

Physicochemical Properties of the Seed Kernels and the Oil of Custard Apple (*Annona squamosa* L.)

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Abstract: Custard apple (*Annona squamosa* L.) seed kernel and the extracted oil were characterized for their physicochemical properties. Crude ether extract was found to be the main component where, the seed kernels had 31.22%. Moreover, protein content was 20.01%. On the other hand, the crude fiber and total ash were 15.43 and 1.89%, respectively. Total phenolic compounds, antioxidant activity and IC₅₀ of CASKF were 42.02 mg GAE/ 100g, 87.55% and 22.84 µg/ml, respectively. The results indicated that CASKF is rich in content of K, P, Ca, Mg and Na. Nevertheless, very low levels of Cd and Pb were detected. The amino acid composition of the defatted CASKF indicated that glutamic, aspartic, alanine, leucine and arginine were the predominant amino acids. The total amount of essential amino acids in the defatted CASKF was 37.77 g /100g protein (-) which is higher than that reported in FAO/ WHO pattern. The dominant fatty acids of custard apple seed kernel oil were oleic (49.75%), Linoleic (22.50%), palmitic (15.06%) and stearic and (4.63%). The oil could be classified as a semi-dry oil. Total lipid fractions consisted mainly of nine classes in which triacylglycerols were the major class.

Keywords: Custard Apple, *Annona Squamosa*, Chemical Composition, Oil Characteristics.

1. Introduction

The custard apple (*Annona squamosa* L.) from Annonaceae family a fruitful in equatorial and semitropical areas, adapted to the climatic states of China, Africa, South America, Australia, India, Mexico, (-)United states, Thailand and Egypt. Increase consumption of custard apple is related to the medicinal and nutritional properties, as well as its pleasant flavor. The fruit is rich in vitamins A, B, C, E and K, antioxidants, polyunsaturated fatty acids and the presence of essential minerals. In addition, this fruit included assortment of compounds which are responsible for antifeedant, antimalarial, cytotoxic, immuno suppressive, anti HIV and antiplatelet aggregation activities [1, 2]. The fruit is easily broken or cut open, uncover the snow-white, Juicy flesh, of pleasant flavor and delicious, sub-acid flavor and containing many hard, brown or black bean-like glossy seeds [3]. Many domestic names have developed for the fruit. In English, it is

most vastly known as sugar apple or sweetsop as well as a custard apple. In the middle East, it is called qishta, ishta or ashta [4]. Custard apple fruits are convenient for treatment because of its high sugar content and delicate flavor. The fruit is marketed as fresh or frozen pulp, strained juice and frozen concentrates which have been preserved as various juice blends, ice cream, sherbets and nectars [3].(-) The seeds have major insecticidal properties and could be used for removing head lice [5]. The seeds are also utilized as a pesticide in the field of agriculture [6], or have antimicrobial properties [7-9]. In addition, Koushik *et al.* [10] stated that the extracts of custard apple seeds could also be included in drugs that treat inflammation caused by fungi.

(-)Sugar apple seed oil can be used to produce biodiesel [11]. There was no interest from food processing workers in the use of Egyptian custard apple seeds (*Annona squamosa* L.) or oil extracted from the seeds in food processing.

Therefore, in this study physical properties, chemical composition, total phenolic compounds and antioxidant

activity of seed kernels of Egyptian custard apple were studied.

In addition, some physical and chemical properties of oil extracted from seed kernels were evaluated. This study is therefore of great importance in assessing the nutritional value of custard apple seed kernels and its extracted oil.

2. Materials and Methods

2.1. Materials

Custard apple (*Annona squamosa* L) fruits were procured from the local market of Alexandria, Egypt. The fruits studied were in full maturity. The fruits were washed with running water then sliced and the seeds were separated with a steel spoon. The seeds were collected and cleaned from the residue of fruit, then washed completely with distilled water and air dried. The dried seeds were manually dehulled with a sharp knife. The resultant seed kernels were ground and sieved through 60 mesh sieve. The obtained flour (CASKF) was stored in tightly Kilner jar at $4 \pm 2^\circ\text{C}$ until used. Figure 1 shows the general appearance of custard apple fruit, cross section and the seeds.

