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Full Length Research Paper

Proximate composition, mineral nutrient and fatty acids of the seed of ilama, *Annona diversifolia* Saff

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The seeds of *Annona diversifolia* were analyzed to establish their chemical composition and nutritional properties in order to investigate the possibility of using them for human and/or animal consumption. The average composition of the kernel was determined to be: 15.40±0.17% of crude protein, 25.03±23% of carbohydrate, 27.43±0.18% of oil, 2.18±0.17% of ash and 62.58±0.25% of crude fiber. While the averages of the shell composition, were: 6.37±0.21% of carbohydrate, 0.86±0.14% of ash and 83.22±0.15% of crude fiber. The mineral content in mg/100 g of kernel: shell was found to be potassium (356.4:568.4), sodium (35.5:21.6); calcium (203.3:184.5); magnesium (4.5:2.0). Investigation of micronutrients in mg/100 g dry matter for kernel: shell gave for iron (21.4:62.5); zinc (21.2:57.1) and copper (24.1:9.8). The manganese determined in kernel was 19.2, and 3.77 for the shell. The oil content in the kernel had higher percentage of unsaturated fat acids (78.39%) than saturated ones (21.61%). The result of the proximate, mineral nutrient composition and fatty acid distribution of this seed and seed oil may offer a scientific basis for use of the seeds and oils in human diet.

Key words: *Annona diversifolia*, Ilama seeds, proximate, mineral composition and fatty acids content.

INTRODUCTION

Annona diversifolia Saff (Annonaceae) is a Mexican indigenous tree, that has many local names such as ilama, ilama zapote, ilamazapotl (Nahuatl language), izlama, hilama, papausa, papauce and zapote de vieja (Ruiz and Morett, 1997). This fruit-tree, which is very highly regarded in its area of origin, has not been studied as it deserves, since it is virtually planted exclusively by indigenous people. It is about 12 cm long, has white, pink or reddish flesh, with a typical aroma and a sweet, exquisite flavor which, according to most people, is superior to that of the cherimoya (*Annona cherimolla*). It is very resistant and sometimes completely immune to attack from seed-boring insects. The leaves of this plant are used by the people because of their antiinflammatory and analgesic properties (Ruiz and Morett, 1997). In a previous work, it was also demonstrated that the ethanol extract from the leaves of *A. diversifolia* (González-Trujano et al., 1998) and palmitone, isolated as the active compound (González-Trujano et al., 2006), had the anticonvulsant and depressant activity on central nervous system (CNS). Also, two acetogenins known as laherradurin

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Table 1. Physical characteristics[a] of *Annona diversifolia* seeds.

Parameter	Whole seed	kernel
Weight (g)[a]	1.43±0.05	0.93±0.03
Length (cm)[a]	2.26±0.04	1.85±0.04
Width (cm)[a]	1.32±0.02	0.93±0.02

[a]Mean of 15 seeds

and cherimolin-2 isolated from seeds of *A. diversifolia* have been analyzed in their *in vitro* and *in vivo* antiproliferative activity on HeLa and SW-480 cells (Schlie-Guzmán et al., 2009). However, nutritional studies as a potential animal feed supplement of this medicinal plant have not yet been undertaken. Here, we report the proximate composition of the seed kernels and shells of *A. diversifolia* and the fatty acids composition in the oil of seed kernels, in order to determine its potential in the area of feed/food industry.

MATERIALS AND METHODS

Collection of seeds

The ilama fruits of *A. diversifolia* were purchased in a market place of Tejupilco, Estado de México, México on September to October 2009. A voucher specimen (AN9702) was deposited at the 'Herbario de Plantas Útiles Efraím Hernández X, Universidad Autónoma Chapingo, Estado de México, México.

Physical properties of seeds

Fifteen seeds of ilama fruits were washed peeled and the pulps squeezed, to remove the seeds. These seeds were cracked using a mechanical cracker, the shells were carefully removed, and the weight, length and width of the whole seeds (kernels + shells) and kernels were recorded.

Proximate composition

The kernels and shells of the seeds were ground, using a mechanical grinder. Moisture, ash, crude protein, oil, crude fiber and ash content were determined in accordance with the standard methods of AOAC (1990). The moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105 °C for 4 h. Ash was determined by the incineration of 2.0 g of each sample placed in a muffle furnace maintained at 550°C for 3 h. Crude protein (% total nitrogen × 6.25) was determined by the Kjeldahl method using 1.0 g samples. Oil was obtained by exhaustive extraction of 2.0 g of each sample in a Soxhlet equipment using ethyl ether as the extractant. Total carbohydrates were determined by difference [100 – (crude fiber + ash + moisture)].

