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**Research Article** 

# Studies of the chemical composition of fruits and seeds of pawpaw (*Asimina triloba* (L.) Dunal)

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An international team of scientists continues to study the resource potential of non-conventional or little-utilized plants. Pawpaw (*Asimina triloba* (L.) Dunal) fruit (pulp and peel) and seeds were analyzed for their nutritional compositions. Seeds exhibited significantly higher levels of crude protein, lipid, and vitamin E (11.82 %, 34.0 %, and 20.80 mg/kg, respectively) than those of the other parts. Sucrose in pulp was 501.40 g/kg, which was the highest among the samples. There is more fructose in the peel 111.90 g/kg. Results revealed that the total amino acids in the seeds, pulp, and peel of *A. triloba* were 144.6, 21.1, and 20.9 g/kg, respectively. Among the different plant parts used in this study, the seeds contained the most abundant essential amino acid. Oleic and linoleic acids in seeds were 40.13 and 38.84 g/100 g, respectively, which were the highest among the pulp and peel. Potassium was the most abundant essential trace mineral element in different parts. This element is present in large ammounts in the peel (15487 mg/kg) and pulp (12198 mg/kg) compared to the seeds (3888 mg/kg). In the seeds, P, Ca and S were higher (1937, 1368, and 1322 mg/kg, respectively) than in pulp and peel (1046, 450, 499 and 831, 837, 646 mg/kg, respectively). The high content of beneficial substances makes it possible to include *Asimina triloba* in the list of species recommended for cultivation on a larger scale and to use its products more widely in dietary nutrition.

Keywords: pawpaw, fruits, seeds, chemical compositions

#### Introduction

The search for new plant species, especially neglected and underutilized plant species that are a valuable source of biologically active compounds, and creation on its basis the new generation of nutritional supplements, has recently become an urgent branch of modern biological science, and one of the most important scientific directions (Brindza et al., 2006, 2016; Klymenko et al., 2017). Guided by these new requirements, an international team of scientists has been studying for several years the content of biologically active compounds in various organs of cultivated new plants to the region (Monka et al., 2014; Ivanišová et al., 2017; Klymenko et al., 2017, 2019; Grygorieva et al., 2018; Horčinová Sedláčková et al., 2018; Grygorieva et al., 2020a, b; Vinogradova et al., 2020). This article focuses on *Asimina triloba* (L.) Dunal.

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© Slovak University of Agriculture in Nitra www.uniag.sk *Asimina triloba* (pawpaw, paw paw, paw-paw, common pawpaw) belongs to the family Annonaceae Juss. native to eastern North America and Canada (Layne, 1996). *A. triloba* fruits are rich in vitamins and minerals (Templeton et al., 2003; Pomper and Layne 2005), are a good source of potassium and several essential amino acids, and they contain significant amounts of riboflavin, niacin, calcium, phosphorus, and zinc (Galli et al., 2007), have a high polyphenolic (Harris and Brannan, 2009; Brannan et al., 2012, 2014) and antioxidant content (Kobayashi et al., 2008; Brindza et al., 2019).

*A. triloba* can be used as an alternative to bananas fruits in most recipes (Jones et al., 1995). Fruits of pawpaw are very fragrant and resemble a combination of aromas of banana and mango, and may be used commercially in cosmetics and skin products (Layne, 1996; Brannan et al., 2012). The extract of unripe *A. triloba* fruit has a value not only as a functional food, but has therapeutic potential for the treatment of cancer as a naturally derived substance that may be less toxic than conventional chemotherapy drugs (Nam et al., 2018b).

Biologically active compounds are not only in fruits, but in different parts of the plant: roots, bark, twigs, leaves, flowers, and seeds (Hui et al., 1989; Zhao et al., 1992, 1993, 1994; Alali et al., 1999; Goodrich et al., 2006; Cuendet et al., 2008; Farag, 2009; Pande and Akoh, 2010). The roots, twigs, flowers, and seeds of *A. triloba* contain acetogenins, which are strong inhibitors of cancer cells (Ratnayake et al., 1992; Woo et al., 1995; Ko et al., 2011; Sica et al., 2016). *A. triloba* leaf essential oil has strong activity against cancer cell lines (Alali et al., 1999; Farag, 2009). *A. triloba* fruit, leaf, bark, and twig extract may be an effective insect feeding deterrent (Rupprecht et al., 1986; Ratnayake et al., 1992; Zhao et al., 1994; Gu et al., 1999; Sedlacek et al., 2010).

