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ORIGINAL PAPER

Annona squamosa and *Catunaregam nilotica* Seeds, the Effect of the Extraction Method on the Oil Composition

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Abstract *Annona squamosa* and *Catunaregam nilotica* seeds and oils were characterized for their approximate analysis and physico-chemical properties. The oil and protein contents were 26.8, 17.5 and 40.0, 22.2%, in *A. squamosa* and *C. nilotica* seeds, respectively. The oils were extracted using cold extraction (CE) and Soxhlet extraction (SE) methods. Fatty acids and tocopherols were determined by GC–MS and HPLC, respectively. Generally the physico-chemical properties and fatty acids were not significantly ($P \leq 0.05$) affected by the extraction methods. The major fatty acids of *A. squamosa* oil extracted by CE and SE were oleic 49.2 and 50.5%, linoleic 22.3 and 22.7%, palmitic 15.6 and 15.2%, and stearic 10.6 and 9.3%, respectively. While the major fatty acids in *C. nilotica* oil extracted by CE and SE were oleic 10.5, and 10.4%, linoleic 63.1 and 63.4%, palmitic 9.7 and 9.8% and stearic 5.1 and 5.4%, respectively. The tocopherol content of CE and SE extracted oils from *A. squamosa* amounted to 16.6 and 15.5 and from *C. nilotica* amounted to 110.5 and 107.7 mg/100 g oil, respectively, with delta-tocopherol as the predominant tocopherol in *A. squamosa* oil, and beta-tocopherol in *C. nilotica* oil. The total amount of amino acids was found to be 7.266 and 14.202 g/100 g protein, in seeds of *A. squamosa* and *C. nilotica,* respectively.

Keywords Amino acids · *Annona squamosa* · *Catunaregam nilotica* · Extraction method · Fatty acids · Physicochemical properties · Seed oil · Tocopherol

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Introduction

Fats and oils are recognized as essential nutrients in both human and animal diets [1]. The Sudan is a large producer of conventional oilseeds such as cotton seed, sesame, ground nut and sunflower. Recently, researchers have started to be greatly concerned about identifying new oil sources from a large number of vegetables, fruits, and wild plants commonly grown in the Sudan [2]. Two of these wild plants are *Catunaregam nilotica* and *Annona squamosa. C. nilotica* belonging to the family Rubiaceae and known locally in Sudan as kerkir. *C. nilotica* is widespread in Central and East Africa as well as in Cameroon and Nigeria [3]. In the Sudan it is found in the South and North Kordofan states. It grows as a medium height shrub (usually <3 m) with grey drupes, stiff spines, and deciduous leaves clustered below the spines. It has a broad range of applications in the indigenous medical system [4]. *Catunaregam nilotica* still grows as a wild plant in different areas in western Sudan states. No research data on its commercial production and its oil composition are available.

Annona squamosa L. (Annonaceae), commonly known as custard apple is a native of the West Indies, it is widely grown throughout the tropics in India and popularly

cultivated in the north eastern parts of Thailand, mainly for its edible fruit. Its seed is well known for killing head lice but there has been no report on the active component [5]. The plant is deciduous and small; reaching a maximum of 6 m in height with many lateral branches, it grows well in regions of medium humidity. Its seeds comprise 30% of its fruit's weight, which is edible [6]. The oil content of these seeds varies from 14 to 49% having a large amount of unsaturated fatty acids, with a higher concentration of oleic acid. *A. squamosa* seed oil was reported to be use in the soap and plasticizer industry as well as in alkyd manufacturing, the seeds are acrid and poisonous. The bark, leaves and seeds contain the alkaloid anonaine [7]. It has been reported that *A. squamosa* seeds contain 23% oil of which 9.8% is a hydroxyl acid, the oil contains 38.6% saturated fatty acids and 61.4% unsaturated fatty acids (29.0% oleic and 32.0% linoleic:), with a saponification value of 191.8, a refractive index of 1.4826 [8], a specific gravity of 0.927, an iodine value of 88.0 and 1.5% of unsaponifiable matter [9]. Studies have revealed that the oil of *A. squamosa* is somewhat viscid in appearance, pale yellow in color and with a persistent smell. The oil is freely soluble in petroleum ether [9]. Its studies revealed that the different extraction methods of oil produce oils with different physicochemical properties [10].

