Investigation and Comparison of Lipid Content and Nutritional Composition of Different Organs of *Brahea armata*– S. Watson, Family Arecaceae, Growing in Egypt

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Abstract: The present study was carried out to characterize the lipoidal matter of different organs (leaves, pollen gains and fruits) of *Brahea armata* – S. Watson and to evaluate their nutritive and amino acid composition. Saponification of petroleum ether extract of the leaves, pollen grains and fruits yielded 57.6%, 39.5% and 2.9% for the unsaponifiable matters and 32.54 %, 2.38% and 65.1% for the fatty acids, respectively. The total identified unsaturated fatty acids ranged from 7.87% to 83.61% in the three investigated organs, being most prominent in the fruits saponifiable matter, while the total saturated fatty acids ranged from 16.39% to 92.13%, in which the leaves saponifiable matter is the richest. The pharmacopoeial constants of the pollen grains and fruits showed higher ratio of total protein (8.6%), crude fat (1.3%), ash (8.72%) and moisture (49.64%) in pollens than those of fruits (5.5%), (0.93%), (4.2%) and (5.88%), respectively. But, the other parameter, total carbohydrate is higher in fruits (83.49%) than in pollens (32.36%). Total percentage of non essential amino acids in the both organs of *Brahea armata*, 70.62% and 60.25% for pollen and fruit, respectively, were higher than percent of essential amino acids 29.38% and 39.75% for the same organs, respectively. The predominant macro-element of minerals in both these organs was potassium (K), followed by sodium (Na). Among the micro-elements, iron (Fe) represents the highest percent (95 and18 mg/100g) in the pollens and fruits, respectively. These results showed that the fruits of *Brahea armata* are nutritious and can play a major role in human nutrition and health, so they are edible.
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Keywords: *Brahea armata*; Lipid content; Pharmacopoeial constants; Amino acids; Minerals; Edible fruits.

1. Introduction

The genus *Brahea* (Arecaceae) in the major group Angiosperms (flowering plants) comprises about 12-16 species native to Baja California, Mexico, and Central America [1] and it is known as Hesper Palm. All Hesper Palms have large, fan-shaped leaves [2]. They are grown as ornamental plants for their beautiful appearance so used for decoration in streets and gardens. Reviewing the current literature, no information could be traced concerning the investigation of the lipoidal matter of any *Brahea* species and the study of their nutritional composition. So, the present study is the first report of characterization of lipid content and determination of nutriontional status of the most widespread *Brahea* species in Egypt, *Brahea armata*- S. Waston. However, there are many *Brahea* species with edible fruits include *Brahea dulcis* (Mart.) Becc., is a widespread fan palm found in Mexico and Central America, the edible fruit of which are sweet and of an agreeable flavour. Guadalupe palm, *Brahea edulis*, native to Guadalupe Island off the coast of Baja California, also has edible fruit and it is said to taste similar to dates. Also, *Brahea aculeata* from western Mexico is said to have edible fruit [3].

Brahea armata -S. Watson, blue hesper palm, which is native to northern Baja California and north-western Mexico [1]. This palm is now pantropic in distribution and is abundantly available in Egypt. *B. armata* has blue costapalmate leaves and cream colored flowers. The fruit is ovid, shiny, and brownish-yellow with white stripes or speckles [4]. The Yuman Indians grind the seeds of this palm into a meal for consumption [5]. They also eat the fruit and use it for making beverages (i.e. edible fruits) [6].

The objective of the present work was to determine the lipid content of different organs of *Brahea armata* –S. Waston with respect to their chemical composition, the mineral content and amino acid composition. The preliminary phytochemical screening of the petroleum ether extracts of different organs of the plant revealed that the petroleum ether extract of the three organs are rich in lipid content and sterols and/or triterpenes. Therefore, it was deemed of interest to carry out comprehensive study using GLC analysis of lipoidal matter to identify the main components.

2. Material and Methods

2.1. Plant material

Different organs (leaves, pollen grains and fruits) of *Brahea armata*- S. Waston used in this study were collected during the year (2012-2013) from certain gardens of Cairo, Egypt as Orman Garden. The plant was kindly verified by Dr. Mohamed EL- Gibali, Senior Botanist National Research Center, Cairo, Egypt. Voucher specimens of this specie were air-dried, powdered and packed in an air-tight plastic container for further analysis.