Mineral composition

Samples were digested by using conc. nitric acid and conc. hydrochloric acid. Atomic absorption spectrophotometer was used (GBC, model 932 AA) to determine calcium, magnesium, iron, copper, manganese, zinc, sodium and potassium averages.

Analysis of fatty acids composition

A transesterification reaction was performed in order to yield fatty acid methyl esters (FAMEs) sample (Om-Tapanes et al., 2008; Da Silva et al., 2010). A mixture of KOH (0.14 g) in anhydrous CH_3OH (5 ml) was added to 20 ml of the oil. The mixture was heated at 65°C for 2 h with continuous stirring. Upon termination of the reaction, the product mixture was transferred to a separating funnel where it was allowed to stand for one day. Afterward, the remaining glycerol lower layer was separated by gravity. In order to avoid an emulsification after the transesterification reaction, 0.1% aqueous citric acid was used as a washing solution to remove catalyst. Finally, fatty acid methyl esters (FAMEs) were dried with anhydrous sodium sulfate to afford 10 ml of a FAMEs mixture. These FAMEs were analyzed by gas chromatography (GC Agilent 6890) and ATSilar Column (30 m · 0.25 mm ID) with a flame ionization detector (FID). The column temperature gradient ranged from 170 to 240°C and hydrogen, at a flow rate of 1.8 ml per min, was used as a carrier gas. A standard fatty acid methyl ester mixture was run and retention times were used in identifying the sample peaks. Fatty acid levels were estimated as area percent of total peak area of methyl esters.

Statistical analysis

Results were expressed as the mean of three separate determinations except for mineral elements and fatty acids. Data were analyzed using student's t-test and significance was accepted at $P < 0.05$.

RESULTS

Physical characteristics

The average weight, length and width of *A. diversifolia* seeds were estimated and are listed in Table 1. The average weight of 15 seeds was 1.43±0.05 g for whole seed and 0.93±0.03 g for kernel. The average length and width of the seeds ranged from 2.26±0.04 cm to 1.32±0.02 cm for whole seed and 1.85±0.04 cm to 0.93±0.02 cm for the seed kernel.

Proximate analysis

The average composition of the seeds of *A. diversifolia* is shown in Table 2. There were differences between kernels and shells. The proximate analysis of the kernels

Table 2. Proximate composition[a] of *Annona diversifolia* seeds (g/100 g dry mater).

Parameter	Kernel	Shell
Moisture	9.61±0.33	7.85±0.26
Ash	2.18±0.17	0.86±0.14
Crude protein (N × 6.25)	15.40±0.17	Negligible
Oil yield	27.43±0.18	1.60±0.11
Crude fiber	62.58±0.25	83.22±0.15
Carbohydrate[b]	25.03±23	6.37±0.21

[a]Means of triplicate analysis, [b]Calculated by difference.

Table 3. Mineral composition of *Annona diversifolia* seeds (mg/100 g dry matter).

Element	Kernel	Shell
Potassium	356.4	568.4
Calcium	203.3	184.5
Magnesium	4.5	2.0
Sodium	35.5	21.6
Zinc	21.2	57.1
Copper	24.1	9.84
Manganese	19.2	3.77
Iron	21.4	62.5

Table 4. Fatty acid profiles of *Annona diversifolia* seed oil (area %).

Fatty acid	Area %
Palmitic acid (C16:0)	16.40
Stearic acid (C18:0)	5.22
Oleic acid (C18:1)	70.42
Linoleic acid (C18:2)	7.97

was: 15.40±0.17% of crude protein, 25.03±23% of carbohydrate, 27.43±0.18% of oil, 2.18±0.17% of ash and 62.58±0.25% of crude fiber. While the averages composition of the shells was: 6.37±0.21% of carbohydrate, 0.86±0.14% of ash and 83.22±0.15% of crude fiber. According to data in Table 2, *A. diversifolia* kernels contain high concentration of lipids. Gas chromatography analysis was made in order to investigate the fatty acid profile. The kernels and shells of *A. diversifolia* had fairly high crude fiber content. The mineral composition of the *A. diversifolia* seeds showed that the most significant mineral element is potassium which is as high as 356.4 in kernels and 568.4 mg/100 g in shells, respectively (Table 3).