This paper deals with the seed proximate analysis, amino acids, fatty acids and tocopherol composition of *A. squamosa*, and *C. nilotica* seed oil obtained by two methods of extraction, and describes for the first time these parameters in *C. nilotica* seed.

Materials and Methods

Samples, Solvents, and Reagents

All solvents used were of analytical grade. Petroleum ether, *n*-heptane, ethanol and diethyl ether, carbon tetrachloride, HCL, NaOH, HNO_3, and H_2O_2 were obtained from Merck (Darmstadt, Germany). Fruits of *A. squamosa* and *C. nilotica* were collected from the Khartoum central market and the Alfola local market, Sudan, respectively. The outer dried surrounding pith of the fruit was removed and the seeds were air-dried in an oven at 40 °C to reach a constant moisture content (6.5%). The dried seeds were cracked manually, the shells carefully removed and the kernels thus obtained were crushed and ground in a grinding mill (Petra electric, Burgau, Germany) the ground kernels with particle size of 0.5 mm were used for oil extraction. The kernels obtained were stored at 4 °C until further investigations.

Methods

Approximate Analysis of Seed

Moisture and Volatile

The moisture content of the samples was determined by the air oven method according to the AOCS Official Method [11].

Crude Protein

The crude protein analysis of the two samples was carried out and nitrogen content was determined by the semi-micro-Kjeldahl digestion, distillation and titration method, as described by the Official Methods of Analysis [12].

Crude Fiber

The crude fiber of the two samples was determined according to the AOCS official method [13].

Ash

The sample was ashed in a muffle furnace at 550 °C for 3 h or more and allowed to cool and weighed following the AOCS official method [14].

Oil Extraction

Two methods were used for oil extraction
- Cold solvent extraction
- Soxhlet extraction

Cold Solvent Extraction

The powdered sample (250 g) was put in conical flasks (1 L), and petroleum ether (b.p. 60–80 °C) was added. The solvent/sample ratio was 2:1(v/w); the mixture was put in an automatic shaker (IKA, KS 501, Staufen, Germany) for about 16 h at room temperature. The mixture was then filtered twice using glass wool. The clear filtrate was concentrated using a rotary evaporator to remove the solvent from the oil. The oil was allowed to stand in the open air at room temperature to ensure removal of all solvent from the oil. The oils obtained by cold extraction from *A. squamosa* (ASCE) and *C. nilotica* (CNCE) was then kept in dark bottles and stored at 4 °C for further analysis.

Soxhlet Extraction

The oils from the seeds of the two samples were extracted exhaustively with petroleum ether (b.p. 60–80 °C)

analytical grade in Soxhlet extractors according to the AOCS official method [15]. The oils obtained by Soxhlet extraction from *A. squamosa* (ASSE) and *C. nilotica* (CNSE) was then kept in dark bottles and stored at 4 °C for further analysis.

Oil Physicochemical Analysis

Physical Parameters

Specific Gravity

The AOCS official method [16] was followed for determination of the specific gravity of the oils at 60 °C.

Refractive Index

The AOCS official method [17] was followed to determine the refractive index of the oils at 30 °C.

Chemical Parameters

The following chemical parameters: acid value, peroxide value, saponification value, and the amount of unsaponifiable matter were determined according to AOCS official methods [18].

Fatty Acid Analysis

The fatty acid composition of *A. squamosa* oil (ASO) and *C. nilotica* oil (CNO) were determined following the ISO method [19]. In brief, one drop of the oil was dissolve in 1 mL of *n*-heptane, 50 µl 2 M sodium methanolate in methanol were added, and the closed tube was agitated vigorously for 1 min. After addition of 100 µL of water, the tube was centrifuged at 4,500*g* for 10 min and the lower aqueous phase was removed. After adding 50 µL 1 M HCl to the heptane phase, the two phases were mixed for a short time and the lower aqueous phase was rejected.