2.2. Study of lipid content

2.2.1. Preparation of lipoidal matter

About 50 g of each of air-dried powdered leaves, pollens and fruits of the plant under investigation were separately exhaustively extracted with petroleum ether (40-60) and the solvent in each case was distilled off under reduced pressure. The mark left in each case was dried and kept for further investigation. The yield of lipoidal matter was 2.198 g, 1 g and 3.147g a residues of leaves, pollen grains and fruits representing about 4.4%, 2% and 6.3% in the three organs, respectively.

2.2.2. Preparation of unsaponifiable Matter (USM) [7]

About 1g of the prepared lipoidal matters of each organ was saponified by heating under reflux using 10 ml alcoholic KOH (10%) and 4 ml of benzene for 24 hours to ensure complete hydrolysis. After distillation of ethanol under reduced pressure, the residue of each organ was mixed with 100 ml cold water, and the mixture was transferred to a separating funnel and shacked with successive portions of diethyl ether (50ml x 4). The combined ethereal extract of each organ containing the unsaponifiable matter was collected, washed several times with distilled water till free from alkalinity and dehydrated over anhydrous sodium sulphate, filtered and the solvent was evaporated to dryness to give yellowish brown solid residue (USM) in a yield of 1.75g, 1.2g and 0.088g representing 57.6%, 39.5% and 2.9% of the leaves, pollen grains and fruits, respectively.

2.2.3. Isolation of the fatty acids from the saponifiable fractions

The aqueous alkaline solutions were separately acidified with dilute HCL (10%) to liberate the free fatty acids (FA) and were extracted with diethyl ether (5× 50 ml) till exhaustion. The ethereal extracts were combined, washed with distilled water till free from acidity then dried over anhydrous sodium sulphate and evaporated to dryness. The fatty acids (oily residues) obtained in each case (1.37g, 0.1g and 2.74g representing 32.5%, 2.4% and 65.1% in three organs, respectively.

2.2.4. Preparation of the fatty acid methyl esters (FAME) [8]

Aliquots of the fatty acid mixtures (0.3g) were separately, dissolved in 10 ml methanol, 0.5 ml of sulfuric acid was added and the mixture was refluxed for one hour on a boiling water bath. The cooled mixture was diluted with 20ml of water and then extracted with ether (5×40ml). The combined ethereal extracts were washed with water till neutral to litmus paper, dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure. The dried residue was, in each case, saved in a dessicator for GLC analysis.

2.2.5. GLC analysis of the USM and FAME

USM analysis was carried out on Hewlett-Packard HP-6890 N network GC system equipped with a FID detector. Analysis was performed on an HP-5 column; using N_2 as a carrier gas, injection temperature 250^{o}C, detector temperature 300^{o}C. Aliquots, 2 µL each, of 10% chloroform solutions of the analyzed USM and reference samples were co-chromatographed. Identification of the components was based on comparison of the retention times of their peaks with those of the available authentic samples. The relative amount of each component was calculated via peak area measurement by means of computing integrator**.**

FAME were analysed on a pye Unicam 304 series gas chromatograph equipped with a FID detector. Analysis was performed on 10% PEGA (on chromosorb W-AW, 100-120 mesh) column; using N_2 as carrier gas, injection temperature 250^{o}C, detector temperature 280^{o}C. Aliquots, 2 µL each, of 10% chloroform solutions of the analyzed FAME and reference fatty acid methyl esters were co-chromatographed. Identification of the fatty acids (FA) was carried out by comparing the retention times of their methyl esters with those of the available reference fatty acids similarity analyzed.

2.3. Nutritional status of Brahea armata

2.3.1. The pharmacopoeial constants

Certain pharmacopoeial constants of the air-dried powdered pollen grains and fruits of this specie were determined according to the methods outlined by AOAC (1980) [9]. All the methods used in estimating the chemical composition of the plant samples were standard methods of the Association of Official Analytical Chemists (1980) where:

- Total protein was determined by the Micro-Kjeldahl method, which involves digestion or oxidation of the sample with conc. H_2SO_4 followed by steam distillation. Ammonia formed at high PH was received in 4% boric acid solution then titrated with 0.1N HCL. The percent of protein was obtained by multiplying N% by the factor of 6.25.
- Determination of total crude fat depends on the difference in weight between the original sample and the hexane-extracted sample in soxhlet apparatus.

- Total ash obtained by heating a crucible containing the sample in a muffle furnace at 550 ^{o}C overnight (till constant weight).
- Total carbohydrate content (as % of dry weight) was obtained by difference.
- Total moisture was estimated (for the fresh samples) by heating at 105^{o}C overnight (till constant weight).

