}	319.13±18.98^{b}	37.63±2.99^{d}	618.03±26.85^{a}
	Phenylalanine	44.56±1.00^{c}	54.05±3.19^{c}	186.43±11.18^{b}	27.74±9.09^{c}	269.22±31.32^{a}
	Lysine	58.49±7.22^{c}	76.29±4.22^{c}	224.15±19.50^{b}	29.69±2.86^{c}	377.95±53.90^{a}
	Tryptophan	12.81±0.06^{d}	21.24±1.43^{c}	54.82±2.24^{b}	n.d.	81.28±4.06^{a}
Total essential amino acid		290.61±14.24^{cd}	394.92±15.39^{c}	1277.14±50.19^{b}	155.71±18.59^{d}	2193.45±180.37^{a}
Nonessen- tial amino acid	Aspartic acid	108.60±5.61^{c}	129.84±9.32^{c}	377.76±35.85^{b}	47.31±2.85^{d}	916.53±14.65^{a}
	Serine	78.90±3.25^{c}	86.72±6.19^{c}	219.74±19.80^{b}	35.01±2.75^{d}	453.11±48.66^{a}
	Glutamic acid	117.83±4.56^{c}	113.99±9.26^{c}	391.55±29.92^{b}	67.68±4.17^{c}	1396.27±79.28^{a}
	Proline	531.95±10.98^{b}	n.d.	n.d.	163.25±13.14^{c}	553.79±19.15^{a}
	Glycine	54.44±3.55^{c}	66.45±4.59^{c}	213.49±17.31^{b}	29.08±1.59^{c}	463.64±54.54^{a}
	Alanine	52.68±0.40^{c}	67.23±4.50^{c}	223.80±18.37^{b}	67.14±4.96^{c}	429.05±6.62^{a}
	Cystine	n.d.	n.d.	n.d.	n.d.	50.63±5.15
	Tyrosine	25.05±1.31^{c}	34.61±8.35^{c}	126.52±6.44^{b}	16.34±0.97^{c}	252.18±30.65^{a}
	Histidine	93.47±15.84^{d}	116.89±3.12^{c}	165.65±10.24^{b}	44.03±5.69^{e}	236.86±20.16^{a}
	Arginine	30.42±5.91^{c}	46.96±4.48^{cd}	214.67±18.80^{b}	n.d.	754.99±35.72^{a}
Total nonessential amino acid		1093.36±11.04^{c}	662.68±49.61^{d}	1933.20±153.62^{b}	469.83±16.95^{d}	5507.05±290.43^{a}
Extra amino acid	Taurine	n.d.	n.d.	n.d.	n.d.	n.d.
	GABA	7.78±0.12^{e}	25.54±0.44^{c}	39.61±0.71^{b}	9.85±0.18^{d}	41.90±0.46^{a}
Total extra amino acid		7.78±0.12^{e}	25.54±0.44^{c}	39.61±0.71^{b}	9.85±0.18^{d}	41.90±0.46^{a}
Total amino acid		1391.76±25.38^{c}	1083.15±64.81^{c}	3249.95±203.90^{b}	635.38±28.79^{d}	7742.41±470.12^{a}
Total EAA /Total AA (%)		20.87±0.64^{e}	36.49±0.84^{b}	39.33±0.90^{a}	24.46±2.13^{d}	28.31±0.64^{c}

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test (P<0.05).
n.d. = not detected.
GABA = γ-aminobutyric acid; EAA = essential amino acid; AA = amino acid.

Minerals

Tab. 5 presents the mineral content of pawpaw tree according to its different parts. Potassium was identified as having the highest level in all tissue; its content in roots, twigs, leaves, fruit, and seeds was measured as 215.21, 193.98, 376.93, 239.36, and 289.17 mg%, respectively. The pawpaw leaves contained the highest amount of potassium, which contrasts the reported potassium content (128.33 mg/100 g) in *Celosia argentea* L. leaves (USUNOMENA and SAMUEL, 2016). Typically, minerals such as magnesium, sodium, and zinc were detected at a significantly higher level in roots than in the other pawpaw parts (P<0.05), and the mineral content (except potassium) were remarkably lower in fruit than in the other parts (P<0.05). The calcium content ranged from 8.15 mg% to 153.41 mg% and exhibited the lowest and highest levels in the fruit and twigs, respectively. The calcium content of the pawpaw leaves was also high at 141.47 mg%, which is higher than the calcium content (123.40 mg/100 g) reported in kale (AGARWAL et al., 2017). Copper was only observed in pawpaw seeds, and there was little or no selenium detected in any of the samples. Plant roots not only provide support to a plant, but also play an important role in water and mineral transport. Some minerals absorbed from roots are transported to the twigs or leaves, whereas others are stored in the roots. This transport and storage of minerals in plants are known to differ according to the growth season (MITCHELL, 1936). Accordingly, it is thought that the distribution of minerals in pawpaw tissues may depend on factors like these.