About 20 mg of sodium hydrogen sulfate (monohydrate, extra pure, Merck, Darmstadt, Germany) was added and centrifugation was carried out at 4,500*g* for 10 min. The top *n*-heptane phase was transferred into a vial and injected into a Varian 5890 gas chromatograph equipped with a capillary column Cp-Sil 88 (100 m long, 0.25 mm i.d., film thickness 0.2 µm). The temperature was from 155 °C heated to 220 °C (1.5 °C/min), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume <1 µl. The peak areas were computed by integration software and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization. All analyses were done in triplicate.

Tocopherols

For determination of tocopherols (TOC) a solution of 250 mg of studied samples oil in 25 mL *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with an L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system; 20 µL of the samples was injected by a Merck 655-A40 Autosampler onto a Diol phase µL 3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 + 1, v/v) [20].

Amino Acid Composition Using an Amino Acid Analyzer

Preparation of Hydrolysate Sample

The content of dry matter and total *N* were determined according to procedures described by the Association of Official Analytical Chemists [12]. The content of amino acids (except for tryptophan) in defatted seeds was determined using an Amino acid Analyzer (L-8900 Hitachi-hitech, Japan) under the experimental conditions recommended for protein hydrolysates. Samples containing 5.0 mg of protein were acid hydrolyzed with 1.0 mL of 6 N HCL in vacuum-sealed hydrolyzed vials at 110 °C for 22 h. The ninhydrine was added to the HCL as an internal standard. Hydrolysates were suitable for analysis of all amino acids. The tubes were cooled after hydrolysis, opened, and placed in desiccators containing NaOH pellets under vacuum until dry (5–6 days). The residues was then dissolved in a suitable volume of a sample dilution Na–S buffer, pH 2.2 (Beckman instrument), filtered through a Millipore membrane (0.22-µm pore size) and analyzed for amino acids by ion-exchange chromatography in a Beckman (model 7300) instrument, equipped with an automatic integrator. The nitrogen in the amino acids was determined by multiplying the concentration of individual amino acids by corresponding factors calculated from the percentage *N* of each amino acid [21]. The ammonia content was included in the calculation of protein nitrogen retrieval, as it comes from the degradation of some amino acids during acid hydrolysis [22, 23]. The ammonia nitrogen content was calculated by multiplying the ammonia content by 0.824 ($N = 82.4\%$ NH_3).

Statistical Analysis

The analyses were performed with three replicates. The mean values and standard deviation (means ± SD) were calculated and tested using the Student's t test ($P < 0.05$).

Statistical analysis of variance (ANOVA) was performed on all values using the statistical program Statgrafics Statistical Graphics System version 4.0 [24].

Results and Discussion

Proximate Analysis of Seeds

Table 1 shows the proximate analysis of *A. squamosa* and *C. nilotica* seed kernels, it is clear that the two samples show high levels of protein and oil content. The oil content of *C. nilotica* seed kernels using cold and Soxhlet extraction methods was significantly ($P > 0.05$) higher (40, 41.2%) than that of *A. squamosa* (26.8, 27.5%). The difference in oil content between cold extraction, and Soxhlet extraction method was not significant and can be attributed to the fact that during Soxhlet extraction, the high temperature employed in solvent evaporation may have caused sample heating which will allow oil droplets to come out of the sample easier. There is no research data, as far as we know, available in the oil content of *C. nilotica* seed. The oil content of *A. squamosa* kernels in this study was higher than that reported by Ansari et al. [8] who reported 23% oil content, but it was in the range of 14–49% which was reported by Morton in 1987 [7]. The oil content of *C. nilotica* kernels was higher than most Sudanese conventional oilseeds (cottonseed, sunflower and groundnut), while that of *A. squamosa* was lower than that of cottonseed, sunflower, sesame and groundnut [25]. These results indicated very clearly that the seeds from these two trees form a potential source of oils and fats. Therefore, from an economic point of view, the production of oil from such seeds could be of interest.

