

GC-MS Bioactive Compounds Characterization and Antioxidant Activities of Defatted and Un-Defatted Cashew (*Anacardium Occidentale L.*) Kernel Flour

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Abstract

This study investigates the bioactive compounds of defatted and un-defatted cashew kernel flour. Kernel was removed from the nuts, cleaned, dried and milled into flour. Kernel flour was defatted using n-hexane and petroleum ether. Samples A (Un-defatted cashew kernel flour), Samples B (Defatted cashew kernel flour using n-hexane for cold pressed extraction) and Samples C (Defatted cashew kernel flour using petroleum ether for hot pressed extraction). The defatted and un-defatted cashew kernel flour were analyzed for ferric reducing anti-oxidant property (FRAP), total phenol, α -amylase, α -glucosidase, 1,1-diphenyl-2 picrylhydrazyl (DPPH) and iron II chelation. Gas chromatography-mass spectrometry (GC-MS) was used to determine the bioactive compounds in the samples. The result of the analysis showed that FRAP content ranged from 5.89 to 20.00 mg AEE/g, total phenol ranged from 24.2 to 64.4 mg GAE/g, α -amylase from 56.54 to 68.57µg/ml, α -glucosidase ranged from 53.8 to 73.00 µg/ml, the inhibitory concentration (IC₅₀) of iron (Fe²⁺) chelation ranged from 0.042 to 0.049 mg/mL and IC₅₀ of DPPH ranged from 0.119 to 0.148 mg/mL. The total number of bioactive compounds observed in sample A, B and C were 42, 36 and 33 respectively. The result has shown that the presence of bioactive compounds in cashew kernel flour suggests its vast pharmacological and medicinal potentials hence; this study may be useful to explore the pharmacological and therapeutic activity.

Keywords: Cashew Nuts; Cashew Kernel; Antioxidant Properties; Bioactive Compounds; GC-MS

Introduction

Bioactive compounds are usually derived from plants these includes alkaloids, terpenoids, flavonoids, nitrogen-containing compounds, phenolics [1]. These has been researched on to be antioxidant, anti-inflammatory, immunostimulatory, anticancer, antimicrobial in nature thereby its consumption prevents the occurrence of degenerative diseases such as cardiovascular diseases, hypertension, diabetics etc. and it helps to promote a healthy life style [2].

Antioxidants are compounds that delay or neutralize free radicals in the body system. They offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and by many other mechanisms to prevent disease progression [3]. They are widely used as food additives to provide protection against oxidative degradation of foods by free radicals [4] and could also combat oxidative stress in human [3]. Antioxidants can be classified as either synthetic or natural antioxidant. Natural sources of antioxidant are found in fruits and vegetables with the highest percentage of antioxidant concentrated in the kernel of nuts, peels of fruit, juice of fruits and vegetables [5-7].

Nuts are dry one seeded fruit of tree or shrubs consisting of an edible kernel in a hard shell that protects the embryo from the surrounding environments [7]. A nut is a specific type of seed that has an external wall and does not open to release its seed at maturity. Nuts are oily and crunchy; it played an important role in diets of many cultures due to its high energy and nutritional value as well as its huge variety of flavors and unique taste [8]. Its consumption had been linked with several health benefits during the last decades due to its particular nutritional composition. Nuts are known to contain a high content of unsaturated fatty acids (USFA), vitamins, minerals, amino acids, phytosterols and fiber. Consumption of nuts or its incorporation into a healthy diet is associated with a reduced risk of life threaten diseases such as cardiovascular disease [8]. It also decreased the risk of metabolic syndrome [9], diabetes and stroke. Furthermore, researchers have reported that nuts had been found to improve mental health [10], increase bone mineral density and decrease the risk of depression [11]. Edible nuts include Cashew nut, Ground nut, Almond nuts, Chinese nut, Conophour nut etc.

Cashew (*Anacardium occidentale* L.), belongs to the Anacardiaceae family and is an evergreen tree, native to northeast region of Brazil which expanded spontaneously to American, India, Africa and Asia. Cashew trees produces a soft, shiny, and juicy fruit known as cashew apple which bears a single-seeded nut in its bottom covered with a hard gray shell [7]. Cashew tree (*Anacardium occidentale*) is a tropical evergreen tree native to South America in the genus *Anacardium* that produces the cashew seed and the cashew apple accessory fruit [12]. Major cashew producing region in the world are West Africa, South East Asia and Africa. In Africa, Nigeria is one of the major countries producing cashew, cashew nut is gaining it important in Nigeria as an export-oriented cash crop since the last three decades; it has become an important source of income to the country because they earned about \$250 million from cashew export in the year 2022 [13]. It is a commercial crop cultivated in about twenty-seven (27) states of the country including Federal Capital Territory [13]. Cashew is widely cultivated in Nigeria especially, the Igala part of Kogi State Nigeria. The fruit of this tree is known as Cashew fruit. The fruit has an oval or pear-like shaped that developed from the pedicel and the receptacle part of the cashew flower. The fruit consist of the cashew apple and the cashew seed is commonly considered a snack nut (cashew nut) eaten on its own, used for food recipes, or processed into cashew cheese or cashew butter [14-15]. National Cashew Association of Nigeria (NCAN) regulates cashew trade in Nigeria. Thus, agents licensed by NCAN help Nigerian cashew farmers profitable. A lot of these agents use their in-depth knowledge of market trends to ensure deals between cashew nut growers in Nigeria and international buyers benefit parties involved.