2.2. Chemicals

All chemicals used were of analytical grade and were purchased from El-Gamhouria Co. for Chemical and Medical Requisites, Alexandria, Egypt. DPPH reagent (1,1-diphenyl-2-picrylhydrazyl) was obtained from Sigma Company, Germany.

2.3. Methods

Physical properties including seed index, bulk density, hull and kernel percentages and seed dimensions including length, width and thickness were determined [12]. Proximate analysis of CASKF including moisture, crude protein (N x 6.25), crude ether extract, total ash and crude fiber were carried out according to AOAC [13]. procedures unless otherwise stated. Nitrogen free extract (NFE) was calculated by difference. Total phenolic compounds were determined by Folin-Ciocaltu reagent [14] after extraction with 70% ethanol [15].



Figure 1. General appearance and cross section of custard apple (*Annona squamosa* L.) fruit and the seeds.

2.4. DPPH Radical Scavenging Activity

Antioxidant activity was estimated by assessing the free radical activity of the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical [16]. (-) 0.3 ml methanolic extract was added to 2.7 ml DPPH 0.1 mmol in methanol solution. Then the reaction mixture was well mixed and incubated for approximately 30 min at room temperature in the dark. Absorbance was measured at 517 nm using a UV-Vis Spectrophotometer. The antioxidant activity was calculated as a percentage of inhibition DPPH from the following equation:

$$\text{Inhibition (\%)} = \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \times 100$$

Where: A sample is the absorbance of sample.

A DPPH is the absorbance of the control (DPPH solution).

The IC_{50} is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by 50%. The IC_{50} of the sample was derived from the % scavenging activity vs. concentration plot and is expressed as mg/ml.

2.5. Minerals and amino acids

Fe, Mg, Ca, Zn, Cu, Mn, Cd and Pb were estimated using Perkin Elmer Atomic Absorption Spectrophotometer, while, Na and K were determined using flame photometer. Total phosphorus (P) was assayed colorimetrically at 630 nm using a Spectrophotometer [13]. Amino acids were analyzed in the hydrolyzate using Amino Acid Analyzer (Biochrom 30). Amino acid composition were expressed, as g /100g protein. Tryptophan was (-)colorimetrically determined in the alkaline hydrolysate [17]. Amino acid score (AAS) was calculated from the essential amino acid (EAA) content to the total EAA content in 1 g sample protein divided by the same EAA content in the reference FAO/WHO [18] pattern. From the AAS, the limiting AA in the sample which had the lowest values AAS could be established [18].

2.6. Lipid Profile

Chloroform: methanol (2:1) was used to extract the total lipids [19]. The total lipid extract was divided into different classes using a TLC method [20] on glass plates (20 x 20cm) pre-coated and loaded with 0.25 mm silica gel, G-60. The rising solvent system used was petroleum ether: diethyl ether: glacial acetic acid (70:30:2 V/V/V). After completing the solvent rise process, the plate was air-dried and the separated spots were shown by iodine vapor. Lipid categories were determined by their R_f values [21].

2.7. Fatty Acid Composition

(-)Fatty acid methyl esters utilizing 1% sulphuric acid in absolute methanol were prepared [22]. Gas chromatographic analysis was carried out using ACME. Equipped with a splitless injector and flame ionization detector. Nitrogen was used as a carrier gas. The components were separated on a

30-cm Sp-2380 fused- silica capillary column. The detector temperature was set at 260°C. The injector temperature was set at 220°C and in split mode (Split ratio 80:1). Total program time was 30 min. Fatty acid composition was expressed as percentage of the total fatty acids.

Identity properties of the oil extracted from CASKF Which included refractive index (RI) at 25°C, Specific gravity (Sp. Gr.) at 25°C, iodine value (IV), saponification value (SV), peroxide value (PV as mEq O₂ / kg) free fatty acid (FFA) as% oleic acid, unsaponifiable matter (%) and color measured by using Lovibond Tentometer were determined [23].

2.8. Statistical Analysis

SPSS software was used to calculate percentages, arithmetic averages and standard deviation [24].

3. Results and Discussion

Physical properties of custard apple fruits, seeds and seed kernels.