Oil Physicochemical Properties

The oils obtained from *C. nilotica* and *A. squamosa* kernels were odorless, of good color, and of good appearance. The physicochemical properties of the oils are represented in Table 2. The two oils were significantly different ($P \leq 0.05$) concerning their physcio-chemical properties as *A. squamosa* oil showed higher values of the refractive index, acid value, peroxide value, saponification value and specific gravity than *C. nilotica* oil. From Table 2, the *Annona squamosa* oil saponification value which gives an indication of the nature of the fatty acids in the fat, was lower than that (186.4) reported by Morton [7]. The refractive index, specific gravity, saponification and acid values of *A. squamosa* oil were different from those (1.475, 0.927, 183.0, and 10.0, respectively) reported by Rafeeg [9]. From Table 2 it can be summarized that the method of extraction only significantly affects ($P \leq 0.05$) the acid value, and no significant changes were observed in the refractive index, peroxide value, saponification value and specific gravity of

Table 1 The proximate analysis of seeds of *Annona squamosa* and *Catunaream nilotica*

Sample	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fiber (%)	CHO (%)
Annona squamosa	6.7 ± 0.2[a]	26.8 ± 0.4[a]	17.5 ± 0.2[a]	2.2 ± 0.1[a]	16.8 ± 0.2[a]	30.0 ± 0.3[a]
Catunaregam nilotica	6.4 ± 0.1[a]	40.0 ± 0.6[b]	22.2 ± 0.3[b]	2.8 ± 0.1[a]	14.0 ± 0.2[b]	14.6 ± 0.2[b]

* All determinations were carried out in triplicate and mean values ± standard deviations (SD) are reported. [a,b]Values with different superscript letters within a row indicate a significant difference at $P > 0.05$

Table 2 Physicochemical properties of *Annona squamosa* and *Catunaregam nilotica* oil

Physicochemical parameters	ASCE	ASSE	CNCE	CNSE
Oil content (% w/w)	26.8 ± 0.1[a]	27.5 ± 0.1[a]	40.0 ± 0.2[b]	41.2 ± 0.2[b]
Refractive index (40 ± 1 °C)	1.470 ± 0.005[a]	1.470 ± 0.005[a]	1.475 ± 0.003[b]	1.475 ± 0.003[b]
Acid value (mg KOH/g)	0.89 ± 0.01[a]	1.54 ± 0.1[b]	0.61 ± 0.01[c]	0.22 ± 0.01[c]
Peroxide value (mequiv O_2/kg oil)	1.0 ± 0.2[a]	0.9 ± 0.1[a]	0.8 ± 0.1[a]	1.0 ± 0.2[a]
Saponification value	185.7 ± 0.21[a]	184.5 ± 0.11[a]	182.6 ± 0.1[b]	181.4 ± 0.1[b]
Specific gravity (30 ± 1 °C)	0.816 ± 0.001[a]	0.816 ± 0.001[a]	0.818 ± 0.001[b]	0.818 ± 0.001[b]

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are presented. [a,b,c,d]Values with different superscript letters within a row indicate significant differences at $P > 0.05$

ASCE Annona squamosa cold extraction, *ASSE Annona squamosa* Soxhlet extraction, *CNCE Catunaregam nilotica* cold extraction, *CNSE Catunaregam nilotica* Soxhlet extraction

the two oils. Compared with the Codex standards [26] for cottonseed, sunflower, sesame and groundnut oils the *A. squamosa* and *C. nilotica* oils showed lower values for specific gravity, refractive index and saponification values.