Despite the importance of *A. triloba* as a nutritional and medicinal plant in the conditions of Ukraine, this species is very little spread. Thus, the objective of the present study was to investigate and compare the nutritional compositions of pulp, peel, and seeds of *A. triloba*.

# Material and methodology

# **Biological material**

*A. triloba* (Figure 1) seeds and fruits (pulp and peel) (Figure 2) were collected in September 2020 from the trees growing in an M.M. Gryshko National Botanical Garden (Kyiv, Ukraine; 197 m a.s.l.).

### Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany), and CentralChem (Slovakia).

#### Phytochemical analyses

# Determination of dry matter, ash, and protein content

Total dry matter, ash, and protein content were determined according to EN method (CSN EN 12145, 1997). Total lipid content was determined according to the methods specified in ISO method (ISO 659:1998).

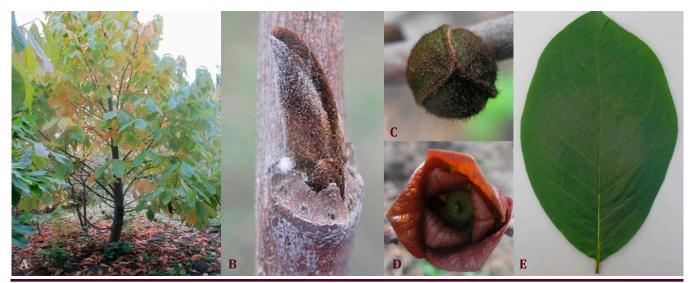


Figure 1 Tree (A), vegetative bud (B), generative bud (C), flower (D), and leaf (E) of Asimina triloba (L.) Dunal



Figure 2 Fruits and seeds of Asimina triloba (L.) Dunal

#### **Determination of saccharides**

For the determination of saccharides, 1 g of a sample was extracted with 10 mL of extraction the solution (ultrapure water and ethanol mixed in a ratio of 4 : 1) in a 50 mL centrifugation tube placed on a vertical shake table (GFL, Germany). After 1 hr of extraction, the samples were centrifuged for 4 min at 6000 rpm in a centrifuge (EBA 21, Hettich, Germany); the supernatant was filtered using a filter with 0.45 mm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with ultrapure water. An Agilent Infinity 1260 liquid chromatography (Agilent Technologies, USA) equipped with ELSD detector was used for the determination of saccharides. A Prevail Carbohydrates ES column (250/4.6 mm) was used as a stationary phase and acetonitrile (VWR) mixed with water in 75: 25 volume ratio was used as the mobile phase.

#### **Determination of carotenoid**

Total carotenoid content expressed as betacarotene was analyzed at a wavelength of 445 nm spectrophotometrically (VIS spectrophotometer UV Jenway Model 6405 UV/VIS). Sample (1 g) was disrupted with sea sand and extracted with acetone until complete discoloration. Petroleum-ether was added and then water with the purpose of the separation of phases. After the separation, the petroleum ethercarotenoid phase was obtained and the absorbance was measured (ČSN 560053, 1986).

#### **Determination of mineral contents**

Sample for elemental analysis was prepared using the wet ashing method in a microwave oven (Milestone 1200, Milestone, Italy). Total of 0.25 g sample matrix was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha Ltd, Czech Republic) and hydrochloric

acid (2 mL) (Analytika Praha Ltd, Czech Republic). Then decomposition sample was filtered using a filter with 0.45 mm pore size and filled up to 25 mL in a volumetric flask with ultrapure water. Elemental analysis was performed using ICP-OES (Ultima 2, Horiba Scientific, France) according to the procedure described by Divis et al. (2015).