Physical properties of custard apple fruits, its seeds and seed kernels are shown in (Tables 1 and 2). It can be noted that fruit weight was 141.25 g. On the other hand, the weight of pulp, hulls and seeds were 77.24, 32.08 and 18.71 g, respectively which represented 54.68, 32.08 and 18.71%, respectively of the total weight of the fruit. Kad *et al.* [12] found that the weight of fruit was 238.68 g. Also they found that peel, capillary pulp, gritty pulp and seeds were 46.77,

35.36, 11.63 and 6.24%, respectively.

As shown in (Tables 1 and 2), seed weight, seed index and bulk density of custard apple seeds (CAS) were 0.2649, 26.44 (g/100g seeds) and 0.49 g/cm³. After dehulling, these values were 0.209 g, 20.896 (g/100 seed kernel) and 0.504 g/cm³, respectively. The data also indicated that CAS dimensions (length, width and thickness) were 1.519, 0.704 and 0.514 cm, respectively. On the other hand, after dehulling these values decreased to 1.148, 0.554 and 0.464 cm, respectively. The percentages of hulls and kernels of CAS were 33.50 and 66.5%, respectively. Comparing with the results obtained in the present study, Kad *et al.* [12] found that length, breadth and thickness of *annona squamosa* seeds were 13.69, 8.29 and 6.1 mm, respectively at the initial moisture content (15.40% d.b.) and decreased with decrease in moisture content to 12.50 and 10.25% (d.b.) They mentioned that the bulk density, was 0.642 g/cm³. This value increased to 0.661 and 0.684 g/cm³ when the moisture content decreased from 15.4% to 12.5 and 10.25%.

Table 1. Physical properties of custard apple (*Annona squamosa* L.) fruit.

Property	Value*
Weight of fruit (g)	141.25 ± 3.06
Weight of hull (g)	45.31 ± 1.62
% of hull	32.08 ± 1.35
Weight of pulp (g)	77.2 ± 2.06
% of pulp	54.68 ± 1.63
Weight of seeds (g)	18.71 ± 0.54
% of seeds	13.24 ± 0.82

* Mean of three determinations ± SD.

Table 2. Physical properties of custard apple seeds and kernels.

Property	Seeds	Kernels
Weight of seed or kernel (g)	0.264 ± 0.001	0.209 ± 0.07
seed or kernel index (g/100 seed or kernel)	26.44 ± 1.03	20.896 ± 0.073
Bulk density (g/cm ³)	0.487 ± 0.03	0.504 ± 0.006
Seed dimensions (mm)		
Length	15.19 ± 0.02	11.48 ± 0.03
Width	7.04 ± 0.01	5.54 ± 0.02
Thickness	5.14 ± 0.01	4.64 ± 0.01
Hull%	33.50 ± 1.63	
Kernels%	66.50 ± 1.82	

* Mean of three determinations ± SD.

A proximate chemical composition, total phenolic contents and antioxidant activity of CASKF are shown in (Table 3). Crude ether extract of CASKF was found to be the main component. The seed kernels had 31.22% crude ether extract. This high content of oil reflects the importance of using such seed kernels for oil production. On the other hand, CASKF had about 20.01% crude protein, which possessed medium concentration between cereals, legumes and other oilseeds. It can be also noted that CASKF contained 15.43% crude fiber. Removal of hulls reduced the fiber content and thus concentrate both the crude oil and protein. The data in Table 3 revealed also that total ash content of CASKF was 1.89%. On the other hand, the calculated NFE was 31.45%. Comparing with the results obtained in the present study, Hassan *et al.* [25] found that on dry weight bases, ash

content, crude lipid, crude protein, crude fiber and available carbohydrate contents were 2.78, 44.0, 4.43, 36.33 and 12.45%, respectively. Further, Mariod *et al.* [26] stated that the oil and protein contents of *Annona squamosa* seeds were 26.8 and 17.5%, respectively. Furthermore, Mariod *et al.* [27] found that *Annona squamosa* seeds had 6.7% moisture, 26.8% fat, 17.5% protein, 2.2% ash, 16.8% fiber and 30.0% carbohydrates. Also, Shardul *et al.* [28] found that the seeds of *Annona squamosa* contained 1.22% moisture by Karl-Fischer method and 1.46% by loss on drying method. The total ash content was 2.39%. The results in Table 3 showed that CASKF contained considerable concentration of phenolic compounds being 42.02 mg GAE/ 100g. It has been reported that CASKF contained different type of phenolics. These compounds had anticancer effects [29].