Fatty Acid Composition

The fatty acid composition of *A. squamosa* and *C. nilotica* oils determined by GCMS is shown in Table 3. The major fatty acids in the two oil samples were palmitic (16:0), stearic (18:0), oleic (18:1n-9), and linoleic (18:2n-6) acids, the two oils were significantly ($P \leq 0.05$) different in their fatty acid composition. The extraction method did not affect the fatty acid composition of the two samples. In *A. squamosa* oils, C18:1 was the most dominant fatty acid; it was 49.2 and 50.5% in ASSE and ASCE, respectively, linoleic acid (C18:2) was the second most dominant fatty acid it was 22.3 and 22.7% in ASSE and ASCE, respectively. In *C. nilotica* oils, C18:2 was the most dominant fatty acid; it was 63.1 and 63.4% in CNCE and CNSE, respectively, followed by oleic acid which was found to 10.5 and 10.4% in CNCE and CNSE, respectively. Palmitic (C16:0) and stearic (C18:0) acids exhibited the third and fourth highest fatty acid contents in the four oils, palmitic acid was 15.6 and 15.2% in ASSE and ASCE, respectively and 9.7 and 9.8%, in CNCE and CNSE, respectively. While stearic acid was 10.6 and 9.3% in ASSE and ASCE, respectively, and 5.1 and 5.4%, in CNCE and CNSE, respectively. The striking feature of the four seed oils was the relative high level of polyunsaturated fatty acids (PUFA) which accounted for 71–74% of the total identified fatty acids, and the high linoleic acid content *C. nilotica* kernel oil makes it nutritionally valuable. The remaining fatty acids contributed only a few percentage points to the total fatty acid percent. Differences in the fatty acid composition of the seed oil may exist even within the same variety. For example, Ansari et al. [8] and Ahmad et al. [27] reported that the dominant fatty acids in the seed oil of *A. squamosa* Linn. were oleic (37.0%), palmitic (25.1%) and linoleic (10.9%). While Rafeeq et al. [9] reported 29.0% oleic, and 32.0% linoleic. The results of the three research groups differ significantly with our results with respect to the major fatty acids in the seed oil of *A. squamosa*.

Table 3 Fatty acid composition (% of total) of *Annona squamosa* and *Catunaregam nilotica* oils

Fatty acid	ASCE	ASSE	CNCE	CNSE
10:00	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	3.7 ± 0.4^{b}	4.1 ± 0.2^{b}
14:00	0.7 ± 0.2^{a}	0.6 ± 0.3^{a}	0.9 ± 0.2^{b}	0.8 ± 0.1^{b}
16:00	$I5.6 \pm 0.5^{a}$	15.2 ± 0.5^{a}	9.7 ± 0.4^{b}	9.8 ± 0.4^{b}
18:00	10.6 ± 0.4^{a}	9.3 ± 0.4^{a}	5.1 ± 0.2^{b}	5.4 ± 0.2^{b}
18:1n-9	49.2 ± 0.6^{a}	50.5 ± 1.2^{a}	10.5 ± 0.5^{b}	10.4 ± 0.7^{b}
18:2n-6	22.3 ± 0.5^{a}	22.7 ± 0.6^{a}	63.1 ± 0.7^{b}	63.4 ± 1.3^{b}
18:3D 9,12,15	ND	ND	0.4 ± 0.1^{a}	0.6 ± 0.1^{a}
20:00	1.3 ± 0.1^{a}	1.5 ± 0.1^{a}	5.9 ± 0.3^{b}	5.3 ± 0.2^{b}
22:1 n:13	ND	ND	0.7 ± 0.1^{a}	0.2 ± 0.1^{a}
Unsaturated FA (%)	71.5	73.2	74.3	74
Oleic/linoleic ratio	2.2	2.2	0.16	0.16

Each value is the mean ± SD of triplicate determinations. ND = not identified, ^{a,b}values with different superscript letters within a row indicate significant differences at $P > 0.05$