Cashew nut is one of the most important products of cashew tree, it has a heart – like shape and it is widely grown in the tropical countries of the world and it is one of the most important edible nuts in the international trade [7]. Its industry was ranked third in the world production of edible nuts with world production of 3,971,046 tonnes in 2017. West Africa provides nearly half (49%) of the world total production of cashew nuts [16]. Cashew nut is often used in baking and confectioneries because it an excellent source of minerals (such as copper, phosphorus, magnesium, manganese and zinc), rich source of vitamins (such as pantothenic

acid, pyridoxine, riboflavin and thiamin). Also, it has about high (82%) unsaturated fatty acid which makes it good for health and most especially for the wellbeing of human heart. Though, cashew is known to have some anti – nutritional factors and components (such as tannin and oxalates) which can affect it utilization. However, methods used for its processing help to reduce the percentage concentration of these anti-nutrients to minimal level that makes it safe for human consumption. Researchers have worked tremendously on cashew kernel; [17] reported on the nutritional composition of cashew kernel flour; [18] worked on phenolic lipids, saturated and unsaturated fatty acid of cashew kernel while [12] gave a report on some identified bioactive compounds in cashew kernel but there are limited information on the defatted and un-defatted cashew kernel flour hence this study will be carried out in order to determine the identify and characterize the bioactive and antioxidant capacities of defatted and undefatted cashew nut (*Anacardium occidentale L*) kernel flour.

Materials and Methodology

Source of Materials

Cashew nuts were obtained from Omachalla Commodities Limited Egume, Kogi State, Nigeria. Reagent used (n-hexane and petroleum ether) for defatting process were of analytical grade and purchased from a renowned chemical store in Enugu State, Nigeria.

Sample Preparation

Preparation of Un-Deffated (Raw) Cashew Kernel Flour

The processing of cashew nut into cashew kernel flour was carried out as described by [19] with slight modifications as shown in Figure 1. Cashew nut was sorted, (to remove visual defects, insects or damaged seeds, extraneous materials etc.), it was cut into halves using the manual cashew kernel cutter to separate the nuts from the kernels. After cutting; the kernels were pulled out and dried in green house for 72 h. The covering testa were removed by squeezing and then winnowed to obtain cream colour kernel. The kernel was then broken into smaller pieces with the use of mortar and pestle, the chopped kernels were milled into fine flour using Kenwood blender (model: BLP41.A0WH) and kept in an air tight polytene bag for further analysis

Preparation of Defatted Cashew Kernel Flour

Cashew kernel flour was deffated as shown in Figure 3.1. Cashew flour obtained above was defatted using two different solvents i.e., the n-hexane (cold pressed) using muslin cloth and petroleum ether (hot pressed) using Soxhlet apparatus. The defatted cashew kernel flour was then dried in green house for 12 h for the complete removal of the solvent. The defatted cashew kernel flour was screened through a sieve with mesh size 0.5 mm and kept in an air tight polytene bag for further analysis. The pictures of the samples are shown in plate 1.



Cashew nuts



Plate 1: Pictures of the cashew nuts, cashew kernel nuts, defatted and un-defatted cashew kernel flour

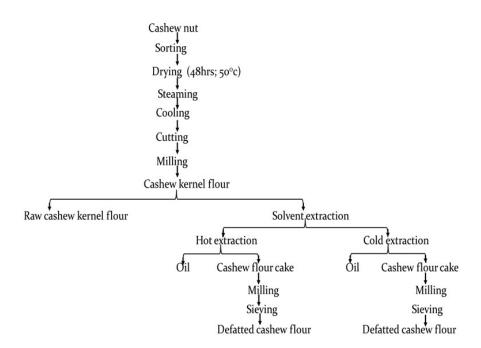


Figure 3.1: Processing flow chart of Cashew kernel flour Source: [19]

Identification of Bioactive Compounds in Defatted and Un-Defatted Cashew Kernel Nut Flour Using Gas Chromatography-Mass Spectrometry (GC-MS) The bioactive profiles of the defatted and un-defatted cashew kernel nut flour extracts were determined by Gas Chromatography -Mass Spectrometry (GC-MS) model QP210 plus Shimadzu, Japan. The procedure for the analysis included the following details. Column temperature was set at 80°C, injection temperature at 250°C, pressure at 108.0 kPa, total flow was at 6.2 ml/min and linear velocity at 46.3 cm/s. The start time was 3.00 min and end time was 28.00 min. The compounds were identified using molecular weight and formula of the compounds and the retention time. Compound identification was obtained by comparing these values and the spectral data with those of authentic compounds from the library data of the corresponding compounds using automated Shimadzu software.

Some Antioxidant Properties of Defatted and Un-Defatted Cashew Kernel Nut Flour

Determination of Total Phenol Content

The total phenol content of the defatted and un-defatted cashew kernel nut flour was determined as reported by [20] and its content was calculated and recorded as mg Gallic Acid Equivalent (mg GAE/g).

Determination of Total Flavonoid Content

The total flavonoid content of the defatted and un-defatted cashew kernel nut flour was determined using the method described by [20], and its content was calculated and recorded as mg Quercetin Equivalents per gram (mg QE/g).

Determination of Ferric Reducing Antioxidant Property (FRAP)

FRAP of the defatted and un-defatted cashew kernel nut flour extract was determined by assessing the ability to reduce the FeCl3 solution as described by Oyaizu [21]. About 0.25 ml of the extract was mixed with 2.5 ml of 200 mM of Sodium phosphate buffer at pH 6.6 and 2.5 ml of 1% potassium-ferricyanide (KFC). The mixture was incubated at 50°C for 20 min, thereafter 2.5 ml of 10% trichloroacetic acid (TCA) was also added and centrifuge at 2,000 rpm for 10 min, 5 ml of the supernatant was mixed with equal volume of water (5 ml) of distilled water, and 1 ml of 0.1% of ferric chloride (FeCl₃). The absorbance was measured using a spectrophotometer at 700 nm and ferric reducing antioxidant property was subsequently calculated and recorded as mg Ascorbic Acid Equivalent per gram (mg AEE/g).