The results in Table 3 also showed that CASKF had high percentage of antioxidant activity being 87.55%. These results confirmed the possibility of using CASKF as an antioxidant source. IC₅₀ of CASKF was 22.84 (µg/ml). In accordance with the results obtained in the present study, Kothari and Seshadri [30] and Biba *et al.* [2] stated that the seed extract of *Annona squamosa* showed highest antioxidant activities and phenolic content. The seeds may be a good source of antioxidants that may have therapeutic uses. Kadarani *et al.* [31] found that seeds of *Annona squamosa* had higher total phenolic concentration and antioxidant activity than the peels of this fruits. Vijayaraghavan *et al.* [32] showed that the seeds of *Annona squamosa* recorded the most effective DPPH radical scavenging activity (77.14%) being close to synthetic antioxidant (BHT) as positive control (76.3%) This is because the seed extract of *Annona squamosa* was rich in glycosides, alkaloids, phenols, tannins and saponins.

Table 3. Proximate chemical composition, total phenolis and antioxidant activities of custard apple seeds kernel flour.

Component	Value*
Moisture (%)	6.80 ± 0.30
Crude ether extract (%)	31.22 ± 1.27
Crude protein (%)	20.01 ± 0.73
Total ash (%)	1.89 ± 0.02
Crude fiber (%)	15.43 ± 1.66
Nitrogen free extract (%)**	31.45 ± 0.89
Total phenolic content (mg GAE/100g)	42.02 ± 0.13
Antioxidant activity (%)	87.55 ± 1.23
IC ₅₀ (µg/ml)	22.87 ± 0.43

* Mean of three determinations ± SD (on dry weight basis).

** By difference.

Mineral content of CASKF are shown in (Table 4). As shown in the Table, CASKF is a good source of macro elements such as k, P, Ca, Mg and Na. low levels of Fe, Zn, Mn and Cu were present. On the other hand, very low levels of Cd and Pb were also detected. The aforementioned data are more or less in accordance with those reported by Amoo

et al. [33], Hassan *et al.* [25] and Souza *et al.* [34]. The latter showed that the mineral composition of flour from residual *Annona squamosa* responds to more than 20% of the daily intake of nutrients, highlighting the Cu, Fe, Mn, Zn, Ca and Mg.

Table 4. Mineral content of custard apple seed kernel flour.

Element	mg/100g*
K	363 ± 9.56
Mg	98 ± 6.63
Na	61 ± 1.88
Ca	280 ± 8.31
Zn	2.84 ± 0.23
P	328 ± 4.21
Cu	1.09 ± 0.23
Pb	0.38 ± 0.003
Fe	3.05 ± 0.17
Mn	2.93 ± 0.31
Cd	0.13 ± 0.005

* Mean of three determinations ± SD (on dry weight basis).

Amino acid composition of the defatted CASKF is presented in (Table 5). The results indicated that the predominant amino acids were glutamic (13.13), aspartic (9.03), alanine (7.84), leucine (7.15) and arginine 6.29 g/100g protein. Relatively, small amount of the other amino acids listed in Table 5 were also found. Table 5 also found that the total amount of essential amino acids in the defatted CASKF was 37.77 g/100 g protein. The results indicated that the protein of the defatted CASKF is deficient in methionine and lysine as compared with FAO/WHO requirement pattern (Table 5). Thus, the first limiting amino acid was methionine and lysine. The obtained results agreed well with those reported by Mariod *et al.* [26, 27]. They reported that the amino acid content of *Annona squamosa* seeds showed a high difference when compared with egg, sesame and broad bean amino acids. They also reported that all the essential amino acids with the exception of tryptophan were found to be present in high amounts when compared to that of the three different foods mentioned above.

Table 5. Amino acid composition and chemical score of defatted custard apple seed kernel.