Fatty acids

The unsaturated fatty acid (UFA) content was higher than the saturated fatty acid (SFA) content in all samples, and the difference between UFA and SFA was more pronounced in pawpaw seeds. In contrast, the difference between the two contents in fruit was negligible (Tab. 6). Significant differences were observed for the ratio of total UFA to total SFA in different pawpaw tissues, with the highest ratio found in seeds and the lowest in twigs (P<0.05). Various SFAs such as caproic (C6:0), caprylic (C8:0), capric (C10:0), and lauric (C12:0) acids were only identified in fruit, which is consistent with a previous report for tropical fruit pulps (COIMBRA and JORGE, 2012). The primary fatty acids included palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2, n-6), which were significantly higher in the seeds (P<0.05). In particular, the contents of oleic and linoleic acids contained in the seeds were 5905.11 and

Tab. 5: Mineral content in various parts of *Asimina triloba*.

Minerals (mg% fresh weight)	Roots	Twigs	Leaves	Fruit	Seeds
Ca	99.74±2.12^{c}	153.41±11.25^{a}	141.47±2.66^{b}	8.15±0.29^{e}	57.51±1.09^{d}
Cu	n.d.	n.d.	n.d.	n.d.	0.57±0.08
Fe	2.77±0.65^{c}	5.16±0.20^{b}	6.25±0.22^{a}	0.29±0.02^{e}	2.13±0.12^{d}
K	215.21±1.73^{d}	193.98±5.75^{e}	376.93±6.36^{a}	239.36±3.00^{c}	289.17±5.74^{b}
Mg	140.24±0.78^{a}	87.55±4.59^{c}	97.69±1.14^{b}	10.93±0.18^{d}	93.77±2.07^{b}
Na	49.79±0.47^{a}	8.52±0.47^{b}	0.92±0.06^{c}	n.d.	n.d.
Zn	1.28±0.07^{a}	0.61±0.02^{c}	0.48±0.03^{d}	n.d.	0.98±0.04^{b}
Se	Tr	Tr	Tr	n.d.	n.d.

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test (P<0.05).
n.d. = not detected.
Tr = trace.

Tab. 6: Fatty acids component in various parts of *Asimina triloba*.