Determination of 1, 1-Diphenyl-2 Picrylhydrazyl (DPPH) Free Radical Scavenging Ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by [22]. Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Determination of Fe²⁺ Chelation Assay

The Fe²⁺ chelating ability of both extracts were determined using a modified method of [23]. Freshly prepared 500 μ M FeSO₄ (150 μ l) was added to a reaction mixture containing 168 μ l 0.1 M Tris–HCl at pH 7.4, 218 μ l saline and the extracts (0–25 μ l). The reaction mixture was incubated for 15 min at 37 °C, thereafter, 13 μ l of 0.25 % Otho-phenanthroline (w/v) was added. The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating ability was subsequently calculated.

Statistical Analysis

Data were obtained in triplicate; one-way analysis of variance (ANOVA) was used to analyze the result using Statistical Package for Social Sciences (SPSS) version 21. Means were separated using Duncan's new multiple range test (DNMRT). Statistical signifi-

cance was accepted at $p \le 0.05$.

Results and Discussions

Antioxidant Properties of Deffated and Un-Defatted Cashew Kernel Flour

The Antioxidant properties of deffated and un-defatted cashew kernel flour presented in Table 1. The Antioxidant properties of defatted and un-defatted cashew kernel flour were presented in Table 1. FRAP content of defatted and un-defatted cashew kernel flour ranged from 5.89 to 20.01 mg AEE/g. The lowest value of FRAP was observed in sample B (cold pressed defatted cashew kernel flour) with 5.89 mg AEE/g while sample A (un-defatted cashew kernel flour) had the highest value of FRAP with 20.01 mg AEE/g. Total phenol content of defatted and un-defatted cashew kernel flour) with 24.2 to 63.4 mg GAE/g, the lowest value of total phenol was observed in sample C (hot pressed defatted cashew kernel flour) with 24.2 mg GAE/g while sample A had the highest value of total phenol with 63 mg GAE/g. Total flavanol content of defatted and un-defatted cashew kernel flour) with 11.9 to 27.1mg QE/g, the lowest value of total flavanol content was observed in sample C (hot pressed defatted cashew kernel flour) with 11.9 mg QE/g while sample A (un-defatted cashew kernel flour) had the highest Total flavanol with 27.1 mg QE/g. Flavonoids have been reported to possess antioxidant, antimicrobial, anticarcinogenic, antitumor, anti-inflammatory allergenic, and antidiarrheal properties [24-25]. Phenols are important indicator of antioxidant in food, it is a natural source of antioxidant in functional foods [26]. Phenols have been reported to possess antioxidant, antibacterial and antifungal activity [27]. All these facts may be attributed to the biological activities of cashew plant. Antioxidants are capable of scavenging free radicals, chelate metals catalysts, reduce α-tocopherol radicals, activate antioxidant enzymes and inhibit oxidases [28].

Sample	FRAP (mg AEE/g)	Total Phenol(mg GAE/g)	Total Flavanol(mg QE/g)
А	20.00 [±] 1.2	63.4 [°] ±1.0	27.1 [°] ±2.0
В	5.89 [°] ±1.0	29.1 [°] ±0.3	14.4 [°] ±1.2
С	14.02 ^b 1±0.4	24.2 [°] ±1.1	11.9 [°] ±0.1

Table 1: Antioxidant's properties of defatted and un-defatted cashew kernel flour

Means in the same column not followed by the same letter are significantly different at 5% level of significance.

Sample A-Un-defatted cashew kernel flour Sample B-Defatted cashew kernel using cold pressed extraction Sample C- Defatted cashew kernel using hot pressed extraction

FRAP- Ferric Reducing Antioxidant Power

The *Invitro* A-Amylase Inhibitory and A-Glucosidase Inhibitory Activities of Defatted and Un-Defatted Cashew Kernel Flour

The *Invitro* α -amylase inhibitory and α -glucosidase inhibitory activities of defatted and un-defatted cashew kernel flour is shown in Figure 2. The result revealed that the extracts of defatted and un-defatted cashew kernel flour inhibited α -amylase activity dosedependently (56.54 to 68.57 ug/mL). The lowest value of α - se was observed in sample C (hot pressed defatted cashew kernel flour) with 56.54 ug/mL while sample A (un-defatted cashew kernel flour) had significantly (P < 0.05) highest α -amylase inhibitory property value of 68.57 ug/mL. α - glucosidase content of defatted and un-defatted cashew kernel flour ranged from 53.87 to 73.18 ug/mL. The lowest alpha Amylase was observed in sample C (hot pressed defatted cashew kernel flour) with 53.87 ug/mL while sample A had the highest Alpha glucosidase with 73.18 ug/mL.

Inhibition of enzymes involved in the hydrolysis of carbohydrates such as a-amylase and a-glucosidase has been exploited as a ther-

apeutic approach for controlling postprandial hyperglycemia [29]. Pancreatic α -amylase is involved in the breakdown of starch into disaccharides and oligosaccharides before intestinal a-glucosidase catalyzes the breakdown of disaccharides to liberate glucose which is later absorbed into the blood circulation. Inhibition of these enzymes would slow down the breakdown of starch in the gastro-intestinal tract, thus reducing postprandial hyperglycemia [30]. This trend of the a-amylase inhibitory property of the extracts defatted and un-defatted cashew kernel flour agreed with their phenol content Table 1.