Amino acid (g/100g protein)*	Value	FAO/WHO pattern**	Chemical score ***
Aspartic acid	9.03 ± 0.30		
Threonine	4.28 ± 0.10	3.40	125.88
Serine	3.95 ± 0.20		
Glutamic acid	13.13 ± 0.50		
Proline	5.68 ± 0.12		
Glycine	5.17 ± 0.10		
Alanine	7.84 ± 0.20		
Cystine	1.09 ± 0.12		
Methionine	2.31 ± 0.10	2.50	92.40+
Cystine + Methionine	3.40 ± 0.10		
Valine	4.47 ± 0.20	3.50	127.71
Isoleucine	3.12 ± 0.20	2.80	111.43
Leucine	7.15 ± 0.30	6.60	108.33
Tyrosine	3.45 ± 0.20		
Phenylalanine	4.41 ± 0.30		
Tyrosine + Phenylalanine	7.86 ± 0.20	6.30	124.76
Tryptophan	1.38 ± 0.12	1.10	125.45
Histidine	1.83 ± 0.10	1.90	96.32

Amino acid (g/100g protein)*	Value	FAO/WHO pattern**	Chemical score ***
Lysine	5.37 ± 0.20	5.80	92.59+
Arginine	6.29 ± 0.30		
Total EAA	37.77	33.90	

* Mean of three determinations ± SD (on dry weight basis).

** Pattern for 2 – 5 years old child

*** g of EAA in 100 g protein of the sample divided by the g of the same EAA in 100 g protein of the FAO/WHO standard pattern × 100.

+ the first limiting amino acid.

The results of the fractionation of the total lipid classes of CASKF are shown in (Figure 2). The total lipids included mainly of eight fractions of acylglycerol and non-acylglycerol fractions in addition to the polar lipid class located on the base line. Triacylglycerol class was found to be the maior fraction of CASK oil. The other classes can be arranged, based on their Rf as follows: monoacylglycerols, 1,2 and 2,3 diacylglycerols, sterols, 1,3 diacylglycerols, unknown, free fatty acids, triacylglycerols hydrocarbons and sterolesters based on the front line. The results obtained in the present study are more or less similar to that of other oil seed resources such as cantaloupe seeds [35], date pits [36], dehydrated mushroom flour [37], flaxseed [38] and okra seeds [39].

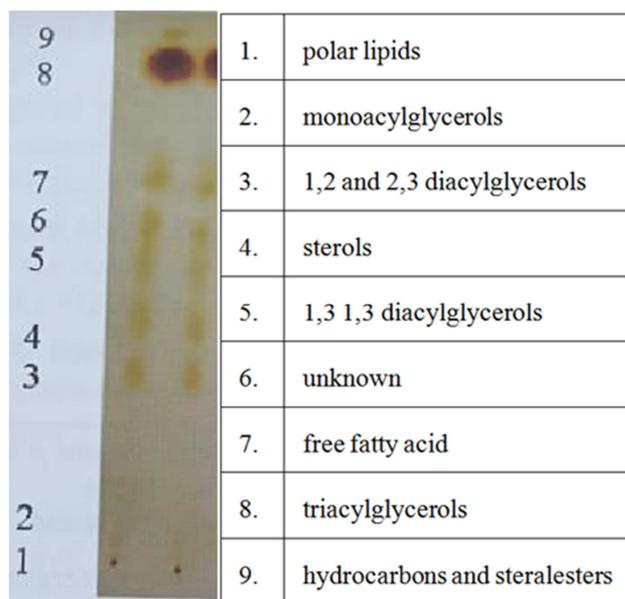


Figure 2. Thin layer chromatogram of total lipids of custard apple seed kernel oil.