Fatty acids (mg% fresh weight)	Roots	Twigs	Leaves	Fruit	Seeds
C6:0	n.d.	n.d.	n.d.	1.61±0.19	n.d.
C8:0	n.d.	n.d.	n.d.	9.73±0.51	n.d.
C10:0	n.d.	n.d.	n.d.	1.43±0.15	n.d.
C12:0	n.d.	n.d.	n.d.	1.53±0.02	n.d.
C13:0	n.d.	n.d.	3.00±0.00	n.d.	n.d.
C14:0	n.d.	n.d.	n.d.	6.00±0.24^{a}	4.70±0.06^{b}
C15:0	n.d.	4.00±0.00^{b}	n.d.	n.d.	5.36±0.08^{a}
C16:0	70.33±0.58^{d}	89.33±0.58^{c}	158.00±1.00^{b}	25.36±0.84^{e}	917.11±4.61^{a}
C16:1	n.d.	4.00±0.00^{c}	4.00±0.00^{c}	6.51±0.08^{b}	137.85±1.28^{a}
C17:0	n.d.	3.00±0.00^{b}	2.00±0.00^{c}	n.d.	11.16±0.33^{a}
C18:0	7.00±0.00^{d}	10.00±0.00^{c}	19.67±0.58^{b}	4.79±0.27^{e}	373.13±2.33^{a}
C18:1	46.00±1.00^{b}	92.33±0.58^{b}	57.33±9.45^{b}	48.02±1.83^{b}	5905.11±54.30^{a}
C18:2, n-6	140.33±0.58^{b}	n.d.	143.00±1.73^{b}	9.79±0.37^{c}	8045.56±76.80^{a}
C20:0	2.67±0.58^{c}	3.00±0.00^{c}	5.00±0.00^{b}	n.d.	31.37±0.37^{a}
C18:3, n-3	19.33±0.58^{d}	73.33±0.58^{c}	401.33±5.69^{a}	18.59±0.65^{d}	233.63±1.92^{b}
C20:1	2.00±0.00^{c}	n.d.	2.67±0.58^{b}	n.d.	48.55±0.46^{a}
C22:0	n.d.	4.00±0.00^{b}	n.d.	0.85±0.06^{c}	7.03±0.10^{a}
C20:3, n-3	3.00±0.00$^{n.s.}$	n.d.	3.00±0.00	n.d.	n.d.
C22:1, n-9	n.d.	3.33±0.58	n.d.	n.d.	n.d.
C20:4, n-6	n.d.	n.d.	2.33±0.58	n.d.	n.d.
C24:0	n.d.	5.00±0.00^{b}	9.33±0.58^{a}	0.68±0.04^{c}	4.69±0.06^{b}
C24:1	n.d.	3.00±0.00	n.d.	n.d.	n.d.
Total SFA	80.00±0.00^{d}	118.33±0.58^{c}	197.00±1.73^{b}	51.98±1.93^{e}	1354.56±6.65^{a}
Total UFA	212.67±1.53^{c}	176.00±1.73^{cd}	613.69±4.16^{b}	82.90±2.88^{d}	14375.96±134.77^{a}
Total UFA/Total SFA	2.63±0.02^{c}	1.49±0.01^{e}	3.12±0.01^{b}	1.59±0.00^{d}	10.61±0.08^{a}

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test (P<0.05).
n.d. = not detected.
n.s. = not significant.
SFA = saturated fatty acid; UFA = unsaturated fatty acid.

8045.56 mg%, respectively, which was more than 120 times and 820 times the oleic and linoleic acids contained in the fruit, respectively. The highest linolenic acid (C18:3, n-3) content was measured in the leaves (401.33 mg%). The contents of linolenic acid in other pawpaw parts were in the following order: seeds (233.63 mg%) > twigs (73.33 mg%) > roots (19.33 mg%) > fruit (18.59 mg%). The content of arachidonic acid (C20:4, n-6) in the leaves was 2.33 mg%, and it was not found in the roots, twigs, fruit, or seeds.

In addition to its antihypertensive effects, oleic acid has been known to be effective in reducing low density lipoprotein cholesterol and increasing high density lipoprotein cholesterol (Terés et al., 2008). Cunnane and Anderson (1997) and Ruthig and Meckling-Gill (1999) speculated that mild skin enlargement and hair loss were found in rats with linoleic acid deficiency, and PAN et al. (2012) reported that linolenic acid, a plant-derived omega-3 (n-3) fatty acid, reduces the risk of cardiovascular disease. Arachidonic acid protects the brain from oxidative stress and activates syntaxin-3, which is involved in the growth and repair of nerve cells (Darios and Davletov, 2006). In this regard, it was previous found that the mental development index was improved in infants with arachidonic acid, and supplementation of arachidonic acid has been shown to be effective in reducing symptoms and delaying progression in the early stages of Alzheimer's diseases (Birch et al., 2000; Calderon-Garciduenas et al., 2004). Consequently, it is expected that pawpaw leaves and seeds rich in UFA such as oleic acid, linoleic acid, linolenic acid, and arachidonic acid will elicit beneficial physiological effects.