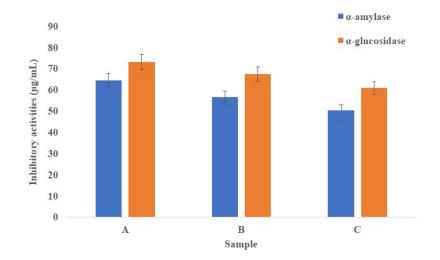


Figure 1: Invitro α-amylase inhibitory and α-glucosidase inhibitory activities of defatted and un-defatted cashew kernel flour

Sample A-Un-defatted cashew kernel flour Sample B-Defatted cashew kernel using cold pressed extraction Sample C-Defatted cashew kernel using hot pressed extraction

In order to determine the effectiveness of each extract, the IC_{50} of defatted and un-defatted cashew kernel flour was determined and the results were presented in Table 2. The IC_{50} DPPH ranged from 119.14 to161.66 µg/g, the lowest value of IC_{50} was observed in Sample C (hot pressed cashew kernel flour) with 119.14 mg/mL while sample B (cold pressed cashew kernel flour) has the highest IC_{50} value of 161.66 µg/g. The iron chelation content of defatted and un-defatted cashew kernel flour ranged from 41.99 to 49.24 µg /mL the lowest value of iron chelation was observed in sample C (hot pressed defatted cashew kernel flour) with 41.99 µg /mL while sample A (un-defatted cashew kernel flour) has the highest iron chelation with 49.24 µg /mL.

 IC_{50} implies the concentration of the sample required to obtain a 50% antioxidant effect [31]. It is a typically used parameter to express the antioxidant capacity and to compare the activity of different compounds. A lower value of IC_{50} corresponds to a higher antioxidant activity of the sample.

Sample	DPPH(µg/g)	Fe [°] Chelation(µg/mL)
А	119.14	49.24
В	161.66	46.23
С	148.46	42.99

Table 2: The Inhibitory concentration (Ic50) of defatted and un-defatted cashew kernel flour

Sample A-Un-defatted cashew kernel flour

Sample B-Defatted cashew kernel using cold pressed extraction

Sample C-Defatted cashew kernel using hot pressed extraction

Fe²⁺ - Iron chelation

DPPH - 2, 2-diphenyl-1-picrylhydrazyl.

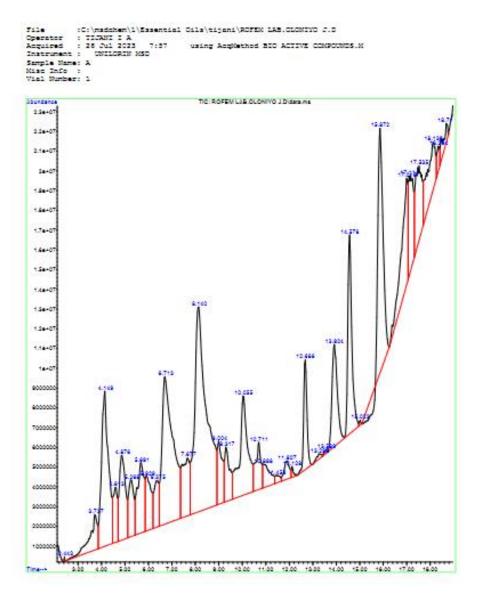


Figure 2: Gas Chromatography-Mass Spectrometry (GC-MS) Sample a (Un-defatted cashew kernel flour)

S/N	RT	Names of Bioactive Compounds	CAS #
1.	2.792	3-Oxabicyclo [3.3.0] octane-2,7-dione,4-methoxy	1000155-61-7
2.	2.961	9-Amino-7-Mercapto-5, 6, 8, 10-tetraaza-benzo [b] fluoren-II-one	100274-60-9
3.	3.074	Benzene, 2-azindo-1-methyl-3-nitro-eta	016714-18-4
4.	3.299	Cyclopentassiloxane, decaethyl-Benzoic acid	000541-02-6
5.	3.524	Piperanzin-1, 4-diiiumi, 4 di(2-chloro-ethyl1)-1,4-diethyl- dichloride.oxalychloride	1000273-25-6
6.	4.257	Silane, dimethyl-fumaric aid, di(2-methoxyethyl)ether	001112-39-6
7.	4.623	l-Methioninol 1,5-Hepadyne	002899-37-8
8.	4.933	1H-pyrrole, 1-methyl-1H-pyrrole	000096-54-8