#### **Determination of amino acids**

Amino acids were determined by ion-exchange liquid chromatography (Model AAA-400 amino acid analyzer, Ingos, Czech Republic) using post-column derivatization with ninhydrin and a VIS detector. A glass column (inner diameter 3.7 mm, length 350 mm) was filled manually with a strong cation exchanger in the LG ANB sodium cycle (Laboratory of Spolchemie) with average particles size 12  $\mu$ M and 8 % porosity. The column was heated within the range of 35 to 95 °C. The elution of the studied amino acids took place at a column temperature set to 74 °C. A double-channel VIS detector with the inner cell volume of 5 µL was set to two wavelengths: 440 and 570 nm. A solution of ninhydrin (Ingos, Czech Republic) was prepared in 75 % v/v methyl cellosolve (Ingos, Czech Republic) and in 2 % v/v 4 M acetic buffer (pH 5.5). Tin chloride (SnCl<sub>2</sub>) was used as a reducing agent. The prepared solution of ninhydrin was stored in an inert atmosphere  $(N_2)$  in darkness at 4 °C. The flow rate was 0.25 (mL/ min) and the reactor temperature was 120 °C.

#### Statistical analysis

Basic statistical analyses were performed using PAST 2.17. Data were analyzed with ANOVA test and differences between means compared through the Tukey-Kramer test (p < 0.05). The variability of all these parameters was evaluated using descriptive statistics.

# **Results and discussion**

In the course of the study of the nutritional properties of *A. triloba* for a recommendation to use it as a raw material for industry, it is crucial to identify the chemical composition of the different organs of the plant.

The total protein content was 11.82, 3.64, and 3.98 % in seeds, pulp, and peel, respectively (Table 1); the total lipid content was 34.0, 1.12, and 3.89 %, respectively. According to Nam et al. (2018a) *A. triloba* seeds also showed significantly higher levels of crude protein and crude lipid.

Chitturi et al. (2013) evaluated the protein levels and antioxidant potential of air-dried medicinally significant domestic fruit peels and their extracts.

Monosaccharide analysis of neutral carbohydrate part showed the presence of fructose (4.20, 87.10, and 111.90 g/kg, respectively) and sucrose (24.30, 501.40, and 227.50 g/kg, respectively) in seeds, pulp, and peel, respectively, while other saccharides, such as maltose and lactose were found only in low amounts only (<0.5 g/kg). Nam et al. (2018a) showed that fructose, glucose, and sucrose showed the highest levels in the fruit of *A. triloba* at 1691.35 mg%, 2148.20 mg%, and 9321.24 mg%, respectively.

The free sugar content, as has been demonstrated by Andersen (1986), depends on the following climatic characteristics: temperature, rainfall, relative humidity, and degree of light. Therefore, the phytochemical composition of *A. triloba*, including free sugars is expected to vary according to cultivation conditions. At the same time, the intensity of sweetness from free sugars and their composition will differ depending on the plant organs.

Carotenoids are widely distributed in nature. They are diverse in structure and in their function for human health. Carotenoids are useful for the prevention of certain types of cancer. They can prevent photosensitization in some skin diseases and increase immune response in infections. Their anti-aging effects on the human body are also known (Kurahashi et al., 2009).

A. triloba contains beta carotene in seeds, pulp, and peel (4.80, 6.60, and 12.70 mg/kg, respectively). As shown from the results, beta carotene accumulated in peel 2 times more than in pulp and approximately 3 times more than in seeds. Differents fruits investigation on  $\beta$ -carotene content showed that in the peel this vitamin concentrated the most. Ghosh et al. (2019) believe the presence of  $\beta$ -carotene in fruit peel wastes might be a contributing factor for its antioxidant activities. Citrus reticulata Blanco (Ghosh et al., 2019), Mangifera indica L. (Ranganath et al., 2018; Ghosh et al., 2019), Musa acuminata Colla (Budhalakoti, 2018), Malus domestica Borkh. (Delgado-Pelayo et al., 2014) have a high in  $\beta\text{-carotene}$  content and they also show potential antioxidant activity. According to Arora et al. (2008), banana peel could be a potential source of carotenoids. The content of carotenoids and  $\beta$ -carotene depends