Tocopherols

The tocopherol content of freshly extracted oils of *A. squamosa* and *C. nilotica* is shown in Table 4. Among the tocopherols identified, alpha-tocopherol was 4.9, 4.4, 31.6 and 28.5 mg/100 g in ASCE, ASSE, CNCE and CNSE, respectively, and delta-tocopherol was 11.7, 11.0, 8.4, and 10.5, respectively. Beta and gamma-tocopherols were found only in *C. nilotica* oil and beta-tocopherol was abundant amounting to 65.7 and 63.8 mg/100 g in oil extracted using cold extraction and Soxhlet extraction methods. The total tocopherol amount was significantly different in *A. squamosa* and *C. nilotica* oils, and that method of extraction had no significant effect on theamount of tocopherol. *Catunaregam nilotica* oils (CNCE and

Table 4 Tocopherol content (mg/100 g) of *Annona squamosa* and *Catunaregam nilotica* oils

Sample	α-T	β-T	T	T	Total
ASCE	4.9 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	11.7 ± 0.3^{a}	16.6 ± 0.4^{a}
ASSE	4.4 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	11.0 ± 0.3^{a}	15.5 ± 0.3^{b}
CNCE	31.6 ± 0.5^{b}	65.7 ± 0.6^{b}	4.7 ± 0.2^{b}	8.4 ± 0.3^{b}	110.5 ± 0.6^{c}
CNSE	28.5 ± 0.4^{c}	63.8 ± 0.6^{c}	5.1 ± 0.2^{c}	10.5 ± 0.3^{c}	107.9 ± 0.6^{d}

All determinations were carried out in triplicate and mean values ± standard deviations (SD) arer given. ^{a,b,c,d}Values with different superscript letters within columns indicate significant differences at $P > 0.05$

ASCE Annona squamosa cold extraction, *ASSE Annona squamosa* Soxhlet extraction. *CNCE Catunaregam nilotica* cold extraction, *CNSE Catunaregam nilotica* Soxhlet extraction

Table 5 Amino acid composition of *Annona squamosa* and *Catunaregam nilotica* seed compared with egg, sesame and bean (g per 100 g protein)

Amino acid	*A. squamosa*	*C. nilotica*	Egg[a]	Sesame[a]	Broad Bean[a]
Threonine	0.324 ± 0.1	0.738 ± 0.4	0.634 ± 0.3	0.763 ± 0.3	0.159 ± 0.1
Methionine + cystine	0.106 ± 0.1	0.206 ± 0.1	0.717 ± 0.5	0.988 ± 0.6	0.071 ± 0.1
Valine	0.642 ± 0.2	1.031 ± 0.5	0.174 ± 0.1	0.985 ± 0.6	0.374 ± 0.2
Isoleucine	0.464 ± 0.2	0.822 ± 0.5	0.778 ± 0.5	0.773 ± 0.5	0.222 ± 0.1
Leucine	0.845 ± 0.3	1.608 ± 0.6	1.091 ± 0.4	1.433 ± 0.4	0.389 ± 0.1
Phenylalanine + tyrosine	0.671 ± 0.2	1.311 ± 0.6	1.224 ± 0.5	1.614 ± 0.5	0.368 ± 0.1
Histidine	0.139 ± 0.1	0.695 ± 0.6	0.301 ± 0.1	0.523 ± 0.2	0.126 ± 0.1
Lysine	0.407 ± 0.2	0.823 ± 0.4	0.863 ± 0.5	0.585 ± 0.3	0.338 ± 0.1
Arginine	0.704 ± 0.4	1.085 ± 0.6	0.754 ± 0.6	2.586 ± 1.0	0.760 ± 0.5
Total EAA	4.302	8.319	6.536	10.25	2.807
Aspartic acid	0.684 ± 0.3	1.464 ± 0.7	0.892 ± 0.6	0.094 ± 0.1	0.659 ± 0.2
Glutamic acid	0.995 ± 0.5	1.876 ± 0.6	0.121 ± 0.1	0.366 ± 0.1	0.781 ± 0.3
Serine	0.299 ± 0.1	0.655 ± 0.4	0.672	0.072 ± 0.1	0.240 ± 0.1
Glycine	0.392 ± 0.1	0.834 ± 0.1	0.302 ± 0.1	0.054 ± 0.1	0.201 ± 0.1
Alanine	0.594 ± 0.2	1.058 ± 0.7	0.503 ± 0.2	0.140 ± 0.1	0.255 ± 0.1
Total NEAA	2.964	5.883	2.49	1.391	2.136
Total amino acids	7.266	14.202	9.026	11.641	4.943