Table 3: Bioactive compounds composition of undefatted cashew kernel flour

9.	5.637	Furfural	000098-01-1
10.	6.088	1-Methyl-2-ethyl pyrazolium bromid 7-Azabicyclo [4.1.0] heptane	1000144-05-7
11.	6.285	Azacycloride can-2-one 6, 8-dioxa-3-thibicyclo (3,2,1)octane	000947-04-6
12.	6.538	2-furancarboxaldehyde	000620-02-0
13.	6.848	Cyclopentasilohexane, decamethyl 3,4 –dihydroxybenzyl alcohol	000541-02-6
14.	7.581	Isobutyraldehyde, propylhydrazone pentyl acetoacetate	020607-78-7
15.	8.144	16-Hydroxyhexadecanoic acid	000506-13-8
16.	8.257	Galacto-heptulose	1000130-14-5
17.	8.398	4H-pyran-4-one, 2, methoxy-6-phenyl ethamine	004225-43-8
18.	8.567	Trans-1-methoxy-3-methy/l-1-butene	000067-47-0
19.	9.102	L 5-Hydroxymethlfurfural thipheae	1000139-44-0
20.	9.271	Acetic acid	000079-11-8
21.	9.581	2,6-Difluoroaniline	005509-65-9
22.	9.947	Octadecanoic acid	000367-30-6
23.	10.088	Dodecanoic acid, methyl ester Decanoic acid	000111-82-0
24.	10.229	Methyl-6-0-[methylpropyl]-beta-d-galactopyranooside	1000125-15-17
25.	10.623	Cyclopropaneoctanal,2-octyl-oleic acid	056196-06-6
26.	10.792	4-chloropyridine	000626-61-9
27.	11.046	Octadecanoic acid	000057-11-7
28.	11.158	Dodecanoic acid	000143-07-7
29.	11.581	Octadecane, 1-(ethenyloxy)-9	00930-02-9
30.	11.75	Spiro [2,3] hexane-5-carboxylic acid	1000152-25-7
31.	11.975	Methyl-2-0-ethyl.alpha d-glucopyranoside	015064-82-1
32.	12.37	n-Hexadecanoic acid	000057-10-3
33.	12.736	d-erythro-pentose	000533-67-5
34.	12.877	d-erythro-pentose, 2 deoxy-2H-1,2,3-Triazol-4-amine	000533-67-5
35.	13.102	Hydrazine, N-(3,5-dibromobenzylidene)-N-[(ethylyhio)amino methylidene	1000189-65-1
36.	14.032	Hexadecanoic acid, methyl ester	000112-39-0
37.	14.37	2-6, 17-octadecadien-1-oi-acetate	086252-73-5
38.	14.623	Fumeric acid, 2-heptyl tridecyl ester	1000348-63-0
39.	15.271	n-Hexadecanoic acid	000057-10-3
40.	16.004	9-octadecanoic acid (2)-, ethylester	000112-62-9
41.	17.215	Oleic acid	000112-80-1
42.	17.891	Hexadecanal, 2-methyl	055019-46-0

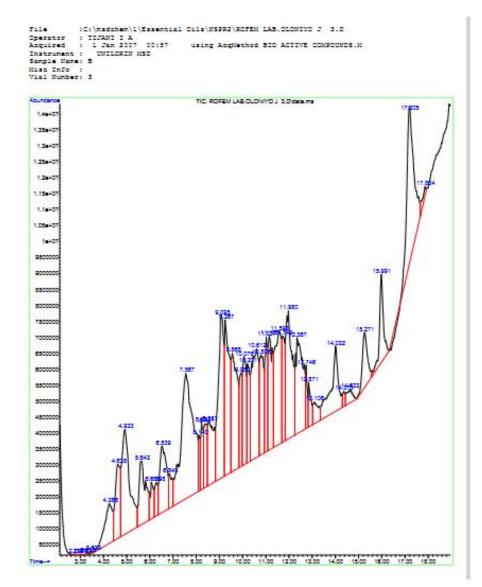


Figure 3: Gas Chromatography-Mass Spectrometry (GC-MS) Sample B (Cold pressed defatted cashew kernel flour)

Table 4: Bioactive compo	ounds of composition	of cold pressed defatted c	ashew kernel flour
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S/N	RT	Names of Bioactive Compounds	CAS #
1.	2.736	3-pyridinecarboxylic acid,1,2-dihydro-2-oxo-	0000609-71-3
2.	3.102	N-Ethyl-2-thiophen-2-ylthioacetamide	1000311-85-1
3.	3.524	Benzene,1-[(2-chloroethyl]sulfonyl)]-4-nitro	006461-63-8
4.	4.313	ТАІР	017088-37-8
5.	4.675	Methanamine, N-methoxy	001117-97-1
6.	4.933		000096-54-8
		, 1-methyl	

7.	5.665	Furfural	000098-01-1
8.	6.708	Methyl 5-piperidino-4-ketocaproate	018904-36-4
9.			
).	7.271	Pyrazole-5-carboxylic acid	1000261-73-1
10.	7.609	Propanoic acid, 2,2-dimethyl-2-ethyl-hexayl ester	016387-17-1
11.	8.567	Hydrazinecarbothiocimide, 2-[1(4-nitrophenyl)ethylidene]	005424-45-3
12.	8.764	Benzaldehyde, 4-brow	001122-91-4
13.	9.764	5-Hydroxymethlfurfural	000067-47-0
14.	9.637	Chloromethane	000074-87-3
15.	10.06	Dodecanoic acid,methl es	000111-82-0
16.	10.454	Morpholine,4-acetyl	001696-20-4
17.	10.539	N-(S-methyl-3-isoxazoly)phthalimide	091377-59-2
18.	10.792	Trichloroacetic acid,3-methyl but-2-enyl ester	1000299-25-5
19.	10.99	Acetic acid,chorol	000079-11-81
20.	11.215	Dodecanoic acid	000143-07-7
21.		2,3-dimethyl-3-heptene	
22.	11.919	Methyl tetradecanoate	000124-10-7
23.	12.342	n-hexadecanoic acid	000057-10-3
24.	12-348	6H-furo{3,2:4,5}imidazo{1,2-g}purine-8-methanol,4-amino-6a,7,8,9a-tetrehydro-7-hydroxyl-{6aS-(6a alpha,7,alpha,8beta,9a alpha)}	033962-27-5
25.	12.623	3-methyl-3-hexane	003404-65-7
26.	13.215	9-octadecenoic acid,(E)	000112-79-8
27.	13.328	Nickel,{(1,2-eta)-1,3-butadiyne}	113726-07-1
		{1,3-propanediybis{bis(L-methylethyl)prosphine}-p,p}	
28.	14.004	Hexadecanoic acid;methyl ester	000112-39-0
29.	14.398	(E,Z)-N-(2,7-Dimethyl-2,7-octadien-1-amine	077984-60-2