**Table 1**Contents of some phytochemical compounds of Asimina triloba (L.) Dunal

Components	Seeds (x̄ ±S <sub>x</sub> )	Pulp (x̄ ±S <sub>x</sub> )	Peel ( $\bar{x} \pm S_x$ )
Total dry matter (%)	96.73 ±3.06	87.07 ±2.68	84.12 ±2.33
Total content of protein (%)	11.82 ±0.12	3.64 ±0.05	3.98 ±0.18
Total content of ash (%)	1.47 ±0.04	4.27 ±0.09	4.04 ±0.05
Total content of lipids (%)	34.0 ±1.02	1.12 ±0.06	3.89 ±0.07
Beta carotene (mg/kg)	4.80 ±0.05	6.60 ±0.09	12.0 ±0.09
Saturated fatty acids (g/100 g oil)	8.90 ±0.03	30.50 ±0.60	25.70 ±0.10
Monounsaturated fatty acids (g/100 g oil)	32.10 ±0.16	28.0 ±0.09	24.90 ±0.11
Polyunsaturated fatty acids (g/100 g oil)	46.60 ±1.13	18.20 ±0.13	23.70 ±0.09
Fructose (g/kg)	4.20 ±0.04	87.10 ±0.21 111.90 ±1.16	
Maltose (g/kg)	<0.5	<0.5 <0.5	
Sucrose (g/kg)	24.30 ±0.10	501.40 ±12.18	227.50 ±9.32
Lactose (g/kg)	<0.5	<0.5 <0.5	
Vitamin A (retinyl acetate) (mg/kg)	<0.1	<0.1 <0.1	
Vitamin E (α-tocopherol) (mg/kg)	20.80 ±0.08	8.80 ±0.11	19.10 ±1.09

Note:  $\bar{x}$  – arithmetic mean;  $S_x$  – standard error of the mean

on many factors such as degree of maturation, type of soil, climatic conditions, etc. (Dhandapani et al., 2017). Fruits and vegetable processing industries produce huge waste in the form of peels, seeds, liquid, and molasses which are a good source of carbohydrates, proteins, fibres, vitamins, and minerals (Kaur et al., 2018).

The major quantitative tocopherol in *A. triloba* seeds, pulp, and peel was  $\alpha$ -tocopherol (20.80, 8.80, and 19.10 mg/kg DWP, respectively). The oil contents were 34.0 (seeds), 1.12 (pulp), and 3.89 % (peel) dry weight plant material. The majority of plant oils are accumulated in the seeds (Bates et al., 2013) and this parameter is controlled by genetic effects (embryonic, cytoplasmic, maternal) and interactions between the genotype and environment (Liu et al., 2014).

The composition and amount of free amino acids varied among the different parts (Figure 3). The taste, flavour, and quality of various foods are directly related to the quantity and quality of free amino acids that accumulate naturally in nutrition products (Kabelova et al., 2008; Sinesio et al., 2009). Amino acid analysis has shown that the studied A. triloba seeds, pulp, and peel contained 18 amino acids (9 essential and 9 nonessential). The contents of the total amino acid of the seeds were significantly higher than those of the pulp and peel, which is consistent with previous studies that found that most of the amino acids of the seeds of A. triloba was in higher levels than those in the fruit (Nam et al., 2018a). The total amino acids in the seeds, pulp, and peel of A. triloba were 144.6, 21.1, and 20.9 g/kg, respectively. The glutamic acid exhibited the

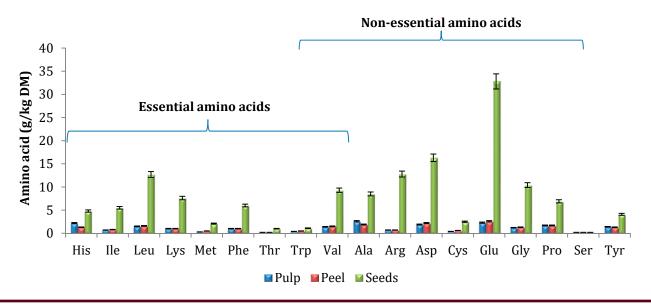
highest concentration in seeds with the highest mean content among the tested amino acids, accounting for more than 22.68 % of the entire amino acid profile. Alanine, glutamic acid, and histidine were the major amino acids detected in the peel, and glutamic acid, and aspartic acid were the main amino acids found in the pulp.