Fatty acid composition

The fatty acids found and determined as FAMEs in the oil of kernels were oleic (70.42%), linoleic (7.97%), palmitic (16.40%) and stearic (5.22%) acids. The oil of the kernels had higher percentage of unsaturated fatty acids (78.39%) than saturated ones (21.61%) (Table 4).

DISCUSSION

The physical characteristics of *A. diversifolia* seeds are shown in Table 1. The values of the average of whole seed length and width found are larger than that found in seeds of soursop (*Annona muricata*) (Fasakin et al., 2008). The moisture content of the kernels and shells from *A. diversifolia* seeds was 9.61±0.33 and 7.85±0.26 g/100 g dry mater (Table 2), respectively, this low moisture level implies that seeds can be stored for longer time without spoilage (Onyeike et al., 1995). Ash content was higher, 2.18±0.17 g/100 g dry matter, in the kernels than in shells. This value is in agreement with the reported for *A. muricata* seeds (2.29 %) (Fasakin et al., 2008), this content is also an index for the quality of feeding materials used for poultry and cattle feeding (Adewuyi et al., 2010).

The crude protein content in kernels was 15.40±0.17 g/100 g determined in dry matter, this content of seeds is higher when it is compared with common cereals like whole wheat flour, parboiled rice and eggs in which case the protein content is 8.55, 7.7 and 12.6%, respectively (Eknayakea et al., 1999) that makes *A. diversifolia* seeds great supplement to cereal based diets. The high crude fiber content in kernels is 62.58±0.25 and 83.22±0.15 g/100 g for shells. These averages are nutritionally significant because fiber helps to maintain the gastro-intestinal tract health (Ajayi et al., 2006). The mineral composition in the kernels and shells showed that potassium is: 356.4 and 568.4 mg/100 g in dry matter, respectively (Table 3). This is nutritionally relevant considering that potassium plays a principal role in neuro-muscular functions. The high quantity of potassium, magnesium and calcium and the extra quantity of sodium plus the contents of essential elements iron, manganese, zinc and copper allow the seeds to be considered as excellent sources of bioelements (Saura-Calixto and Cafiellas, 1982). Therefore these seeds can be recommended to be used in the preparation of diets for persons with low levels of these mineral elements. It is of capital importance to indicate that zinc and copper have a range of intake over which their supply is adequate to the body (Cu 1.5 to 3 mg/day, Zn 12 to 15 mg/day). However, beyond this range, deficiency and toxic effects are observed because a high supplementation of copper had been related with liver damage (García-Rico et al., 2007).

The oil content of the seeds of *A. diversifolia* was 27.43±0.18 and 1.60±0.11 g/100 g dry matter (Table 2) for kernels and shells, respectively. The high content of unsaturated fatty acids in the seed oil (Table 4) and in the light of the beneficial effects of such unsaturated fatty acids in health and disease, the oils which are rich in linoleic and oleic acids might be acceptable as substitutes for unsaturated oils and could be exploited for nutritional advantage. This is of alimentary importance since, linoleic acid (7.97%), undoubtedly one of the most important polyunsaturated fatty acids in human food because of its prevention of cardiovascular disease (Omode et al., 1995), is present in the seed oil. Additionally a potent proapoptotic capacity of oleic acid (found in 70.42%) has been developed in different types of cultured neuronal cells (Zhu et al., 2005). The sample of *A. diversifolia* transesterified oil exhibited more than 98 wt% in methyl esters, these results were in agreement with those reported for other vegetable oils that presented typical fatty acid composition (Da Silva et al., 2010).

Conclusions

In this study, protein, oil and crude fiber of the kernels and shells of *A. diversifolia* seeds were determined. Protein content of seeds is high when compared with common cereals, the high crude fiber content for kernels and shells are of nutritional significance since fiber helps to maintain the health of the gastro-intestinal tract. The mineral composition showed that potassium is high; this is nutritionally important considering the fact that potassium plays a principal role in neuro-muscular function. The oil of seeds has higher percentage of unsaturated fatty acids than saturated ones, this is very important in human food because of its prevention of cardiovascular disease. The overall results of this work may offer a scientific basis for use of the seeds and oils in human diet.

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