30.	14.652	3-(E)-Hexen-2-one,(5S)-5-(ct-butoxycarbonxy –(R)-alanyl)amino acid	1000164-14-6
31.	14.736	3-chloro-N-(2-methyl-4(3H)-oxo-3-quinazolinyl)-2thianaphenecarboxamide	304882-71-1
32.	15.271	n-Hexadecenoic acid	000057-10-3
33.	15.976	12- octadecenoic acid, methyl ester	056554-46-2
34.	17.215	9-octadecenoic acid (z)-,2hydroxylethyl ester	004500-01-0
35.	17.835	S-(2-(N,N-Dimethylamino)ethyl)N,N-dimethylcarbamoyl thiocarbohybroximate	1000212-14-4
36.	18.342	Oleoyl chloride	000112-77-6

Table 5: Bioactive Compounds Composition of Hot – pressed Defatted Cashew Kernel Flour

S/N	RT	Names Of Bioactive Compounds	CAS #
1.	2.454	1,3-Dioxocane,5,5,6,6,7,7, -hexafluoro	036301-46-9
2.	3.75	Ethylamine, N-methyl-N-hexadecyl	066997-40-8
3.	4.144	Propanoic acid	0000540-73-8
4.	4.623	Glycine, N-methyl-N-methoxycarbonyl, 1-, hexadecyl ester	1000320-61-5
5.	4.877	Furfural	000098-01-1
6.	5.271	Lexoglucosenone	037112-31-5
7.	5.693	Furancarboxaldehyde, 5-methyl	000620-02-0
8.	5.919	2-Furanone, 2,5-dihydro-3, 5-dimethyl	1000196-88-1
9.	6.37	2-cyclopenten-1-one, 2-hydroxy-3-ethyl	000080-71-7
10.	6.708	Isobutyraldyhde, Propylhydrazone	020607-78-7
11.	7.665	Cycloheptasiloxane, tetradecamethyl	000107-50-6
12.	8.144	5-Hydroxymethyl furfural	000067-47-0
13.	9.018	Tridecanoic acid, methyl ester	001731-88-0
14.	9.327	Cycloactasiloxane, hexadecane-ethyl	000556-68-3
15.	10.06	Diethyl phthalate	000084-66-2
16.	10.708	Methyl tetradecanoate	000124-10-7
17.	10.905	2-chloro-3-[4-chloro-2-(chloro-ethyl)-1, 3-oxazol-5-yl]-1H-indol-1-carboxylic acid, 2,2,2-trichloroethyl ester	350816-73-4
18.	11.468	L-valine, N-(4 ethylbenzoyl)-1 undecyl ester	1000346-64-4
19.	11.919	Tetradecanoic acid	000544-63-8
20.	12.116	Trisiloxane,1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]	003555-47-3
21.	12.68	Haxadecanoic acid, ethyl ester	0001112-39-0
22.	13.299	1,2,3-propanetriol, 1-acetate	000106-61-6
23.	13.581	Phthalic acid, 2,7-dimethyloct-7-en-5-yn-4-yl isohexyl ester	1000315-49-8
24.	13.919	n-Hexadecanoic acid	000057-10-3

25.	14.567	9-octadecenoic acid (2) -, methyl ester	000112-62-9
26.	15.018	Cyclooctasiloxane, haxadecamethyl	000556-68-3
27.	15.863	Cis-Vaccenic acid	000506-17-2
28.	17.018	Dodecanoic acid, 1,2,3-propanetriyl ester	000538-24-9
29.	17.102	Colchiceine, N-deaceto-N-TFA-2-deethyl-2-acety	1000126-10-6
30.	17.525	1,4-Benzenediamine	000106-50-3
31.	18.145	Diltiazem	042399-41-7
32.	18.37	3-[7-Bromo-5-(2-chloro-phenyl)-2-0	1000318-52-1
33.	18.708	1-Nonene, 2-ethyl-3-(methoxymethoxy)	1000197-21-7

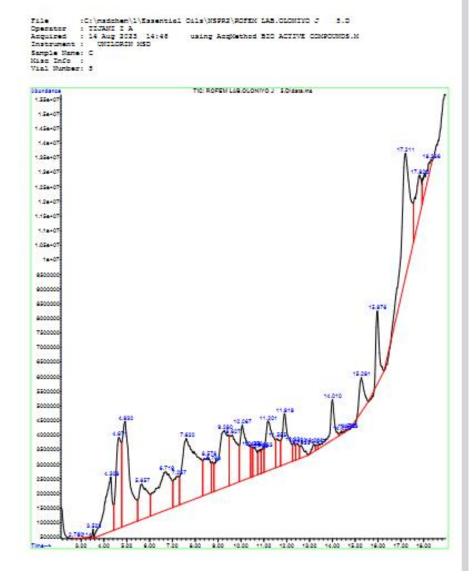


Figure 4: Gas Chromatography-Mass Spectrometry (GC-MS) Sample C (Hot pressed defatted cashew kernel flour)