All determinations were carried out in triplicate and mean values ± standard deviations (SD) are reported

[a] Source: Paul A.A and Southgate DAT (1979) In: McCance RA, Widdowson EM (eds) The composition of foods, 4th edn. The Foods Standard Agency (London), Royal Society of Chemistry, Cambridge

CNSE) showed higher amounts of tocopherols 110.5, 107.9 mg/100 g, respectively, compared to other common oils such as sesame (33–101), groundnut (17–130) and sunflower (44–152 mg/100 g) oils [26]. The main tocopherol of the *A. Squamosa* oils was delta-tocopherol, which constituted more than 70% of the total tocopherols. In the case of *C. nilotica* oils β-tocopherol was the predominant one constituting more than 59%. The other tocopherols in the oil of the two samples were below 1 mg/100 g each.

Amino Acid Profile

The amino acids profile of *A. squamosa* and *C. nilotica* seed, analyzed by amino acid hydrolysis is presented in Table 5. Values are given for 16 different amino acids, and sums for essential amino acids (EAA) and non-essential amino acids (NEAA). However, another essential amino acid tryptophan, which acts as a precursor of the vitamin niacin and the neurotransmitter serotonin, cannot be determined because it is very labile in an acid environment and is completely destroyed by acid hydrolysis (HCl 6 N) [28]. To determine tryptophan, base hydrolysis or enzymatic hydrolysis should be used [29]. The total amount of the essential amino acids (phenylalanine, leucine, valine, threonine, isoleucine, methionine, tyrosine, histidine, arginine, cystine and lysine) found in *A. squamosa* and *C. nilotica* seeds were 4.302 and 8.319 g/100 proteins, respectively, and differed significantly. The percentage of sulfur-containing amino acids (methionine and cystine) in *A. squamosa* seed was 0.106, while in *C. nilotica* seed was 0.206/100 g, which was the lowest among the others. The percentage of aromatic amino acids (phenylalanine + tyrosine) was 0.671 and 1.311/100 g protein in *A. squamosa* and *C. nilotica*, respectively.

Annona squamosa and *Catunaregam nilotica* seeds when compared with egg, sesame and broad bean showed a great difference in their amino acids contents, because those are known as a source of protein [30]. All the essential amino acids with the exception of tryptophan which was not analyzed were found to be present in high amounts in *C. nilotica* seed, when compared to that of three different foods in Table 5. The total amount of the essential amino acids of *A. squamosa* seeds comprised about 59.2% of the total amino acids and that of *C. nilotica* seeds was 58.6%. The individual essential amino acids of *C. nilotica* seeds were higher somewhat than that of egg and broad bean but egg was higher in methionine + cystine and isoleucine amino acids.

Conclusions

The seed kernels of *A. squamosa* and *C. nilotica* collected in the Sudan have a high potential from an economic aspect

because of their high protein and oil content. The oils obtained from the two samples were significantly ($P \leq 0.05$) different in their physicochemical properties, fatty acid and tocopherol composition, the major fatty acids in the two oil samples were oleic, linoleic, palmitic and, stearic acids. Delta-tocopherol was the predominant tocopherol in *A. Squamosa* oil, while beta-tocopherol was the predominant tocopherol in *C. nilotica* kernel oil. The extraction method had nor affect on the fatty acid, nor on the tocopherol composition of the two samples. The amino acid profile of the defatted seed kernels as analyzed by amino acid analyzer indicated that the total amount of amino acids of *A. squamosa* was 2.964/100 g protein and that of *C. nilotica* was 5.883 g/100 g protein. The essential amino acids represent 59.2 and 58.6% of the total amino acids in *A. squamosa* and *C. nilotica* seeds, respectively.

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