2.3.2. Estimation of amino acids

Determination of all amino acids other than tryptophan of two organs (pollen grains and fruits) of *Brahea armata* was carried out according to the method described by Winder and Eggum (1966) [10]. These amino acids are aspartic acid, thereonine, serine, glutamic acid, praline, glycine, alanine, cystine, valine, isoleucine, leucine, tyrosine, phenylalanine, histadine, lysine and arginine.

2.3.3. Determination of Mineral Element

Mineral elements were determined by atomic absorption spectrometry adobting the reported methods [11]. Dry ashing was carried out by ashing 1 g of pollen grain and fruit samples in a muffle furnace at 500 ^{o}C overnight. The ash of each sample was allowed to cool then was dissolved in 5 ml 20% HCL (with slight warming). The solution of each was then filtered through an acid-washed Whatman filter paper no. 1 in a volumetric flask 50 ml, washing the filter paper several times with deionized water. Dilution of each sample with deionized water to specific volume was then carried out after which each element was determined spectrophotometrically in the samples solution. The concentration of each element was determined from the standard calibration curve, obtained using known concentrations of standard solutions prepared from stock solutions. For Ca and Mg, the sample, blank and standard solutions contained 1 % w/v Lanthanum to avoid anionic interferences.

The mineral elements determined were; Manganese (Mn), Copper (Cu), Potassium (K), Sodium (Na), Iron (Fe), Chromium (Cr), Magnesium (Mg), Nickel (Ni) and Calcium (Ca).

2.3.4. The Contribution of essential nutrients of pollens and fruits to their RDA values for adults

The contribution of the levels of essential nutrients of pollen grains and fruits of *Brahea armata* under investigation to their RDA (Recommended Dietary/Daily Allowances) values for adults established by the Food and Nutrition Board (1980) [12].

3. Results and Discussion

3.1. Characterization of lipid of Brahea armata

Saponification of pet-ether extracts of three organs (leaves, pollen grains and fruits) of this specie yielded 57.6%, 39.5% and 2.9% for USM, respectively; 32.5%, 2.4% and 65.1% for fatty acids (FA) of *Brahea armata*, respectively. The GLC analysis of USM of these three organs showed that the percentage of total identified sterols was higher in fruits (79.92%) than those of pollen grains (72%) and leaves (15.9%). While, the percentage of the total identified hydrocarbons of leaves (84.1%) was higher than those of both pollens (28%) and fruits (20.08%). Stigmasterol was the major one reaching 38.62% in the pollen grains, of lower occurrence in fruits unsap. (7.60%) and in traces in the leaves unsap. (0.50%). While, β- sitosterol was of lower occurrence in the unsap. of leaves and pollens (2.77% and 1.74%, respectively). 5α-Cholesterol was found in lower amount in leaves only (0.46%) and Cholesterol was of lower amount in pollens only (1.77%). N-heptacosane was the major hydrocarbon (10.63%) in the pollens and n-octacosane (10.31%) in the leaves. N-hexacosane and n-octacosane were of lower occurrence in fruits unsap. representing, (0.67% and 1.24%), respectively. From the overall results it is evident that the pollen unsap. is the richest in its sterols and hydrocarbones composition. (Table 1).

The GLC analysis of the identified fatty acid methyl esters of leaves, fruits and pollen grains of *Brahea armata* showed that the total identified unsaturated fatty acids ranged from 7.87% to 83.61% in the three investigated organs, being most prominent in the fruits saponifiable matter, while the total saturated fatty acids ranged from 16.39% to 92.13%, in which the leaves sap. matter is the most richest. Oleic acid was the major unsaturated fatty acid while; pentadecanoic acid was the major saturated fatty acid, being most predominant in the fruits sap. matter (38.98% and 7.07%, respectively). Behenic acid was the major saturated fatty acids (11.88%) in leaves. While, the rest of both saturated and unsaturated fatty acids were found in lower amount in the three organs. (Table 2)

These polyunsaturated fatty acids (PUFAs) cannot be made in the body and must be provided by diet and are known as essential fatty acids. Within the body, they can be converted to other PUFAs such as arachidonic acid, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), which are important for maintaining the membranes of all cells; production of prostaglandins which regulate many body processes include inflammation and blood clotting. On the other hand they play very important role to enable the fat-soluble vitamins A, D, E and K to be absorbed from food and regulating body cholesterol metabolism [13].