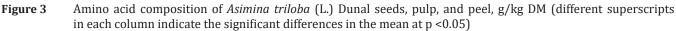
Our data coincide with those of Nam et al. (2018a) that glutamic acid was the major amino acid measured in seeds having the highest level of 1396.27 mg%.

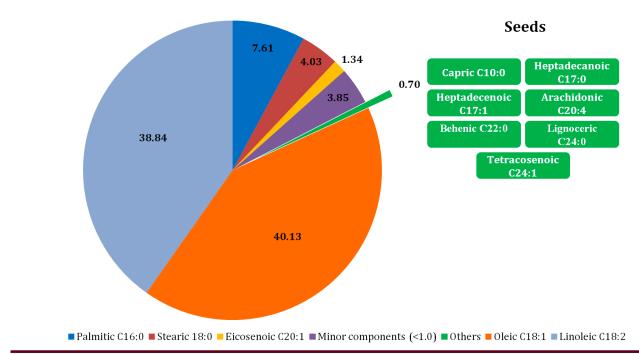
Fatty acids play an important role in human health. Thus, they are valuable in the prevention of cardiovascular diseases, coronary heart disease, and cancer. Their role is also important in the prevention of inflammatory, thrombotic and autoimmune diseases, hypertension, type 2 diabetes, kidney diseases, rheumatoid arthritis, and ulcerative colitis (De Caterina et al., 2000; Abedi and Sahari, 2014). Lauric, palmitic, linolenic, linoleic, oleic, stearic, and myristic fatty acids have antibacterial and antifungal properties (McGaw et al., 2002; Seidel and Taylor, 2004). In this regard, we determined the fatty acid content in various organs of *A. triloba*.

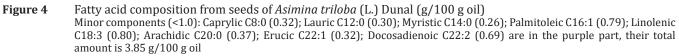
Total fatty acids varied in different parts of *A. triloba* (Figure 4, 5, 6). The unsaturated fatty acid content was higher than the saturated fatty acid content in all samples, and the difference between unsaturated fatty acid and saturated fatty acid was more pronounced in pawpaw seeds. In contrast, the difference between the contents in pulp and peel was negligible.

In the present study, *A. triloba* contained palmitic acid, oleic acid, linolenic acid, and linoleic acid. Oleic









acid and linoleic acid in the seeds samples were 41.59 and 40.25 %, respectively (Figure 4). Palmitic acid and stearic acid were the minor fatty acids in leaves, accounting for 7.89 and 4.18 % of the total fatty acids, respectively. Unsaturated fatty acids were also predominant in seeds, which accounted for 86.23 % of total fatty acid while saturated fatty acids accounted for 13.77 %.

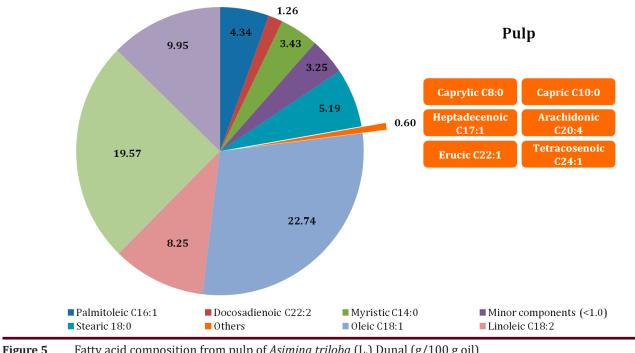
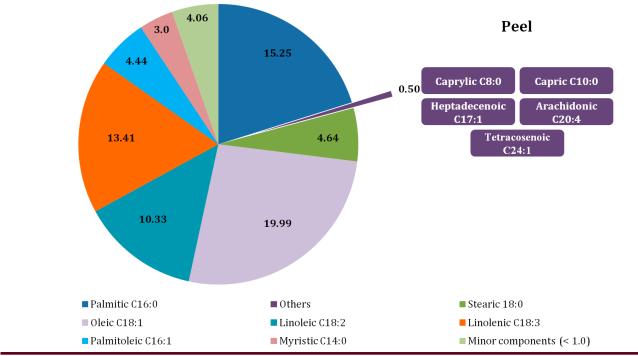
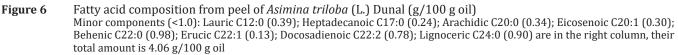