Coating material: Silica gel G Merk type 60

Solvent System: petroleum ether: diethyl ether glacial acetic (70:30:2 v/v/v)

Detection: Exposure to iodine vapour

The physicochemical characteristics of the oil extracted from CASKF are presented in Table 6. The color of the crude oil was light yellow and smelled acceptable and liquid at room temperature and did not contain high free fatty acids (1.68 as% oleic acid) and unsaponifiable matter (1.21%). The results of the refractive index and the specific gravity obtained were within the standard of most crude vegetable oils [40]. The value of saponification in oil was slightly high

(189.21) and the value of iodine was low comparatively (90.55). Thus, this oil is categorized as semi-dry oil such as cottonseed, sunflower oil and maize. The value of peroxide in crude oil was low (0.82 mEq O₂/kg oil) indicated its high stability to retrogradation. Mariod *et al.* [26, 27] and Lokhande *et al.* [41] showed that the results of the present study are extremely similar in terms of physical and chemical properties of oil extracted from the seeds of *Annona squamosa*. Onimawo [42] Indicated that if was removed seamy odors from the oil of sour soup, was deodorized it can be valid as a source of oil for both domestic and industrial uses.

Table 6. Physicochemical properties of custard apple seed kernel oil.

Parameters	Value*
Acid value mg KOH/g oil	0.85 ± 0.11
Free fatty acids as% oleic acid	1.68 ± 0.20
Iodine value	90.55 ± 1.32
Unsaponifiable mater (%)	1.21 ± 0.30
Saponification value	189.21 ± 2.16
Peroxide value (m Eq O ₂ /kg oil)	0.82 ± 0.10
Refractive index at 25°C	1.4668 ± 0.10
Specific gravity at 25°C	0.8175 ± 0.06
Lovibondcolour (1 in.cell)	
Yellow	35 ± 0.0
Red	3.5 ± 0.01
Blue	0.3 ± 0.01

* Mean of three determinations ± SD.

Fatty acid composition of CASK oil are presented in (table 7). The percentage of the unsaturated fatty acids was 72.25% of the total fatty acids. The main unsaturated fatty acids were oleic (49.75%) followed by linoleic (22.50%). On the other hand, the saturated fatty acids represented 27.75% of the total fatty acids. The saturated fatty acids were found to be composed of palmitic acid (15.06%), followed by stearic acid (11.63%) and trace amounts of C14:0, C 20:0 and C12:0. The existence of high content of unsaturated fatty acids especially oleic acid demonstrated to be a highly nutritious oil and can be used to decrease high level of blood cholesterol. As shown in (Table 7), the ratio of unsaturated to saturated fatty acids was 2.60. This ratio is quite similar to the common crude oil such as corn, cottonseed and sunflower seed oils.

In accordance with the results obtained here, Mariod *et al.* [26, 27] found that the fundamental fatty acids of *Annona squamosa* oil were C16:0, C18:0, C18:1 and C18:2. They stated that the extraction method did not impact the fatty acid structure. C18:1 was the most dominant fatty acid followed by C18:2. On the other hand, they reported that C16:0 and C18:0 acids exhibited the third and fourth highest fatty acid

contents.

Acda [43] pointed out that saturated and unsaturated fatty acids identified from *Annona squamosa* seeds were biologically active ingredients responsible for the expulsion of certain insects. [44] Showed that the fatty acid composition of *Annona squamosa* L. seed kernels were C18:1 (47.4%), C18:2 (22.9%), C18:0 (13.6%) and C16:0 (12.1%). C18:1 and C18:2 together constituted 70.3% of the total UFAs followed by the SFAs (25.7%).

Table 7. Fatty acid composition (% of total) of custard apple seed kernel oil.

Fatty acid	% of total*
C 12: 0	0.21 ± 0.07
C 14: 0	0.52 ± 0.05
C 16: 0	15.06 ± 0.20
C 16: 1	ND***
C 18: 0	11.63 ± 0.32
C 18: 1	49.75 ± 0.95
C 18: 2	22.50 ± 0.11
C 18: 3	ND***
C 20: 0	0.33 ± 0.02
TSFAs (S)	27.75
TUFAs (U)	72.25
U/S ratio**	2.60: 1.0

* Mean of three determinations ± SD.

** Unsaturated/saturated ratio.

*** Not detected.

4. Conclusions

It can be concluded that custard apple seed kernel is a good source of crude oil and crude protein as well as some macroelements. Total essential amino acids was higher than that reported in FAOL WHO pattern. The extracted oil can be classified as a semi-dry oil. In general, custard apple seed kernel oil and its defatted flour appear to be useful in some food applications. Therefore, much research has to be done to explore digestibility, functional properties and the applications of custard apple seed kernel oil and defatted flour in some functional food products.

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