3.2. Chemical composition

Table 3 shows the chemical composition of the pollen grains and fruits of *Brahea armata*. Total protein, fats, ash and moisture percentages were

higher (8.6%, 1.3%, 8.72 %and 49.64%) in pollen grains than that (5.5%, 0.93, 4.2% and 5.88%) of fruits, respectively. Total carbohydrates content was higher (83.49%) in fruits than those (32.36%) of pollen grains. These Data suggested that both pollen grains and fruits can be used as a high source for nutrition.

3.3. Amino acid composition

Total percentage of non essential amino acids in the both organs of *Brahea armata*, 70.62% and 60.25% for pollen and fruit, respectively, were higher than percent of essential amino acids 29.38% and 39.75% for the same organs, respectively. Proline was the highest percent of amino acids in pollen grains (950.52mg/100g) followed by aspartic acid (562.48mg/100g). Proline (654.34mg/100g) was highest percent of amino acids in fruits followed by glutamic acid (630.08mg/100g). Nearly Total amino acids percent in fruits were much higher than these of pollen grains. (Table 4)

So, fruits of *Brahea armata* have a high nutritional value than pollen grains where they have a high percentage of the following amino acids [14]:

- Aspartic acid that is of a highly importance that helps in the protection of the liver by aiding the removal of ammonia, is involved in DNA and RNA metabolism as well as it is involved in immune system function by enhancing immunoglobulin production and anti- body formation.

- Glutamic acid was found to play an important role in increasing energy, accelerating wound healing and ulcer healing and detoxifing ammonia in the brain by forming glutamine, which can cross the blood-brain barrier, which glutamic Acid cannot do.

-Tyrosine is the precursor to thyroxine and growth hormone, increases energy and improves mental clarity and concentration.

3.4. The Mineral content

Mineral element analysis of both pollen grains and fruits showed that potassium (K) was the major composition of macro-elements in (2985 and 1412 mg/100g) both organs followed by sodium (Na) (652 and 387 mg/100g), respectively. Chromium (Cr) was the minor micro-element (1mg/100g) in pollen grains but chromium (1mg/100g) and nickel (Ni) (1mg/100g) were the minor micro elements in fruits. Among the micro-elements, iron (Fe) represents the highest percent (95 and 18 mg/100g) in both organs, respectively (Table 5). So, pollen grains of *Brahea armata* contain a high concentration of mineral elements that play important role in our life such as:

- Calcium, potassium and Magnesium which are important for metabolism in human cells [15-17].

-Magnesium and calcium that they are essential for healthy bone development and for energy metabolism.

-The high potassium content is suitable for people with hypertension [18].

3.5. *The Contribution of essential nutrients of pollens and fruits to their RDA values for adults*

Table 6 shows that pollen grains and fruits posses a high nutritional value, since each 100g of the dried plant covers at least 25 % of adults daily requirements for most nutrients.

Table 1: Results of GLC analysis of the identified unsaponifiable matter of leaves, pollen grains and fruits of *Brahea armata*.

Items		**RR_t**	**Organ**		
			Leaves	**Pollen grain**	**Fruits**
Hydrocarbon & sterols content (Relative %)	n-Henicosane	0.51	2.51	---	---
	n-Docosane	0.55	2.98	---	---
	n-Tricosane	0.60	0.52	---	---
	n-Tetracosane	0.63	2.05	---	---
	n-Pentacosane	0.66	0.59	---	---
	n-Hexacosane	0.85	0.76	1.37	0.67
	n-Heptacosane	0.86	---	10.63*	---
	n-Octacosane	0.90	10.31*	4.36	1.24
	5α-Cholesterol	0.79	0.46	---	---
	Cholesterol	0.93	---	1.77	---
	Stigmasterol	1	0.50	38.62*	7.60*
	β-sitosterol	1.05	2.77	1.74	---
	% of identified Hydrocarbon		84.1	28	20.08
	% of identified Sterol		15.9	72	79.92

RR_t = Retention time relative to Stigmasterol (pollen grains)

* Major components

Table 2: Results of GLC analysis of the identified fatty acid methyl esters of leaves, pollen grains and fruits of *Brahea armata*.