Vitamins

β-Carotene was the major vitamin in pawpaw tree (Tab. 7). Pawpaw leaves contained the highest level of β-carotene (3931.35 μg/100 g) among all samples, which is much higher than the β-carotene content (1957-2631 μg/100 g) measured in corn rocket, mountain lettuce, and poppy (Maurizi et al., 2015). The β-carotene content contained in the twigs was similar to that in the fruit (84.18 μg/100 g versus 82.82 μg/100 g, respectively). Pawpaw roots exhibited a remarkably lower level of β-carotene (7.06 μg/100 g). In the twigs, vitamin B_1 (thiamine) was measured in the highest amount (at 96.30 μg/100 g). The vitamin B_1 content in the other pawpaw parts were in the following order: roots (53.35 μg/100 g) > seeds (50.09 μg/100 g) > leaves (10.60 μg/100 g), and it was not observed in the pawpaw fruit. The vitamin B_2 (riboflavin) content contained in pawpaw twigs, leaves, and seeds were 85.36, 51.54, and 82.73 μg/100 g, respectively, and none was detected in the roots and fruit. Vitamin B_3 (niacin) was only detected in the roots, and vitamin B_{12} (cyanocobalamin) was not detected in any of the samples. Vitamin B_5 (pantothenic acid) was observed in all samples, and vitamin B_6 (pyridoxine) was detected in all samples except seeds; its content varied according to the specific tissue. The vitamin C (ascorbic acid) and vitamin E (tocopherol) contents in leaves were 3.70 mg/100 g and 4.04 mg/100 g, respectively, which are significantly higher level than those of roots, twigs, fruit, and seeds ($P<0.05$). β-carotene, a precursor of vitamin A, is known to be a functional compound with antioxidant, anticancer, and anti-aging properties and plays a useful role in the retina of human eye (Jang et al., 2016). Vitamins C and E also have antioxidant effects on account of their ability to reduce reactive oxygen species, and an antihypertensive effect on account of their action on redox-sensitive vascular tissue (Chen et al., 2001). As a result of this study, we identified that abundant levels of β-carotene, vitamin C, and vitamin E were contained in the leaves of pawpaw. Consequently, pawpaw leaves are expected to be a useful material for producing functional foods.

Conclusions

The present study investigated the nutritional compositions in different parts of pawpaw (*A. triloba*) tree. Specifically, the roots, twigs, leaves, seeds and fruit were found to contain an abundance of essential nutrients such as amino acids, minerals, and vitamins required in the body. However, their amounts showed considerable variation

Tab. 7: Vitamin content in various parts of *Asimina triloba*.

Vitamins	Roots	Twigs	Leaves	Fruit	Seeds
β-carotene (μg/100 g FW)	7.06±0.10^{d}	84.18±0.17^{b}	3931.35±12.85^{a}	82.82±1.50^{b}	70.45±1.53^{c}
B_1 (thiamin) (μg/100 g FW)	53.35±3.23^{b}	96.30±0.93^{a}	10.60±0.59^{c}	n.d.	50.09±2.59^{b}
B_2 (riboflavin) (μg/100 g FW)	n.d.	85.36±2.42^{a}	51.54±2.52^{b}	n.d.	82.73±8.77^{a}
B_3 (niacin) (μg/100 g FW)	15.94±8.26	n.d.	n.d.	n.d.	n.d.
B_5 (pantothenic acid) (μg/100 g FW)	5.64±0.91^{d}	136.09±1.82^{a}	114.14±1.07^{b}	13.29±0.64^{c}	6.71±2.99^{d}
B_6 (pyridoxine) (μg/100 g FW)	28.60±0.54^{a}	12.78±0.98^{b}	2.20±0.13^{d}	10.38±1.92^{c}	n.d.
B_{12} (cyanocobalamin) (μg/100 g FW)	n.d.	n.d.	n.d.	n.d.	n.d.
C (ascorbic acid) (mg/100 g FW)	0.59±0.05^{e}	0.80±0.03^{d}	3.70±0.02^{a}	0.98±0.06^{c}	1.29±0.08^{b}
E (tocopherol) (mg/100 g FW)	0.19±0.01^{d}	0.61±0.08^{c}	4.04±0.47^{a}	Tr	1.28±0.05^{b}

Results are presented as mean±SD (n = 3).
Means with different letters in the same row are significantly different by Duncan's multiple range test ($P<0.05$).
n.d. = not detected.
Tr = trace.

among the distinct pawpaw parts. High levels of amino acids and fatty acids were measured in the seeds, and minerals and vitamins were found to be rich in the pawpaw roots and leaves. Overall, these results provide basic data for improving the sitological value of pawpaw and will promote further studies on the nutritional compositions of other plants and their components.

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