The GC -MS chromatogram of sample A (un-defatted cashew kernel flour), sample B (cold pressed deffated cashew kernel flour) and sample C (hot-pressed defatted cashew kernel flour) were shown in figure 2, 3 and 4 respectively with distinct peaks. The names of the identified bioactive compounds by GC-MS were presented in Table 3, 4 and 5 for Sample A (un-defatted cashew kernel flour), sample B (cold pressed deffated cashew kernel flour) and sample C (hot-pressed defatted cashew kernel flour) respective-

ly. It was observed that forty-two (42) bioactive compounds were revealed in Sample A (Undefatted cashew kernel flour), hot pressed defatted cashew kernel flour revealed thirty-six (36) bioactive compounds while thirty-three (33) bioactive compounds were identified in hot pressed cashew kernel flour by GC-MS. The peaks observed in the chromatogram were integrated and compared with the database of spectrum of known components stored in the GC-MS library. The main bioactive compounds observed in the three samples were Propanoic acid, 9-octadecenoic acid (Z), 5-hydroxylfurfural, Isobutyraldyhde Propylhydrazone, Methyl tetradecanoate, n-Hexadecanoic acid, Dodecanoic acid, 1H-pyrrole, Acetic acid and Hydrazine. Furfural, 5-Hydroxymethyl furfural, n-Hexadecanoic acid, Dodecanoic acid was observed in both defatted and un-defatted samples; Isobutyraldyhde Propylhydrazone was observed in un-defatted sample and hot-pressed defatted sample; Propanoic acid, Methyl tetradecanoate, 9-octadecenoic acid was observed in undefatted and hot-pressed defatted sample; while 1H-pyrrole, Acetic acid, Hydrazine was observed in the both defatted samples. 5-hydroxymethylfurfural (HMF) is an important marker of food processing [32-33] and food storage extensively used as an important parameter to retain food freshness and quality [33]. It has been reported by [34-35] that propanoic acid (PA) lowers the level of fatty acids in the liver and plasma, reduces the rate of food intake, exerts immunosuppressive actions and possibly improves tissue insulin sensitivity. Hence, high consumption of propanoic acid rich foods might be considered beneficial for the prevention of type 2 diabetes and obesity. Hexadecanoic acid is also known as Palmitic acid which is a most common saturated fatty acid in nature [36] comprising 20-30% of the lipids with 16-carbon chain. Dodecanoic acid also known as Lauric acid, is a saturated fatty acid with a 12-carbon atom chain, thus having many properties of medium-chain fatty acids [37]. Dodecanoic acid is a plant metabolite, an antibacterial agent and an algal metabolite [38].

Conclusion

Sample A (un-defatted cashew kernel flour) has the highest values in ferric reducing antioxidant property, total phenol, total flavanol, iron chelation, alpha amylase, alpha glucosidase and low in DPPH. The un-defatted cashew kernel flour has the highest antioxidant properties compared to the defatted cashew kernel flour. The main bioactive compounds present in both defatted and un-defatted cashew kernel flour observed were Furfural, 5-Hydroxymethyl furfural, n-Hexadecanoic acid, Dodecanoic acid. Most of the identified bioactive compounds in this study are fatty acid in nature and they are known to be antioxidant, anti-inflammatory and they are associated with the reduction of in cardiovascular disease they are rich in oleic acid and is responsible for reducing blood pressure. However, the presence of these bioactive compounds in *cashew kernel flour* suggests its vast pharmacological and medicinal potentials hence, this study may be useful to explore the pharmacological and biosynthetic activity.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Rebecca Olajumoke Oloniyo conceived and designed the research, analyzed the data and wrote the paper. Hannah Ojonemile Mepa performed the experiments. Rebecca Olajumoke Oloniyo and Cornelius Ojo Orishagbemi supervised the research. All of the authors have read and approved the final manuscript. This manuscript has not been submitted to, nor is under review at another journal or other publishing venue.

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References

1. Saboon Chaudhari SK, Arshad S, Amjad MS, Akhtar MS (2019) Natural compounds extracted from medicinal plants and their applications. Natural Bio-active Compounds: Production and Applications, 1: 193-207.

2. Afreen AB, Rasool F, Fatima M (2023) Bioactive properties of brown seaweed, Sargassum wightii and its nutritional, therapeutic potential and health benefits: A review. Journal of Environmental Biology, 44: 146-58.

3. Koren D, Kun S, Hegyesné Vecseri B et al. (2019) Study of antioxidant activity during the malting and brewing process. J Food Sci Technol 56: 3801–9.

4. Gulcin I (2012) Antioxidant activity of food constituents: an overview. Arch Toxicol, 86: 345-91.

5. Choudhary R, Singh A, Upadhyay A, Singh R, Thangalakshmi S et al. (2023). Exotic god fruit, persimmon (Diospyros kaki): Pharmacological importance and human health aspects. eFood, 4: e52.

6. Sruthi P, Naidu MM (2023) Cashew nut (Anacardium occidentale L) testa as a potential source of bioactive compounds: a review on its functional properties and valorization. Food Chemistry Advances, 100390.

7. Sruthi P, Roopavathi C, Naidu MM (2023) Profiling of phenolics in cashew nut (Anacardium occidentale L.) testa and evaluation of their antioxidant and antimicrobial properties. Food Bioscience, 51: 102246.

8. Kris-Etherton PM, Zhao G, Binkoski AE (2018) The effects of nuts on coronary heart disease risk. Nutrition Reviews, 59: 103-11.

9. Fernández-Montero A, Bes-Rastrollo M, Beunza JJ, Barrio-Lopez MT, de la Fuente-Arrillaga C et al. (2013) Nut consumption and incidence of metabolic syndrome after 6-year follow-up: the SUN (Seguimiento Universidad de Navarra, University of Navarra Follow-up) cohort. Public health nutrition, 16.

10. Bizzozero-Peroni B, Fernández-Rodríguez R, Martínez-Vizcaíno V, Garrido-Miguel M, Medrano M et al. (2023) Nut consumption is associated with a lower risk of depression in adults: A prospective analysis with data from the UK Biobank cohort. Clinical Nutrition, 42: 1728-36.