Items		RRT*	Organs		
			Leaves	pollens	Fruits
Fatty acid content (Relative %)	Pentadecanoic $C_{15:0}$	0.35	-	-	7.07*
	Palmitoleic $C_{16:1}$	0.60	1.09*	-	-
	Stearic $C_{18:0}$	0.82	-	1.53	0.79
	Oleic $C_{18:1}$	0.45	-	-	38.98*
	Linoleic $C_{18:2}$	1	0.56	0.84	1.13
	Behenic $C_{22:0}$	0.40	11.88*	-	-
	Tricosanoic $C_{23:0}$	0.39	0.55	-	-
	Tertacosanoic $C_{24:0}$	0.61	6.88	-	-
	Carboceric $C_{27:0}$	0.90	-	2.27*	-
	Saturated fatty acid		92.13	81.90	16.39
	Unsaturated fatty acid		7.87	18.10	83.61

RR_t = Retention time relative to Linoleic acid
*Major components

Table 3: General chemical analysis of pollen grains and fruits of *Brahea armata*.

Item Type of analysis	Pollen grain	Fruits
Total protein (as % of dry weight)	8.6	5.5
Total crude fat (as % of dry weight)	1.3	.93
Total ash (as % of dry weight)	8.72	4.2
Total carbohydrates (as % of dry weight, by difference)	32.36	83.49
Total moisture (as % of fresh weight)	49.64	5.88

Table 4: Amino acids analysis of pollen grains and fruits of *Brahea armata*.

Items		RRT***	Organ	
			Pollen grains	Fruits
Amino acids content (relative%)**	Aspartic acid	0.56	562.48	586.32
	Thereonine*	0.71	104.88	191.84
	Serine	0.78	195.04	257.2
	Glutamic acid	0.86	410	630.08
	Proline	1	950.52	657.34
	Glycine	1.22	97.52	271.52
	Alanine	1.27	225.76	343.6
	Cystine *	1.39	7.92	0.0
	Valine *	1.55	161.28	266.96
	Isoleucine *	1.79	135.2	235.12
	Leucine *	1.84	234.08	457.6
	Tyrosine	1.99	230.96	445.04
	Phenylalanine*	2.05	267.36	365.44
	Histadine *	2.42	189.04	265.2
	Lysine *	2.58	183.04	433.36
	NH_4^+	2.75	994	1007.84
	Arginine *	2.96	242.64	554.24
	%of essential amino acid		29.38	39.75
	% of non essential amino acid		70.62	60.25

*Essential amino acids
**percent mg/100 g of powder
*** Relative retention time to praline (pollen grains)

Table 5: Mineral element analysis of pollen grains and fruits of *Brahea armata*.

Items			Organ	
			Pollen grain	Fruit
Mineral content (relative %)	Macroelements*	Mg	372	140
		Na	652	387
		Ca	586	209
		K	2985	1412
	Microelements*	Cr	1	1
		Mn	3	2
		Cu	2	3
		Fe	95	18
		Ni	2	1

*concentration (mg/ 100g dry weight)

Table 6: The contribution of the levels of essential nutrients in pollen grains and fruits of *Brahea armata* under investigation to their RDA (Recommended Dietary/Daily Allowances) values for adults established by the Food and Nutrition Board (1980).

Essential nutrient		RDA value for adults	Percentage coverage of daily needs for 100g of the dried powder	
			Pollen grains	Fruits
Protein		34-56 g/day	15. 36 – 25.29%	9.8 –16.18 %
Essential amino acids	Thereonine	27mg/g protein	45.17 %	Much higher than 100%
	Cystine and Methionine	25mg/g protein	3.68%	0.0%
	Valine	32mg/g protein	58.60 %	Much higher than 100%
	Isoleucine	25mg/g protein	62.88 %	Much higher than 100%
	Leucine	51mg/g protein	53. 37 %	Much higher than 100%
	Phenylalanine and Tyrosine	47mg/g protein	66.14 %	Much higher than 100%
	Histadine	18mg/g protein	Much higher than 100%	Much higher than 100%
	Lysine	55mg/g protein	38.7 %	Much higher than 100%
	Arginine	27mg/g protein	Much higher than 100%	Much higher than 100%
Carbohydrates		130 g/day	25 %	64.22 %
Minerals	Mg	240-420 mg/day	88.57% - Much more higher than 100%	33.33- 58.33 %
	Ca	1,000-1,300 mg/day	45.1-58.6 %	16.1-21 %
	K	240-420 mg/day	Much more higher than 100%	Much more higher than 100%
	Fe	8-18 mg/day	Much more higher than 100%	100- Much more higher than 100%

4. Conclusion

The results obtained from this study supported that fruits of *Brahea armata*- S. Waston are edible as they contained higher amount of unsaturated fatty acids. These unsaturated fatty acids always play an important role in the metabolism of living organism. Moreover, these fruits also contain higher amount of carbohydrates, a high number of amino acids as well as macro and micro nutrients which they may take part in the buildup of human good health.

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