Figure 5Fatty acid composition from pulp of Asimina triloba (L.) Dunal (g/100 g oil)<br/>Minor components (<1.0): Lauric C12:0 (0.42); Heptadecanoic C17:0 (0.27); Arachidic C20:0 (0.30); Eicosenoic C20:1 (0.90);<br/>Behenic C22:0 (0.75); Lignoceric C24:0 (0.61) are in the right column: are in the right column, in the purple part, their total<br/>amount is 3.25 g/100 g oil





As reported by Nam et al. (2018a) the contents of oleic and linoleic acids contained in the seeds were 5905.11 and 8045.56 mg%, respectively, which was more than 120 times and 820 times the oleic and linoleic acids contained in the fruit, respectively.

Oleic acid and palmitic acid in pulp accounted for 28.66 and 24.67 % of total fatty acids, followed by linolenic acid and linoleic acid, accounting for 12.54 and 7.89 % of total fatty acids, respectively (Figure 5). Unsaturated fatty acids were also predominant in pulp, which accounted for 60.88 % of total fatty acid while saturated fatty acids accounted for 39.12 %.

In peel, oleic acid, palmitic acid, linolenic acid, and linoleic acid accounted for 26.23, 20.01, 17.59, and 13.55 % of total fatty acids, respectively (Figure 6). Stearic acid and palmitoleic acid were the minor fatty acids in the peel, accounting for 6.09 and 5.83 % of the total fatty acids, respectively. Unsaturated fatty acids were also predominant in the peel, which accounted for 65.70 % of total fatty acid while saturated fatty acids accounted for 34.30 %.

Oleic acid was present in the largest amount in the seeds, pulp, and peel. Oleic acid belongs to the omega-9 fatty acids. Omega-9 fatty acids (MUFAs) have one double bond per one molecule of a fatty acid. Oleic acid (18:1 n-9), as the major MUFAs, is the main characteristic of the Mediterranean Style Diet, which is high in olive oil (Rustan and Drevon, 2005). The monounsaturated fatty acids (MUFAs) at normal amounts in the diet do not affect blood cholesterol levels (Hegsted et al., 1993). When polyunsaturated fatty acids in the diet are replaced by monounsaturated fatty acids, such as oleic acid, they do not increase blood cholesterol levels (cholesterolemia). However, increased MUFAs content in the diet lower than LDL- and total cholesterol and increases HDL-cholesterol (Mattson and Grundy, 1985), and thus MUFAs reduce the risk of cardiovascular disease.

The palmitic acid, stearic acid, and myristic acid belong to the saturated fatty acids (SFAs). SFAs increase and are the primary determinants of serum cholesterol. The polyunsaturated fatty acids (PUFAs) actively lower serum cholesterol (Hegsted et al., 1993). Dietary omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFAs), including alpha-linolenic acid, docosapentaenoic acid, and eicosapentaenoic acid, are most studied. The anti-inflammatory and hypotriglyceridemic effects of these fatty acids are well known, whereas pro-inflammatory properties have been recognized in their dietary counterparts, the  $\omega$ -6 PUFAs (D'Angelo et al., 2020).

In order to comply with plant food safety requirements, the concentration of heavy metals in plants must be evaluated. Toxic elements including mercury, lead, and cadmium can be present in trace amounts. Major mineral elements such as iron, copper, zinc, and manganese should not exceed critical thresholds, as excessive concentrations are also toxic (Lekouch et al., 2001).