11. Rivas A, Romero A, Arcas M, Monteagudo C, Feriche B et al. (2013) Mediterranean diet and bone mineral density in two age groups of women. International Journal of Food Sciences and Nutrition, 64: 155–61.

12. Trox J, Vadivel V, Vetter W, Scherbaum V, Ute Gola et al. (2010) Bioactive Compounds in Cashew Nut (Anacardium occidentale L.) Kernels: Effect of Different Shelling. Methods Journal of agricultural and food chemistry. Journal of Agricultural and Food Chemistry, 58: 5341-46.

13. This day (2023) Federal Government: Nigeria earned \$250 million from cashew export in 2022. www.thisdaylive.com 2023/01/25

14. Mitchell JD, Pell SK, Bachelier JB, Warschefsky EJ, Joyce EM et al. (2022) Neotropical Anacardiaceae (cashew family). Brazilian Journal of Botany, 45: 139-80.

15. Malik JA, Bhadauria M (2020) Cashew Nut (Anacardium occidentale). Antioxidants in Vegetables and Nuts-Properties and Health Benefits, 539-57.

16. Babatunde OP, Adeigbe O, Sobowale O, Muyiwa A, Balogun S (2023) Cashew Production and Breeding in 5 West African Countries. Journal of Scientific Research and Reports, 29: 28-39.

17. Shobha SV, Krishnaswamy PR, Ravindranath B (2012) Phenolic lipid composition during development of cashew. Phytochem, 31: 2295–7.

18. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM (2016) Fatty acid profile, tocopherol., squalene and phytosterol content of Brazil, pecan, pine, pistachio and cashew nuts. Int. J. Food Sci. Nutr, 57: 219–28.

19. Govindaraju R, Ramasamy G (2021) Chemical Characterization and Physico Chemical Properties of Cashew nut (Anacardium occidentale) Oil and Cashew nut Shell Liquid. Journal of Compliance Engineering, 12: 1 – 5,

20. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food chemistry, 91: 571-7.

21. Oyaizu M (1986) Antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr, 44: 307-15.

22. Gyamfi MA, Yonamine M, Aniya Y (1999) Free-radical scavenging action of medicinal herbs from ghana: Thonningia sanguine on experimentally-induced liver injuries. Gen Pharmacol, 32: 661-7.

23. Minotti G, Aust SD (1987) The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. Journal of Biological Chemistry, 262: 1098-104.

24. Omoba, O.S., Obafaye, R.O., Salawu, S.O., Boligon, A.A., & Athayde, M.L. (2015). HPLC-DAD Phenolic Characterization and Antioxidant Activities of Ripe and Unripe Sweet Orange Peels. Antioxidants 4: 498-512.

25. Oloniyo RO, Omoba OS, Awolu OO (2021) Biochemical and antioxidant properties of cream and orange-fleshed sweet potato. Heliyon, 7: e06533.

26. Helkar PB, Sahoo AK, Patil NJ (2016) Review: Food industry by-products used as a functional food ingredient. International journal of waste resources, 6: 1-6.

27. Obafaye RO, Omoba OS (2018) Orange peel flour: A potential source of antioxidant and dietary fiber in pearl-millet biscuit. Journal of Food Biochemistry.

28. Jia W, Wu X, Kang X (2023) Integrated the embedding delivery system and targeted oxygen scavenger enhances free radical scavenging capacity. Food Chemistry: X: 100558.

29. Mahomoodally MF, Subratty AH, Gurib-Fakim A, Choudhary MI, Nahar Khan S (2012) Traditional medicinal herbs and food plants have the potential to inhibit key carbohydrate hydrolyzing enzymes in vitro and reduce postprandial blood glucose peaks in vivo. The Scientific World Journal.

30. Kwon SJ, Hwang SJ, Jung Y, Park HG, Kim GH et al. (2017) A synthetic Nitraria alkaloid, isonitramine protects pancreatic β--

cell and attenuates postprandial hyperglycemia. Metabolism, 70: 107-15.

31. Khatoon M, Islam E, Islam R, Rahman AA, Alam AK et al. (2013) Estimation of total phenol and in vitro antioxidant activity of Albizia procera leaves. BMC research notes, 6: 1-7.

32. Pérez-Burillo S, Jiménez-Zamora A, Párraga J, Rufián-Henares JA, Pastoriza S (2019) Furosine and 5-hydroxymethylfurfural as chemical markers of tea processing and storage. Food Control, 99: 73-8.

33. Choudhary A, Kumar V, Kumar S, Majid I, Aggarwal P et al. (2021) 5-Hydroxymethylfurfural (HMF) formation, occurrence and potential health concerns: Recent developments. Toxin Reviews, 40: 545-61.

34. Sa'ad H, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K (2010) Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1801: 1175-83.

35. Pradhan S, Panchali T, Paul B, Khatun A, Rao Jarapala S et al. (2020) Anti-obesity potentiality of Tapra fish (Opisthopterus tardoore) oil. Journal of Food Biochemistry, 44: e13448

36. Krishnaveni M, Dhanalakshmi R, Nandhini N (2014) GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of Gossypium seeds. Int. J. Pharm. Sci. Rev. Res, 27: 273-6.

37. Anzaku AA, Akyala JI, Juliet A, Obianuju EC (2017) Antibacterial activity of lauric acid on some selected clinical isolates. Ann. Clin. Lab. Res, 5: 1-5.

38. Shah Z, Badshah SL, Iqbal A, Shah Z, Emwas AH (2022) Investigation of important biochemical compounds from selected freshwater macroalgae and their role in agriculture. Chemical and Biological Technologies in Agriculture, 9: 9.