The average contents of the elements in the different parts of *A. triloba* are shown in Table 2. Macroelement and trace element concentrations in the leaf samples revealing the following trend: Ca > P > Mg > K > S > Fe > Zn > Al > Mn > Cu > Na > Se > Cr > Ni > Pb > As > Hg > Cd. These elements were also detected in seeds samples according to the following order: K > P > Ca > S > Mg > Fe > Zn > Cu > Na > Mn > Al > Ni > Se > As > Cr > Pb > Cd > Hg. In the pulp samples, the following concentrations were observed: K > P > S > Ca > Mg > Fe > Na, Cu > Al > Zn > Mn > As > Cr, Se, Ni > Pb > Cd > Hg. In the peel samples, the following result was obtained: K > Ca > P > S > Mg > Fe > Na > Cu > Al > Zn > As > Cr, Se, Ni > Mn > Pb > Cd > Hg.

For all of these elements, the required amount of the individual's daily intake (mg/day) has been determined (Anke et al., 2002). The average concentrations of Hg, As, and Pb analyzed in *A. triloba* were below the maximum allowable levels.

Table 2 shows that mineral elements are higher in the peel than in the other parts of *A. triloba*. K was the most abundant essential trace mineral element in different parts. There is more of this element in the peel (15487 tmg/kg) and pulp (12198.0 mg/kg) than in the seeds (3888.0 mg/kg). In the seeds, P, Ca and S were higher than in pulp and peel. Seeds and pulp contribute moderate trace elements because of their high water content. Nam et al. (2018a) report that seeds (289.17 mg% fresh weight) have more potassium content than fruits (239.36 mg% fresh weight).

The many reports proposed that the chemical composition content may differ according to several factors such as growing season, cultivars, and climatic conditions (Rohloff et al., 2005; Jang et al., 2011; Nwofia et al., 2012). Therefore, the present study suggests that the chemical composition content of *Asimina triloba* can depend on their different parts, as well as these other factors.

Our conclusion provides additional information to elucidate the medical functions and nutritional properties of *A. triloba*. The fruits of *A. triloba* can also be recommended for use as a potential functional food, nutraceutical, or dietary food supplement in processed form.

Minerals	Seed ( $\bar{x} \pm S_x$ )	Pulp (x̄±S <sub>x</sub> )	Peel ( $\bar{x} \pm S_x$ )
Р	1937.0 ±122	1046.0 ±75	831.0 ±48
К	3888.0 ±215	12198.0 ±334	15487.0 ±361
Ca	1368.0 ±96	450.0 ±28	837.0 ±31
S	1322.0 ±99	499.0 ±31	646.0 ±45
Fe	26.0 ±1.02	25.0 ±1.12	31.0 ±1.09
Mn	7.1 ±0.5	1.1 ±0.02	$1.5 \pm 0.01$
Mg	1021.0 ±65	396.0 ±35	573.0 ±47
Na	8.0 ±0.7	5.0 ±0.2	11.0 ±0.4
Al	1.1 ±0.01	$4.4 \pm 0.2$	$3.7 \pm 0.2$
Cr	<0.2	<0.2	<0.2
Cu	14.0 ±0.5	5.0 ±0.3	4.0 ±0.2
Zn	17.0 ±0.4	3.0 ±0.1	$3.0 \pm 0.2$
Se	0.25 ±0.05	<0.2	<0.2
As	<0.3	<0.3	<0.3
Cd	0.050 ±0.002	<0.01	<0.01
Ni	0.74 ±0.08	<0.2	<0.2
Hg	0.009 ±0.0001	0.007 ±0.0002	0.006 ±0.0001
Pb	<0.1	<0.1	<0.1

 Table 2
 Mineral composition of different parts of Asimina triloba (L.) Dunal (mg/kg)

Note:  $\bar{x}$  – arithmetic mean;  $S_x$  – standard error of the mean

#### Conclusion

Diverse amino acids, fatty acids, and mineral elements that are essential to human health were in *Asimina triloba*. The glutamic acid, oleic acid, linoleic acid, palmitic acid, K, P, Ca, and S were found in relatively high levels. High potassium content was found in all the plant parts of *A. triloba*. Considering these results, *A. triloba* fruits and seeds can contribute to the coverage of nutritional recommendations in human nutrition and some nutritional requirements in the human diet.

#### Acknowledgments

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